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Skin Cancer after Organ Transplantation

edited by
The SCOPE
Collaborative Group

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Steven T. Rosen, M.D., *Series Editor*

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Skin Cancer after Organ Transplantation

The SCOPE Collaborative Group

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Foreword

The life-promoting and life-enhancing benefits of solid organ transplantation are a major and fascinating medical advance, but come at the cost of the lifelong immunosuppression needed to prevent rejection of the donated organ. This induction and maintenance of impaired immunological surveillance is paralleled by significant increases in the incidence of specific cancers, of which skin cancers are numerically way out in front. Prolonged waiting times for organ transplantation, an increasing average age of recipients, and the improving long-term graft and patient survival are closely related to this trend towards steadily increasing rates of post-transplant malignancies and have shifted the concerns of the global transplant community towards the possibilities of post-transplant cancer.

Already the most common cancer in fair-skinned populations, keratinocyte skin cancers are increased a further 100 fold in organ transplant recipients.

Individual high-risk patients demonstrate accelerated carcinogenesis and may develop very large numbers of (predominantly) squamous cell carcinomas, tumours that are more likely to behave aggressively or metastasize in the context of a suppressed immune system.

This book explores the pathogenesis of transplant skin malignancies, including the immunological basis and contribution from specific drugs. Experts in the field recommend management strategies for preventing and treating transplant skin malignancies, with always an emphasis on a multidisciplinary approach. As scientists and clinicians strive together to develop effective pathophysiological concepts and clinical strategies in the face of this accelerated carcinogenesis, there is a real opportunity not only for advances in the treatment of transplant-related skin malignancies but also for translating these findings into effective skin cancer control in the general population.

Following the age of striving for sufficient prevention of acute rejection by developing ever more effective immunosuppressive agents, transplant medicine now has to face the challenge of direct and indirect consequences of lifelong impaired immunity. All disciplines in medicine are invited to contribute their knowledge, innovation, and strategies to aid transplant medicine in the rewarding struggle against malignancies in organ transplant recipients.

Peter Neuhaus

Acknowledgements

The management of skin cancer in organ transplant recipients is an evolving medical speciality, influenced by intensive clinical and basic research efforts in recent years, and inspired by the escalating burden of transplant-associated skin malignancies.

This monograph shall serve as an effective resource for an interdisciplinary readership and aspires to translate insights from basic skin cancer research into the practical steps in skin cancer prevention, diagnosis, and management as it specifically relates to organ transplant recipients. New diagnostic and therapeutic standards are included and commented on. Separate chapters have been devoted to skin cancer prevention and to the essential role of clinical studies in providing an evidence base and in improving treatment outcome.

The clinician will find interdisciplinary, personal experiences together with new concepts for his daily interaction with organ transplant patients.

We are indebted to our co-editors: Sylvie Euvrard, Charlotte Proby, Jan-Nico Bouwes Bavinck, Ed Geissler, Paul Harden and Jaques Dantal for their excellent cooperation and endeavour in producing this monograph. We are indebted to Petter Gjersvik for his invaluable editorial advice and skilful contribution to many of the topics presented herein.

The editors would like to thank all authors for their outstanding contributions to this monograph and for their interest and endeavour in the field of transplant oncology.

The publication of this monograph would not have been possible without the dedication, hard work and enthusiasm of the project coordinator, Birgit Hinrichs.

The skin cancer burden in organ transplant recipients is a growing challenge for us all.

Berlin, Germany

Eggert Stockfleth
Claas Ulrich

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Introduction – Historical Perspective

Georgios Katsanos and Vincent Donckier

"She was of divine race, not of men, in the fore part a lion, in the hinder a serpent, and in the middle a goat, breathing forth in terrible manner the force of blazing fire. . . ." This description by Homer of the mythical creature called Chimera is one of the first known bibliographic references supporting the idea of beings made out of several creatures joined together in a single one. The concept of combining parts of different bodies into one functioning entity is a very old one, expressed mainly in the forms of myths and incarnated via fearsome monsters (chimera), seductive legends (mermaids), luring nymphs (sirens), and many more.

This fictional concept started to materialize initially by the work of an Indian surgeon, Sushruta (1000 BC), who developed a technique to reconstruct large nasal defects by skin grafts, a technique still used in modern plastic surgery. Sushruta was the first surgeon ever recorded to perform transplantation with homologue tissue transfer in the form of skin grafts.

Tissue restoration is found again in the literature in 15 A.D. in the form of a miracle. St. Agatha was sentenced to "be bound to a pillar and her breasts be torn off with iron shears." She endured this prolonged and horrific torture, and she was left in a dungeon to die, only to be visited by St. Peter, who restored her breasts.

The first reference to organ transplantation for therapeutic purposes comes from China, where Hua-To (136–208 A.D.) allegedly replaced diseased organs with healthy ones in patients under analgesia. In the year 300 A.D., Cosmas and Damian performed the miracle of grafting a cadaveric limb onto a person with a diseased leg, marking the first reference to cadaveric grafts. In 1200 A.D., St. Anthony of Padua reported grafting the foot of a young man who had deliberately mutilated himself. All these references depict the development of the concept of organ and tissue transplantation and its evolution from myth, legend, and rumor through the centuries.

The voyage from fiction and myth to reality proved to be a long one, as the dark ages cast a thick shadow upon all scientific development. In the 16th century, the Italian surgeon Gasparo Tagliacozi revived the ancient Indian method of nose

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reconstruction and further developed it by using skin grafts from the inner arm in a two-stage reconstruction. The 17th century is marked by the work of John Hunter, an extraordinary experimental surgeon from Scotland who worked with autografts. One of his famous experiments was the autotransplantation of a cock's claw to his comb.

In 1901, Karl Landsteiner, an Austrian physician, described the first three human blood groups, A, B, and O, and 1 year later, Decastrello and Sturli found the fourth blood type, AB. Landsteiner received the Nobel Prize for his work in 1930. By 1907 blood transfusion became safe, as Reuben Ottenberg performed the first blood transfusion using blood typing and cross-matching.

In the beginning of the 20th century, a famous figure of surgery appeared in the literature, named Alexis Carrel. Born in Lyon in 1873 and trained in France, this skilled experimental surgeon wrote in 1906: "The question of the transplantation of organs in man is a very serious one and difficult, for will the transplanted organ remain and function normally for a long period of time? Another difficulty would be that of finding organs suitable for transplantation into man. A process of immunization would no doubt be necessary before the organs of animals would be suitable for transplantation into man. Organs from a person killed by accident would no doubt be suitable." Carrel described a technique of effective vascular terminoterminal anastomosis, resolving some major technical difficulties of organ transplantation such as graft thrombosis, opening thus the gates for the realization of organ grafting.

This technical advance marks the beginning of a new era in transplantation, the era of multidisciplinary medicine. Surgeons soon realized that overcoming technical difficulties of surgical practice was just the beginning of a difficult journey as immunological issues began to emerge. Remarkably also, in 1910 Carrel intuitively described the problem of graft rejection: ". . .the changes undergone by the organ would be due to the influence of the host, that is, biological factors." From there on, biology, medicine and surgery would have to advance side by side in order to achieve the miracle of transplantation in the form we know it today.

In 1914, Murphy observed that rejected organs are infiltrated by lymphocytes, and with subsequent experimental studies he showed that lymphocytopenia inhibits rejection. Murphy was one of the first researchers to implicate the role of cellular immunity in the rejection process.

In the beginning of World War II, a British medical researcher named Peter Medawar, intrigued by the treatment of burned aviators, focused his research on their treatment with skin grafts. Essentially, when comparing the fate of skin graft taken from the patient itself (autograft) or from another person – the donor – (allograft), Medawar clearly identified the phenomenon of rejection and paved the way for a comprehensive approach to transplantation immunity. By extending his curiosity to animal models, Medawar later demonstrated in mice that full acceptance of foreign skin graft (allograft) could be actively induced by neonatal injection of hematopoietic cells from the donor strain. These pioneer works build the fundamental grounds for the concept of self- and non-self immune recognition and subsequently, for the definition of transplantation tolerance. In the same period, Australian Frank Macfarlane Burnet published his conclusions on immune tolerance and rejection. Medawar and Burnet shared the Nobel Prize for their work in 1960.

The foundations of modern immunology having been laid, the necessity of immunosuppression became evident. The first method of immunosuppression was total body irradiation, characterized by Murray as "blunt and unpredictable." In 1962, the discovery of azathioprine by Nobel Prize laureates Gertrude Elion and George Hitchings and then the discovery of cyclosporine 10 years later, in 1972, by the Swiss biochemist Jean-François Borel, marked the beginning of a new saga, that of organ replacement.

The first organ to be successfully transplanted was the kidney. In 1954, Murray successfully performed kidney transplantation between two monozygotic twins with excellent results. In 1958 Murray, in Boston, and Hamburger, in Paris, started performing a series of human kidney transplantations, initially using total body irradiation as immunosuppression and later the available immunosuppressive drugs.

The success of kidney transplantation sparked the hopes of replacing other organs, and in 1966 W.D. Kelly performed the first human, whole-organ pancreatic transplantation for treatment of type 1 diabetes mellitus. However, this important breakthrough was marked initially by poor results, and very few pancreatic transplantations were performed until 1978, when the combination of newer immunosuppressive drugs and innovative surgical methods yielded acceptable results.

The first human lung transplantation was performed by D. Hardy and his colleagues at the University of Mississippi Medical Center in 1963. The 58-year-old patient died 8 days after the operation of renal complications. Seven years later, Belgian doctors of the University of Ghent performed a successful pulmonary transplantation in a patient with end-stage lung disease. Their patient survived for 10 months.

In 1963, Thomas Startzl performed the first orthotopic liver transplantation. Initial results were disappointing, but Startzl's perseverance and extraordinary surgical skills prevailed, and liver transplantation became a reality. In 1967 Christian Barnard performed a cardiac transplantation in a 54-year-old patient. The operation was successful, and the transplanted heart functioned for 18 days, when the patient succumbed to pneumonia.

Although small bowel transplantation was first performed before 1970, the ubiquitous rejection and total graft failure at the time discouraged the surgical community. However, with the cyclosporine revolution the interest in small bowel grafting was revived and along with the modern immunosuppressive agents, the first successful small bowel transplantation with long-term survival was performed in Germany in 1988 with a graft survival of 4 years.

An important date is the year 1967, when Jan van Rood founded Eurotransplant in an effort to coordinate and optimize organ allocation. The model of Eurotransplant is to establish a central registration of patients on waiting lists and then organize transparently the organ allocation according to equitable medical criteria.

Somehow victims of their success, transplant programs rapidly evolved, and new medical and ethical problems emerged, such as organ shortage, the need to define donor legislation, and priority criteria. In 1984, the National Organ Transplant Act in the United Kingdom laid solid foundations in the medico-legal aspect of human transplantation, setting an elaborate frame for further development in this field by

establishing the Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients. Yet, major concerns remain concerning illegal or unethical activities, such as organ trafficking or transplant tourism.

After a long voyage through the centuries and with the contribution of great minds, organ transplantation is now a reality in every day medical practice. In multidisciplinary coordinated efforts, involving surgeons, physicians, anesthesiologists, immunologists, and researchers across the world, many obstacles have been tackled. Later advancements have come from the technical side, such as the development of living donor transplantations, but also from the pharmalogical side, including the discovery of tacrolimus in 1990, daclizumab in 1997, and sirolimus in 1999. Major challenges have still to be faced, notably, the long-term toxicity of immunosuppressive agents and the problem of organ shortage. These are the key points to improve long-term quality of life of transplant recipients but also to reduce the mortality while waiting for transplantation. As a matter of fact, chronic immunosuppression now represents the leading cause of morbidity and mortality after organ transplantation. Many efforts are currently being made to design new therapeutic strategies, aiming at reducing or discontinuing post-transplant immunosuppression, establishing the so-called transplantation tolerance. In parallel, great hopes are also generated by stem cell researchers as an alternative to whole organ transplantation. Scientists at the University of Minnesota managed to create a functioning rat's heart from the animal's stem cells in the beginning of 2008, opening a door to custom organ creation from the recipient's cells, alleviating any need for immunosuppression.

The future of transplantation is colorfully depicted by the quote of Dr. Doris Taylor of the University of Minnesota: "...What we've done, is hopefully open a door to the idea that we can actually begin to build not just pieces of tissue and organs, but build organs...our hope is that if you need it, we can make it."

Skin Cancer After Transplantation: Where Did We Come From, Where Do We Go?

Robin Marks

When Paul Gerson Unna first described a possible relationship between sunlight and development of cutaneous epithelioma, he would have had no idea of the impending public health epidemic of these tumours to be seen in the 100 years following his publication.

The incidence of sun-related skin tumours, including melanoma, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC), has been increasing in virtually every fair-skinned population in which they have been studied throughout the world. Nonmelanoma skin cancers (SCC and BCC) are now the most common cancers in Australia, occurring at least three times more commonly than all other cancers combined. By virtue of their number, they now comprise the biggest burden of all cancers to the health budget in Australia. Variations on this exist in many other countries where there are fair-skinned populations exposing large amounts of their skin to hot sunny climates. In Australia, the latest data suggest that at least two of three people born in the country will eventually develop one of the nonmelanoma skin cancers (NMSCs).

There has been increasing awareness of the public health implications of skin cancer, as was initially reported in the incidence data. The mortality from NMSC has been traditionally very low, with the majority being from SCC. Many organisations have started public health programs on prevention and early detection of skin cancer. Much research is being done into the basic pathogenesis of these tumours, and our knowledge has expanded enormously. There is also much work being done on new forms of treatment, particularly topical treatments, which will gradually replace surgery over time.

In the public health area there have been some remarkable changes in knowledge, attitudes, and behaviours in the sunlight in some countries, Australia in particular. There are early data suggesting a reversal in the increasing incidence and mortality caused by melanoma in younger cohorts in Australia and a similar change in incidence of BCC. But does this mean that we can sit back and relax with the reassurance that it will all be over soon? Of course the answer is no. There is a "new kid

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on the block" – organ transplantation – and this has brought a new dimension to the epidemic of skin cancer.

Whether or not people develop a skin cancer is a combination of their genetic susceptibility and the circumstances in which they have lived their life. Even if they do achieve the right combination to initiate the cellular changes in keratinocytes that we recognise as dysplasia, a variety of mechanisms will act to control further tumour development, immunological mechanisms in particular. A reduction in, or a lack of, these immunological control mechanisms will inevitably lead to an increased ease of induction of what we recognise as invasive cancer. And that is exactly what is being found in patients who have undergone organ transplantation. The immunological surveillance and control currently reduced to prevent transplant rejection is the same as that preventing tumour formation. Thus, predictably, successful organ transplantation is followed by an increased risk of skin cancer, particularly SCC.

Following organ transplantation, it is not just the formation of one or two tumours that is the concern. Very large numbers of tumours, SCCs in particular, develop over time in those at risk. It creates an enormous challenge to everyone involved, both patients and those responsible for their care. So where do we go from here? What can be done?

There are different approaches to disease control. The first and perhaps the most ideal would be to reduce an individual's genetic susceptibility to develop the disease, in this case skin cancer. Ironically, at the moment this is the most difficult of the approaches, as it is the area in which we have the least knowledge and the least ability to bring about the changes necessary.

Another problem with this simplistic-sounding approach is that by the time many people require their organ transplantation, they have often gone a long way along the pathway that leads to tumour formation. This means, for example, that they may have actinic keratoses already and thus reducing genetic susceptibility would occur too late.

Another approach might be to develop more targeted, or more specific, immunosuppression. Ideally, this would reduce the risk of transplant rejection but would not reduce tumour rejection. There is a promise of this with, for instance, the mTOR inhibitors, but a long-term benefit in skin cancer reduction is not yet proven and must be balanced against other, possibly less favourable, drug characteristics.

The public health approach to skin cancer control would comprise the two classical components. The first component is to deal with the problem people have now, that is, incipient or overt tumours. These must be detected early, either in the "precancerous" stage, or very early in the truly invasive phase, thus allowing an easy cure to be achieved with relatively simple treatment.

The second component of a public health approach is the long-term goal of trying to prevent skin cancer: This is to reduce environmental exposure to the carcinogen that precipitates the tumours in susceptible people: sunlight. The ideal here is to commence photoprotection at a very early pretransplant stage and to continue it to an almost obsessional degree post transplant. Complications of excessive photoprotection, such as vitamin D deficiency, could be easily overcome through dietary vitamin D supplementation.

The final approach is the “when all else fails-approach.” There is the need for better skin cancer treatments that are effective, simple, ideally applied by the patient themselves, and that are not too expensive. As transplant patients frequently have diffuse sun-related changes in their skin, it is necessary to take a broader view of the therapeutic approach. Some people have termed this “treating the field” rather than just treating individual tumours if or when they become clinically apparent.

So, in summary, the need for a book such as this one is a clear indication that the development of skin cancer in patients undergoing organ transplantation is not just a problem now. It also will be an increasing problem in the future, as an increasing number of people are treated with this therapeutic approach to organ failure.

There is no doubt that there have been very many advances over the years in all the components underpinning successful organ transplantation. It is to be hoped that, by exploring all or many of the possibilities to deal with skin cancer outlined here, this side effect of organ transplantation will become less of a problem in the future.

Part I
Transplant Medicine

De Novo Post-Transplantation Malignancies: Incidence and Risk Factors

Jacques Dantal

Introduction

An increased incidence of cancer in immunodeficient and immunosuppressed patients is now well established. Improvements in transplantation procedures and immunosuppressive therapies have resulted in better short-term and long-term graft survival, but immunosuppression exposes patients to long-term complications [1]. Malignancies are becoming the greatest limiting factor for patient and graft survival following kidney transplantation, even as incidence of death related to cardiovascular diseases and infections is decreasing [2]. Cancers are frequently more aggressive in transplant patients and are more likely to be fatal than would be expected in patients who have not undergone transplantation [3].

The majority of information concerning cancer in transplant patients comes from registries such as the CTTR (Cincinnati Transplant Tumor Registry), created by Penn in 1970 [4], or the ANTR (Australian and New Zealand Transplant Registry [5] in the case of kidney transplantation, and from many single-center [6–9] or regional [10–12] registries studies. Nevertheless, the majority of studies reporting on the incidence and risk factors for de novo cancers post transplantation have used different control populations and methodologies and have focused on the most frequent type of tumors, which are virus-related cancers such as nonmelanoma skin cancers.

Overall Incidence of De Novo Cancer After Organ Transplantation

It is clear that de novo post-transplantation malignancies are a problem shared by all transplant patients regardless of the organ that has been transplanted.

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Kidney Transplantation

The global reported cancer incidence in renal transplant recipients ranges from 2.3% to 31%, depending on the report in question (for review, see reference [13]). This large variation is mainly caused by differences in the length of follow-up. In fact, the incidence is clearly underestimated in some reports because of the lack of long-term follow-up and the absence of systematic detection of cancer, especially cancers affecting the skin. For example, a maximum cumulative incidence above 75% and 33% has been observed in patients followed for more than 30 years, for skin and non-skin cancers, respectively [14]. Nevertheless, a precise assessment of cancer incidence is difficult in the context of small cohorts or single-center studies, and cancer incidence must not be calculated as a global percentage that is biased by the large group of recently transplanted patients.

More informative results from two large studies were reported recently. Kasiske et al. compared the incidence of cancers by linking the data from the United States Renal Data System (USRDS) and the Medicare billing claims for cancer [15]. This study was performed over a 3-year follow-up period, among three large populations (more than 35,000 transplant patients, the general population, and patients awaiting a kidney transplantation) between 1995 and 2001. In transplant recipients, the cumulative incidence of nonmelanoma skin cancer (NMSC) and non-skin cancer (but including melanoma and Kaposi's sarcoma, KS) was 7.43% and 7.45%, respectively. Compared to the general population, the risk of cancer was increased for all types of tumors, but when compared to the waiting list patients, an increased risk of cancer emerged for only some types, mainly virus-related cancers (NMSC, KS, and non-Hodgkin's lymphoma).

The second study, involving more than 28,500 Australian renal transplant recipients and linking data from ANTR and the Australian National Cancer Statistics Clearing House, found similar results [16]. The overall incidence of cancer, excluding NMSC and those known to be related to end-stage renal disease, was clearly increased after transplantation [standardized incidence ratio (SIR): 3.27; 95% confidence interval (CI): 3.09–3.46]. Compared to the general population, the risk of cancer was slightly increased during end-stage renal failure and dialysis (SIR: 1.16; CI 1.08–1.25; and SIR: 1.35; CI: 1.27–1.45, respectively). In addition, most cancers with a risk in excess of 3 were suspected to be of viral origin (see below).

Heart and Lung Transplantation

The Registry of the International Society for Heart and Lung Transplantation showed that 3.1%, 16.1%, and 26.2% of heart transplant recipients presented malignancies after 1, 5, and 8 years of follow-up, respectively [16], and 4%, 18%, and 30% of lung transplant recipients presented malignancies after 1, 7, and 9 years, respectively. The authors suggested that the risk of developing NMSC was greater in heart than in kidney transplant patients [17], but that subsequent recurrence of

NMSC was more frequent in kidney recipients [18]. The results from the Collaborative Transplant Study (CTS) demonstrated that, relative to the general population, the risk of non-Hodgkin's lymphoma was highly increased in lung as well as in heart transplant recipients compared to recipients of kidneys from deceased donors (5-year relative risk at 239 and 58.6 compared to 12.3) [19].

Liver Transplantation

An increased incidence of de novo cancer has also been reported after liver transplantation. However, very few studies have assessed the extent of the increased risk compared to the general population. One recent study reported the observed cancer incidence in a cohort of 1,778 patients transplanted in England and Wales between 1982 and 2004, compared to a matched control group [20]. In this publication, 7.9% of all patients developed some form of cancer (median follow-up of 65 months) and had an increased incidence for all types of tumors (SIR: 2.07; CI: 1.74–2.44). Another study, in the United States, reported a 3.16-fold increase in cancer incidence (skin carcinoma excluded from the analysis) in patients surviving for more than 5 years after liver transplantation compared to the general population [21]. Finally, the cumulative incidence of de novo cancer at 5, 10, and 15 years after liver transplantation was 2%, 6%, and 15%, respectively [22], apparently lower than that reported for other types of organ transplantation.

Type of Malignancy

Once the post-transplant cancer incidence has been established, it is crucial to ascertain the distribution pattern of the different types of neoplasia. In the CTTR, 40% of the tumors registered affected the skin, 11% were lymphoproliferative disorders, 4% were KS, 4% affected the cervix and kidney, and 3% were vulva and perineum cancers [23, 24]. Although all types of malignancies were reported in the transplant population, a specific pattern could be distinguished in these immunosuppressed patients. Compared to the general population, cancer distribution in patients from the CTTR registry showed an increase from 6% to 24% for lymphomas, from almost 0% to 4% for KS, from 2% to 5% for kidney cancer, and from 0.7% to 3% for vulva cancer. A similar pattern of distribution was reported for all the registries [25, 26].

Perhaps the most interesting analyses come from large cohort studies of transplant patients in comparison with matched control populations (the general population or patients awaiting transplantation or under dialysis). These studies enable calculation of the risk ratio (RR) or standardized incidence ratio (SIR). In the USRDS/Medicare analysis concerning renal transplant recipients [15], the incidence of common cancers, such as those of the breast, prostate, lung, or colon, was roughly 2-fold higher than that observed in the general population after 3 years of

follow-up. Nevertheless, when compared to patients on the transplant waiting list, the incidence of most malignancies was similar, with the exception of KS (9-fold increase), non-Hodgkin's lymphoma (3.3-fold increase), NMSC (2.6-fold increase), and melanoma and cancer of the mouth (2.2-fold increase in both cases). Renal carcinomas were also increased (by 39%) as well as leukemia and esophageal cancers. Finally, prostate and ovarian cancers were less frequently observed after renal transplantation than during the period on the waiting list.

The major shortcomings of this study are a short duration of follow-up (3 years) and a possible bias in the population studied, not reflecting the whole transplant population, but rather patients who have Medicare as their primary provider (47% of the whole population). In another study, an increased incidence of only a few types of cancer was reported before renal replacement therapy and for some patients during dialysis, whereas an increased incidence of a wide range of types was observed after kidney transplantation [29]. The cancers that were found to have an increased incidence during end-stage renal disease, but before dialysis, were limited in type, including non-Hodgkin's lymphoma and KS, suggesting some degree of immune deficit resulting from renal failure or a role of the immunosuppressive medication given to treat certain renal diseases.

Some publications concerning the dialysis period reported no [27] or only a slightly increased [28] cancer risk. In a large international collaborative study, after a mean follow-up of 2.5 years, the cancer incidence was found to be increased in patients undergoing dialysis, especially for cancers of the kidney (RR: 3.6; CI: 3.45–3.76), bladder (RR: 1.5; CI: 1.42–1.57), and thyroid and other endocrine glands (RR: 2.28; CI: 2.03–2.54) [28]. Among the cancers found to have an increased incidence in dialysis patients, those of viral etiology were very common (KS, liver, cervix, tongue) [29]. In addition, the risk of cancer was particularly high in young dialysis patients (less than 35 years of age; RR: 3.68; CI: 3.39–3.99).

After transplantation, no cancers have been reported as having a lower incidence than that observed in the general population. A risk similar to the general population has been frequently observed for the more common cancers such as prostate and breast cancer [29]. In contrast, an increased incidence of prostate cancer (SIR: 3.6; CI: 1.55–7.06) was observed in a French cohort of renal transplant recipients in comparison to the age-matched general population [30].

It has been clearly demonstrated that, after transplantation, cancers known or suspected to be related to viral agents are the most representative types [29]. Among the 18 types of cancer with a relative risk above 3, more than 50% are related to viral infection: KS caused by human herpesvirus 8, non-Hodgkin's lymphoma, and Hodgkin's disease related to Epstein–Barr virus, liver cancer related to hepatitis B or C, and the large group of cancers related to human papilloma virus (NMSC, tongue, mouth, vulva, vagina, and penis). For some other frequent localizations, the evidence for human papilloma virus involvement is limited or inconclusive (nasal cavity, esophagus, eye, salivary gland), and only for a few remaining localizations is the association with a viral infection not actually suspected. In addition, other cancers related to human papilloma virus infection were also significantly increased with a relative risk below 3 (cervical and anal cancers), lending credence to a pivotal

role for viral infection in the development of cancer in immunosuppressed transplant recipients.

The incidence of cancers known to be associated or at the origin of end-stage renal failure, such as urothelial and kidney cancers or myeloma, was increased for all the periods studied (before dialysis, during dialysis, and after transplantation). Finally, analysis of these three periods of renal disease revealed that preexisting personal factors and/or end-stage renal failure or the dialysis procedure itself were not involved in the increased cancer incidence observed after kidney transplantation.

Although the incidence of NMSC, KS, and lymphoproliferative disorders was also increased regardless of the type of transplantation, the incidence of some other types of cancer, such as those related to organ-specific diseases, could also be increased after immunosuppression. After heart transplantation, the incidence of bronchogenic carcinoma remains controversial. Some authors have reported an incidence similar to that observed in the general population [31]. Lung cancer is one of the most common causes of cancer-related deaths in the United States. Accordingly, lung cancer in transplant patients would be expected to occur on the basis of chance alone. Nevertheless, other authors have reported an increased incidence of lung cancer in transplant patients [32]. This type of cancer occurred in 0.28% to 4.1% of heart and lung transplant recipients, and the risk was approximately 20 to 25 times that of the general population [33, 34]. In addition, one study reported an increased incidence of primary lung cancer after single lung transplantation compared to a matched population of bilateral lung recipients with comparable native disease, age, and tobacco history [34]. However, these cancers were frequently diagnosed after systematic X-ray examination whereas chest CT screening is recommended in high-risk patients (>10 pack/year smoking history) [35].

In some publications, colon and oropharyngeal cancers are reported as having a high overall incidence subsequent to liver transplantation. Colon carcinoma represents 3% to 14% of all tumors observed with an incidence of less than 1% in most of the series reported, but the relative risk could be as high as 12.5 times that observed in the general population [22]. In the liver transplant population, this cancer could be related to the initial hepatic disease (i.e., primary sclerosing cholangitis), which is frequently associated with inflammatory bowel disease, especially ulcerative colitis. Although this subgroup of patients is at a high risk of developing colorectal carcinoma [36], the incidence of colic carcinoma is not increased in all studies of transplant recipients [37]. Oropharyngeal cancers presented an incidence ranging from 0.2% to 1.5% and represented up to 21.9% of the overall tumors in liver transplant patients [38,39]. These types of tumors are related to alcohol consumption and tobacco history and were only reported in patients requiring transplants for alcoholic liver cirrhosis [40]. When compared to nontransplant patients with similar risk factors, these cancers do not occur more frequently after liver transplantation [41]. Finally, after renal transplantation, hepatocarcinoma is a long-term complication for patients with hepatitis B and/or C infection [42], but after liver transplantation recurrence of viral infection is the main problem, and an increased incidence of de novo cancer is still questionable [43, 44].

Risk Factors for De Novo Cancer After Organ Transplantation

Post-transplant de novo malignancies are the result of complex interactions between immunological and nonimmunological factors. As for the general population, many conventional risk factors, such as age, gender, cigarette smoking, and sun exposure, contribute to the incidence of cancers. Age, which is known to be associated with a decrease in immunosurveillance, is a strong predictor of skin and non-skin malignancies after renal transplantation [12, 45]. In addition, being over the age of 60 is an independent risk factor for non-Hodgkin's lymphoma in transplant patients [45]. Moreover, the overall risk of developing a cancer after transplantation is thought to correlate closely with cumulative exposure to and type of immunosuppressive medication (as described in more depth in the section by E. Geissler in this volume). Finally, there is a strong association with nonimmunological factors such as individual risk factor and environmental exposure in the genesis of cancer for transplant recipients.

All data collected to date from single-center and registry studies indicate that transplant recipients are at risk of developing the types of cancer that have an established or suspected viral etiology [29]. Even when excluding these cancers from the analysis, the risk of cancer remains at least twice that observed in age-matched controls. Certain viral infections are clearly linked to the development of specific types of malignancies. For example, Epstein–Barr virus (EBV) is associated with post-transplantation lymphoproliferative disorders [see reference [46] for review], human herpesvirus 8 is frequently associated with Kaposi's sarcoma [see reference [47] for review], human papilloma virus is associated with a large variety of epithelial cancers (skin, cervix, penis, or anogenital carcinomas) [see reference [48] for review], and hepatitis virus B and C are linked to the development of hepatic cell carcinoma [see reference [49] for review]. All these viruses share the capacity to control cell cycle and division, as well as escape from apoptosis, thus sustaining transformation and cell growth. In addition, proliferating tumor cells can easily escape from T-cell surveillance when this is impaired by the immunosuppressive therapies. As a consequence, intense immunosuppression is the main risk factor for virus-related cancers. Post-transplant lymphoproliferative disorders (PTLD) are more frequently observed after T-cell depletion by antithymocyte globulins [19] or treatment with Orthoclone OKT3 [50], and cancers (mainly skin cancers) are more frequently observed in patients exposed to a high versus low regimen of cyclosporin A [6] or when exposed to a triple drug regimen combining cyclosporine A, azathioprine, and corticosteroids [51]. The role of immunosuppression is described in this book in more detail by P. Harden.

The origin of the initial disease could also influence the incidence of cancer. Patients who have renal failure as a consequence of type 1 diabetes have a relatively lower risk of cancer (RR: 0.11; CI: 0.03–0.47) [45]. Nevertheless, more studies are required to confirm this observation and to put forward possible explanations. Patients with a history of analgesic abuse [52] or use of Chinese herbs [53] are at a high risk of uroepithelial carcinoma. Some rare primary diseases, especially von Hippel–Lindau and Denys–Drash syndrome, are associated with an intrinsically

higher risk of developing Wilms' tumor [54, 55]. For these patients, genetic predisposition plays a role in the occurrence of de novo post-transplantation malignancies, although this hypothesis could be put forward for more common cases where the occurrence of different types of tumors (mainly skin and others cancers) occurred in the same patient [56, 57].

Cigarette smoking is also a well-known risk factor in the general population, and of course the risk of cancer is increased in immunosuppressed organ recipients with concurrent tobacco use [58]. Tobacco has a central role in the etiology of cancers of the lung, head, and neck [59], urinary tract (such as renal cell carcinoma) [60], bladder [61], and pancreas [62], and acts synergistically with alcohol, for oral and esophageal cancers, and probably with human papilloma virus (HPV) infection for some others cancers such as those affecting the cervix [63]. After renal transplantation, patients who smoke more than 25 packs per year at the time of transplantation present a relative cancer risk of 2.26 compared to their non-smoker counterparts (CI 1.51–4.32) [45]. Although active programs against smoking are able to decrease smoking rates, no studies have clearly analyzed the potential effects of smoking cessation before or after organ transplantation. Such analyses are difficult due to the relatively small groups of patients, and it has been suggested that giving up smoking more than 5 years before transplantation does not influence the risk of post-transplantation malignancies [45].

The type and frequency of malignancies vary widely between geographic regions. These differences may be explained by sun exposure, phototype, and prevalence of viral infections. The relationship between sun exposure and skin cancers, which is well established in the general population, also exists in transplant patients. The risk of developing skin carcinoma is extremely high in Australia and in the fair-skinned Caucasoid population [64], while these carcinomas are infrequent in the Asian population [65]. In Japan, the incidence of skin cancer is very low and Kaposi's sarcoma is almost absent. Here the most frequently observed carcinomas are those affecting the digestive organs, which is in accordance with the high incidence of these cancers in this country [66]. In the Chinese population, the distribution pattern of cancer after kidney transplantation was also found to be different from that observed in Western countries; bladder and renal cancers were the most frequent, followed by liver carcinoma (high prevalence of hepatitis B and C in South East Asia), but with no skin cancers [67]. In Saudi Arabia, Kaposi's sarcoma is the most frequent of the malignancies, which can be explained by the high prevalence of human herpesvirus 8 (HHV8) infection [68].

The high prevalence of transplant patients with a history of cancer is a growing problem. A waiting period of at least 2 years and up to 5 years is proposed to avoid any cancer recurrence [69]. This waiting period is not necessary for any types of in situ cancer, basal cell carcinoma, and incidentally discovered renal cancer. The global frequency of recurrence is 21% [70], with highest recurrence rates for breast carcinomas (23%), symptomatic renal carcinomas (27%), sarcomas (29%), bladder carcinomas (29%), nonmelanoma skin cancers (53%), and multiple myeloma (67%). The problem of recurrence is different from that of de novo cancer. It was recently suggested that, in patients suffering from a first cancer before

transplantation, the incidence of a second de novo cancer is the same as that of a first de novo cancer [71].

An association between splenectomy and cancer was reported in one study in patients with more than 10 years of follow-up after transplantation [45]. Such an association has been evoked in nontransplant patients, with an increased risk being reported in some studies [72] but not recognized in other more recent studies [73, 74]. In addition, despite a limited number of cases, there is no correlation between tumor risk and HLA mismatches [12], panel reactive antibodies [12], number of acute rejection episodes, or their treatment [45].

Conclusion

The cumulative incidence of cancer has continued to grow even in the late post-transplantation period. The age of transplant recipients and long-term graft survival have both increased during the last decade. If these trends continue, and long-term graft success is obviously a major goal, this will lead to an expected increased frequency of cancer. Understanding the relative risks and identifying the causes of the increased risks are critical to reduce the rising impact of cancer on the morbidity and mortality of transplant recipients. Nevertheless, a long-term follow-up may be necessary to define the effects of a particular factor, mainly immunosuppression, on the incidence of de novo cancer post transplantation.

Efforts to reduce immunosuppression, particularly for patients over the age of 45 years, and prevention of viral infections as well as efforts to discourage cigarette smoking, may help to reduce the risk of cancer after transplantation. Cancer prevention is now a major goal in the care of transplant recipients and defines new strategies such as limitation of associated risk factors, tailored immunosuppressive strategies (risk of allograft rejection/risk of cancer), and early diagnosis through regular and appropriate screening programs before and after transplantation.

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Immunosuppression

Edward K. Geissler

History of Transplant Immunosuppression

Transplantation began as a clinical discipline with basic science seeking to explain, improve, and prevent consequences of the clinical practice. The primary consequence is the well-known outcome of immunologically mediated organ transplant rejection. Indeed, basic science has been evolving, along with clinical practice, to find strategies aimed at downregulating the immune system to a level that will prevent allograft rejection. This is commonly referred to in the transplant world as "immunosuppression."

In general, organ transplantation and the development of immunosuppression can be separated into four discernible stages:

1. The discovery of the alloimmune response and development of surgical methods to transplant organs
2. Focus on discovering donor/recipient differences and inhibiting the immune system to prevent rejection
3. Developing and using more selective immunosuppression
4. Using strategies to minimize immunosuppression and engineer clinical tolerance

We are perhaps entering a fifth parallel stage, where approaches are being sought to address the long-term consequences of lifelong immunosuppression, including post-transplant malignancy.

Near the beginning of what is considered the modern organ transplantation era, Peter Medawar's experimental studies of skin grafts in the 1940s and early 1950s provided a basis for basic transplantation research, showing that the immune system was responsible for allograft rejection [1,2]. This work led to the modern theory that donor-specific tolerance was possible if donor hematopoietic cells were engrafted into recipients before solid organ transplantation. Essentially, if the hematopoietic system of the transplant recipient could be engineered to contain donor and recipient cells ("chimera"), a solid organ allograft from the same donor would no longer be

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recognized as foreign. This breakthrough in transplant medicine remains a cornerstone for research today. However, as is described later in this chapter, achieving a state of chimerism in recipients comes at a price that remains generally unacceptable in clinical transplantation. Hence, we continue to rely on immunosuppressive drugs to prevent allograft rejection.

From a technical perspective in the history of transplantation, methods were in place to surgically transplant kidney, heart, and liver by 1968. The first basic immunosuppressants (steroids, antilymphocyte globulins, and azathioprine) were discovered in the late 1950s and early 1960s, which allowed organs and hematopoietic tissue to be transplanted with some degree of success. Critically, however, determining or predicting outcome had not been possible because antigenic differences between donor and recipients were not fully understood. Critical advances led to the identification of the human leukocyte antigen (HLA) system by the late 1960s, which allowed for tissue typing so that the degree of the rejection response could be predicted, and the close matching required for bone marrow transplantation was made possible. Unfortunately, acute rejection remained a common important problem for more than a decade. The next advancements came from the discovery of distinct cell signaling pathways for controlling cell behavior that opened the way for development of more specific immunosuppressive drugs.

The first, and foremost, of this new age of immunosuppressive drugs was cyclosporine. Cyclosporine came regularly onto the clinical scene in the early 1980s, dramatically improving the risk-to-benefit ratio in organ transplantation. Besides the fact that acute rejection episodes could be kept under control with cyclosporine, the drug exhibited a more acceptable side-effect profile. The discovery of tacrolimus a decade later gave yet another option to control acute rejection, with seemingly even fewer side effects. As is discussed in more detail, cyclosporine and tacrolimus both function to inhibit calcineurin, defining, therefore, a class of immunosuppressants.

The development of mycophenolate mofetil (CellCept, Roche), and more recently the enteric-coated formulation of mycophenic acid, Myfortic (Novaris), has added to our armamentarium of immunosuppressive drugs that can enhance graft survival and reduce acute rejection episodes. Newer immunosuppressive drugs are continually being developed with the goal of maintaining graft function with a minimum of side effects. These advances have fundamentally changed the focus in transplant medicine from controlling acute rejection to developing strategies to prevent chronic rejection and engineer tolerance. However, what has also become apparent from this more recent era is that although acute rejection is largely controllable, there are long-term consequences of immunosuppressive drug use that reduce recipient survival, including in particular cardiovascular disease and malignancy.

One solution to the problem of long-term immunosuppression is to move away from using general pharmacologic immunosuppressive drugs and to develop strategies for inducing immunological clinical tolerance. The idea of inducing immunological tolerance is not new and, in fact, has been a central focus of research in transplantation since the early work of Medawar. Indeed, if the recipient's immune system can be stably manipulated so immunological cells no longer attack and damage the transplanted allograft, the current problems facing transplant recipients seem

to fade away. Although tolerance is plausible, as immunological tolerance can be engineered in specific animal models and because clinical tolerance has been documented in a few transplant patients, the underlying factors remain unknown and are not reproducible in humans. The complexity and heterogeneity of the human HLA system, coupled with the outbred nature of humans, makes reliably engineering tolerance an extreme challenge. A second approach that is being slowly, and cautiously, attempted is to combine tolerance strategies with minimized immunosuppression. However, as is discussed later in this chapter, a major question remains: Can we identify transplant recipients who could benefit from reduced immunosuppression and who are at increased risk for rejection? Nonetheless, at present we remain reliant on the continuous use of immunosuppressive drugs at some level to protect patients from transplant rejection.

The purpose of the following review is to provide perspective on how immunosuppressive drugs function to inhibit the immune system and to understand the consequences of their long-term use, including the development of cardiovascular complications, diabetes, chronic rejection, infectious disease, and malignancy. Of particular relevance to dermatologists is the development of nonmelanoma skin cancer, which is dramatically increased in transplant recipients. Moreover, according to the most recent Australian New Zealand Data Registry, deaths in transplant recipients from cancer have exceeded those from cardiovascular disease. In this respect, research is discussed that indicates malignancy may be reduced by selective use of certain classes of immunosuppressants. Suppressing the immune system has a broad spectrum of effects that we can only begin to understand by studying the specific effects of individual drugs.

Pharmacologic Immunosuppression

Calcineurin Inhibitors

The discovery of cyclosporine in 1972, which is derived from the soil fungus *Tolypocladium inflatum*, marked the beginning of a new era in organ transplantation. Although not originally recognized for this purpose, a few years later, in 1976, Borel and colleagues [3] described potent immunosuppressive effects of cyclosporine. Calne et al. were quick to recognize the phenomenal effects of this drug, and they performed the first testing in a small cohort of human renal transplant recipients that showed great promise with regard to efficacy [4]. These results led to larger international trials that basically confirmed their findings, which ushered in a whole new perspective in organ transplantation by the early 1980s. Moreover, the excellent results obtained in renal transplant recipients with cyclosporine gave incentive for expanding the transplantation of other organs, such as heart, liver, pancreas, and lung.

Cyclosporine functions as an immunosuppressant by inhibition of interleukin (IL)-2 gene transcription. More specifically, the hydrophobic cyclosporine molecule

passes through the cell membrane, where it associates with cyclophilin and inhibits a calcium/calmodulin-dependent phosphatase, calcineurin; hence, the designation calcineurin inhibitor (CNI). Binding of cyclosporine to cyclophilin allosterically interferes with the ability of this complex to dephosphorylate nuclear factor of activated T cells (NFAT), which, when dephosphorylated, can enter the nucleus and cause transcriptional upregulation of proinflammatory mRNAs, including IL-2. The end effect is inhibition of T-cell activation, producing a potent immunosuppressive effect (Fig. 1). A second important cytokine that is affected by cyclosporine is transforming growth factor-β (TGF-β). In this case, TGF-β transcription is increased, which has been implicated with the chronic and progressive fibrosis that is associated with its use [5]. Interestingly, TGF-β has also been associated with the development of malignancy in transplant recipients [6, 7].

Cyclosporine is very effective at preventing the acute immunological response against transplanted organs, and the use of this class of CNIs continues to dominate transplant medicine in terms of usage in allograft recipients. A second CNI, often used instead of cyclosporine, is called tacrolimus. Tacrolimus, similar to cyclosporine, blocks IL-2 gene transcription by inhibiting the phosphatase activity of calcineurin; however, this is accomplished through an interaction with FK-binding protein 12 (FKBP12). Considered as a class of immunosuppressive drugs, CNIs have contributed in a large part to 1-year graft survival rates near 90% (renal). Therefore, within most professional circles debating issues in transplantation, the

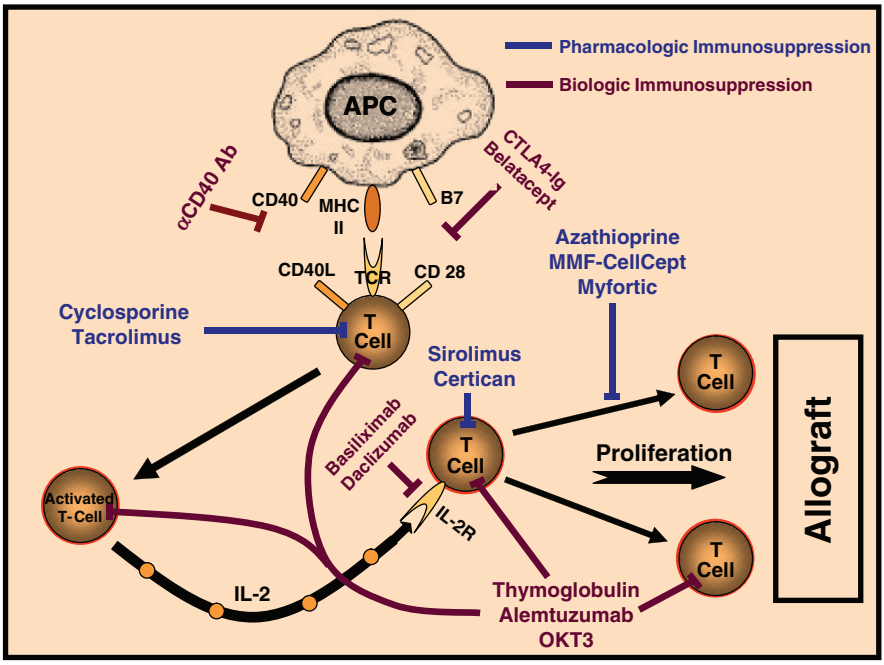

Fig. 1 Pharmacologic and biologic immunosuppression

treatment of acute rejection has been effectively minimized with the introduction of CNIs.

The more urgent question now has become how to reduce or eliminate the short-term and long-term side effects of CNI usage. Of primary concern is the development of hypertension and delayed graft function in the short term and nephrotoxicity, chronic allograft nephropathy (CAN), diabetes, and malignancy in the longer term. Ironically, transplanted kidneys often develop damage that appears to be directly related to the use of CNIs. The causes for early nephrotoxicity are not completely understood but relate in part to renal vasomotor effects that contribute to decreased creatinine clearance and systemic hypertension. It has also been reported that CNIs may directly damage renal tubular cells and cause arterial and arteriolar lesions. What remains unknown are the long-term effects of early delayed graft function, nephrotoxicity, and lifelong immunosuppression.

Renal allograft loss is attributed most commonly to cardiovascular disease and CAN. In the case of CNIs, CAN is likely a combination of both nephrotoxicity and chronic rejection, leading to glomerulopathy and arterial intimal thickening. At present, nephrotoxicity and chronic rejection remain unsolved problems with CNIs without a positive prospective. Furthermore, the development of malignancies in transplant recipients under CNI use has emerged as an increasing cause for morbidity and mortality. Cyclosporine has been shown to promote cancer cell invasiveness [6], metastasis [7], and tumor angiogenesis [8]; tacrolimus has demonstrated similar effects on tumor cells [9]. These effects appear to be dose related as patients on lower levels of CNIs are less likely to develop malignancy [10]. In summary, CNIs have proven to be excellent immunosuppressive agents in preventing acute rejection. However, having largely overcome this problem, we are now faced with a higher standard of improving long-term graft function and reducing systemic complications, including cardiovascular disease and malignancy.

In recognition of the problems associated with CNIs, a number of options are being actively explored. A first option is the possibility of discontinuing the use of CNIs at a later time after organ transplantation. The possible success of such an approach depends on whether transplant recipients on CNIs develop immunological tolerance to their allograft and whether new immunosuppressive drugs, which are not known to be nephrotoxic, can be substituted. Regarding the complete removal of CNIs, as well as an other immunosuppression, evidence thus far indicates that the risks outweigh the benefits. Results in uncontrolled trials using such extreme measures of CNI withdrawal have revealed a high incidence of acute rejection [11].

This finding brings to the forefront at least two other possible solutions. First, as mentioned, CNIs could be used early after transplantation and replaced in the longer term with other immunosuppressants, including mycophenolic acid prodrugs or mammalian target of rapamycin (mTOR) inhibitors (as is discussed later). Several trials are underway to withdraw CNIs, with the hypothesis that these less nephrotoxic compounds will improve long-term graft function. Although early results from these trials indicate promising results, we still do not know if the long-term results will be improved, or even acceptable. A second option is to administer CNIs at very low levels, so as to decrease side effects, by increasing doses of mycophenolic acid

prodrugs or mTOR inhibitors. Once again, however, long-term data are needed to test the risk-to-benefit profiles of this strategy, particularly with regard to antirejection efficacy and the whole new set of risk factors associated with the non-CNIs. Yet another option is to use antilymphocyte antibodies in the very early phase after organ transplantation, starting CNIs only after renal function is already recovered in the allograft. This strategy would help to avoid the early delayed graft function associated with CNI use, which could potentially increase long-term survival. In summary, the long-term results from CNI use necessitate that we consider limiting the implementation of CNIs, particularly in relation to renal transplantation.

Mycophenolic Acid Prodrugs

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA) that has potent immunosuppressive effects via several mechanisms. MPA has a long history, as it was discovered in 1893 by Gosio to have antibacterial activity [12] and was found more recently, in 1969, to be an inhibitor of inosine-5′-monophosphate dehydrogenase (IMPDH) [13]. IMPDH is a critical enzyme for synthesis of de novo guanosine nucleotides, which is an essential salvage pathway for specific cells. Importantly, MPA is a potent inhibitor of the type II isoform of IMPDH, which is the primary form expressed in activated T and B cells, resulting in lymphocyte inhibition. The effect is rather selective in that most other cells in the body express the type I isoform of IMPDH, for which MPA is fivefold less inhibitory. Lymphocyte dependence on de novo guanine nucleotide synthesis, combined with their expression of the type II enzyme isoform, make them exquisitely sensitive to MPA and thus an effective therapeutic means to inhibit immune responses.

The development of an MPA prodrug with good bioavailability properties, MMF (RS-61443), brought the concept from the laboratory to the clinical transplant setting in 1991 by Sollinger and Colleagues [14]. Preliminary trials in the first half of the 1990s indicated that MMF (developed by Roche as CellCept) could be effectively used to prevent and reverse allograft rejection in humans [15]. Most recently, another prodrug of MPA has been developed and used clinically; it is referred to as enteric-coated mycophenolate sodium (EC-MPS; myfortic, Novartis).

The primary positive aspects of MPA prodrugs stem from the fact that they provide immunosuppression, without being associated with nephrotoxicity or development of diabetes. In contrast to CNIs, MPA does not decrease IL-2 production, but rather upregulates the cyclin-dependent kinase inhibitor $p27^{kip1}$, interfering with cytokine-dependent signals necessary for early to mid G_1 phase events in T-cell proliferation. This, combined with blockage of de novo guanine nucleotide synthesis, provides a relatively potent and specific inhibition of lymphocyte stimulation. Furthermore, MPA has been shown to promote T-cell apoptosis, and among other effects, suppresses dendritic cell maturation, which could help reduce the inflammatory immune response to transplanted tissues. Another attribute of MPA prodrugs is that they do not cause an increase in TGF-β. As mentioned previously, TGF-β

is not only an important mediator of collagen deposition associated with CAN and other atherosclerotic disorders, it is a factor that promotes neoplastic cell aggressiveness [6]. At least theoretically, the use of MMF should indeed reduce nephrotoxicity and even possibly mitigate the problem of cancer in transplant recipients [16]. The attenuation of vascular disorders to some degree could provide an additional advantage to patients, because, simply stated, cardiovascular disease remains a primary cause of deaths in transplantation.

As with all immunosuppressive agents, MPA prodrugs also have side effects that must be considered. Most often occurring is the development of gastrointestinal symptoms and bone marrow suppression. It should also be noted that MPA prodrugs are used in the vast majority of patients as part of a multidrug immunosuppressive strategy to prevent acute allograft rejection, being combined with CNIs or mTOR inhibitors. Caution must be taken, therefore, in concluding whether MPA prodrugs will provide significant long-term beneficial effects with regard to organ function. Furthermore, the use of MPA prodrugs in a monotherapy immunosuppressive regimen remains questionable, although reports in liver transplant recipients suggest this may be possible [17]. Monotherapy with MPA prodrugs is most often considered when patients have a high risk for renal dysfunction; in this case, the relative risk of immunological rejection may be acceptable, considering the high likelihood of functional kidney loss. Most indications are, however, that MPA prodrugs will need to be combined with other forms of immunosuppression to provide adequate graft protection.

In terms of risk for malignancy in transplant recipients under treatment regimens using MPA prodrugs, answers remain forthcoming. Interestingly, MMF was originally developed as an antineoplastic agent because of its general antiproliferative action [18], although it was not taken beyond the experimental stage of development. Since these early studies, MMF has been shown to inhibit the development of lymphoid-derived tumor cells [19], and with specific regard to multiple myeloma, it induces caspase-dependent apoptosis of tumor cells [20]. Most recently, our research group has shown that MMF can have some inhibitory effects on tumor growth and development, but the effects appear to be small and perhaps will not prove to outweigh the negative effects of the immunosuppression component on antitumor immunity [16]. In support of these experimental data, one report on transplant registry data indicates a trend toward a decreased risk for malignancy when MMF is used clinically [21]. However, more experimental and clinical studies are clearly needed to better determine the effects of MPA prodrugs on cancer in transplant recipients.

m-TOR Inhibitors

mTOR inhibitors consist of rapamycin (Rapamune/sirolimus, Wyeth) and its derivatives (CCI779, Wyeth; RAD001/everolimus, Novartis), and analogue ap23573 (ARIAD Pharmaceuticals). Sirolimus and everolimus are the only two compounds presently approved for use in organ transplant recipients.

Rapamycin was originally discovered through a research program for antimicrobial agents from natural resources [22]. A soil sample collected at Easter Island (Rapa Nui), containing a strain of *Streptomyces hygroscopicus*, was shown to exhibit antifungal properties. Hence, the active substance was named rapamycin. Rapamycin is a lipophilic macrocyclic lacton with early described antimicrobial effects, but later it was discovered that this compound has profound immunosuppressive activity. Rapamycin was first characterized in 1975, and since that time its structure, biosynthesis, and binding partner(s) have been investigated [22]. By an interesting coincidence, rapamycin binds to intracellular FKBP12, the same molecule to which the CNI tacrolimus binds. Although the tacrolimus/FKBP12 complex forms a ternary complex with calcineurin, as described earlier, the rapamycin/FKBP12 complex has a different target, namely mTOR, and consequently a completely different mode of action. mTOR is a 289-kDa intracellular protein that has a pivotal regulatory role for cell growth and proliferation in many cell types. Although mTOR inhibition interferes with numerous intracellular pathways, one of its best known effects is G_1 cell-cycle arrest [23]. Owing to rapamycin's diverse effects on cell processes such as cell growth, angiogenesis, and survival, possible therapeutic applications are broadly based. Paradoxically, although originally examined because of its antifungal action, enthusiasm for rapamycin's therapeutic usefulness against infections was hindered by its potent immunosuppressive effect. The immunosuppressive action of rapamycin was insightfully pursued by Suren Sehgal [24], as he envisioned application in transplantation medicine.

The mechanism of rapamycin's immunosuppressive effect stems from its interaction with, and resulting inhibition of mTOR, downregulating p70S6 kinase activity and subsequent translation of specific mRNAs required for cell-cycle progression from G_1 to S phase. One of the most important cytokines that relies on this cell proliferation signaling pathway, at least in terms of the immune response, is IL-2. Therefore, unlike the CNIs, T cells can be activated in the presence of rapamycin but are subsequently unable to proliferate in response to IL-2, producing a therapeutic immunosuppressive effect. In 2000, after undergoing extensive testing, the mTOR inhibitor sirolimus received approval for use in renal transplant patients, particularly in light of evidence for low nephrotoxicity [25]. Although recent studies suggest sirolimus-containing regimens may reduce nephrotoxicity [26], this issue remains a significant concern when used in combination with CNIs [27,28]. At present, mTOR inhibitors are being extensively tested to explore strategies to reduce the interactions with CNIs that can cause nephrotoxicity, and importantly, to investigate other more positive activities of these drugs, including antifibrotic and vascular protective properties and, critically, their anticancer effects.

In contrast to CNIs mTOR inhibitors appear to affect a wide range of normal cell types ranging from cells of the immune system to smooth muscle cells [29] to endothelial cells [8]. These effects on smooth muscle and endothelial cells appear to be important for blood vessels, as rapamycin has been very successfully incorporated into vascular stents to prevent vascular reocclusion [30]. Ironically, although rapamycin has this protective effect on blood vessels, one of the most common side effects of mTOR inhibitor use is hyperlipidemia. The question then becomes

whether the protective action on blood vessels offsets the potential detrimental effects of lipidemia. The answer to this question will be the subject of further investigations.

Another general feature of mTOR inhibitors is their most recently uncovered anticancer activities. In fact, decades ago, rapamycin was shown to have direct cytotoxic effects on tumor cells, although the drug seemingly possessed less than desirable potency at high doses. The apparent weak cytotoxicity and its subsequently described immunosuppressive activity undoubtedly reduced early enthusiasm for its use in oncology [24]. However, a recent surge in scientific reports over the last few years from our own group [8, 31, 32], and now many others, have shown that rapamycin can be used experimentally at low doses as a very effective antitumor agent, in the face of its immunosuppressive effect. At the center of this activity is the fact that the mTOR pathway controls many signaling pathways that cancer cells and growing tumors need. For instance, rapamycin is an effective antiangiogenic agent through its ability to not only inhibit the transcription of vital factors, such as vascular endothelial growth factor (VEGF), but it also prevents signaling of VEGF to endothelial cells, thus blocking tumor blood vessel formation at multiple levels.

Other critical angiogenesis pathways are also influenced by mTOR, including hypoxia inducible factor-1α (HIF-1α). In addition to inhibiting angiogenesis, mTOR inhibitors have been shown to increase apoptosis of tumor cells [33], and control the activities of upstream dysregulated genes such as PTEN, AKT, TSC-1/2, and HER-2 [34]. Rapamycin has also been found to inhibit tumor metastasis through upregulation of E-cadherin [7]. Therefore, although the thought of using an immunosuppressive agent for treating cancer would have been considered incongruous some years ago, there is a growing optimism for its use in oncology.

What is especially relevant is its use in organ transplantation to reduce the problem of cancer. This is an extraordinary situation, as we need both immunosuppression to protect the graft and an anticancer effect that can reduce the alarming problem of cancer in this patient population. At least experimentally, mTOR inhibitors are uniquely suited for this task. Ongoing clinical studies are now being conducted to test this hypothesis. Because skin cancer is particularly relevant in immunosuppressed transplant recipients, clinical studies investigating this issue, which is discussed in more detail elsewhere in this book, are of a high priority.

Azathioprine

Azathioprine was one of the first immunosuppressants developed that allowed for successful renal transplantation in humans. Azathioprine is an imidazole derivative of 6-mercaptopurine that is more potent than its parent compound. Its immunosuppressive activity is derived from disruption of DNA and RNA synthesis, interfering, therefore, with lymphocyte proliferation in response to alloantigen activation. However, because azathioprine's effect is not specific for lymphocytes, other bodily systems are adversely affected, including particularly hematopoiesis. Another

significant drawback observed in the early years of immunosuppression was that although azathioprine was quite effective at preventing rejection, it proved ineffective at reversing acute rejection, hence the relatively poor allograft survival rates reported before the cyclosporine era. After the advent of cyclosporine, azathioprine continued to be used in combination therapy regimens, but its use has diminished substantially since the introduction of MMF, which is a good substitute because of its reduced side effects. Nonetheless, azathioprine continues to be used in some clinical transplant protocols.

From the perspective of post-transplant cancer, the influence of azathioprine is not clear. Although azathioprine disrupts cell division, which might be considered an attribute that would inhibit cancer cells, most evidence suggests the drug is rather associated with cancer development [35], both in patients who were treated for autoimmune disease and in transplant recipients. Besides the increased risk for lymphomas, azathioprine use correlates with the occurrence of a broad variety of solid neoplasms, including squamous cell carcinoma [36, 37]. However, because azathioprine is nearly always used in combination with other immunosuppressive substances, and at less intensive doses than needed in earlier times, the assessment of tumor development in the current transplant situation has become difficult. Therefore, a clear correlation of azathioprine with cancer development in transplant patients has not been established.

Corticosteroids

Corticosteroids have been used as a general immunosuppressant for many years and continue to be used extensively today. Glucocorticoids are normally synthesized in the adrenal cortex and naturally possess antiinflammatory properties that can be used to treat autoimmune disease, allergic reactions, and protect against allograft rejection. The mechanisms behind the antiinflammatory action include inhibition of arachidonic acid metabolism (thromboxane, prostacyclins), multiple effects on dendritic cell (antigen presentation) function, lymphotoxicity, and, importantly, a decrease in transcription of IL-1, thereby reducing IL-1-dependent activation of lymphocytes. This broad spectrum of effects results in potent antiinflammatory effects, but corticosteroids are relatively poor at immunosuppression per se. On the other hand, during acute rejection episodes, high-dose bolus steroids are very effective at reversing rejection.

Side effects from steroid therapy are multiple, and severe to the point that steroid avoidance is common practice. Among the side effects are osteoporosis, iatrogenic diabetes, obesity, hypertension, and water retention that creates a Cushingoid appearance. Interestingly, steroids have been associated with the occurrence of cancer [38], but ironically, it is also well known that steroids are actually used to treat certain types of cancer, including lymphomas. Unfortunately, because steroid therapy is used essentially only to support other immunosuppressive therapy against transplant rejection, its own effective role in cancer development is difficult to assess.

FTY720

FTY720 is a synthetic structural analogue of myriocin that shares structural and functional homology with sphingosine-1-phosphate (S1P), a natural ligand to several G protein-coupled receptors. Mechanistically, FTY720 is novel in that a primary mode of action is through sequestration of lymphocytes into secondary lymphoid organs, without affecting lymphocyte functions or properties [39]. FTY720 acts as an agonist and signals the sphingosine-1-phosphate (S1P) receptor family of molecules on lymphocytes, increasing their intrinsic mobility and responsiveness to chemokines [40]. The redirection of lymphocytes is toward the peripheral lymphoid tissues, thus trapping T cells away from the transplanted allograft and producing a novel form of immunosuppression.

Clinically, FTY720 combined with cyclosporine or everolimus has been tested for the treatment of allograft rejection. However, the substance has recently failed in phase III clinical trials and is no longer planned for use in organ transplantation. Nonetheless, its apparent mode of action has opened up other novel therapeutic possibilities for its use, including cancer treatment. Interestingly, FTY720 reduces integrin expression patterns on tumor cells, which has been shown to prevent adhesion and migration of tumor cells to extracellular matrix proteins [41]. In this same study, FTY720 suppressed the growth of murine breast cancer tumors. Furthermore, FTY720 has been shown to promote the apoptosis of tumor cells by inhibiting AKT activation [42], which is a key intracellular signaling molecule affecting cell apoptosis and proliferation. It is yet to be shown, however, whether FTY720 can be effective in a human cancer setting.

Antibody-Based Therapies

OKT3

OKT3 refers to a monoclonal antibody that binds to the ε-chain of the CD3 complex on T cells. Binding to the CD3 complex causes T-cell receptor internalization and ultimately depletion of these lymphocytes from the circulation. The primary application of this antibody is for difficult-to-reverse acute rejection episodes, but its use often can cause a cytokine release syndrome that has severe side effects, including fever and pulmonary edema. Although this agent continues to be used against recalcitrant rejection, a certain risk for development of post-transplant lymphoproliferative disease has been reported [43].

Thymoglobulin

There are many paralleling aspects to OKT3 with the use of thymoglobulin. Thymoglobulin is derived from rabbits with specificity against human thymocytes. This

antibody preparation is used both for induction therapy (near the time of transplantation) and as a treatment option for steroid-resistant rejection. In general, its side-effect profile is similar to OKT3 but typically milder in intensity. Most recently, the interest in using antibody induction therapy has increased in order to avoid early CNI use and because of the desire to develop strategies to induce immunological tolerance (as is discussed below), which may be best accomplished together with minimum concurrent pharmacologic immunosuppressive drugs immediately after transplantation.

IL-2 Receptor Antibodies

Another class of antibodies that serve clinically to prevent organ rejection is the anti-IL-2 receptor antibodies, basiliximab and daclizumab. Basiliximab is generated as a chimeric protein in which the murine variable regions are conjugated to the human immunoglobulin constant region of IgG_1; in slight contrast, daclizumab is almost completely humanized (IgG_1).

The basis for their effectiveness relates to the specificity of the monoclonal antibodies to the α-chain of the IL-2 receptor, which is expressed only on activated T cells; the β- and γ-chains are constitutively expressed on T cells. Therefore, these antibodies specifically target those T cells activated, and in the transplant situation, are lymphocytes primarily reacting to the allograft. The distinct advantage of this more selective targeting is that there is very low toxicity and fewer side effects in comparison to either OKT3 or thymoglobulin. Indeed, basiliximab and daclizumab have been shown to be effective against acute rejection in the early transplantation setting [44]. One possible disadvantage of targeting the IL-2 receptor, and more specifically CD25 (α-chain), is that the CD25-positive cell population is thought to harbor a large number of T regulatory cells capable of inducing allograft tolerance. However, studies are needed to test whether establishment of immunological tolerance is hindered in any respect by using these monoclonal antibodies.

Advanced Experimental Immunosuppression

Campath-1H (Alemtuzumab)

In 1984, Waldmann et al. gave the first description of an anti-CD52 monoclonal antibody that exhibited a profound ability to deplete lymphocytes [45]. Later, a humanized rat IgGκ monoclonal antibody was developed, which is referred to as Campath-1H, or alemtuzumab. With this anti-CD52 antibody, human lymphocytes, monocytes, macrophages, and thymocytes are massively depleted after treatment. Depletion of lymphocytes by greater than 99% can be seen, and the depletive effect is not only in the circulation, it affects cells in the peripheral lymph nodes as well. Notably, plasma cells and memory lymphocytes appear to be spared from the depletive effects of alemtuzumab. In terms of the depletion duration, monocytes

(3 months) recover before B cells (12 months), but T cells do not recover to normal levels for 3 years or more. In fact, because of this thorough depletive effect, and CD52 expression on certain malignant lymphoid cells, alemtuzumab has been approved for several years to treat lymphoma.

From an immunosuppressive perspective, not only was it proposed that the obvious effects of lymphocyte depletion with alemtuzumab would be effective, but also early hope was directed toward the potential for the drug to provide a window-of-opportunity for tolerance development after transplantation. This concept is based on the notion that after such a complete depletion following allografting, newly arising lymphocyte populations would recognize the transplant as "self" – hence, the potential for establishment of tolerance. Unfortunately, although alemtuzumab has been used successfully in conjunction with lower-dose CNIs to prevent rejection, tolerance has not been observed in this patient population. Speculation on the cause for this disappointing lack of tolerance is directed mostly toward the survival of memory T cells. The survival of memory T cells could, on the other hand, be related to the very low rate of infections found in alemtuzumab-treated patients. Patients on alemtuzumab could receive an additional benefit because CNIs can be reduced and steroids tapered away. Moreover, in a select group of patients experiencing delayed graft function, where CNIs are temporarily withheld, alemtuzumab provides an umbrella of protection to the kidneys from rejection.

The negative aspects of alemtuzumab use have not been fully addressed as studies have rarely been followed up for more than 5 years. So far, notable side effects may include more episodes of late rejection and a report of episodes of autoimmune disease (although not confirmed in additional studies); although cancer development was a serious concern, an increase in malignancy rate has not been reported until now. Alemtuzumab has a promising future, but more studies are needed to secure its place in transplantation medicine.

Costimulation Blockade (Balatacept)

It has long been recognized that the proper activation of T cells first requires the interaction of the T-cell receptor with a major histocompatibility complex (MHC) molecule on an antigen-presenting cell. A second critical signal required for T-cell activation involves "costimulatory" signals between the T cell and the antigen-presenting cell; one of these signals is between the CD28 receptor on T cells and CD80 (B7-1) and CD86 (B7-2) on the antigen-presenting cell. Importantly, there exists a homologue to CD28, called CTLA-4, which is a transiently expressed negative regulator on T cells that competes with CD28 for binding to B7 molecules. As such, one means of inhibiting the immune response is to simulate the activity of CTLA-4 by producing the same molecule in a soluble form that can compete with CD28 for B7 molecules.

The most well developed soluble CTLA-4 to date is called belatacept (LEA29Y), formed as a soluble recombinant immunoglobulin fusion protein with an extracellular domain of CTLA-4 conjoined with the Fc portion of IgG_1. Phase II trials with

this biological molecule have shown it to be effective in combination with MMF, corticosteroids, and basiliximab [46]. Renal function, compared to the same regimen where cyclosporine substituted for belatacept, showed a significant improvement. Side effects normally associated with CNI use were notably reduced (hypertension, hyperlipidemia, and diabetes). However, this agent is still in the testing phase, and long-term data are needed to fully evaluate its comparative efficacy. Although evidence for development of immunological tolerance was an original aim, the substance needs to be applied indefinitely, and thus allograft tolerance has not been shown to occur.

Tolerance Induction

Background

A recognized primary goal in organ transplantation, now for more than 50 years, has been to design protocols to induce immunological tolerance to an allograft. Before delving into this subject, it is appropriate to assign a definition to the term *tolerance*. From a more clinical perspective, tolerance can be defined as long-term function of an allograft, without the use of continuous immunosuppression, where immunity to infectious agents is maintained. Therefore, the immune response is more or less specifically downregulated to the allograft antigens but not to other foreign molecules.

Owing to the high basic standard of this definition, one questions whether there is enough evidence to suggest tolerance is even possible. Indeed, tolerance can be achieved, as was recognized as long ago as 1945, when Ray Owen observed that dizygotic cattle twins (that uniquely share a blood supply in utero) "tolerate" each other's blood cells into their adult life [47]. A few years later, Billingham, Brent, and Medawar reported their Nobel prize-winning work showing that infusion of allogeneic bone marrow into newborn mice (creating cellular chimerism) allows for skin from the bone marrow donor to be later transplanted successfully onto the recipient [1]. This is a principle that continues to hold true today, as is discussed in the next section. Moreover, it has been demonstrated in unique cases, where transplant recipients elected to have their immunosuppression completely withdrawn, that tolerance to the donor allograft can occur for over 30 years. This finding has been correlated, at least in part, by Will Burlingham's research group with the development of microchimerism in the recipient [48].

Bone Marrow Transplantation-Chimerism

The greatest question in transplantation research is how tolerance could be reliably created in transplant recipients. Scientists have tried to induce tolerance, both in the laboratory and in the clinic. In the laboratory, tolerance can be achieved with a high

success rate with different strategies, but in the clinic, there are critical limiting factors. The only successful attempts in the clinic have come from re-creation of the chimerism concept through bone marrow transplantation.

A good example comes from a study by Cosimi and Sachs, where a limited number of patients with multiple myeloma have received both a bone marrow transplant and a kidney allograft [49]. Although the chimeric state was not maintained indefinitely in recipients with this protocol, the patients have maintained their functional tolerance state for several years, with longer-term results pending. However, serious safety concerns regarding graft-versus-host disease, and recipient conditioning, make this strategy not presently acceptable for generalized use. Other attempts to create chimerism in human transplant recipients, for instance, using total lymphoid irradiation, have met with only limited success. Therefore, although the concept of inducing tolerance by creating a chimeric recipient is scientifically sound, application of such a strategy in humans is far from common practice.

Lymphocyte Depletion and Costimulation Blockade

As previously mentioned in this chapter, another strategy for inducing tolerance has been to reset the immune system by depleting T cells near the time of transplantation. The concept is logical, in that depletion of potential alloreactive T cells, and subsequent repopulation with developing T cells exposed to donor antigens (theoretically, now recognized as "self"), should conceivably produce a state of nonresponsiveness to the allograft. Unfortunately, attempts to deplete T cells with antibodies such as alemtuzumab have not produced tolerance. As pointed out before, it is possible that the lack of tolerogenic success with alemtuzumab stems from the fact that memory T cells are not depleted. Therefore, the research community is actively searching for T-cell depletion schemes that would be effective against memory cells.

However, each scheme has its negative aspect, and depletion of memory cells could have far-reaching effects as well, in that immunity to other alloantigens, such as infectious agents, could additionally be lost. Trials have also been initiated using thymoglobin induction with a tapering to low doses of pharmacologic immunosuppressive drugs, such as tacrolimus, but chronic allograft loss was noted later in these uncontrolled studies.

Other promising approaches have so far met with a similar lack of tolerance success. For instance, there was great hope in the use of costimulatory blocking agents for induction of tolerance. These agents performed well in experimental animal models and were able to induce an apparent tolerance to organ allografts. The two primary targets up until now have been the CD28-B7 and CD40-CD40L pathways of costimulation, as already discussed. Although belatacept appears to work well as an immunosuppressant, tolerance in transplant recipients is not evident. Tolerance has also not been observed with CD40 pathway blockade in nonhuman primates [50], and early discontinuation of the human trial because of reported thromboembolic

events did not allow for hints as to whether immunological tolerance occurred at some level [51]. A general conclusion from these initial human studies involving lymphocyte depletion and costimulation blockade does not indicate that a state of tolerance to human organ allografts will be as easily achieved as indicated in small animal models.

Pharmacologic Immunosuppression Minimization

The concept of "prope" tolerance, coined by Calne et al. [52], is based on the concept that lymphocyte depletion strategies, combined with minimized immunosuppression, could be a more reasonably effective strategy for organ transplant recipients. In fact, this general concept of reducing, but not eliminating, immunosuppressive drugs has gained favor with the lack of solid evidence in humans that tolerance can be reliably produced by blocking costimulation, or by early elimination of T cells. Rather, a more pragmatic approach would be to reduce early expansion of alloreactive cells with lymphocyte-depleting strategies, so that a minimal level of general immunosuppression could control long-term perturbances in alloreactivity that might occur.

There is already evidence that this might be a viable strategy. As described earlier, Knechtle et al. have shown that although alemtuzumab induction therapy does not appear to produce a tolerant state [53], maintenance immunosuppression (sirolimus) can be effective at a minimum level. Similar results have been reported in other studies using alemtuzumab in combination with tacrolimus and MMF [54], or with thymoglobulin in combination with CNIs [55]. There is promise for "prope" tolerance, but further testing is necessary in the long term.

One important aspect to the success of immunosuppression minimization is to increase our ability to detect when a state of immunological tolerance exists in a patient. In other words, can we identify patients for whom it is safe to reduce, or even eliminate, general immunosuppression? The standard method used thus far involves allograft biopsies, but these require invasive procedures that have an inherent risk. What is needed are noninvasive tests for immune status and tolerance. A number of possible ideas have been studied [56], including a trans vivo delayed-type hypersensitivity test, where cells from the recipient are challenged in an immunodeficient mouse with donor antigen. With this test, it can be determined if the recipient has a regulated immune response to prevent activity against donor antigens, while maintaining a normal response to third-party antigens [57]. Other tests being developed utilize urine samples to look for RNA species or chemokines that reflect an active immune response associated with ongoing subclinical rejection.

The drawback with any method, however, is the metastable nature of these responses; an accurate assessment can probably only be made with measurements over a significant time period. Nonetheless, to take the next step forward in deciding which patients are best suited to minimize immunosuppression requires a reliable test. This is a clear realization in the transplant community, and therefore a strong emphasis is being placed on this research aim.

Cell Therapy

In a much earlier developmental phase is the possible use of cell products to promote tolerance in transplant recipients (Fig. 2) [58]. One of the promising cell products is T regulatory cells. In the past several years there has been a deluge of papers regarding the potential for T regulatory cells in controlling immune reactions. Experimental data no longer leave this issue in question; however, the selection of specific human T-cell subsets for this purpose, and the need for their in vitro expansion, are currently a limiting issue that requires further research before their therapeutic use is safe and feasible.

The primary advantages of using T regulatory cells therapeutically is that they can conceivably be generated to downregulate responses to specific donor antigens, and these cells have the ability to migrate to anatomic sites where they can exert local immunosuppressive effects. On the other hand, besides working out conditions for their purification and expansion, it will be necessary to test whether they can be used in combination with immunosuppressive agents, and if lymphocyte depletive agents will eliminate them after injection. Whether T regulatory cell therapy can become a clinical reality with a beneficial effect is currently unknown, but the transplant research community is focusing significant efforts into developing this strategic approach for future application.

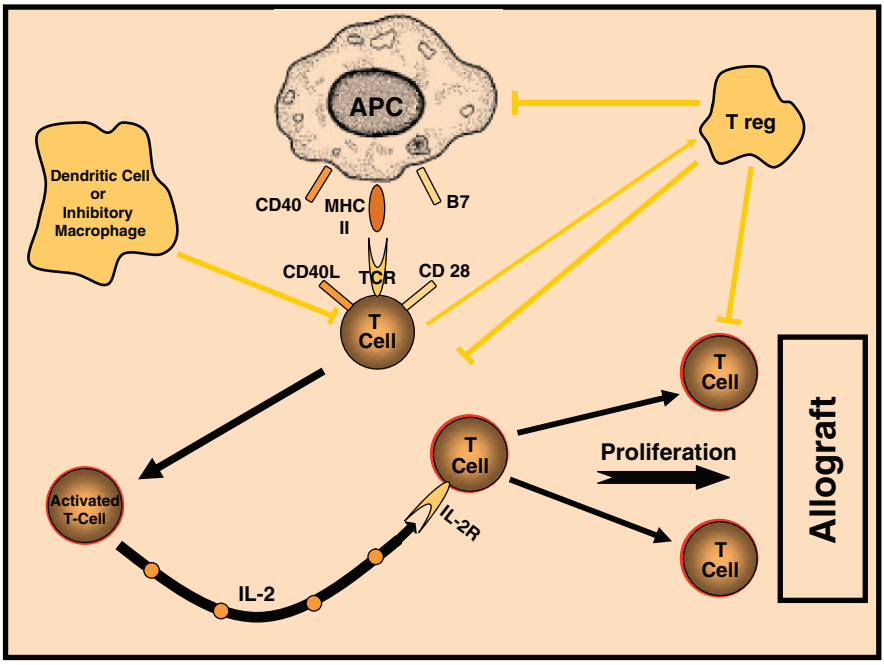

Fig. 2 Immunosuppression by cell therapy

Another concept being developed by Thomson and colleagues [59], and others, is the potential for the use of dendritic cells as a therapeutic product for tolerance induction. It has been known for many years now that dendritic cells have potent suppressive functions on T cells. The question now becomes if these cells can be utilized effectively in the setting of organ transplantation. Furthermore, many variations of suppressive dendritic cells have been described, so another issue becomes which cell population to choose for therapeutic use in humans. One positive, and rather advanced, aspect is that "stimulatory" dendritic cells have already been successfully administered to more than 1,000 cancer patients for the purpose of vaccination, with a very good safety record [60]. Therefore, the decision as to which "suppressive" dendritic cells to use in transplantation, and their ultimate efficacy, as well as the specificity of their activity toward an organ allograft, will determine their future for tolerance induction as a cellular product. Clinical trials using this class of cells are an exciting possibility in the near future.

Finally, regarding cell therapy, an inhibitory macrophage population has been identified that shows potential for tolerance induction [61]. The work with these cells is in a very early stage, but results thus far from our workgroup and the workgroup of Fändrich and colleagues (Kiel, Germany) suggest that the these donor-derived monocyte-derived macrophages have impressive potential for inhibiting human alloimmune responses to donor antigens. A pilot clinical trial conducted by Fändrich's group does not indicate any safety concerns at this early stage with the injection of the cells [62]. The efficacy of the donor-derived cells in an organ transplant setting is yet to be determined. Our own mechanistic investigation on the corresponding cell from mice has shown that these inhibitory macrophages profoundly delete activated lymphocytes, and, most impressively, surviving lymphocytes are highly enriched for T regulatory cells [61]. Moreover, we have found that these autologous inhibitory macrophages are effective at resolving ongoing autoimmune colitis in various mouse models. For now, the full potential of these cells as inducers of tolerance is only beginning to be elucidated, but as with the other tolerance-inducing strategies discussed in this chapter, merit further study with the overall aim of reducing or eliminating our current reliance on pharmacologic immunosuppression in organ transplant recipients.

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Skin Immune System

Jan D. Bos and Rosalie M. Luiten

Functions of the Skin

The skin is, in weight, the largest organ of the human body. Its primary role is that of a physical and biological barrier. This principal function is most apparent in the skin's relative lack of permeability for agents from outside, including microbes and parasites, but also for water and water-soluble compounds. The resistance to exogenous influences is mainly the result of the physicochemical properties of its outermost layer, the corneal layer of the epidermis (stratum corneum). In addition to its function as a barrier against potentially harmful outside effects, the skin also serves to maintain the homeostasis of the "milieu intérieure" by preventing desiccation.

Its physical strength depends largely on the vivid and dynamic connective tissue called the dermis, which is (cells not counted) mainly composed of intertwining collagen and elastin fibres. Other major physiological functions include maintenance of body temperature, regulation of stable blood circulation, and production of endocrine mediators. The skin also serves as an outpost of the central nervous system by its dense network of peripheral neural receptors and nerves, with countless axons ending unmyelinated in the epidermis. Psychological and social functions are so obvious that they do not need further explanation.

Sunlight, especially its short-wave ultraviolet radiation portion, is another physical force that the skin must meet. Photons induce the formation of damaging free radicals, but a variety of defence mechanisms has evolved including free radical-trapping molecules, thiols, melanin, and enzyme systems that can almost perfectly absorb and eliminate these DNA-damaging, potentially carcinogenic elements.

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To counter all this, a DNA-repair system that is present in all cells effectively repairs DNA damage and is most efficient in epidermal keratinocytes. These cells are the primary possible victims of sun exposure in man and have much more efficient DNA-repair reserves as compared to, for example, T cells. Deficiency or malfunction of various elements of this DNA-repair system leads to variants of xeroderma pigmentosum, in which patients develop all kinds of cutaneous malignancies early in life.

In addition to induction of free radicals, sun rays (especially the ultraviolet spectrum), may be absorbed by many other chromophores including *trans*-urocanic acid (trans-UCA), many cell membrane lipids, haeme rings in papillary dermis erythrocytes, haemoglobulin, and DNA itself.

Finally, the skin has a complicated defence function that is best denominated as "immunological." Its capacity to discern self from nonself is indeed challenging to the imagination when one considers the rich variety of exogenous substances to which it is continuously exposed. The immunological function of the integument, related to both physiology and pathology, is the subject of the present chapter. Emphasis is given to the role of the skin immune system (SIS) in immunosurveillance, especially in the prevention and elimination of malignancies and its possible role in eliminating oncogenic viruses.

The Skin: Concepts of Immunological Functioning

The development of concepts of the skin as an organ of immunity is of historical interest. According to Silverstein [1], Alexandre Besredka was perhaps the first to realize the existence of organ-specific immunity, early in this century. While working in the Institut Pasteur with the cellular immunologist Ilya Metchnikoff, Besredka wrote at least two books on the subject, but the skin apparently escaped his attention. In 1970, Fichtelius and coworkers published a classic article in which they suggested that the skin is to be considered as a *first-level lymphoid organ*, comparable to the primary lymphoid tissue thymus [2]. They referred to lymphoepithelial microorgans in the skin of newborns and human foetuses, which were detected at orifices of the body such as under the nails, in the preputial fornix, in the fornix vaginae, at the conjunctival fold, around the glandular tissue of the external ear canal, around the pilosebaceous units of the lower ear lobes as well as scrotal skin, and around the primitive mammary gland tissue. These collections of lymphocytes were suggested to reflect educational lymphoid environments in which systemic immunity to exogenous agents was formed and in which cells were educated to discern self from nonself antigens. Their localization near the openings of the body suggests that these sites are particularly vulnerable.

In adults, these lymphoid accumulations may recur and are then diagnosed as benign lymphoproliferative skin tumors (lymphadenosis cutis benigna). For dermatologists, this still forms a most attractive hypothesis as to the origin of certain benign cutaneous lymphomas. The concept of the skin as a first-level lymphoid organ, however, has as yet not been further substantiated.

Can the skin then be a *second-level lymphoid organ*? It has been suggested that in the classical type IV hapten-induced contact hypersensitivity reaction, sensitization may take place entirely within the skin, without the need for the involvement of regional skin draining lymph nodes. However, such a phenomenon of "peripheral" sensitization has yet to be definitively proven to be a common event in vivo. Confirmation of this concept would categorize the skin as a secondary lymphoid organ such as lymph node tissue and spleen.

At the present time, however, there is no definite evidence for the skin to be either a primary or secondary lymphoid organ. The observations by Fichtelius and coworkers do not exclude a primary lymphoid role during embryogenesis or later in foetal life.

If then the integument is not part of the central organs of the immune system, many features of it have led to the development of concepts that try to give the skin its deserved and distinct place in immunology. A variety of models has been proposed to seize that special place. These are, in order of their appearance in the literature: SALT, SIS, DMU, and DIS.

Streilein, in 1978, coined the term *skin-associated lymphoid tissues (SALT)*, which embraces epidermal keratinocytes, intraepidermal Langerhans' cells (LCs) as antigen-presenting cells, the skin-homing T lymphocytes assumed to exist since the first observations on cutaneous T-cell malignancies, the endothelial cells of the skin directing these skin-seeking cells into the dermis, and the skin-draining lymph nodes, being the specific localization of induction of immunity by antigens that have been processed and transported by LCs [3]. Later, Streilein extended his concept of SALT by defining two subsystems entitled endoSALT and exoSALT [4]. In this subdivision, dendritic epidermal T-cell receptor (TCR) γδ-expressing T cells are crucial. These dendritic TCR γδ cells are very common in the epidermis of mice and other animals and might serve a function in primary immune defence. However, a comparable human equivalent does not seem to exist, indicating that such a subdivision is less attractive for the envisioning of the role of human skin in immunological defence [5, 6].

In 1986, we proposed *skin immune system (SIS)* as the term for the complexity of immune response-associated cells present in normal human skin [7]. By making a qualitative inventory of cell types present in normal human skin, it became evident that approximately half of them have immune functions and thus are part of the immune system (Table 1). Such a simple observation emphasizes the important role of the integument in immune responses. The concept of SIS was later extended by adding its humoral constituents [8–10]. Table 2 summarizes both the cellular and humoral constituents of SIS as we presently recognize them.

Since the introduction of the SALT concept, several authors have entirely focused on the epidermis and suggested it to be an immunological organ with its combination of keratinocytes, dendritic cells, and T lymphocytes. Obviously, concepts focusing solely on the epidermis are incomplete as they exclude the major site of immunological action in skin. The preferential distribution of T cells, monocytes, mast cells, endothelial cells, and most other cellular constituents of the skin immune system can be found in the dermis, especially in its papillary part. Thus, Sontheimer

Table 1 Normal human skin: overview of cell types present and differentiation between primarily immune-response associated and not primarily immune-response associated cells [modified from [53]]

Immune response associated	Not immune response associated
Keratinocytes	Merkel cells
Immature dendritic cells (LCs)	Melanocytes
T lymphocytes and their subpopulations	Fibroblasts/fibrocytes/myofibroblasts
Vascular and lymphatic endothelial cells	Pericytes
Granulocytes	Eccrine glandular cells
Tissue macrophages	Apocrine glandular cells
Monocytes	Sebocytes
Mature tissue (myeloid) dendritic cells	Schwann cells
Mast cells	Smooth muscle cells

Table 2 Cellular and humoral constituents of the Skin Immune System (SIS) [modified from reference [53]]

Cellular constituents	Humoral constituents
Keratinocytes	β-defensins, cathelicidins
Immature dendritic cells (LCs)	Complement and complement regulatory proteins
Mature tissue (myeloid) dendritic cells	Mannose binding lectins
Monocytes/macrophages	Immunoglobulins
Granulocytes	Cytokines, chemokines
Mast cells	Neuropeptides
Vascular and lymphatic endothelial cells	Eicosanoids and prostaglandins
T lymphocytes and their subpopulations	Free radicals

in 1989 gave his definition of the *dermal microvascular unit (DMU)*, which was to point to the very centre of immunological reactivity in most immune-mediated skin diseases [11]. Directly around the postcapillary venules of the papillary layer of the dermis, we find accumulations of T cells, monocytes and tissue macrophages, mast cells, and dendritic cells. All elements of immune reactivity are present, and it is no surprise that most inflammatory skin diseases show expansion of the cellular elements of the DMU. Thus, the DMU might be considered to be a subsystem of the SIS.

Nickoloff [12] then proposed the term *dermal immune system (DIS)* to be the cellular and humoral counterpart of SALT [12]. It included fibroblasts, mainly because they are intrinsically related to homeostasis of other skin components such as epidermis. With the exception of SALT lymph nodes and DIS fibroblasts, these two concepts might also be considered as functional subsystems of SIS. The conceptual differences in terms of components between SALT, DMU, DIS, and SIS are given in Table 3.

Table 3 A comparison of the proposed constituents of the skin-associated lymphoid tissues (SALT), the dermal microvascular unit (DMU), the dermal immune system (DIS), and skin immune system (SIS)

	SALT	DMU	DIS	SIS
Keratinocytes	+	−	−	+
Langerhans' cells	+	−	−	+
Epidermal T lymphocytes	+	−	−	+
Dermal T lymphocytes	−	+	+	+
Mast cells	−	+	+	+
Vascular endothelial cells	+	+	+	+
Lymphatic endothelial cells	+	−	−	+
Tissue dendritic cells	−	+	+	+
Monocytes/macrophages	−	+	+	+
Fibroblasts	−	−	+	−
Granulocytes	−	−	−	+
Free radicals	−	−	−	+
Secretory immunoglobulins	−	−	−	+
Complement factors	−	−	−	+
Eicosanoids	−	−	−	+
Cytokine network	−	−	+	+
Coagulation/fibrinolysis system	−	−	−	+
Neuropeptides	−	−	+	+
Skin draining lymph nodes	+	−	−	−

The Skin Immune System (SIS)

Resident, Recruited, and Expanding Cell Populations

The concept of the SIS might be regarded as a rather static one. Of course, there is intense and vivid activity in it, which can best be illustrated by looking at its cellular constituents. Some of them are resident; others can be recruited and stay or die within the skin. They may expand in both benign and malignant ways. Despite some overlap between these categories, it is helpful to make this subdivision because it is a reflection of essential steps in the development of inflammatory and immune-mediated events.

Immunocompetent cells of the skin may also be divided in cells of the innate skin immune system as well as cells of the adaptive skin immune system, each having recruitable, expanding or growing, and resident subpopulations (Table 4). Eosinophilic granulocytes, for example, are a recruited cellular subpopulation, being present within the skin in certain pathological states only. Another example is the influx of neutrophilic granulocytes that is seen in a natural situation, such as after ultraviolet B irradiation of the skin [13], and in many pathological conditions such as in acute infections. Tissue macrophages (histiocytes) are generally believed to be resident, although they are bone marrow derived. Other cellular components of SIS may be only formed from precursor cells in pathological conditions. T lymphocytes

Table 4 Innate, bridging, and adaptive cells of the skin immune system, divided over resident, recruited, in situ, maturated, and outgrown populations [substantially modified from reference [53]]

	Resident	Recruited	Maturation, growth, malignancy
	Keratinocytes		Papilloma Basal cell carcinoma Spinal cell carcinoma
		Natural killer cells Natural killer T cells	Cutaneous T-cell lymphoma
INNATE		Eosinophilic granulocytes	Hypereosinophilic syndrome
		Neutrophilic granulocytes	Acute infections Subcorneal pustulosis
	Macrophages	Monocytes	Epitheloid cells Multinucleated giant cells
	Endothelial cells –Vascular –Lymphatic	Endothelial cell precursors	Teleangiectasia Kaposi sarcoma
INNATE + BRIDGING	Dendritic cells (DCs) – Langerhans' cells – Dermal DCs	DCs – Plasmacytoid DCs – IDECs – TIP DCs	Histiocytosis X
	T lymphocytes – Helper T cells – Cytolytic T cells – Regulatory T cells	T lymphocytes – Helper T cells – Cytolytic T cells – Regulatory T cells	Cutaneous T-cell lymphoma
ADAPTIVE		B lymphocytes	Plasma cells Lymphadenosis cutis Benigna Malignant B-cell lymphoma
	Mast cells	Basophils	Mastocytoma Mastocytosis

IDEC, inflammatory dendritic epidermal cells; TIP, TNF-α-INOS producing.

are the best example of a cellular constituent of SIS that is assumed to be moving, especially to the skin from the secondary immune organs, the skin-draining lymph nodes.

In addition to the cellular constituents of the SIS, a wide variety of inflammatory and immune mediators are present within the normal integument. A part of them reach the skin by the circulatory route, while many are constitutively produced within the organ itself.

Innate and Adaptive Compartments

An objective approach to the description of the cellular and humoral elements of the SIS would then divide them over innate and adaptive subsystems, as described earlier. Both innate and adaptive subsystems are involved in the immunosurveillance of the SIS against pathogens and/or abnormal cells [14]. Innate immunity of the integument is represented by a number of biochemical and physical factors, some of them secreted by the sebaceous and sweat glands, orchestrating cellular constituents such as phagocytes and killer cells in eliminating the invading potentially harmful compounds and microorganisms. The adaptive subsystem would then include those elements that are essential in the second line of host defence against pathogens as well as in the preservation of a natural homeostasis by limiting sensitization to autoantigens.

Dendritic cells, perhaps dermal dendritic cells more than epidermal Langerhans' cells [15], seem to form a bridge between the innate and adaptive immune systems [16]. By expression of Toll-like receptors (TLR), they can recognize molecular patterns of microbial agents and thereby be activated, leading to the production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α. In this process, the activation of immature dermal dendritic cells and/or epidermal Langerhans' cells can be further enhanced by factors of the innate subsystem, such as proinflammatory cytokines TNF-α and interleukin (IL)-1α that are produced by keratinocytes upon contact with pathogens.

The activated dendritic cells gather (foreign) antigens from the skin environment and undergo maturation as they migrate to the skin-draining lymph nodes to initiate adaptive immune responses. Maturation includes the upregulation of the expression of HLA and costimulatory molecules, required for efficient priming of effector T lymphocytes. In particular, Langerhans' cells also lose the expression of E-cadherin during activation, which facilitates their exit from the epidermis by the decreased adhesion to the epidermal keratinocytes. In the lymph nodes, skin-derived antigen-presenting cells prime naïve T cells for the pathogens that are present in the skin and simultaneously confer skin-homing capacity to the T cells. The primed T cells will subsequently migrate to the skin and mediate specific, adaptive immune responses in the skin. In contrast, in normal noninflamed skin, factors that cause activation and/or maturation of dendritic cells or Langerhans' cells are absent, leading to decreased antigen presentation to T lymphocytes. If immature dendritic cells reach the lymph node and present (auto)antigens to T lymphocytes, they will confer a more regulatory phenotype to the primed T cells rather than effector function. This process, called peripheral tolerance, is important to maintain homeostasis in the skin.

Although most T-cell priming will likely occur in the skin-draining lymph nodes, it cannot be excluded that activated Langerhans' cells and dendritic cells may also present antigens to resident T cells when passing the papillary dermis on their way from the epidermis to the lymph nodes.

The Distinction Between Cutaneous and Systemic Immunity

One might argue that the simple presence of T lymphocytes, dendritic cells, mast cells, and monocytes in the environment of vascular endothelial cells is the result of a random distribution of tissue-infiltrating T lymphocytes rather than specific targeting to the skin. In other words, these are normal elements of connective tissues, and there is no difference between the dermis and the supporting connective tissue of other organs.

However, there are a few distinctions that can be made. First of all, the skin shares only with the eyes its almost continuous exposure to light and ultraviolet irradiation. During evolution, this has led to adaptation as exemplified by the presence of melanocytes. Another presumably adaptive evolutionary development is reflected in the relative insensitivity of keratinocytes to the DNA-damaging effects of ultraviolet (UV) exposure. Especially, their capacity to recover from damage by UV is high as compared to other immune response-associated cells. The study of UV effects on cutaneous immune function is the major focus of interest in photoimmunology. It is generally believed that UV has an immunosuppressive effect on cutaneous immune responses, but there are immunostimulatory effects as well. The precise pathways by which these effects occur include changes in the production of cytokines by keratinocytes and other skin cells, alteration of adhesion molecule expression, and impaired antigen-presenting function of dendritic cells and Langerhans' cells, leading to the generation of regulatory/suppressor T lymphocytes and decreased cell-mediated immunity [17].

A second major distinction is that the skin is continuously exposed to an infinite variety of antigens, either in the form of infections or from the environment, such as from plants or chemicals. Although the skin shares this characteristic with the pulmonary and gastrointestinal tracts, there is clearly a distinction in the way the skin has developed its immune responses to these antigens. In pulmonary and gastrointestinal immunology, the term *mucosal immune systems* (MIS) has developed. A major characteristic is the directly submucosal localization of lymphoid accumulations. These extranodal lymphoid tissue accumulations are thought to have a major role in the production of secretory IgA, independent of systemic immunity. However, although secretory IgA has been detected in human cutaneous excretions, the quantity is not comparable, and subepidermal extranodular lymphoid tissues are not part of normal human skin. Thus, the innate and adaptive subsystems of SIS are highly distinct from those of MIS.

A third major characteristic of SIS, which sets it apart, is the presence of Langerhans' cells. These cells are immature dendritic cells that in themselves constitute a subset of antigen-presenting cells (APCs), often referred to as professional APCs. Dendritic cells have the unique capacity to induce primary immune responses, and they are divided into those homing to the connective tissues (tissue dendritic cells), the lymphoid organs (lymphoid dendritic cells), and those homing in epithelia (epithelial dendritic cells: Langerhans' cells). Although we are not entirely certain as to why the epidermis would need such a dense infiltration of these dendritic cells, it is evident that these cells have a major role in inducing primary immune responses.

There is now evidence that, in terms of activation, dendritic cells respond differently to various pathogens. If pathogens activate dendritic cells, these dendritic cells will be more effective in priming adaptive immunity, whereas in the absence of activation, immature dendritic cells generally induce T-cell tolerance. In this way, it is in fact the dendritic cell that makes the distinction as to what antigens are to be recognized and which ones can be discarded. During priming of T cells, the specificity of the T cells will further determine the magnitude of the T-cell response. Such a function of dendritic cells is discordant with previous paradigms of immunity, which gave T cells the exclusive role of being the cells making such an important distinction. Manipulation of dendritic cell activation may therefore represent an effective way to either increase tolerance in transplantation [18] or increase immunity in tumor therapy (described later).

In addition to these three major points of difference between cutaneous and systemic immunity, one might point to the existence of organ-specific T lymphocytes. These are assumed to move between the lymphoid organs and their natural home base. These skin-seeking T cells, so long suspected to be there because of the existence of cutaneous T-cell lymphomas, have now been further defined with their skin-specific homing address.

Immunosurveillance by the Skin Immune System

The immunosurveillance function of the SIS ensures effective immunity against pathogens in the skin at three levels; increasing exposure of naïve T cells to antigens present in the skin by the sentinel function of Langerhans' cells and dermal dendritic cells, targeting the effector response to the skin area where the antigen was encountered, and, last, mediating spreading of the memory T cells to other skin areas.

It has been estimated that the number of T cells in normal human skin is higher than the number in peripheral blood. Clark et al. arrived at a total of 2×10^{10} of T cells in the normal skin of an adult [19]. This finding indicates the magnitude of SIS as an organ-based immune response complex, as there are twice as many T cells in normal human skin as compared to the circulation (1.2×10^{10}). Most of them are Th1 memory effector T cells (circulating between blood and skin), with smaller subsets of central memory T cells (circulating between blood, lymph nodes, and skin), Th2 cells, and functional regulatory T cells.

T cells are present in very small numbers in normal human epidermis. They are also regularly present in normal human dermis, where small clusters of T cells can be detected around the postcapillary venules [20]. It is known that T cells as they occur in the peripheral circulation have a subset, estimated as 16%, which undergo skin tissue-specific lymphocyte recirculation. The identification of cutaneous lymphocyte antigen (CLA) on a subset of circulating memory T cells, and the expression of its ligand E-selectin on endothelial cells of the dermis, gave rise to a series of studies that have further elucidated this phenomenon [21].

The very existence of tissue-specific homing T cells is thought to serve different purposes: increase effectiveness of regional immune responses; decrease possibility of tissue antigen cross-reactivity; and allow functional immune specialization of particular tissues, that is, the skin. The precise mechanisms of cutaneous T-cell homing have been described, and the different adhesion molecules and chemokines involved have now been partly characterized [21]. Murine studies have shown that the tissue microenvironment of the dendritic cell determines the T-cell trafficking to the organ of dendritic cell origin [22]. In the case of dermal dendritic cells or Langerhans' cells, T cells acquire skin-homing capacity during priming, by the expression of CLA and chemokines receptors CXCR3, CCR4, and CCR10.

The subsequent process of T-cell homing to the skin can be divided into different stages. Endothelial cells express different adhesion molecules that have roles in the different stages of T-cell adherence and transendothelial migration into the dermis. Tethering occurs when CLA expressed on microvilli of fast-moving T cells binds to E-selectin present on the luminal surface of endothelial cells. Subsequent rolling, arrest, and transendothelial migration occur through binding of various adhesion molecules on endothelial cells and their counterstructures on T cells (VLA-4/VCAM-1, LFA-1/ICAM-1).

Migration of T cells through the connective tissue of the dermis is in part dependent on binding of T cells to counterstructures on matrix proteins.

Chemokines and their receptors have been identified as key elements of this process, adding tissue specificity to the migration of T-cell subpopulations. Skin-derived dendritic cells and Langerhans' cells instruct the T cells to home to the skin by inducing the expression of CLA and chemokine receptors CCR4, CXCR3, and CCR10. This chemokine receptor profile corresponds to many of the chemokines that are produced in human skin, especially in inflammatory states. Local chemokine production in the skin directs the migration of T cells to the skin tissue site where the antigen was encountered. Recognition of the endothelial cell-expressed chemokine TARC (CCL17) by T-cell receptor CCR4 forms an integral part of the rolling and migration process. After arrival in the dermis, monocyte-derived chemokine MDC (CCL22) activates the migration of T cells by binding to CCR4. Finally, subsequent intraepidermal immigration is stimulated by keratinocyte-derived chemokine CTACK (CCL27) and CXCL10 (IP10) that selectively bind to CLA+ T-cell chemokine receptors CCR10 and CXCR3. Especially, the CCR10/CTACK (CCL27) interaction is an important feature of the SIS, as CTACK is exclusively produced in the skin, and not in other organs, and CCR10 expression on T cells is restricted to the CLA+CD4+ subset.

Skin T-cell homing is thought to be particularly necessary for immunosurveillance, serving effective acquired responses to microbial infestation, and preventing the development of or eliminating different cutaneous, particularly keratinocyte, malignancies. In contrast, T-cell homing is seen as disadvantageous in T-cell-mediated skin diseases, of which there are many. Knowledge of the molecular processes involved in T-cell homing may be of use in different situations. Detection of circulating adhesion molecules has been found to be correlated with disease activity in a variety of skin diseases, for example, atopic dermatitis [23]. Upregulation of

adhesion molecules in disease states can be used for advanced diagnostic imaging. Understanding of chemokine and adhesion molecule genetic polymorphisms might contribute to our understanding of the variability that skin diseases have in different individuals affected. And finally, adhesion molecules and chemokines might form targets of therapy.

The new insights into the mechanisms of cutaneous T-cell homing thus have resulted in the development of innovative therapeutic approaches aimed at preventing skin T-cell immigration. However, in immunotherapy of malignancies, one might want to increase organ-specific homing of tumor-specific T cells, for example, following vaccination or adoptive transfer of ex vivo expanded T cells [24–26].

Immunosurveillance Against Tumors and Oncogenic Viruses

There are at least three lines of evidence which indicate that when the skin immune system is malfunctioning, malignancies and premalignancies may arise. First, experimental animal models have shown that UVB-exposed mice are unable to reject transplanted syngeneic skin tumors, whereas nonexposed littermates do reject these tumors [27]. The immunosuppressive effect of UVB radiation was identified to occur through the induction of suppressor cells. Depletion of these suppressor T cells restored tumor rejection, indicating that UV-induced immunosuppression is not caused by the deletion of effector cells that recognize tumor antigens. Schwartz et al. have shown in murine models that the induction of suppressor/regulatory T cells by UVB radiation is the result of UV-induced DNA damage in the Langerhans' cells, causing impaired antigen presentation and the induction of regulatory T cells [28]. These regulatory T cells migrate to the skin tissue and locally suppress effector T-cell activity. Regulatory T cells following UV exposure have also been identified in human skin and were shown to suppress the elicitation phase of nickel-contact hypersensitivity [29]. The finding that UV exposure results in local immunosuppression and an increased incidence of malignancies indicates that the maintenance of immunosurveillance in the skin is important to prevent the development of malignancies.

A second line of evidence is the observation that patients who are iatrogenically immunosuppressed, such as after organ transplantation, develop malignancies (including skin) in frequencies much higher than nonimmunosuppressed individuals. As a result, it is expected that cancer will surpass cardiovascular complications as the leading cause of death in transplant patients within the next two decades. In patients receiving single immunosuppressive regimens, such as in psoriasis patients prescribed cyclosporin, there is an increase in cutaneous malignancies, especially spinal cell carcinoma. However, the highest increases in cutaneous malignancies, mainly spinal cell carcinoma, but also basal cell carcinoma and melanoma, occur in combined immunosuppressive regimens. Calcineurin inhibitors (cyclosporin, tacrolimus) and azathioprine have been linked with post-transplant malignancies, while newer agents such as mycophenolate mofetil and sirolimus have not and

may even have antitumor properties. The types of malignancies in renal transplant patients vary geographically [30]. The aetiology of post-transplant malignancy is believed to be multifactorial and generally involves impaired immunosurveillance of neoplastic cells as well as depressed antiviral immune activity. Indeed, a number of common post-transplant malignancies are virus related. The precise role of papilloma viruses in post-transplant development of spinal cell carcinoma, however, has not as yet been precisely defined [31].

The third line of evidence that immunosurveillance against cutaneous malignancies indeed exists is that, in patients infected by human immunodeficiency virus (HIV), subsequent virus-induced immunosuppression leads to increased frequency and/or an altered course of malignancies. Malignant melanoma and squamous cell carcinoma are examples of cutaneous malignancies that have a more aggressive course in patients with HIV. Others, such as basal cell carcinoma, appear more frequently in this population but do not seem to be more aggressive [32]. It was Kaposi's sarcoma that led to the initial recognition of a new sexually transmitted immunosuppressive disease, now known as HIV infection. This entity is seen as a subform of the malignancy first described by Moritz Kaposi in the 19th century. It is known to be related to human herpesvirus type 8, which takes its chance in immunosuppression, such as caused by HIV. Especially, viruses are associated with the development of malignancies in immunocompromised patients (Table 5). The exact role, however, of papilloma viruses in spinal cell carcinoma of the skin remains to be established. Basal cell carcinoma is not known to be virus associated but is mainly the result of cumulative damage by UV irradiation.

Now that we have a more precise definition of the elements of the human skin immune system, a logical question remains which of these elements plays a central role in immunosurveillance against tumors [33]. It is generally assumed that acquired immunity mediates immunosurveillance against tumors. However, early immunotherapy studies have shown some antitumor effects of natural killer cells or IL-2-activated killer cells (LAK cells) in mediating tumor regression, indicating that innate immunity may also contribute to immunosurveillance [34].

In melanoma patients, the clinical observations of spontaneous tumor regression and/or periods of stable disease suggest the presence of immunosurveillance against melanoma. Spontaneous T-cell responses that specifically recognize melanoma cells and which can mediate tumor cell lysis ex vivo are frequently found in melanoma

Table 5 Viruses known to be associated with malignancies in man

DNA viruses	Malignancy
Epstein–Barr virus (EBV)	Lymphoma
Human papilloma virus subtypes	Cervical carcinoma, anal carcinoma
Hepatitis B virus (HBV)	Hepatocellular carcinoma
Herpes simplex virus 8 (HHV-8)	Kaposi's sarcoma
RNA viruses	**Malignancy**
Adult T-cell leukaemia virus (HTLV)	Leukaemia
Hepatitis C virus (HCV)	Hepatocellular carcinoma

patients, indicating the immunogenic character of melanoma. Analyses of these spontaneous immune responses in melanoma patients have identified a series of antigens, including melanocyte differentiation antigens, cancer-testis antigens, and antigens from mutated gene products [35]. These antigens are either self-antigens that are overexpressed on tumor cells, in the case of melanocyte differentiation antigens, embryonic antigens that are not expressed on normal cells except for testis (cancer-testis antigens), or newly arisen antigens from gene mutations. All three categories can be recognized by the immune system, leading to antigen-specific immune responses. Knowledge of tumor-specific antigens in basal cell and spinal cell carcinoma, such as mutated p53, is limited, however.

In most melanoma patients the immunosurveillance that is provided by spontaneous immune responses fails to prevent disease progression, which may result from insufficient levels of immunity or mechanisms of the tumor cells to escape attack by immune cells (described in next section). Melanoma patients with increased levels of immunity, as measured by the presence of autoimmunity and especially autoimmune vitiligo, show less relapse of the melanoma and experience prolonged survival, indicating that increased levels of immunity can indeed be effective to prevent tumor growth [26, 36].

Immunotherapy studies of vaccinating melanoma patients with melanoma antigens have shown that providing the immune system with the antigen alone is not sufficient to activate effective antitumor immune responses. Antigens must be presented to the immune system by professional antigen-presenting cells that are appropriately activated to prime effective immune responses which can mediate tumor regression. As many tumor antigens are self-antigens, the existing immunological tolerance to self-antigens limits their immunogenicity.

UV radiation and chemicals that cause skin cancer both interfere with the antigen-presenting function of dendritic cells and Langerhans' cells, indicating the central role of these cells in skin cancer immunosurveillance. The dendritic cells, and in the skin also the Langerhans' cells, are therefore the key elements that determine the outcome of T-cell responses against tumors. The induction of effective T-cell responses requires mature, activated dendritic cells that express high levels of MHC–antigen complexes and costimulatory molecules and produce cytokines such as IL-12 [37]. Failure of T-cell responses to mediate tumor regression in patients may result from impaired priming of T cells by immature or insufficiently activated dendritic cells. Indeed, dendritic cells and plasmacytoid dendritic cells (pDC) found in melanoma displayed a predominantly immature phenotype, indicating defective dendritic cell maturation at the tumor site [38]. In these melanomas, the infiltrating T cells consisted of only naïve, resting CD27-and CD45RA-expressing T cells, which suggests insufficient T-cell activation by the immature dendritic cells.

The requirement of mature, but not immature, dendritic cells for effective priming of human T cells in vivo was demonstrated in a vaccination study of melanoma patients, in which only mature dendritic cells pulsed with gp100 or tyrosinase peptide were able to induce T-cell responses [39]. Maturation of dendritic cells can greatly be improved in vivo by administering molecularly defined triggers of dendritic cell activation, such as agonistic anti-CD40 antibody or TLR ligands CpG and

detoxified lipopolysaccharide (LPS) (MPL) [40,41], which increased the efficacy of immunotherapy in animal models. Upon dendritic cell activation, T cells that had previously been inadequately stimulated by the tumor were now activated to migrate into the tumor tissue and mediate tumor regression.

Lack of proper dendritic cell activation by tumor cells may be one of the main reasons why the natural antitumor T-cell response is insufficiently effective. Moreover, the activation conditions during dendritic cell maturation determine polarization of the T-cell response, which allows choosing specific dendritic cells activation conditions in an immunotherapy setting to induce the desired type of T-cell response that can effectively mediate tumor regression [42].

Immune Escape Mechanisms of Tumors

Phenotypic changes occurring in tumor cells may allow the escape from recognition or killing by infiltrating T cells, and thereby decrease the efficacy of T cells to mediate tumor regression in vivo. Immune escape mechanisms of tumor cells include the downregulation of antigen and HLA expression, secretion of immunomodulatory cytokines by the tumor, the induction of immunological tolerance, counterattack of the tumor by deleting infiltrating T cells, and the resistance to apoptosis [43]. A decreased expression of HLA–peptide complexes or costimulatory molecules by the tumor results in impaired tumor recognition by T cells and impaired T-cell activation. In addition, tumors may downregulate T-cell activation by the secretion of the immunomodulatory cytokines transforming growth factor (TGF)-β, IL-10, the production of indoleamine 2,3-dioxygenase (IDO), or the expression of molecules of the B7 family, B7-H1 and B7-DC, that bind to their receptor PD-1 on the T cells. This mechanism causes repression of local T-cell function or tumor vascularization. Moreover, lack of VCAM-1 expression on endothelial cells in tumors inhibits infiltration of T cells in the tumor tissue. Alternatively, the expression of FasL (CD95L) by tumors may actively induce apoptosis in infiltrating T cells, leading to T-cell deletion.

Even when activated effector T cells are found in the tumors, killing of tumor cells by these T cells can still be impaired at the level of apoptosis induction in the tumor cells [43] by the expression of antiapoptotic molecules or the downregulation and mutation of proapoptotic molecules. Cytotoxic T cells can induce apoptosis by the death receptor pathway or by the granzyme B/perforin pathway, which trigger different signaling pathways of apoptosis induction. Apoptosis induction through ligand binding to death receptors (CD95 or TRAIL) is mediated by intracellular adaptor proteins that recruit initiator procaspase-8 to form the death-inducing signalling complex (DISC). At the DISC, procaspase-8 is cleaved into active caspase-8, which in turn can activate caspase-3 and other effector caspases, leading to apoptosis. In addition, caspase-8 can induce apoptosis by the cleavage of Bid and the activation of the mitochondrial pathway of apoptosis induction. The cellular FLICE inhibitory protein, cFLIP, interferes with the activation of caspase-8 at the DISC

and thereby blocks the initiation of the death receptor pathway. cFLIP is frequently expressed in human melanoma and inhibits death receptor-induced apoptosis of tumors in mice [44, 45], whereas sensitivity to granzyme B-mediated apoptosis remains intact. Increased resistance of melanoma to CD95-induced apoptosis or drug resistance may also arise from the downregulation of death receptors or the altered expression of apoptosis-regulating proteins, such as members of the Bcl-2 family [46], inhibitor of apoptosis protein (IAP) Livin/ML-IAP [47], or the proapoptotic molecule Apaf-1 [48]. Because chemoresistance is frequently observed in melanoma treated with immunotherapy, it is relevant to determine whether these mechanisms of apoptosis resistance affect the susceptibility of these tumors to T-cell-induced apoptosis in situ.

The granzyme B pathway of apoptosis induction targets caspase-3 directly or indirectly through the mitochondria, initiating the caspase cascade resulting in DNA fragmentation and apoptosis [49]. The protease inhibitor (PI)-9 can effectively and irreversibly inactivate granzyme B in vitro and prevents T-cell-mediated killing via the granzyme/perforin pathway [50, 51]. PI-9 expression is found in a subset of human tumors, and overexpression of the murine counterpart SPI-6 confers immune escape of murine tumors [52]. The relative contribution of the various apoptosis resistance mechanisms on the efficacy of tumor killing by CTL is of prognostic value for the clinical outcome of immunotherapy.

Conclusions

In conclusion, we believe it is essential for our understanding of cutaneous immunology to keep in mind what distinguishes the skin from other organs. From such a platform of specific immunophysiology of the skin, one might try to understand its dysregulations as we know them in the form of a surprisingly large number of inflammatory and immunodermatological diseases, and in immunosurveillance against tumors and oncogenic viruses. The science of cutaneous immunophysiopathology can best be further developed by taking into consideration the complete picture of cutaneous immunity, as reflected in its large variety of cellular and humoral factors, which are summarized under the heading SIS. Further research on the precise role of distinct elements of SIS in immunosurveillance will provide more insight into novel targets for immunotherapeutic intervention to either decrease or increase immunity in the treatment of inflammatory and autoimmune skin diseases or skin malignancies, respectively.

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Part II
Post-Transplant Cancer

Post-Transplant Skin Cancer: The Influence of Organ and Pre-Transplant Disease

Sylvie Euvrard and Alain Claudy

Post-transplant skin malignancies have been extensively studied [1–6], but the role of the transplanted organ in the occurrence of tumours is still discussed. This chapter reviews the epidemiological, clinical, and therapeutic aspects of skin cancers in populations of kidney (KTR), heart (HTR), and liver transplant recipients (LTR). Furthermore, some data on the impact of the pre-existing disease leading to organ failure are now available [7–10], and we consider here whether certain disorders warrant more intensive dermatological surveillance.

This update takes into account mainly keratinocyte skin cancers (KSC), which represent 95% of all post-transplant skin cancers, and Kaposi's sarcoma (KS). Data on the other types of skin cancers are not sufficient to allow any comparison. Kidney transplant recipients will be considered as the reference population as they have the longest follow-up, currently reaching more than 40 years.

Influence of Organ

Keratinocyte Skin Cancer

Many authors have reported the incidence of skin cancers in KTR in various countries including Europe, North America, and Australia (Table 1). Selected relevant series that have been published these past 10 years have shown that the incidence always increases with time after transplantation and varies in large groups from 0.2% to 2.25% [2, 7] at 1 year to 10% to 17% at 10 years and 40% to 60% at 20 years in the United States and Western Europe [2, 11]. Higher figures are reached sooner in Australia: 7% at 1 year, and 25% to 30% at 5 years [2, 12], the long-term incidence at 20 years (70%–82%) being still higher than in Europe (40%–60%). However, some differences may be observed in countries with similar sun exposure: At 10 years post transplant the incidence was 10.8% to 17% in Italy [3, 13] and 48%

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Table 1 Incidence of skin cancer in series of kidney, heart, and liver transplant recipients according to the length of immunosuppression

Author	Numbers of patients	Country	Year 1	Year 2	Year 3	Year 5	Year 6	Year 7	Year 10	Years 12–15	Year 15	≥20 years
Kidney												
Bouwes Bavinck 1996	1098	Australia	7%		16%	25%		33%		45% year 11	59%	70%
		The Netherlands	0.2%		0.7%	3%		6%		16% year 11	24%	41%
Fortina 2000	228	Italy				6%			17%			
Naldi 2000	1062	Italy							9.7%			
Harden 2001	211	UK				3.2% (<5)		8.4% (5–10)	9.3% (>10)			
Ramsay 2002	398	Australia				29.1% (<5)		52.2% (5–10)	72.4% (10–20)			82.1% (>20)
Fuente 2003	174	Spain			13%		27.5%		48%			
Bordea 2004	979	UK										61%
Kasiske 2004	35, 765	US	2.25	4.95	7.43							
Otley 2006	46, 355	US			1.12%							
Heart												
Espana 1995	92	Spain	4.3%					43.8%				
Lampros 1998	248	USA (Oregon)	3%			21%			35%			
Ong 1999	455	Australia				31%			43%			
Naldi 2000	267	Italy							11.4%			
Caforio 2000	300	Italy				15%			35%			
Fortina 2000	252	Italy				16%			33%			
Otley 2006	8594	US			5.18%							
Liver												
Haagsma 2001	174	The Netherlands				4%			13%			
Otley 2006	8075	US			1.08%							

in Spain [14], but this could be explained by the older age of the Spanish population at transplantation (45 vs. less than 37 years).

The number of publications concerning non kidney transplant recipients is increasing, but longitudinal studies are limited, and there are no data beyond 10 years post transplant [15–18]. Several comparative studies show a 2- to 4-fold-higher risk in HTR as compared to KTR [8, 13, 19–21]. The incidence varies from 3% to 4% at 1 year [15, 17] to 11.4% to 35% at 10 years [3, 13, 18] in Europe and the United States and 43% in Australia [16]. Although the higher prevalence of KSC in HTR has been initially thought to be caused by deeper immunosuppression [19, 21], it seems this would be mainly related to their older age at transplantation as compared to KTR [3, 8, 13]. In a Spanish group of HTR, a high incidence of 43% at 7 years was reported [15]. Indeed, in a recent study, we found that at the occurrence of skin cancer, the dosages of immunosuppressive treatment according to the weight of patients were similar in HTR and KTR [20]. In both KTR and HTR, the risk ratio was reported to be 6-fold- and 12-fold-higher in patients grafted between 35 and 55 years, respectively, and in those beyond 55 years of age as compared to those grafted at less than 34 years [8]. These differences between HTR and KTR could be less pronounced in years to come as the mean age of transplantation in KTR increases.

Few studies have been specifically devoted to skin cancer in LTR [22–25], and most epidemiological data are briefly reported in series dealing with general complications after liver transplantation. The global incidence of skin cancer in various centres, independent of the time of transplantation, ranges from 1.1%–1.6% to 22.5% [22–28]. The two available studies providing time-related incidence show similar figures at 3, 4, and 10 years in LTR as compared to control KTR from the same area [8, 28]. The highest reported incidence per centre (22.5%) comes from a survey performed in Boston where data were collected using a questionnaire that was sent to patients with a median follow-up of 4 years. This study suggests that some KSC in LTR are treated by local physicians and could be under-reported in many series [23].

Kaposi's Sarcoma

The incidence of KS varies from 0.14% to 0.5% in Western countries and the United States to 1.5% in Northern Italy and 4.1% in the Middle East [1, 7]. Several series from France, Italy, and Spain have reported a higher incidence of KS in LTR as compared with KTR [29–32]. In a study performed in Italy, LTR were at 2.7-fold-higher risk of KS than KTR [30], which could be caused by a higher risk of human herpesvirus 8 (HHV8) infection transmission from the graft, which was found to be 40% as compared with 33% for heart and less than 5% for kidney [33]. Although older age at transplantation increases the risk of KS, HTR seem to be involved less often, possibly because viral coinfections are less frequent in HTR as compared to LTR and older KTR, in whom hepatitis viruses are very common.

Influence of End-Stage Disease

Kidney

Data on the impact of the *dialysis period* are controversial. Cancer has been reported to be more common among dialysed patients compared with the general population because kidney failure is associated with abnormalities in the immune system, and the relative risk was found to be higher in younger patients [34]. The improvement in dialysis procedures is probably changing the current data, and a recent study has shown that a longer time on dialysis before transplantation was found to be associated with a lower risk of skin cancer [7].

Table 2 shows the percentage and the risk ratio of skin cancer and non-skin cancer according to the *underlying kidney disease* in several series [7–10]. The two disorders that deserve special mention are diabetes and polycystic kidney disease.

The lower incidence of skin cancer in patients with *diabetic nephropathy* as compared with the other renal diseases had been already mentioned in two series of more than 1,000 patients [35, 36]. Three recent multicentric American studies of patients who received a first kidney transplantation have assessed the impact of the underlying disease leading to end-stage kidney failure and the development of post-transplant skin and non-skin cancer [7–9]. Kasiske et al. [7] examined the 3-year incidence of most major post-transplant malignancies among recipients of both deceased or living donor kidney transplantations in 1995–2001 ($n = 35,765$) using Medicare billing claims. Taking glomerulonephritis as reference, diabetes was found to be protective for both skin and non-skin malignancies, but this was more pronounced for skin. Two other publications used the data from the Transplant Tumor Registry of the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS), which were collected in transplants performed between January 1996 and December 2001 [8, 9]. Data were censored at 963 days for all the patients studied by Kauffman et al. [9] to allow comparable

Table 2 Influence of kidney diseases on the incidence of skin cancers

Authors	Kauffman	Otley	Kasiske	Kasiske	Agraharkar	Agraharkar
Number of patients	33,249	46,355	35,765	35,765	1,739	1,739
Percent (%) or risk ratio (RR)	% of cancers	% and RR of skin cancers	RR non-skin	RR skin	RR all cancers	Without skin
Glomerular diseases	1.29	0.99 (RR 1)	1	1	1	1.3
Tubular interstitial	2.31	1.62 (RR 1.38)	NA	NA	NA	NA
Diabetes mellitus	1.50	0.89 (RR 0.70)	0.82	0.63	1	1
Hypertensive nephro-sclerosis	1.97	0.95 (RR 1.13)	1.06	1.05	1.6	2.3
Polycystic kidneys	2.87	2.52 (RR 1.65)	0.99	1.27	NA	NA
Renovascular and vascular diseases	1.71	1.05 (RR 1.38)	NA	NA	NA	NA
Other diseases	1.37	0.82 (RR 0.97)	1.49	0.89	1.1	1.5

follow-up time, while the follow-up varied from 18 to 90 months for those included in the series of Otley et al. [8]. In both studies, glomerular diseases were also taken as reference. Diabetic nephropathy was associated with the lowest incidence of skin cancer [8], but not for all cancers (including skin and nonskin cancer) where glomerular diseases had the lowest incidence [9]. Agraharkar et al. described a total of 1,979 transplants performed in 1,739 patients from 1967 to 2002 at a single centre in Texas with a mean follow-up of 6.1 years [10]. They classified the end-stage renal diseases into four groups comprising diabetes, hypertension, glomerulonephritis, and miscellaneous. The risk ratio for diabetes was found to be the lowest for all cancers with or without skin cancers. The lower risk of skin cancer in transplant patients with diabetes could be due to a lower absorption of immunosuppressive drugs because of gastroparesis, resulting in decreased blood levels of cyclosporine [37], tacrolimus [38], or mycophenolate mofetil [39]. It has been also speculated that a lower rate of cigarette smoking among diabetics could reduce the overall risk of cancer [7].

The higher trend of *polycystic kidney disease* to malignancy, which has been already suggested [40, 41], has been confirmed (see Table 2) [8, 9]. The results of Kauffman et al. analyse globally all types of "de novo" cancer, including skin cancer, but it seems that the increase would be mainly related to skin cancer [8]. Kasiske, who studied separately the impact of the disease on skin and non-skin cancer, found an increased risk only for skin cancer. A major limitation of these studies is the relatively short duration of follow-up (3 years) in which patients with skin cancer were the oldest patients. Mean age at transplant is significantly older for patients with polycystic kidney disease, and another explanation could be the "overrepresentation" of this disorder where graft and patient survival are better as compared to the other kidney diseases [42]. Thus, there are several confounders that could explain, at least in part, the apparent excess of skin cancer in allograft recipients with polycystic kidney disease.

Heart

To our knowledge, the only work studying the impact of the initial heart disease is provided by the data of the registries of the OPTN/UNOS, where 8,594 HTR were included with a mean follow-up of 1,107 days [8]. Although the highest incidence of skin cancer was described in patients with coronary artery disease (6.72%) as compared with those grafted for cardiomyopathy, valvular diseases, and miscellaneous, multivariate analysis showed that underlying diseases had comparable risks.

Liver

By contrast to the previous organs, it seems that a greater number of authors have endeavoured to assess the impact of the primary liver disease in LTR on the occurrence of cancers [8, 23–26, 28, 43, 44]. Several works have reported a higher global

incidence of cancer in alcoholic cirrhosis [26,44], and it has been shown that alcohol intake may be responsible for genetic alterations [45]. Furthermore, alcohol consumption is often associated with smoking, a well-known risk factor for several types of cancer. A Spanish study has recently reported a twofold-higher risk for skin cancer in a group of 276 patients grafted for alcoholic cirrhosis as compared to 425 grafted for nonalcoholic disease. In this work, the authors mentioned that 85% of the patients with skin cancer from the alcoholic group were smokers as compared to 30% in the nonalcoholic group [25]. However, this study reported a higher rate of basal cell carcinoma (BCC), although smoking is recognised to increase the risk of squamous cell carcinoma (SCC). Primary sclerosing cholangitis has been reported to increase the risk of skin cancer in a series of 151 patients [23], which could be due to the additional immunosuppression given before transplantation to treat occasionally associated inflammatory bowel disease or autoimmune hepatitis [28]. A larger study on 8,594 LTR recorded in the OPTN/UNOS data confirmed that patients with cholestatic liver diseases (including primary sclerosing cholangitis) and cirrhosis had an increased risk of skin cancer [8]. Patients with hepatocarcinoma would also have an increased risk, although this was not statistically significant [8, 43]. Hepatitis C virus, which was reported to play a role in internal neoplasms in LTR [44], was mentioned only in a univariate analysis in one study as a risk factor for skin cancers [23].

Clinical Features

It seems that some clinical differences may be highlighted according to the type of grafted organ.

Keratinocyte Skin Cancers

Location. Although most skin cancers are located on the uncovered areas, it seems that some differences may be observed between HTR and KTR [19]. Indeed, these differences are probably related to the younger age of KTR. Those transplanted before 40 years of age developed most of their lesions on the upper limbs, mainly the dorsum of the hand and the forearm, whereas patients transplanted at an older age have the greater number of lesions on the head. The reasons for these differences remain unclear [19, 46].

Number of lesions. Another difference between HTR and KTR could be the number of lesions, which seems greater in KTR. In one recent single-centre study, we found that the mean number of lesions per patient was increased by a factor of 2 in KTR [20] at 5 years (10 vs. 5). The comparative study performed by Fortina also showed a slightly higher number of tumours in KTR as compared to HTR for similar follow-up [13].

Distribution of lesions. The reversal of the ratio SCC/BCC observed in the transplant population as compared to the control groups seems variable according to the series and increases with sun exposure and the length of follow-up [1]. Although for similar age KTR and HTR show similar figures, several studies mentioned that LTR could have a higher rate of BCC [24, 25, 47–50], as we observed in our patients (unpublished data). This finding seems to be related neither to a higher age of LTR nor to a shorter follow-up.

A number of series reporting the occurrence of skin cancers do not mention keratoacanthoma (KA), probably because lesions of this type are included in the SCC group by several authors. However, KA that can be considered as a well-differentiated SCC has specific clinical and histological features and is regularly reported in many series [11, 12, 16, 19, 20]. In a former study, we noticed that KA were less frequent in HTR as compared to KTR [19]. From a total of 540 skin tumours, the rate of KA was found to be 6% in our KTR versus 1.5% in HTR. In two other studies on KTR performed in Queensland on 361 KTR who had developed 3,979 NMSC, and in UK on 187 KTR with 1065 lesions, KA represented, respectively, 5.7% and 6.6% of all the tumours [11, 12]. In the single other series of 148 Australian HTR who had developed 1,410 skin cancers, KA represented less than 2% of lesions [16].

Course. Reports of aggressive SCC seem more frequent in HTR [51, 52], but it is unclear if heart transplantation increases the risk of aggressive SCC, which is mainly associated with older age.

Kaposi's Sarcoma

The prognosis of KS is related to the existence of visceral involvement, which seems more frequent after liver and heart transplantation [29]. As we mentioned earlier, HHV8 transmission from the graft is higher in nonkidney transplant recipients, while most cases result from reactivation of pre-existing HHV8 infection in KTR. Especially in LTR, this is supported by several reports of grafted liver involvement with a disseminated disease [53–55]; this raises the question of routine screening of liver and heart donors for HHV8 to clinically and biologically monitor patients who have received a graft from a positive donor. A lower rate of survivals in HTR as compared to KTR has been reported [56]. In our experience with 26 transplant patients with KS including 18 KTR, 6 LTR, and 2 HTR, only 2 patients died of disseminated KS, and they were both HTR [32].

Therapeutical and Prophylactic Aspects

Minimisation of immunosuppression is increasingly popular in an attempt to reduce the rate of subsequent skin cancers in all types of organ transplantation [57]. In addition, new strategies with m-TOR inhibitors are emerging, especially in KTR [58, 59], while experience with HTR and LTR seems much more limited.

Prophylaxis of skin cancers is currently performed worldwide in most kidney transplantation centres, and a dermatological examination is proposed once a year for all patients. This procedure allows early detection and treatment of skin tumours and reinforces education about sun protection. Many publications about KTR have been devoted to the need for information on sun protection, and recent reports have shown the better results of a reinforced education using written advice [60, 61]. Dermatological referral has been more recently adopted by cardiologists, but LTR are not yet regularly screened in many centres.

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The Epidemiology of Transplant-Associated Keratinocyte Cancers in Different Geographical Regions

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Introduction

There are believed to be more than 1 million individuals worldwide currently with an organ allograft [1], and a further steep increase in numbers is expected in the next decade. The life-enhancing benefits of organ transplantation are undisputed, but come at a cost. Complications from graft-preserving iatrogenic immunosuppression include a significantly increased risk of malignancy. More than 40 primary malignant neoplasms were reported in the first 4,000 patients to undergo renal transplantation [2], and this early observation has been consistently supported by subsequent studies [3–7].

The overall risk for any cancer is reported to be two- to sixfold greater than in the general population, although for many common cancers, including lung, colon, breast, and prostate, the risk is small or is not increased [6–9]. In contrast, there is a disproportionate increase in the incidence of four tumour types, namely, keratinocyte cancers (KC) (comprising basal cell carcinoma and squamous cell carcinoma), post-transplant lymphomas/lymphoproliferative disorders (PTLD), anogenital dysplasias, and Kaposi's sarcoma, with smaller but significant increases in hepatocellular and renal cancers and some sarcomas [3, 6, 8, 10–12].

In Caucasian adults, KC are overwhelmingly the most common post-transplant tumour [4]. KC are also the most frequent malignancy following paediatric renal transplantation and the second most common (after lymphoproliferative disease) after other transplantations in children [13, 14]. Different post-transplant cancer patterns are seen in other populations. For example, Kaposi's sarcoma is the most common post-transplant skin tumour among organ transplant recipients (OTR) from endemic areas, such as around the Mediterranean and sub-Saharan Africa, or in those of Caribbean origin [5]. Similarly, Kaposi's sarcoma is the most common post-transplant malignancy in Saudi Arabia [15, 16], whereas urogenital cancers

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and hepatoma are the most common malignancies in Taiwan [17, 18]. In a cohort of 542 renal transplant recipients (RTR) from South Africa, the incidence of overall cancer was comparable in white and non white patients, but although KC was the most common cancer in whites, Kaposi's sarcoma was the most common cancer in non whites, in whom it accounted for almost 80% of all cancers [19].

Accumulating evidence suggests that tumours that occur at high frequencies in the transplant population are those associated with a viral aetiology; anogenital cancer is unequivocally associated with human papillomavirus (HPV) infection (specifically, high-risk mucosal types such as HPV 16), non-Hodgkin's lymphoma with Epstein–Barr virus, and Kaposi's sarcoma with human herpesvirus 8 [9]. The viral aetiology of such tumours of the skin is described in later chapters.

Keratinocyte Cancers

Keratinocyte cancers (KC) are the most common cancers in fair-skinned individuals [20–22], and the two main types, basal cell carcinoma and squamous cell carcinoma, are clinically and histologically distinct. Basal cell carcinomas (BCC) are slow-growing tumours that arise de novo and rarely metastasize [23–26]. Squamous cell carcinomas (SCC) grow more rapidly, are associated with Bowen's disease (carcinoma in situ) and with precursor lesions, namely, actinic keratoses (AK), and have a potential for metastasis [27, 28]. BCC are considerably more common in the general population than SCC, with a BCC/SCC ratio in the UK of about 4:1 [29], and also in Australia of 4:1 in 1985, but lowering to 2.5:1 in 1995 [30].

Epidemiological data and laboratory studies strongly implicate ultraviolet radiation (UVR) as the major aetiological factor for KC (reviewed in [31]) and worldwide, the incidence has been increasing dramatically since the 1960s, with an annual increase of 3% to 8% in white populations [29, 30, 32–35], probably as a consequence of greater affluence, sun-seeking behaviour, and ageing populations [36]. More than 1 million cases per year are recorded in the United States [28, 37] and more than 44,000 cases per year in the UK, although precise estimation of incidence trends is very difficult, because KC are not recorded by the majority of cancer registries [38–40]. Based on rates in South Wales where accurate local skin cancer registration does exist, incidence is estimated at up to 265.4 per 100,000 [29]. This rate would equate to 100,000 cases per year in the UK at large, substantially more than the registered 44,000 cases. Although mortality is low [39], KC are a cause of significant morbidity and a major burden on health care resources [31, 39, 41, 42].

Region-Specific Factors in the Epidemiology of Post-Transplant Skin Cancer

Historically, the earliest report of increased cutaneous SCC in OTR came from Australia in the early 1970s when Walder and colleagues reported 7 patients with KC in a group of 51 RTR immunosuppressed for up to 6 years [43]. Many more

SCC than BCC were diagnosed, substantially reversing the ratio seen in the general population. Further reports of KC in transplant patients from the United States and Australia followed [44–51]. These reports confirmed the predominance of SCC over BCC, and many additionally demonstrated a progressive increase in KC incidence with duration of immunosuppression. In fact, many early studies had reported no increased risk for BCC [43, 47, 50], but later studies indicated an excess risk for BCC, of the order of 2- to 10 fold [51–53].

Reports of skin cancer in RTR resident in temperate climates appeared later in the literature. Review to the end of the 20th century shows an excess risk for KC reported from Scandinavia [54, 55], the Netherlands [53], Britain [3, 50, 56–58], and Ireland [59]. Again, a predominance of SCC was found, but many also reported an increased incidence of BCC, especially in populations from southern Europe in Italy and Spain [60, 61]. The overall risk of KC in transplant recipients was reported as 4 times that expected in the Irish population [59], 7 to 17.6 times that expected in Scandinavia [54, 55], and up to 250 times that expected in the Netherlands [53].

Variability of estimates between these studies is likely to reflect many factors, including the case mix of the populations and differences in race, skin type, age, UV exposure, and mean duration of immunosuppression, as well as differences in the methods employed to estimate the occurrence rates of KC. Some studies have reported incidence, others cumulative incidence, others relative risk or the factor by which KC incidence is increased in OTR compared to a specified reference population. Other studies did not report the statistical methods used. One of the main difficulties is the lack of high-quality population-based studies based on national cancer registries and calendar period-specific incidence rates in the general population. Here the Scandinavian countries and Ireland have a great advantage with the reliable reporting of cutaneous SCC (reporting of BCC is more problematic) to a comprehensive national cancer registry [8, 10, 62–64]. In many (most) other countries there are no accurate comparison rates for skin cancer risk in the general population because of their lack of registration. Reporting only the first of multiple tumours in any one individual leads to further underestimation of the true incidence.

We have undertaken a systematic review of all available reports in the literature of skin cancers in solid organ transplant recipients, and reviewed those in which the baseline characteristics have been clearly stated and the authors have reported either a cumulative rate of skin cancer or an incidence rate or a relative risk. Despite the lack of comparative regional data, it has been possible to draw some conclusions about the epidemiology of skin cancers in transplant recipients in different regions of the world.

Search Strategy

An extensive literature search was performed in PubMed using synonyms for relevant words of the clinical question: “What is the incidence of skin cancer post-organ transplantation?” First, a combination of “skin cancer” and “transplantation” (both

as subheadings) was made in which "transplantation" was matched to the subheading "adverse effects and/or complication." Only English articles were included, and case reports and editorials were excluded, resulting in 448 articles. To include articles reporting malignancies in general without skin cancer as a subheading, "transplantation" (matched to "incidence") and "malignancies" were combined, which resulted in 501 articles. Finally, a broad selection was performed (7,786 references) using various terms for "skin cancers" combined with terms for "transplantation" matched to "incidence," resulting in an additional 428 articles. Full details of search synonyms are given in Box 1 (see Appendix).

After exclusion of duplicates, a total of 1,377 articles remained. A selection was made using title and/or abstract, resulting in 329 articles that appeared to match the clinical question of the incidence of skin cancer post-solid organ transplantation. For these 329 articles, full texts were evaluated and scored (A, B, C, D, or no score) where A was a "good article" with cumulative rate of skin cancer given and B was a "sufficient article" without cumulative rate of skin cancer, but with incidence rate or relative risk given. The full scoring system is given in Box 1 (Appendix). Only data from "A" and "B" articles have been used for the tables and graphs shown in this chapter. Complete details on "A" and "B" articles are available at the SCOPE website: http://www.scopenetwork.org/.

Summary of the Evidence

Data from 58 "good" or "sufficient" articles (scoring "A" or "B") were used to compare the incidence of skin cancer after organ transplantation in Australia, USA/Canada, southern Europe, and northern Europe. Comparative rates for SCC, KC, or BCC are shown in Table 1a–c, respectively. Table 2 documents the relative risk of SCC and BCC where this has been directly compared in the same population using the same epidemiological methods. Table 3 shows reported SCC/BCC ratios according to organ transplant type (heart, kidney, liver). Cumulative incidences from different regions of the world are shown graphically (Fig. 1), with population-based standardized incidence ratios (SIR) for SCC and BCC in Fig. 2.

Discussion

The influence of organ and pretransplant diseases on post-transplant malignancy has already been addressed (see preceding chapter by Euvrard and Claudy). Here we focus on the epidemiology of post-transplant skin cancers with reference to region-specific factors. Most of the population-based studies examining the incidence of post-transplant skin cancer pertain to populations of northern Europe, Australia, and the United States, whereas most of the studies from developing countries have not been population based and the number of patients and years of follow-up are limited. Consequently, there is an absence of good comparative data. It is apparent that the highest incidence rates have been reported in Australia [65–67], whereas

Table 1 (a) Risk of SCC in organ transplant recipients[a]; (b) risk of KC in organ transplant recipients[b]; (c) risk of BCC in organ transplant recipients[c]

Country	Authors	Year	Type of Transplant	No of patients	Length of follow up (years)	Incidence/ 1000/year	Ratio[d,e,f] of observed to expected incidence (95% CI)
(a)							
Australia	Ong	1999	Heart	400	5.5 (0.5–13) Median	379	
USA	Hoxtell	1977	kidney	495			36.4 (9.9–93)
Canada	Gupta	1986	kidney	523			18 (17–20)
Spain	Espana	1995	Heart	92	3.6 (0.1–9.5) Mean	29	
Italy	Montagnino	1996	kidney	854			6.2 ($p = 0.002$)
Norway	Gjersvik	2000	kidney	1020			49 (33–70)
The Netherlands	Hartevelt	1990	kidney	764	8.7 (1–21) Mean	7.6	253 (172–334)
United Kingdom	Bordea	2004	kidney	979	7.5 (2–23) Mean	71	
Ireland	Moloney	2006	kidney	1558	5.72 (0–16) Median		82 (73–91)
(b)							
USA	Otley	2005	heart	8594	3.0 Median	52	
USA	Otley	2005	liver	8075	2.9 Median	11	
Spain	Espana	1995	heart	92	3.6 (0.1–9.5) Mean	45	
Spain	Herrero	2005	liver	170	5.2 (0.5–14) Median	43	20.3 (14.7–27.3)
Italy	Naldi	2000	heart	267	2.5 (0.2–10) Median	15	
Italy	Naldi	2000	kidney	1062	4.0 (0.3–26) Median	9.1	
Sweden	Blohme	1984	kidney	129	(3–16) Only range		7.6 (4.1–13.11)[g]
Sweden	Lindelof	2000	kidney	5356	5.6 (0–24) Mean		109 (95–123) men 93 (73–116) women
Sweden	Adami	2003	kidney	5931	6.8 (0–27) Mean		56 (50–63)
The Netherlands	Hartevelt	1990	kidney	764	8.7 (1–21) Mean	9.0	
The Netherlands	Haagsma	2001	liver	174	5.1 (1.5–19) Median		70 (28–144)
UK	Bordea	2004	kidney	979	7.6 (2–23) Mean	141	
Ireland	Moloney	2006	kidney	1558	5.7 (0–16) Median		33.3 (30.3–36.2) Excluding carcinoma-in-situ

Table 1 (continued)

Country	Authors	Year	Type of Transplant	No of patients	Length of follow up (years)	Incidence/ 1000/year	Ratio[d,e,f] of observed to expected incidence (95% CI)
(c)							
Australia	Ong	1999	Heart	400	5.5 (0.5–13) Median	127	
USA	Hoxtell	1977	kidney	495			3.4 (0.7–9.9)
Canada	Gupta	1986	kidney	523			1.4 (0.7–2.2)
Spain	Espana	1995	Heart	92	3.6 (0.1–9.5) Mean	26	
Italy	Montagnino	1996	kidney	854			5.7 ($p = 0.02$)
The Netherlands	Hartevelt	1990	kidney	764	8.7 (1–21) Mean	3.3	10 (6–15)
United Kingdom	Bordea	2004	kidney	979	7.6 (2–23) Mean	22	
Ireland	Moloney	2006	kidney	1558	5.7 (0–16) Median		16 (14–18)

[a] SCC: Squamous cell carcinoma; CI: Confidence interval; No: number;
[b] KC: Keratinocyte Skin Cancers; CI: Confidence interval; No: number;
[c] BCC: Basal cell carcinoma; CI: Confidence interval; No: number;
[d] SCC: Observed incidence in transplant patients divided by the expected incidence in the general population.
[e] KC: Observed incidence in transplant patients divided by the expected incidence in the general population. Blohme used psoriasis patients as "general population."
[f] BCC: Observed (age specific) incidence in transplanted patients divided by the expected incidence in the (age specific) general population.
[g] Estimated.

Table 2 Relative risk of SCC compared with relative risk of BCC

Country	Authors	Year	No of pt	Length follow up (years)	Ratio* of observed to expected incidence (95% CI) SCC	Ratio* of observed to expected incidence (95% CI) BCC
Renal transplant recipients						
USA	Hoxtell	1977	495		36 (9.9–93)	3.4 (0.7–9.9)
Canada	Gupta	1986	523		18(17–20)	1.4 (07–2.0)
Italy	Montagnino	1996	854		6.2 (p = 0.002)	5.7 (p = 0.02)
The Netherlands	Hartevelt	1990	764	8.7 (1–21) Mean	253 (172–334)	10 (6–15)
Ireland	Moloney	2006	1558	5.7 (0–16) Median	82 (73–91)	16 (14–18)

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; CI: Confidence interval; No: number; pt: patients;
*Observed (age specific) incidence in transplanted patients divided by the expected incidence in the (age specific) general population.

Table 3 SCC/BCC ratios

Country	Authors	Year	No of pt	% pt KC	SCC/BCC ratio	Mean no of cancers	Length of follow up (years)	Mean time to presentation (years)
Heart transplant recipients								
Australia	Ong	1999	400	38	3.0		5.5 (0.5–13) Median	
USA	Lampros	1998	248	17	8.6	4.7	5.1 Mean	3.3 Mean
Spain	Espana	1995	92	16	1.5		3.6 (0.08–9.5) Mean	2.6 Mean
Italy	Caforio	2000	300	16	1.4		4.6 (0.1–12) Mean	
Italy	Naldi	2000	267	8.6	1.1		2.5 (0.2–10) Median	
Italy	Fortina	2004	230	21	2.2	2.5	9.1 (3.0–15.6) Mean	5.2 Mean
Renal transplant recipients								
Australia	Bouwes Bavinck	1996	1098	25	2.9	10	5.0 (0–24.3) Median	4.6 (0.3–8.9) Mean
Australia	Ramsay	2002	361	52	2.0	21	7.1 (2.3–13.1) Median	4.2 Median
Australia	Carroll	2003	310	42	3.1		(0->20) only range given	
South Africa	Moosa	2005	185	5.4	1.7	5.9	6.3 Mean	5.3 Mean
Spain	Fuente	2003	174	22	0.7	3.6	6.0 (1–11.7) Median	3.3 Mean
Spain	Marcen	2003	793		1.0		6.3 (0.5–12) Mean+/−SD	9.4 (3.4–16) Mean+/−SD
Italy	Naldi	2000	1062	6.7	2.6		4.0 (0.25–26) Median	
France	Euvrard	1995	580		2.4		Unclear	
Netherlands	Hartevelt	1990	764	6.2	3.6		8.7 (1–21) Mean	9.1 (4.6–14) Mean
UK	Liddington	1989	598	4.8	3.6	3.2	(0–12) Only range	7.1 (3.6–11) Mean
UK	Bordea	2004	979		3.2		7.6 (2–23) Mean	7.8 Mean
Ireland	Moloney	2006	1558		2.6		5.7 (0–16) Median	
Liver transplant recipients								
Spain	Xiol	2001	137	10	4.0	1.5	5.8 (3.6–8.7) Median	
The Netherlands	Haagsma	2001	174	6.9	1.6	1.8	5.1 (1.5–19) Median	
UK	Kelly	1998	888	1.7	4.0		4.4 (2.6–6.2) Mean	

SCC: Squamous cell carcinoma; BCC: Basal cell carcinoma; No: number; pt: patients;
KC: Keratinocyte skin cancers; % pt KC: Percentage of patients with a keratinocyte skin cancer;
SD: Standard deviation; SCC/BCC ratio:

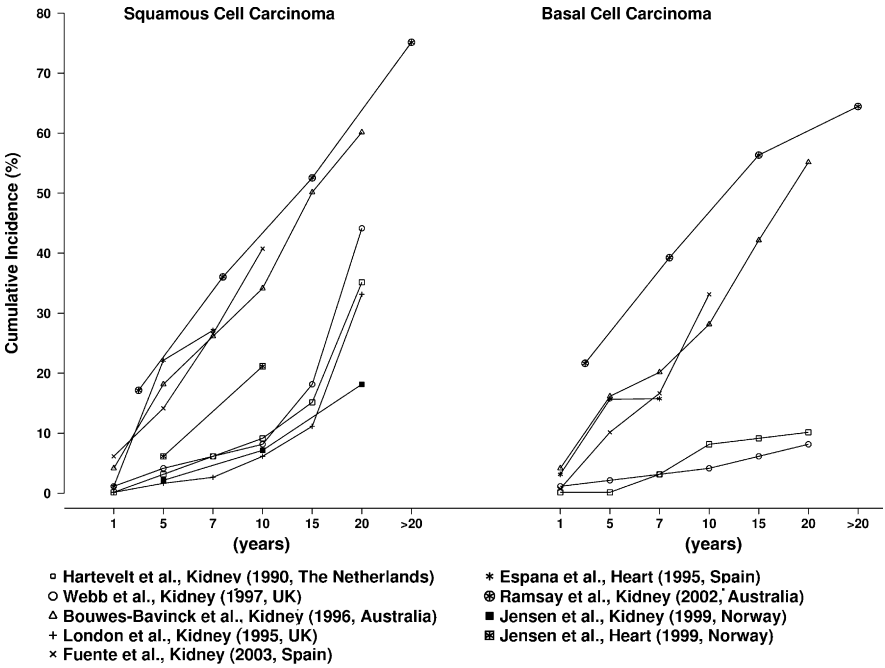


Fig. 1 Cumulative incidence

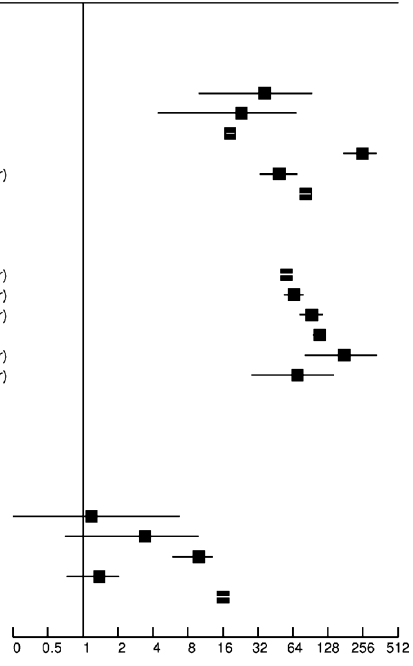
KC is reported infrequently in darker-skinned communities from developing countries. In developing countries, the overall incidence of any post-transplant cancer is generally much lower, with one review finding that only around 5% of 15,825 OTR developed malignancy compared with around 14% of 36,628 OTR from developed countries [19]. Japan and Taiwan seem to differ from both Western countries and the developing world. Two recent studies report little or no KC and no Kaposi's sarcoma [18, 68]. There was one study from South Africa examining skin cancer in 542 RTR [69], but no others from developing countries with data on KC, perhaps reflecting its rarity in these populations post transplantation. This finding accords with the data from South Africa, where skin cancers excluding Kaposi's sarcoma were seen only in patients of European origin [69].

An Increased Incidence of Post-Transplant SCC Occurs in All Areas of the Developed Western World and Is Highest at Low Latitudes

Studies throughout the Western world confirm a greatly increased incidence in cutaneous SCC after organ transplantation and consistently show that this risk increases with duration after transplantation (see Table 1a, Fig. 1). The highest risk is seen for heart transplant recipients in Australia, where an incidence of 379 per 1,000

HISTOLOGY/ORGAN STUDY (YEARS)	COUNT O/E		SIR (95%CI)	No. patients	Country or city / Comparison group	Standardised by sex/age/reference years
SQUAMOUS CELL CARCINOMA						
KIDNEY TRANSPLANTATIONS						
Hoxtell et al, 1977 (68-74)	First	4/0.11	36 (10-93)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72
Kinlen et al, 1979 (70-78)	First	3/0.13	23 (4-68)	3823	UK/Birmingham CR*	Y/5yrs/74
Gupta et al, 1986 (66-84)	NK	NK	18 (17-20)	523	Toronto / Detroit	Y/1yr/77-78
Hartevelt et al, 1990 (66-88)	All	38/0.15	253 (172-334)	764	Leiden / Eindhoven CR	Y/5yrs/83-85
Gjersvik et al, 2000 (83-92)	NK	NK	49 (33-70)	1020	Norway / CR	Y/1yr/calendar(1yr)
Moloney et al, 2006 (94-01)	All	282/3.4	82 (73-91)	1558	Ireland / CR	Y/1yr/94-2001
OTHER ORGANS +/- KIDNEY TRANSPLANTATIONS						
Adami et al, 2003 (70-97)	First	278/4.95	56 (50-63)	5931	Sweden / National CR	Y/1yr/calendar(1yr)
Jensen et al, 1999 (63-92)	First	97/1.49	65 (53-79)	2561	Oslo / National CR	Y/1yr/calendar(1yr)
Lindelof et al, 2000 (W) (70-94)	All	77/0.8	93 (73-116)	5356	Sweden / National CR	Y/1yr/calendar(1yr)
(M)		248/2.3	109 (95-123)			
Gjersvik et al, 2000 (83-92)	NK	NK	177 (81-336)	148	Norway / CR	Y/1yr/calendar(1yr)
Haagsma et al, 2001 (79-96)	First	7/0.1	70 (26-144)	174	The Netherlands / Mixture**	Y/1yr/calendar(1yr)
BASAL CELL CARCINOMA						
KIDNEY TRANSPLANTATIONS						
Kinlen et al, 1979 (70-78)	First	1/0.85	1.2 (0-7)	3823	UK / Birmingham	Y/5yrs/74
Hoxtell et al, 1977 (68-74)	First	3/0.88	3 (0.7-10)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72
Hartevelt et al, 1990 (66-88)	All	18/1.81	10 (6-15)	764	Leiden / Eindhoven CR	Y/5yrs/83-85
Gupta et al, 1986 (66-84)	NK	NK	1.4 (0.7-2)	523	Toronto / Detroit	Y/1yr/77-78
Moloney et al, 2006 (94-01)	All	143/8.9	16 (14-18)	1558	Ireland / CR	Y/1yr/94-2001

SIR & 95% CI



* CR= Cancer Registry; ** Mixture= Eindhoven before 1989 and National Cancer Registry after 1989
Y= yes; NK: not known; O= observed; E= expected; yr= year; No= number; CI= confidence interval

Fig. 2 Standardised incidence ratio (SIR)

person years at risk has been recorded [66]. Similarly, Bouwes Bavinck [65] and Ramsay [70] found equivalent high risks for SCC in the subtropical/tropical Australian state of Queensland, with a cumulative incidence at 20 years of 60% and 75% for those transplanted over 20 years (Fig. 1). Another study from Australia reported lower than expected rates, namely, a cumulative incidence of 38% for SCC at 20 years or more post transplant [71], but this was not a true incidence, rather a predicted incidence based on the number of skin cancers recorded between two different time points using data acquired from the ANZDATA cancer registry. Furthermore, the ANZDATA cancer registry lacks completeness for skin cancer: A previous study found ANZDATA failed to report 28% of post-transplant skin cancers [70] and recorded only the first episode of SCC (or BCC) post transplant.

A study from Spain [72] gave only cumulative incidence up to 10 years post transplant but showed high rates of increase similar to those observed in Australia (see Fig. 1). Studies from the UK [73] and The Netherlands [53] found lower 20-year cumulative incidence rates for SCC, 30% and 35%, respectively. Indeed, Australian rates were consistently higher than rates reported in diverse European centres for which incidence or relative risk estimates were available (see Table 1a). Studies from Spain, UK, and The Netherlands found an incidence for SCC of 29 per 1,000, 71 per 1,000, and 7.6 per 1,000 person-years, respectively [53, 61, 74]. The high incidence in this UK study can be explained by the inclusion of cumulative (multiple) SCC for given individuals, with an average of six tumours per patient [74]. The incidence of the first SCC would be lower by a factor of six, at least.

Cutaneous SCC Is the Dominant Skin Cancer Following Organ Transplantation, and There Is a High Incidence of Multiple Tumours

The most frequently encountered post-transplant skin cancers are SCC, and in some transplant cohorts SCC account for 90% of all skin cancers [75]. Unfortunately, valuable information on SCC incidence in other studies from the United States, Southern Europe, and Northern Europe is missing because they reported only all KC combined (as shown in Table 1b), rather than SCC and BCC separately. The post-transplant KC incidence rate in cardiac transplant recipients (CTR) from the United States and Spain is very similar [61, 76], with 52 and 45 per 1,000 person-years, respectively. Two studies from The Netherlands and Italy [53, 77] found an incidence for post-transplant KC of 9 per 1,000 person-years based their incidence data on the first SCC or first BCC, so their estimates were considerably lower than that reported from Oxford, UK [74], of 141 per 1,000 (also in a cohort of RTR) in which precancerous lesions and multiple tumours were recorded. A high increase in risk (for SCC or KC generally) has been seen in studies from Sweden, Norway, The Netherlands, and Ireland, where there are relatively low rates of SCC in the general population, particularly in younger adults (see Table 1a,b). For example, one study that examined more than 5,000 OTR from Sweden reported a relative risk of 109 for men, 93 for women, and, compared with the general Swedish population,

an overall 100-fold increased risk for SCC [63]. Thus, the increase in risk of skin cancer observed in OTR in North Europe may be higher than that demonstrated in sunny countries such as Australia because the baseline incidence in North Europe is so much lower. Moloney et al., in a population-based study in Ireland over a 7-year period [64], noted age-specific patterns of increase in KC incidence. There was a steady increase from the second year on after transplantation in older RTR (age 50 years or older), but a later and much greater increase in younger RTR (age less than 50 years), reaching incidence rates 200 times those in an age-matched nontransplanted population and peaking 10 to 12 years after transplantation.

The cumulative incidence of KC in renal transplant recipients follows a similar pattern to that seen for SCC, with the highest reported risk in Australia [65, 70] and lower cumulative incidence in Europe [3,53,64,73] and America [78]. A study from South Africa [69] found a cumulative incidence of KC at 10 years post transplant of 7%, but KC was limited to white patients of European origin, who comprised only one-third (34%) of the RTR cohort.

Tumour burden is compounded by the multiplicity of KC in OTR, which can be very high [55, 65, 74, 79]. Bouwes Bavinck et al. [65] found 2,751 KC in 271 OTR in Australia, Bordea et al. [74] reported an average of 6 tumours per patient in the UK, and Blohme and Larko [55] reported 2 patients in Scandinavia with more than 100 skin lesions each.

Apart from geographical influences, the prevalence of patients with multiple skin lesions varies from study to study also because of differences in length of follow-up and age of patients, with a range of 26% to 73% [51, 53, 55, 80, 81]. Careful cataloguing of all KC over a 16-year period in a cohort of RTR from London, UK, found that although two-thirds of skin cancer patients had multiple tumours, it was a minority of these who carried the majority of the tumour burden, with 22 RTR (3.4% of the whole cohort) having 10 or more SCC and accounting for 59% of the total SCC burden. Similarly, 56 RTR (8.5% of cohort) with 4 or more SCC account for 83% of the total number of SCC (Proby and Harwood, unpublished data).

Post-Transplant BCC Show Smaller Increases Than SCC with a Reversal in the BCC to SCC Ratio, Although Regional Differences Exist

BCC are the second most common cancers in fair-skinned OTR, and approximately 30% to 50% of OTR with SCC also have BCC [75]. Although BCC is the most common skin cancer in the general population, all studies of post-transplant skin cancers have shown smaller increases in BCC compared with SCC (see Table 1c). Consequently, when compared with skin cancers in the general population, the ratio of BCC to SCC is usually reversed, although the extent of this reversal differs in various regions of the world (see Tables 2, 3). In The Netherlands [53], a 10-fold increase in BCC was found, compared with a 250-fold increase in risk of SCC,

and a complete reversal in the SCC/BCC ratio from 1:4 to 3.6:1. A reversal of similar magnitude is seen in Australian transplant recipients of European origin, with four studies reporting an SCC/BCC ratio between 2.0 and 3.8 [65, 66, 70, 71]. An early study from the United States [49] reported a 10-fold difference in the relative risk of SCC compared with BCC, and a study from Canada [80] examining relative increase showed a SCC/BCC difference of the same order of magnitude (Table 2).

The magnitude of the SCC/BCC ratio is not always so high, however. Lower SCC/BCC ratios have been reported in studies from Spain and Italy [60, 61, 72, 82–84] (see Table 3). In the UK [73], the risk of SCC post transplant appeared to increase exponentially, whereas the risk of developing BCC seemed to increase linearly with increasing years of immunosuppression. Fuente et al. [72] similarly noted a linear increase in BCC compared to a more exponential increase in SCC, with increasing duration post transplant. Consequently, a gradual increase in the SCC/BCC ratio was observed over time. Studies of skin cancer rates in the immunocompetent general population in Australia have reportedly shown a secular change in the SCC/BCC ratio from 1:4.5 in 1985 [85] to 1:2.5 in 1995 [30]. This finding was speculated to reflect a reduction in incidence of BCC occurring in young people, perhaps the result of improved sun protection from childhood. This dynamic situation, together with latitude differences in the SCC/BCC ratio also seen in Australia [30], suggests that level of sun exposure influences the proportion of BCC and SCC seen post transplant. An alternative explanation for the apparent excess of BCC in transplant populations from Southern Europe is that their darker 'Mediterranean' skin type is relatively protective against SCC in the early post-transplant years but is perhaps less protective against BCC development.

Occurrence of Keratinocyte Cancer Increases After All Types of Solid Organ Transplantation, Although Level of Increase May Vary

When data on post-transplant skin cancer rates are available in both cardiac transplant recipients (CTR) and renal transplant recipients (RTR) from the same centre, the risk appears to be greater after cardiac transplantation [62, 77, 81, 86]. This risk may be partly (or wholly) the result of the generally older age, male predominance, and higher level of immunosuppression of cardiac transplant recipients however and thus it is therefore difficult to directly compare these two groups.

A cancer registry-based study from Italy compared KC risk in 1,062 RTR and 267 CTR [77] and concluded that there was no definite increased risk amongst CTR after adjustment for age at transplantation and sex. Another Italian study [81] and two studies from Norway based on the same data ([62] [see Fig. 1]; [86]) reported an approximately threefold higher risk of skin cancer in CTR compared with RTR, but differed in their interpretation of its significance. Fortina et al. [81] found that organ type was not independently associated with risk after a multivariate analysis;

Gjersvik et al. [86] attributed the increased risk to higher levels of immunosuppression in CTR, whereas Jensen et al. [62] reported a threefold increased risk for CTR even after adjustment for age and immunosuppressive regimen.

The risk for development of KC is also increased in liver transplant recipients (LTR). In similarly large cohorts of OTR from the United States, both followed for approximately 3 years, Otley and coworkers [76] reported an incidence for KC of 52 per 1,000 patient-years for CTR compared with 11 per 1,000 patient-years for LTR. However, a study from Spain with 170 LTR followed for an average of 5 years found an incidence for KC of 43 per 1,000 patient years [87], and an earlier study from The Netherlands reported a relative risk for KC of 70 in LTR [88], similar to increased risks seen in CTR and RTR (see Table 1b). Cumulative incidence for KC up to 15 years post liver transplantation was available in these two studies from Spain and The Netherlands. The pattern of increase with duration of transplant was similar in the two studies and closely resembles patterns seen after CTR and RTR. There may be regional differences, with higher tumour numbers reported from Spain [87], but there are too few studies to confirm this.

Risk Factors for Post-Transplant Keratinocyte Cancer

Many of the studies reviewed examined risk factors for KC development using univariate and/or multivariate analyses (Cox proportional hazard risk models). The most important factors that appeared to favour development of skin cancer were age at transplantation, sex (male), fair skin type, high sunlight exposure (including the presence of actinic keratoses), and length and level of immunosuppression. Few investigators found all these to be independent risk factors, but many of the same factors were reported across a wide range of studies [56,62,64,65,67,72,74,77,82,83]. Ferrándiz et al. [82], in a prospective study examining the first 3 years of immunosuppression in RTR from Spain, found a cumulative risk for KC of 18%, with age at transplantation and occupational sun exposure being significant risk factors. From Italy, Naldi et al. [77] found age at transplantation and male sex to be the most important risk factors. Also from Italy, Caforio et al. [83] found older age at transplantation, fair skin, high sunlight exposure, actinic keratosis, and a high rejection score to be independently associated with an increased SCC risk in CTR. They proposed that a high rejection score in the first year post transplantation might be a useful predictor for patients at risk because cumulative immunosuppressive load is so difficult to calculate. However, other studies have not found an association between the number of rejections and development of KC in transplant recipients [74, 77, 89, 90].

A study based in Queensland, Australia, involving 361 Caucasian RTR found SCC risk was strongly associated with blue eyes, duration of residence in a hot climate, and pretransplantation SCC, whereas high tumour numbers were associated with being born in a place with a hot climate, childhood sunburn, pretransplantation actinic keratoses, and smoking [67]. Meanwhile, in Europe, Bouwes Bavinck et al.

[91] found a strong and significant association with number of keratoses and presence of SCC after controlling for sex, age, and skin type and, in a recent multicentre study, again found that a high number of warty keratoses was a significant and independent risk factor for SCC, OTR with more than 50 keratotic skin lesions having an adjusted odds ratio of 12.1 [92]. All these risk factors are discussed in more detail elsewhere in this book.

Standardized Incidence Ratio (Fig. 2)

Population-based standardized incidence ratios (SIR) were only available from a limited number of studies, with an overrepresentation of Scandinavian countries because of their long history of national cancer registration [8, 10, 12, 62] (see Fig. 2). Cancer registration in Finland, for instance, started in 1952. Studies from The Netherlands used a combination of national and city cancer registries [53, 88]. Kinlen et al. [49] used the Birmingham Cancer Registry as "representative" of the UK population, while a study from Canada [80] used a cancer registry with the same latitude. Hoxtell et al. [49] used the USA Third National Survey to derive their ratios. Those population-based SIR that were available for post-transplant SCC and post-transplant BCC are illustrated in Fig. 2.

Conclusions

Organ transplant recipients are at greatly increased risk of keratinocyte cancers, particularly SCC, compared with their counterparts in the general population; this is true in all regions of the world and has been shown in multiple studies, even though many of these studies are limited by underreporting of KC incidence in the general population. The increased incidence in OTR is particularly notable in younger transplant recipients because post-transplant KC develop on average 20 years earlier than in the general population [20, 66]. SCC show a much greater increase post transplant than BCC, leading to a reversal in the normal SCC/BCC ratio, although region-specific differences in the frequency of post-transplant BCC alter the extent of this reversal. The SCC/BCC ratio is also influenced by time from transplantation because BCC show a steady linear increase compared with an exponential rise in post-transplant SCC. Post-transplant SCC are frequently multiple, leading to a very high burden of disease in some individuals and placing a heavy cost on the affected patients and health care resources alike.

It is difficult to compare studies from different regions of the world because of the diversity of characteristics of study populations (different ages, skin type, immunosuppressive regimens, length of follow-up, epidemiological methods, etc.) as well as the different environmental factors (notably latitude and level of sun exposure). It is clear, however, that post-transplant skin cancer is a major problem in temperate climates and an even greater problem in tropical and subtropical regions. Caucasians living in Australia have the highest incidence of post-transplant KC, and

southern Europe experiences higher rates than northern Europe. The SIR, however, may be at least as high in more temperate countries because of a relatively low frequency of these skin cancers in the general population, particularly in younger age groups. The type of transplantation may influence the extent of the problem, but any organ-specific risk is probably small when age and level of immunosuppression have been taken into account. Cardiac transplantation typically has the highest levels of immunosuppression, and therefore the highest rates of skin cancer, because of the catastrophic consequence of rejecting the donor organ. There is no firm epidemiological evidence for an oncogenic effect of a specific immunosuppressive regimen, and the level of immunosuppression rather than a specific agent may be the more important factor for skin cancer risk. Finally, solar ultraviolet radiation is the principal agent responsible for the development of KC, and it is therefore essential that all future investigations are able to account for its independent influence, even if only at the level of ambient sun exposure.

Appendix

Box 1 Search strategy

In PubMed, an extensive literature search was made using synonyms for relevant words of the clinical question. First, a combination of "skin cancer" and "transplantation" (both as subheadings) was made in which "transplantation" was matched to the subheading "adverse effects and/or complication". Only English or Dutch articles were included, and case reports and editorials were excluded; this resulted in 448 articles.

("Neoplasms, Basal Cell"[Majr] OR "Skin Neoplasms"[Majr] OR "Melanoma"[Majr] OR (("Carcinoma, Squamous Cell"[Majr] OR "squamous cell carcinoma"[ti] OR malignancy[ti] OR malignant[ti]) AND (skin[ti] OR dermis[ti] OR epidermis[ti])) OR melanoma[ti] OR melanoma∗[ti] OR "skin cancer"[ti] OR "skin cancers"[ti] OR "skin tumor"[ti] OR "skin tumors"[ti] OR "skin tumour"[ti] OR "skin tumours"[ti] OR "basal cell carcinoma"[ti]) AND (transplant[ti] OR transplants[ti] OR transplantation[ti] OR transplanted[ti] OR post-transplant[ti] OR "Transplantation/adverse effects"[MAJR] OR "Transplantation/complications"[MAJR])

To include articles that focus on malignancies in general without skin cancer as a subheading, "transplantation" (matched to "incidence") and "malignancies" were combined. Only English or Dutch articles were included, and case reports and editorials were excluded; this resulted in 501 articles.

("Neoplasms"[Majr:noexp] OR malignancy[ti] OR malignant[ti]) AND (transplant[ti] OR transplants[ti] OR transplantation[ti] OR transplanted[ti] OR post-transplant[ti] OR "Transplantation/adverse effects"[MAJR] OR "Transplantation/complications"[MAJR]) AND incidence.

Finally, a broad selection (7786 references) was performed using various terms for "skin cancers" combined with terms for "transplantation" matched to "incidence":

("Neoplasms, Basal Cell"[MeSH] OR "Skin Neoplasms"[MeSH] OR "Melanoma"[MeSH] OR (("Carcinoma, Squamous Cell"[MeSH] OR "squamous cell carcinoma") AND (skin OR dermis OR epidermis)) OR melanoma OR melanoma∗ OR "skin cancer" OR "skin cancers" OR "skin tumor" OR "skin tumors" OR "skin tumour" OR "skin tumours" OR "basal cell carcinoma") AND (transplant OR transplants OR transplantation OR transplanted OR post-transplant OR "Transplantation"[MeSH]) AND (incidence OR incidences OR incidenc∗).

Selection

After exclusion of duplicates, a total of 1,377 articles were found. A selection was made using title and/or abstract; this resulted in 329 articles that matched our clinical question. Full texts were evaluated and scored (A, B, C, D, or no score). The remaining A and B articles were used for tables and graphics in this book.

A (good article) when

- Baseline characteristics are clear
- Cumulative rate of skin cancer is present

B (sufficient article) when

- Baseline characteristics are clear
- Cumulative rate is unclear, but incidence rate or relative risk is given

C (doubtful article) when

- Only the number of patients with skin cancer is given
- No statistical analysis are used

D (insufficient article) when

- It does not match our clinical question

No score

- Paper not relevant at all

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Etiological Factors in Cutaneous Carcinogenesis – An Introduction

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Risk factors for skin cancer in organ transplant recipients largely overlap with risk factors that are known in the nonimmunosuppressed population and consist of a complex interplay between environmental and host-related factors.

Well-known environmental risk factors are exposure to sunlight [1–3], ionizing radiation [4–6], and various chemical carcinogens. Infections with certain viruses are also likely to be involved in skin cancer carcinogenesis. Human herpes virus type 8 is almost always present in Kaposi's sarcomas [7–9]; mucosal human papillomaviruses play an important role in the development of cervical carcinoma and anogenital carcinomas [10–12]; and beta papillomaviruses are thought to play a role in the development of cutaneous squamous cell carcinoma and possibly basal cell carcinoma [13–16].

Increasing age is an important nongenetic, host-related factor for the development of skin cancer [17–19]. Other host-related risk factors for skin cancer include genetic factors such as male sex, fair complexion, inability to tan, and nongenetic factors such as chronic scars and ulcers of the skin [20, 21]. Well-known genes that influence skin cancer susceptibility are, among others, melanocortin 1 receptor variants (MCR1); nucleotide excision repair (NER) genes, involved in xeroderma pigmentosum; the p53 tumor suppressor gene; stat 3 regulated genes (c-*myc*, cdc25A, COX 2); the IkB kinase gene; human leukocyte antigens (HLA); and many other possible genes [22–30].

In organ transplant recipients, long-term immunosuppressive therapy forms one of the most important risk factors [31,32]. Specifically, cumulative doses of immunosuppressive agents play a role, but there may also be differences in the carcinogenic potential of the different immunosuppressive agents. Prospective randomized studies with skin cancer as the final outcome are still lacking, and therefore one has to rely on retrospective follow-up studies with differences in the immunosuppressive regimens. Conclusions based on these types of studies are not always reliable.

Donor-related factors, such as HLA and other antigens that are present in the transplanted organ but not present in the recipient, are additional potential risk

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factors for skin cancer in transplant recipients. HLA mismatching is the best known example that donor-related antigens directly or indirectly may play a role in skin cancer carcinogenesis [33–38]. The HLA in the mismatched organ may exert a direct effect, but may also be associated with higher rates of rejection and, therefore, with more intense immunosuppression, which may increase the risk of skin cancer. The association between HLA mismatching and risk of skin cancer could not be confirmed in all studies. The exact role of HLA mismatching in the risk of skin cancer, therefore, remains still unclear.

In organ transplant recipients, both the nonspecific immunosurveillance against skin cancer and the specific immunosurveillance may be hampered because of a depressed natural killer cell function and a decreased function of CD8-positive cytotoxic T lymphocytes after antigenic stimulation in the context of HLA class I antigens. There are a number of theoretical mechanisms by which HLA antigens may be associated with an increased risk of skin cancer in transplant recipients. HLA plays a pivotal role in the cellular immune response to viral and tumor antigens. The HLA class II antigens are involved in recognizing foreign peptides by CD4-positive regulatory T lymphocytes, whereas the HLA class I antigens mainly serve as restriction elements for the reactivity of CD8-positive cytotoxic T lymphocytes.

Both HLA class II and class I antigens have been found to be associated with skin cancer. HLA-DR7 has been found to be associated with an increased risk of skin cancer [37], suggesting that an impaired response of CD4-positive regulatory T cells in the context of class II antigens may play a role in the etiology of skin cancer. HLA-B27 has been reported to be associated with skin cancer in both the Netherlands and Australia [34, 36, 37]. HLA-A11 has been found to be associated with a decreased risk of skin cancer [34], suggesting that reactivity of CD8-positive cytotoxic T lymphocytes in the context of class I is hampered in these patients. However, in Australia and the northern part of the United States of America, HLA-A11 was associated with an increased risk of skin cancer [33, 36], and other studies did not show any association between HLA-A11 and the risk of skin cancer [35,37–39]. It is not clear whether differences among these studies reflect differences in patient population, differences in environmental antigens (such as viruses), or differences in immunosuppression protocols, in geography and climate, or in methodology, or whether other, still unknown, factors may play a role. Alternatively, individual HLA types may be linked to nonimmune risk factors, such as skin pigmentation [33].

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Photocarcinogenesis – DNA Damage and Gene Mutations

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Ultraviolet Radiation, Skin Cancer, and Immunosuppressants

Ultraviolet (UV) radiation in sunlight is cytotoxic and, in overdosages, clearly detrimental to the skin, as becomes manifest in common sunburn reactions in which epidermal cells die in apoptosis ("sunburn cells") and strong inflammation occurs (vasodilation, extravasation, and infiltrates of leukocytes), turning the skin red (erythema) and swollen (edema). In excessive cases the skin ends up peeling, or it may even develop blisters. Fair-skinned people are clearly most susceptible to these sunburn reactions. Although these dramatic reactions may leave a different impression, it appears that the skin is quite well adapted to the persistent UV challenge in its natural environment – even the excessive reactions may be considered part of a formidable adaptation.

The skin is well equipped to repair the damage inflicted by solar UV irradiation, even damage from excessive exposures. Nevertheless, the repair mechanisms are not perfect, and in the course of time errors may creep in, most specifically, in the repair of the genome of skin cells. An accumulation of such errors in the genetic code may eventually give rise to skin cancer.

Squamous cell carcinomas (SCCs) have been associated with cumulative sun (UV) exposure, but the risk of basal cell carcinomas and cutaneous malignant melanomas shows a most significant increase with intermittent overexposures (i.e., episodes of severe sunburn). Interestingly, it is the risk of SCC that is most strongly increased in organ transplant recipients (OTRs). This risk has been considered an inevitable collateral effect of the immunosuppressive treatment, because animal experiments had shown that skin tumors induced by (chronic) UV exposure were highly antigenic and subject to immunosurveillance and elimination [1]. However, in the late 1980s it became clear that conventional immunosuppressive drugs also adversely affected DNA repair in the skin, which could contribute to the enhanced UV carcinogenesis observed with these drugs [2]. This latter finding did not seem to receive much attention at first, but it now comes into the limelight with the arrival of

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a new generation of immunosuppressants that differ in the mode of action from the earlier generation. If local adverse side effects of immunosuppressants in the skin can be minimized while maintaining adequate immunosuppression, the risk of SCC in OTRs may substantially decrease.

In the following sections, we discuss the UV-related steps in carcinogenesis in skin cells and the potential impact of immunosuppressants on these steps, as schematically represented in Fig. 1.

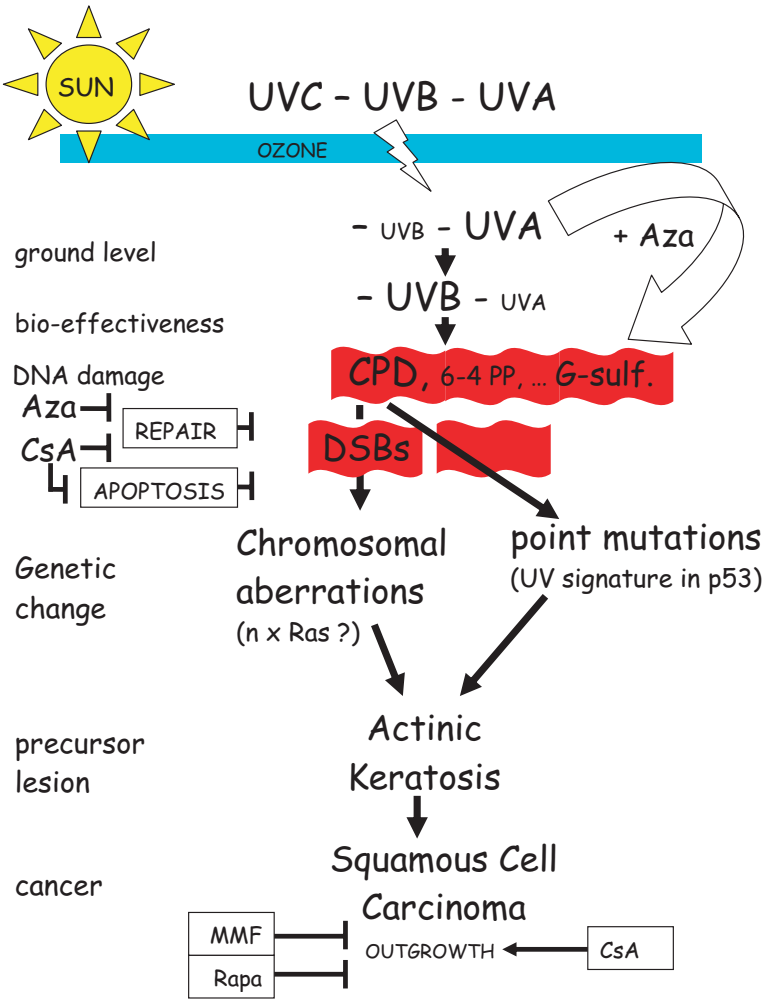

Fig. 1 Scheme of steps in ultraviolet (UV) carcinogenesis, and effects of azathioprine (*Aza*), cyclosporin A (*CsA*), mycophenolate mofetil (*MMF*), and rapamycin (*Rapa*)

UV Radiation and DNA Damage

The shorter the wavelength of UV radiation, the more energy each photon carries. The shortest wavelengths emitted by the sun, in the UV-C band (wavelengths <280 nm), are generally most damaging to organic molecules (specifically those with conjugated bonds and aromatic rings), but this radiation does not reach the Earth's surface. UV-C radiation and a part of the UV-B (wavelengths 280–315 nm) radiation are absorbed in the stratospheric ozone layer. Most of the UV-A radiation (wavelengths 315–400 nm; bordering the visible spectrum, 400–780 nm) passes through the atmosphere on a clear day and comprises more than 95% of the solar radiant UV energy at ground level. The minor fraction of UV-B radiation is, however, largely responsible for the sunburn reaction of the skin and, most likely, also for skin carcinogenicity (i.e., >80% of the effective UV dose stems from the UV-B wavelength band), as both effects appear to be related to the UV-induced DNA damage in the skin [3].

UV-B radiation is absorbed by DNA (owing to an abundance of aromatic rings in its bases), and dimeric lesions are formed at neighboring pyrimidine bases, mostly cyclobutane pyrimidine dimers (CPDs) and, to a lesser extent, 6–4 photoproducts (6–4PPs) in a ratio of about 4:1. The effectiveness of inducing CPDs appears to extend into the UV-A band (especially at wavelengths bordering UV-B) [3, 4], but that of inducing 6–4PPs does not [5]. Although indirect DNA base damage (e.g., 8-oxo-guanosine) from reactive oxygen species becomes relatively more important going from short UV-B to longer UV-A wavelengths [4], the dominant DNA lesion induced by broadband UV-A radiation in the skin is the CPD [5]. CPDs in turn can give rise to frank double-strand breaks (DSBs) in the DNA during replication in S phase [6].

UV-A radiation is far less effective in causing DNA damage than UV-B radiation, but the conventional immunosuppressant azathioprine was found to sensitize the DNA for UV-A radiation [7, 8]. Through the purine synthesis pathway, azathioprine is incorporated in the DNA as a 6-thioguanine pseudo-base, which causes UV-A sensitization and subsequent oxidation to form a guanine sulfonate. Thus, azathioprine enhances DNA damage and increases skin sensitivity to sunburn from UV-A radiation [8].

DNA Repair, Cell-Cycle Arrest, and Apoptosis

CPDs and 6–4PPs (and guanine sulfonates) block transcription and replication of DNA. Hence, the cell needs to repair these lesions to remain functional and viable and to be able to replicate its DNA without errors. Nucleotide excision repair (NER) is primarily responsible for the repair of these lesions. NER entails a cut-and-paste type of mechanism that involves a multitude of enzymes to recognize and cut out an oligomer containing the lesion and to fill in the gap in the DNA strand. There are two distinct pathways of NER: global genome (GG-) NER, operating

on helix-distorting lesions throughout the genome, and transcription-coupled (TC-) NER, that efficiently removes lesions that stall RNA polymerase in transcription. Defects in TC-NER greatly enhance the acute (sunburn) sensitivity to UV radiation, whereas a defect in GG-NER does not. The latter does, however, greatly increase mutagenesis and carcinogenesis, as seen in xeroderma pigmentosum (XP) patients who lack GG-NER [9]. If CPDs and 6–4PPs are not adequately removed from transcribed DNA strands, the cell is more sensitive to cell-cycle arrest and apoptosis. The arrest allows for more time to repair, and apoptosis will kill a cell that may otherwise replicate with an overly damaged genome that may give rise to errors in replication, that is, mutations in genes. If only GG-NER is defective (as in XP-C patients), the alarm signals for cell-cycle arrest and apoptosis will not be enhanced because of the still proficient TC-NER, and the cell may therefore enter replication with damage remaining in its nontranscribed DNA. Replication of this damaged DNA is bound to raise mutagenesis. If not strictly avoiding UV (solar) exposure, XP patients with severe impairment of GG-NER contract multiple skin cancers in childhood and succumb to these cancers before reaching the age of 20 years.

As the conventional immunosuppressants cyclosporin A and azathioprine were found to adversely affect NER [2,10], they can increase UV mutagenesis in the skin, and thus increase the risk of skin carcinomas, SCC in particular [11]. Cyclosporin A also appears to hamper apoptotic responses, which may further enhance UV mutagenesis and carcinogenesis [10]. As discussed earlier, azathioprine leads to thioguanine pseudo-bases in DNA. These pseudo-bases are substrates of mismatch repair (MMR; correcting mismatches between two complementary DNA strands). Through survival and growth advantages, thioguanine bases can introduce a selection pressure for loss of MMR [12]. If this mechanism occurs in the skin of OTRs on azathioprine, the skin carcinoma risk may further increase because MMR also operates on UV-induced DNA damage.

Certain variants (polymorphisms) of genes coding for proteins (XRCC2, XRCC3, ligase IV) involved in the repair of DSBs were found to increase the risk of skin carcinomas [13]. These results agree nicely with the recent finding that people who had had skin carcinomas removed showed an increased susceptibility to DSB induction in their UV-irradiated leukocytes [14].

No data exist on whether novel immunosuppressants, such as rapamycin (Rapa) or mycophenolate Mofetil (MMF), affect DNA repair or apoptosis. Although MMF does not lead to pseudo-bases, its inhibitory effect on the purine synthesis pathway could perhaps have repercussions on filling the DNA gap in NER. The inhibitory effect of Rapa on mTOR in the Akt ("survival") pathway may deregulate cell-cycle control and enhance apoptosis, as found in p53-null cells [15].

UV Radiation and Genetic Alterations

The UV-induced DNA damage may lead to changes in the genome, ranging from subtle point mutations (single base changes) to gross chromosomal aberrations, and even the formation of "micronuclei" (small satellites to the main nuclei). As

mentioned in the previous section, the S phase in the cell cycle appears to be most critical for acquiring these genomic alterations: Damage encountered in template DNA generally poses an obstacle to the replication machinery, which can then mobilize alternative pathways to sidestep the problem, either by error-prone replication over a noninstructive damaged base (lesional bypass) or by pulling in the DNA strands from the other parental allele of the gene to be copied and using these undamaged strands as templates (homologous recombination). The lesional bypass may give rise to point mutations, and the recombination repair may enhance sister chromatid exchange, or may even lead to loss or duplication of chromosome fragments. If the damage is not adequately circumvented, DSBs may occur at stalled replication forks with "less than perfect" patch-ups (e.g., nonhomologous end-joining) which further enhance the risk of gross chromosomal aberrations. Coding errors can be detected and repaired post hoc by comparing complementary strands in MMR.

The UV-induced pyrimidine dimers (CPDs and 6–4PPs) characteristically lead to cytosine (C) to thymine (T) single base changes at dipyrimidine sites (about 70% or more of mutations), and even to CC-to-TT tandem mutations (about 10%) [16]; these are considered UV-signature mutations. Other genetic changes, such as caused by oxidative damage or DSBs, can be induced by many genotoxic agents and are therefore not UV specific.

Genetic Defects in Skin Carcinomas and Precursor Lesions

Skin carcinomas carry UV-signature mutations in the gene that codes for p53 tumor suppressor protein [17]. The benign precursor lesions, actinic keratoses (AKs), of SCCs already show these mutations, and even before any macroscopic lesion is visible, microscopic clusters of cells (clones) appear that overexpress mutant-p53. A close and most likely causal relationship between these early mutant p53 foci and eventual SCCs has been established in experiments with hairless mice [18]. In contrast to SCCs, the mutant p53 foci do not appear to be subject to immunological surveillance and elimination [19], but they do occur in higher frequency in OTRs than in immunocompetent individuals (ICIs) in normal skin neighboring SCCs [De Graaf et al., manuscript in preparation]. Hence, the increase in mutant p53 foci in OTRs is likely to result from local adverse effects of immunosuppressants (viz. azathioprine and cyclosporin A) on the skin cells, and this in turn is likely to contribute to the SCC risk independently of immunosuppression per se.

Similar to the *p53* mutations found in skin carcinomas from ICIs, those in carcinomas from OTRs show the UV signature [20]. In contrast to this earlier study in which no CC-to-TT mutations were found, a recent study on 25 skin carcinomas from 20 OTRs found a rather high percentage (35%) of these tandem mutations among the *p53* mutations [21], reminiscent of what is observed in XPC patients who specifically lack GG-NER. The early study may well have included more OTRs with long-term azathioprine treatment, whereas the OTRs in the more recent study

may have been treated more with cyclosporin A. The authors of the recent study speculate that the increased percentage in tandem mutations that they observed is attributable to a slow repair in combination with a repressed apoptosis, as is caused by cyclosporin A. In the *p53* mutation spectrum they found no deviations that may have arisen from azathioprine-derived photosensitization. However, the total number of mutations studied ($n = 24$) may have been too low to establish this firmly. No apparent effort was made to correlate *p53* mutations to specific immunosuppressive treatments in either study, but the numbers of OTRs were rather small for such analyses.

SCCs and AKs in ICIs carry many chromosomal aberrations [22]. An amplification of the *H-RAS* oncogene has been reported for SCCs from OTRs [23]; that is, multiple copies of the gene are present in the cells of SCCs. This is a chromosomal aberration (repeats of chromosomal fragments). Considering the effects of azathioprine and calcineurin inhibitors such as cyclosporin A, the risk of UV-induced DSBs and chromosomal aberrations may well also be elevated in OTRs.

Tumor Outgrowth

Cyclosporin A has been found to enhance tumor growth and metastases by cellular changes related to increased expression of TGF-β, independent of its immunosuppressive effect [24]. In contrast to the conventional immunosuppressants, the novel drugs mycophenolate mofetil and rapamycin have antitumor effects [25], and rapamycin has been experimentally proven to inhibit angiogenesis and tumor growth while providing adequate immunosuppression to maintain an allograft [26]. Results from our group and the group of Dr. A. Vanbuskirk [manuscripts in preparation] show net inhibitory effects of rapamycin on UV carcinogenesis, specifically on the development of larger skin tumors.

Conclusions

With the successful long-term retention of organ transplantations, adverse side effects, such as an enhanced rate of skin carcinoma development, become more and more pronounced. The traditional notion that the immunosuppressive regimen per se necessarily results in the collateral formation of skin carcinomas is in need of revision: The conventional immunosuppressants, azathioprine and cyclosporine, exert adverse effects on DNA repair and apoptosis in skin cells, which may importantly contribute to the risk of skin carcinomas. A novel generation of immunosuppressants, most notably rapamycin, differs in mode of action from the conventional drugs, and exerts antitumor effects while maintaining adequate immunosuppression for organ transplantation. Although the effects of these novel drugs on UV-induced skin carcinogenesis are not fully studied, the first experimental results hold great

promise for lowering substantially the risk of skin carcinomas in OTRs. Some recent clinical reports appear to point in the same direction [27, 28].

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Ultraviolet-Induced Immunosuppression: Implications for Photocarcinogenesis

Stefan Beissert and Thomas Schwarz

Introduction

Ultraviolet (UV) irradiation can be regarded as one of the most significant environmental factors affecting human life. Although UV irradiation has an essential impact on terrestrial and aquatic ecology and is an essential requirement for the different life forms, particularly the mid wavelengths, UVB (290–320 nm), can also exert deleterious effects on health. The mechanisms underlying the influence of UV radiation on health are not limited to its instrumental role in the development of skin cancer, but also include the profound effects it has on local and systemic inflammatory responses. Analysing the biological effects of UVB irradiation has shown that UV exposure can significantly inhibit immunity.

The implications of the immunosuppressive properties of UV irradiation are manifold because UVB-induced immunosuppression is not only responsible for the inhibition of protective cell-mediated immunity but also contributes to the initiation as well as development and perpetuation of several skin disorders [1–6]. These effects include induction of inflammation and cell death, premature skin aging, exacerbation of infectious diseases, and induction of skin cancer as well as photosensitive diseases such as cutaneous lupus erythematosus (LE), polymorphous light eruption, and solar urticaria. Some of these clinical effects of solar irradiation were already described more than 100 years ago [1].

Therefore, detailed knowledge about the mechanisms underlying UVB-mediated immunomodulation is of utmost importance. Extensive investigations have been performed in the field of photoimmunology within the past three decades, and it has become much clearer by which mechanisms UVB irradiation suppresses immunity [7–12]. Most of the experiments were performed in mice using the contact hypersensitivity (CHS) or delayed-type hypersensitivity (DTH) model to haptens as well as photocarcinogenesis experiments [10–12]. These models have provided important information not only for photoimmunology but also for the field of immunology in

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general. In the following, the effects of UV exposure on the murine and human immune system with regard to the development of UV-induced skin cancer are briefly reviewed.

UV-Induced Local Immunosuppression

Application of haptens onto low-dose UVB-exposed human or murine skin leads to inhibition of the induction of CHS. This effect has also been termed UV-induced local immunosuppression. The UV-induced changes in epidermal Langerhans' cell function, as well as the UV-induced release of soluble immunosuppressive factors [interleukin (IL)-10, tumor necrosis factor (TNF)-α, IL-1α, *cis*-urocanic acid], which influence the local micromilieu, have been proposed to be the major players contributing to this phenomenon [13–20].

More than two decades ago, the observation had already been made that exposure to low-dose UVB irradiation is able to suppress CHS responses to topically applied haptens in certain strains of mice, and following investigations revealed that inherited gene sequences influenced the individual immunological (un)responsiveness [21]. Mouse strains in which immunosuppression was observed were designated UVB susceptible (e.g., C3H/HeN; C57BL/6), whereas strains resistant to the adverse effects of UV irradiation were termed UVB resistant (C3H/HeJ; Balb/c). Additional investigations indicated that the relevant autosomal loci controlling these phenotypes can be confined to the alleles *lps* and *tnf* [23]. Work supporting the relevance of the *tnf* locus was supplied by studies in which inhibition of CHS in UVB-susceptible animals was prevented application of neutralizing anti-TNF-α antibodies [23]. In agreement with these results, a reduced capacity to mount a CHS response when hapten was applied to murine skin following injection of subinflammatory doses of TNF-α has been demonstrated [22–24].

UV irradiation also induces morphological and functional alterations in epidermal Langerhans' cells, leading to their immobilization or, UV dose dependently, to cell death. The involvement of TNF-α in the emigration of Langerhans' cells from UV-exposed skin into the regional lymph nodes has also been reported [25]. However, the role of TNF-α was questioned by the report that normal Langerhans' cell migration was observed in TNF receptor 1 (p55)-deficient mice following hapten application onto unirradiated skin. However, the treatment of these mice with neutralizing anti-TNF-α antibodies still had the effect of reducing Langerhans' cell migration [25–27]. These data suggest that TNF receptor 1 may not be crucial for this process and indirectly implicate the TNF receptor 2 (p75) as being required for Langerhans' cell migration. Because of the possible similarities between UVB- and TNF-α-mediated effects, the same group employed these mouse models to scrutinize known TNF-α signals in UV-induced local suppression [28]. UVB irradiation similarly abrogated CHS responses in both mutant and wild-type mice as well as in TNF-α receptor 1 + 2 double-deficient mice, once again precluding TNF-α receptor 1 as an integral factor in the effects caused by UV irradiation in local cutaneous immunity. In summary, the results obtained from these studies with gene-targeted

mice put the role of TNF-α signalling into a different perspective and suggest a rather minor role, if any, of the classic TNF-α pathway in UVB-induced local immunosuppression, pointing to other substances as key factors in this scenario.

UVB susceptibility and UVB resistance can also be scrutinized to a certain degree in humans [29]. An association between the immunosuppressive effects of UVB and the development of skin cancer was suggested by the finding of a significantly higher incidence of skin tumors in photosensitive patients. In agreement with the evidence provided by the murine models, microsatellite markers and single nucleotide polymorphisms (SNPs) link these phenotypes to the TNF-α locus, pointing to a role of TNF-α or other genes contained in this gene cluster are possible determinants for UVB susceptibility in humans [30]. With the availability of the full human genome, better marker(s) for UV susceptibility could be identified soon and will help to clarify these controversially discussed data.

It is well known that exposure to UVB radiation functionally alters Langerhans' cells in their activity to present major histocompatibility complex (MHC)-dependent antigens [31–37]. Low-dose exposure of Langerhans' cells to UVB also leads to the preferential activation $CD4^+$ cells of the T helper 2 (Th2) subset, but does not result in the activation T helper 1 (Th1) cells [38,39]. In subsequent investigations it was reported that UVB irradiation converts Langerhans' cells from immunogenic to tolerogenic antigen-presenting cells because of induction of specific clonal anergy in $CD4^+$ T helper 1 cells [38, 39]. As hapten sensitization represents a primary syngeneic response and these studies used either allogeneic primary systems or primed syngeneic systems, an extrapolation of these findings to the in vivo situation for hapten sensitization may not be feasible, as neither one of these model systems is an appropriate surrogate for the suppression of a primary immune response.

Langerhans' cells have the ability to present tumor-associated antigens for both the induction and the elicitation of protective immunity. It was shown that the subcutaneous injection of tumor antigen-loaded Langerhans' cells into naïve recipient mice resulted in the development of strong antitumoral immune responses because these animals rejected a subsequent challenge with viable tumor cells. UV irradiation of Langerhans' cells before immunization impaired the induction of antitumoral immunity, leading to the rapid growth of the inoculated tumor cells [40]. In later experiments it was demonstrated that UV-induced keratinocyte-derived IL-10 was able to inhibit the antigen-presenting function of Langerhans' cells [41, 42]. Together, these findings suggest that the UV-induced alternation of Langerhans' cell antigen-presenting function is mediated via the production of IL-10 from keratinocytes.

Mechanisms of UV-Induced Systemic Immunosuppression

Irradiation of mice to larger doses of UVB ($\geq$2 kJ/m^2) inhibits both CHS responses following painting of haptens onto sites not exposed to UV and the induction of DTH responses [10, 11, 19, 20, 40]. As Langerhans' cells critically involved in local

immunosuppression were not altered in their number or morphology in non-UVB-exposed skin areas, these findings suggested effector mechanisms other than those involved in UV-induced local immunosuppression. Several molecular pathways are considered to be involved in this so-called UV-induced systemic immunosuppression including impaired signalling caused by UV-induced mutations of the photoreceptor DNA, conformational changes in the photoreceptor urocanic acid, and the release of a large number of soluble mediators with suppressive properties such as IL-1α, TNF-α, prostaglandin E_2 (PGE_2), and IL-10 [13–15, 41–49].

In particular, the role of IL-10 in UV-induced immunosuppression and regulation of cutaneous immune responses has been emphasized by a number of research groups [41, 42, 45, 46]. Intraperitoneal IL-10 administration was found to inhibit the elicitation phase, but not the induction phase, of CHS responses [48]. On the other hand, both the induction and the elicitation of DTH immunity are suppressed by IL-10 treatment, indicating that CHS and DTH responses are related but distinct immune reactions. Increased concentrations of IL-10 were detected in the serum of UVB-exposed mice, and application of neutralizing anti-IL-10-antibodies significantly inhibited the UV-induced suppression of DTH responses to alloantigens, suggesting that IL-10 functions as a main mediator of UV-induced systemic immunosuppression [10, 12]. These findings are in agreement with the observation that spleen cells from UVB-treated mice were unable to present antigen to Th1 cells, whereas antigen presentation to Th2 cells was even enhanced [48]. Abrogation of both effects was achieved by application of neutralizing anti-IL-10 antibodies. To directly address the role of IL-10 in UV-induced systemic immunosuppression, IL-10-deficient mice were utilized [50]. The induction of DTH responses in IL-10-deficient mice could not be suppressed by UVB irradiation whereas the induction of CHS responses was suppressed following UVB exposure. These data clearly demonstrate the in vivo relevance of IL-10 as a key mediator of UV-induced systemic immunosuppression. Furthermore, since IL-10 is one of the key cytokines involved in the skewing the immune balance toward Th2-like immunity, such findings support the concept that UV exposure inhibits Th1-type immune responses.

To investigate the role of IL-10 during the development of UV-induced skin tumor development (photocarcinogenesis), groups of IL-10-deficient and wild-type mice were chronically UVB irradiated. Importantly, IL-10-deficient mice failed to develop UV-induced skin tumors compared to controls, indicating that IL-10 plays a key role during photocarcinogenesis [51]. Additionally, it was found that basal cell carcinomas are able to produce IL-10 and perhaps this IL-10 production contributes to cancer progression.

The concept of a Th2 shift in systemic immunosuppression is further supported by the observation that immunosuppression is blocked in mice treated with neutralizing anti-IL-4 antibodies [52]. Although UVB radiation does not directly induce the release of this key Th2-cytokine, the IL-4 effects might be mediated indirectly via the UVB-induced release of PGE_2 by keratinocytes. Accordingly, this concept was substantiated by the observation that cyclooxygenase-2 inhibitors blocked IL-4 production following UV treatment, which alludes to the activation of a cytokine cascade (prostaglandin E_2 → IL-4 → IL-10) following UVB exposure

that finally results in systemic immunosuppression [52]. Recent observations in humans revealed that UVB radiation stimulates the immigration of neutrophils into the skin, which could give rise to type 2 T-cell responses in UVB-exposed skin via secretion of IL-4 [53]. Hence, there is substantial evidence that exposure to UVB radiation generates a shift toward a Th2 immune response in vivo, thus explaining the fact that mostly Th1-mediated cellular immune reactions are impaired by UVB radiation.

UV-Induced Antigen-Specific Immunotolerance

Another of the many consequences of UV irradiation for the immune system is that it also interferes with cell-mediated immunity to allergens by inducing antigen-specific tolerance [11, 21]. Mice having received an initial immunization through UVB-exposed skin do not mount an immune response following resensitization with the same antigen at a later time point [21]. These very same mice showed no compromised immune responses upon sensitization against a different unrelated antigen, suggesting that UVB radiation leads to an antigen-specific rather than a general suppression of the immune system. Subsequent investigations revealed that the induction of antigen-specific tolerogenic suppressor/regulatory T cells was the root of the observed immunosuppression and that this also occurred in the model of systemic immunosuppression.

There is also evidence that UVB radiation can impair CHS responses because of antigen-specific tolerance in humans. In about 10% of the human subjects tested, tolerance was induced [29]. This was antigen specific, as they reacted with pronounced CHS responses upon subsequent sensitization with a nonrelated antigen. Even higher percentages of human volunteers developing tolerance when the antigen was initially applied onto skin areas exposed to erythemogenic UVB doses were reported in a further study [54]. These variations may result from the different UV irradiation protocols used. Nevertheless, both reports demonstrate the existence of a subtype of humans who develop tolerance when the sensitizing antigen is first applied onto UVB-exposed skin.

Erythemogenic UVB not only causes the emigration and subsequent depletion of Langerhans' cells in the skin but also results in the infiltration of $CD1a^+$ $HLA\text{-}DR^+$ $CD36^+$ macrophages in the skin [54]. These macrophages are then able to activate autoreactive T cells [55, 56], specifically $CD4^+$ "suppressor-inducer" cells, which in turn induce the maturation of suppressor T cells [57, 58]. Additionally, these macrophages, which also express $CD11b^+$, can release the immunosuppressive cytokine IL-10 at considerable concentrations, probably representing the major source for epidermal IL-10 protein in human UV-exposed skin [59]. This finding is of particular relevance in light of the fact that IL-10 seems to play a major role in UVB-induced immunosuppression. In vitro studies have shown that upon UVB exposure the macrophages infiltrating the epidermis can also induce $CD4^+$ T lymphocytes, which lack the expression of the IL-2 receptor alpha chain

[60]. The downregulation of the IL-2 receptor alpha chain seems to be connected with effects caused by transforming growth factor-β, another immunosuppressive mediator.

UV-Induced Regulatory T Cells

UV-induced skin tumors from UV-suppressed mice grow progressively when transferred into mice immunosuppressed by UV but typically regress when transplanted into immunocompetent mice [61–63]. Furthermore, the transfer of T lymphocytes from UVB-irradiated mice into normal recipients also results in the failure to reject UVB-induced tumors [64, 65]. Analogous results were obtained using the hapten model of sensitization [66, 67] in which injection of T lymphocytes from lymph nodes or spleens obtained from UVB-irradiated and hapten-sensitized mice suppress CHS responses in the recipients. In correlation with the studies previously mentioned, the recipients were still able to generate a normal CHS response to an irrelevant hapten [66, 67]. Taken together, these findings argue that UV-induced tolerance is mediated via induction of hapten-specific suppressor T cells. Because of the poor characterization of the molecular mechanisms and the phenotypes of the cells inducing this active immunosuppression, the term T suppressor cells was almost banned and the entire concept of suppression drawn into question [68, 69]. Yet the persistent hunt for T suppressor cells by investigators not only in the field of photoimmunology finally resulted in the discovery of these regulatory T cells, thus retrospectively justifying both the search and the concept of T suppressor/regulatory cells [68].

Tolerance can be induced by the transfer of lymphocytes in both local and systemic suppression. However, different subsets of T cells seem to be responsible for the immunosuppressive effects. Systemic UVB-induced suppression is mediated by antigen-specific $CD3^+$, $CD4^+$, and $CD8^-$ suppressor cells [45]. The results of a study initiated by Elmets et al. [66] revealed that in the local UV-induced immunosuppression, treatment of cells from UVB-irradiated animals with antibodies directed against Lyt-1 (CD4) completely abrogated their ability to transfer suppression, while treatment of cells with antibodies directed against Lyt-2 (CD8) partially inhibited suppression [66]. Accordingly, Schwarz et al. reported that in the UV low-dose model suppression was prevented when the transferred T lymphocytes were depleted of $CD8^+$ cells [70]. It is important to note that T suppressor cells in this particular experimental design only influence the induction but not the elicitation of CHS, as introduction of UVB-induced T suppressor cells into previously sensitized mice does not affect the CHS response in recipients [71]. This observation might indicate that effector T cells dominate T suppressor cells.

A number of studies have been conducted to further characterize this cell type. Both human and murine $CD4^+$ T cells subjected to chronic activation with CD3 in the presence of IL-10 induce $CD4^+$ T-cell clones with low proliferative capacity, low levels of IL-2, and no IL-4 that are yet able to produce high levels of IL-10 [72]. Studies in SCID mice demonstrated that these antigen-specific T-cell clones are able

to suppress the proliferation of $CD4^+$ T cells in response to antigen and can be used to prevent T-cell-mediated colitis. This particular subset of $CD4^+$ T cells was designated T regulatory cells. Another subset of $CD4^+$ regulatory T cells is characterized by the constitutive expression of the α-chain of the IL-2 receptor (CD25) [73]. Interestingly, $CD4^+$ $CD25^+$ regulatory T cells constitute approximately 10% of all murine peripheral $CD4^+$ T cells. The results of these and other studies have inspired much new research investigating the role of suppressor/regulatory T cells, currently making this area of research one of the most intensively studied subjects in general immunology. Whether the cells are termed regulatory or suppressor is more a matter of semantics, but because of this new breakthrough the concept of T suppressor cells has been redeemed and is now accepted in the immunological community [74].

The first successful cloning of regulatory T cells from UVB-irradiated mice was achieved by Shreedhar et al. [75]. Mice were sensitized with fluorescein isothiocyanate (FITC) following UVB treatment. The T cells cloned from these mice were phenotypically analyzed as $CD4^+$, $CD8^-$, TCR-α/β^+, MHC-restricted T cells specific for the FITC antigen. They secreted IL-10, but not IL-4 or interferon-γ, whereas cells from nonirradiated control animals produced high amounts of interferon-γ and little IL-4 and IL-10 [75]. The cytokine pattern of the UVB-induced cells was related but not identical to that of T regulatory 1 (Tr1) cells. Thus the authors designated these cells T regulatory 2 type cells. In vitro experiments established that these cells have the ability to block antigen-presenting cell functions, including IL-12 production. Even more importantly, injection of these T cells into untreated recipients suppressed the induction of CHS against FITC.

Although many studies previously described regulatory T cells to be of the CD8 type, the aforementioned studies and many more provide increasing evidence that the majority belong to the CD4 type. In this respect, the role of $CD4^+$ $CD25^+$ regulatory T cells in eliciting UVB-induced tolerance remains to be determined. First clues as to the importance of $CD4^+$ T cells in generating UVB-induced immunosuppression were recently found using MHC class II knockout mice. These animals are resistant to the immunosuppressive effects of UVB radiation, indicating that UVB-induced immunosuppression is caused by preferential activation of $CD4^+$ regulatory T cells as a result of deficient priming or expansion of effector $CD8^+$ T cells [76].

UV-induced regulatory T cells also express the B7 family molecule cytotoxic T lymphocyte activation molecule-4 (CTLA-4; CD152) on their surface. CTLA-4 is functionally relevant for immunosuppression as inhibition of CTLA-4 by a neutralizing antibody inhibits the induction of tolerance and immunosuppression following the transfer of T cells [77]. In vitro stimulation of UV-induced regulatory T cells induced the release of IL-2, interferon-γ, and high amounts of IL-10 but no IL-4, a cytokine secretion pattern reminiscent of that of regulatory T cells. Release of IL-10 appears to be functionally relevant because transfer of suppression was inhibited when recipients received neutralizing anti-IL-10-antibodies.

There is evidence for a distinctive heterogeneity of (UV-induced) regulatory cells based on the observation that UVB-induced NKT cells are involved in the suppression of tumor immune responses [78]. NKT cells express intermediate amounts of

T-cell receptor molecules and coexpress surface antigens normally found on natural killer cells (NK1.1, DX5, and Ly49a). Moodycliffe et al. supplied compelling data that UVB-induced regulatory T cells may actually belong to the NKT type and that these cells can suppress both DTH and antitumoral immunity. It remains to be determined to what extent these cells, which have also been detected in UV-exposed humans, play a role in the etiology of tumor progression of UVB-induced skin cancers [79].

Antigen-presenting cells are crucial for the induction of antigen-specific T-cell activation. Besides the interaction of the T-cell receptor and MHC class I/II molecules ("signal 1"), costimulatory molecules ("signal 2") also have to participate in this cell–cell communication for efficient T-cell priming. Among the costimulatory molecules, the B7 family plays a pivotal role, as in this group of receptor/coreceptor pairs, stimulatory as well as inhibitory signal pathways exist. The two oldest "family members" are B7.1 (CD80) and B7.2 (CD86), which bind to CD28 as well as CTLA-4 (CD152). A functional blockade of CD80/CD86 signaling induced by transgenic overexpression of soluble CTLA-4Ig resulted in reduced UV-induced skin tumor development [80]. Additionally, CD80/CD86 inhibition led to impaired UV-induced skewing of immunity toward Th2, as evidenced by the increased interferon (IFN)-γ production of T cells from UV-treated K14-CTLA-4Ig transgenic mice. Since CD80/CD86 can bind to both coreceptors CD28 and CTLA-4, mice deficient for either CD80 or CD86 were chronically UV irradiated to induce skin tumor development. Although $CD80^{-/-}$ mice developed UV-induced skin tumors to a similar extent compared to wild-type mice, $CD86^{-/-}$ mice developed skin tumors significantly earlier. Interestingly, dendritic cells from $CD86^{-/-}$ mice induced markedly less T-cell proliferation compared to controls, suggesting that once again antigen-presenting cells might play a critical role for antitumoral immunity [81].

Besides CD86-mediated signalling, the CD80/CD86-CTLA-4 pathway also regulates the development of UV-induced carcinogenesis, as mice treated with neutralizing anti-CTLA-4 antibodies after each UV treatment showed strongly reduced photocarcinogenesis [81]. Furthermore, anti-CTLA-4 antibody treatment induced strong long-lasting protective antitumoral immunity, as indicated by the rejection of a challenge with viable UV tumor cells. Importantly, anti-CTLA-4 antibodies impaired the suppressor function of UV-induced $CD4^{+}CD25^{+}$ regulatory T cells, suggesting another therapeutic beneficial effect of interfering with CD80/CD86-CTLA-4 signaling. Indeed, a humanized anti-CTLA-4 antibody has been already successfully used to treat melanoma patients [82]. Together, these findings indicate the importance of CD80/CD86-CD28/CTLA-4 pathways for UV-induced skin cancer development and further suggest that interfering with CD80/CD86-CTLA-4 signaling might be beneficial for the treatment of patients with cutaneous malignancies.

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Carcinogenic Mechanisms Related to Immunosuppressive Therapy

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Introduction

Before 1985, azathioprine and corticosteroids were used to suppress the immune response and prevent allograft rejection in organ transplant recipients. Since 1985, the majority of patients have received cyclosporin in combination with azathoprine and/or corticosteroids. More recently, other immunosuppressive agents have been introduced, including tacrolimus, sirolimus, and mycophenolate mofetil [1–3], which are described elsewhere in this book (see Part I).

Excess skin cancers, and indeed other cancers, in organ transplant recipients have been attributed in very large part to chronic suppression of the immune system by drugs used to prevent allograft rejection [4]. Loss of immunocompetence facilitates the frequency and persistence of viral infection, causal in the development of some transplant-associated cancers, including cervical and anogenital cancer, and in post-transplant lymphoproliferative disorders [4]. In addition, it is believed that such loss may reduce both "immune surveillance" and eradication of precancerous lesions, although the mechanism by which this occurs in the immunocompetent host is not well defined [5]. The important contribution of immunosuppression is further highlighted by the similarities between the range of cancers in organ transplant recipients and among human immunodeficiency virus (HIV)-infected individuals [6]. Kaposi's sarcoma, non-Hodgkin lymphoma, liver cancer, and cervical cancer are common in both groups.

In addition to overall intensity of immunosuppressive load contributing to excess skin cancer risk, there is increasing evidence to suggest that some drugs, principally azathioprine and cyclosporin, may also be directly carcinogenic, whereas others, specifically rapamycin, may have antineoplastic properties.

This chapter reviews the evidence for the contributions of overall reduction in immunosurveillance and specific carcinogenic properties of immunosuppressive drugs in the pathogenesis of post-transplant skin cancer.

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General Effects of Immunosuppression on Carcinogenesis

As most organ transplant recipients receive a combination of two or more immunosuppressive agents, it has been difficult to attribute quantifiable risk to any individual immunosuppressive agent. There are currently no data on skin cancer risk associated with the newer agents such as mycophenolate mofetil and tacrolimus, although some evidence suggests that sirolimus may confer a lower risk than standard therapy [7–9].

There is no satisfactory method to quantify immunosuppressive load, and it has therefore not been possible to establish the association between skin cancer risk and intensity of immunosuppression in the laboratory. Surrogate markers of immunosuppression have been employed, including lymphocyte subset analysis, lymphocyte proliferation assays, immunoglobulin levels, and Langerhans' cell density in the skin. Each method provides circumstantial support for the hypothesis that immunosuppressive load per se contributes to the development of skin cancer [10–12].

There is also clinical evidence to support this. For example, some tumours regress on withdrawal of immunosuppression [13] and skin cancer risk generally increases with increased duration of therapy [14]. In addition, triple immunosuppressive therapy is associated with a higher risk for skin cancer than dual therapy [5, 15]. There may also be a dose effect for individual drugs; low-dose cyclosporin regimens are, for example, associated with a lower cancer risk than standard doses [16]. Other studies show that cardiac transplant patients, who generally receive more intense immunosuppressive therapy, have an age- and sex-matched risk of skin cancer that is threefold higher than that of renal transplant recipients [15]. One report suggests that rejection episodes in the first year post transplant may be predictive for patients at higher risk of skin cancer, possibly because they require higher levels of immunosuppressive therapy to maintain graft function [17].

However, the effect of immunosuppressive dose on skin cancer risk for an individual needs careful interpretation because genetic [18] and pharmacokinetic variability may be important potential confounders. For example, the contribution of genetic variation to susceptibility to skin cancer has been investigated with regard to glutathione *S*-transferase genes. Glutathione *S*-transferases are a group of genes that encode enzymes involved in the detoxification of a variety of potentially mutagenic compounds, including ultraviolet radiation (UVR)-induced oxidative stress. Studies have shown that polymorphism in the glutathione *S*-transferases is associated with nonmelanoma skin cancer (NMSC) in organ transplant recipients in both the UK [19] and Australia [20].

Effects of Individual Immunosuppressive Drugs

Glucocorticoids

Prednisolone is the main glucocorticoid used to prevent allograft rejection in organ transplant recipients. It causes blockade of interleukin (IL)-1, -2, -3, -4, and -6,

tumor necrosis factor (TNF)-α, and interferon-γ by inhibition of cytokine gene transcription [1]. This action occurs through binding of the steroid receptor complex to the glucocorticoid response element in the promoter regions of cytokine genes. Prednisolone also exerts its antiinflammatory effect by inhibiting phospholipase A2 and the arachidonic acid cascade and by inhibition of monocyte migration and the synthesis, release, and action of chemotactic factors, permeability agents, and vasodilators [1]. In one study, long-term exposure to prednisolone alone was associated with an increased risk of skin cancer in non-organ transplant recipients [21], but there is no evidence to suggest any directly mutagenic or carcinogenic effect [22]. In mouse models of UV-induced carcinogenesis, for example, prednisolone does not enhance tumour formation [23].

Cyclosporin

Cyclosporin is a nonpolar cyclic oligopeptide. Its immunosuppressive activity is the result of inhibition of T-cell signalling. It binds cyclophilin, an immunophilin, which prevents dephosphorylation of nuclear factor of activated T cells (NF-AT) by the phosphatase calcineurin. Once translocated to the nucleus, NF-AT is responsible for stimulating IL-2 production and, therefore, the subsequent immune response [1]. Other calcineurin inhibitors used to prevent allograft rejection include tacrolimus.

Cyclosporin used alone is associated with an increased risk of keratinocyte skin cancer; a 5-year cohort study of psoriasis patients treated with cyclosporin showed that this increased risk was particularly enhanced in those on treatment for more than 2 years [24]. Early studies comparing the prevalence of cutaneous malignancy in organ transplant recipients receiving both azathioprine and cyclosporin reported varying results [25, 26]. Bunney et al. reported no difference, at least in the early stages of immunosuppression, in the prevalence of skin cancers between cyclosporin- and azathioprine-treated renal allograft recipients [25], whereas Shuttleworth et al. reported a higher prevalence of cutaneous dysplasia in transplant patients receiving cyclosporin [26]. More recent studies report an earlier onset and increased incidence of skin cancer in organ transplant recipients treated with cyclosporin [5, 15, 27, 28].

Evidence suggests that, in addition to being immunosuppressive, cyclosporin may also be mutagenic [29]. In vivo and in vitro studies have shown that calcineurin inhibitors including cyclosporin are associated with delayed repair of DNA damage and apoptosis in skin exposed to UV [27, 30–34] and increased UV sensitivity in human fibroblasts [35]. In one study, p53 mutations were reported in 15 of 25 (60%) keratinocyte skin cancers from immunosuppressed renal transplant recipients. Most (78%) were UV-specific C-to-T transitions at bipyrimidine sites, and, importantly, 35% of these were tandem mutations (including four UV signature CC-to-TT transitions), a significantly higher frequency than that found in the general immunocompetent population. This finding prompted the authors to propose that these mutations may be linked to inhibition of DNA repair by cyclosporin [35]. Inhibition of repair by cyclosporin may result in more cells with unrepaired

UV-induced DNA lesions in which deamination has time to occur and result in the formation of tandem CC-to-TT mutations. These findings not only confirm the importance of UV light as a major risk factor for skin carcinogenesis in transplant recipients on long-term immunosuppression but also highlight the potential importance of cyclosporin-modulated DNA repair in skin carcinogenesis in this patient group.

Cyclosporin may also promote cancer progression. Hojo et al. [36] reported that addition of cyclosporin to cultured adenocarcinoma cells increased their malignant phenotype, mediated through interaction with transforming growth factor (TGF)-β receptor. Adenocarcinoma cells treated with cyclosporin underwent morphological changes characteristic of invasive cells including membrane ruffling, increased motility, anchorage-independent (invasive) growth, and pseudopodial protrusions. Development of this cyclosporin-induced invasive phenotype appears to be related to TGF-β on the basis of several observations. First, cyclosporin stimulated TGF-β secretion in adenocarcinoma cells. Second, in contrast to IgG monoclonal antibodies, anti-TGF-β monoclonal antibodies prevented the cyclosporin-induced alterations. Third, recombinant TGF-β induced morphological alterations similar to those induced by cyclosporin in adenocarcinoma cells. Cyclosporin also induced phenotypic alterations in other cell types including murine renal cell adenocarcinoma cells, mouse mammary gland epithelial cells, and mink lung epithelial cells. Tumour growth was also enhanced by cyclosporin in immunodeficient SCID-beige mice, which were used to minimize the possibility that cyclosporin-induced suppression of the host immune system contributed to tumour progression. This finding suggests that cyclosporin induces tumour cells to produce TGF-β, which promotes cell invasiveness by a cell-autonomous mechanism independent of the immunosuppressant effect of cyclosporin on the host immune system [36].

Azathioprine

Azathioprine was initially introduced for the control of graft rejection after solid organ transplantation. It has since been used in other conditions including inflammatory arthropathies, such as rheumatoid arthritis, and inflammatory bowel disease (Crohn's disease and ulcerative colitis).

In mouse models, azathioprine, but not prednisolone, enhances the frequency of UV-induced tumours in hairless mice [23]. These findings were confirmed in another study in which 57% of mice treated with azathioprine and UVR (280–370 nm; peak, 310 nm) developed cutaneous squamous cell carcinomas (SCCs), compared with 18% treated with UVR alone, but none treated with prednisolone alone or in combination with UVR, suggesting a potential protective effect for prednisolone [37]. These combined data from murine models provide evidence that azathioprine has carcinogenic as well as immunosuppressive potential. Subsequent

studies have confirmed this and have proposed a mechanism of action, and azathioprine is now a recognised carcinogen [38].

The thiopurines, including azathioprine, are prodrugs requiring metabolic activation to thioguanine nucleotides that are, in turn, precursors for 6-thioguanine (6-TG) incorporation into DNA [39]. Azathioprine first undergoes cleavage to generate 6-mercaptopurine (MP). 6-MP and 6-TG are subsequently metabolised to 6-thiodeoxyguanosine triphosphate (6-TdGTP), which produces DNA-TG, believed to be responsible for most of the characteristic biological effects of delayed cytotoxicity and chromosome damage [40].

Modes of Action of Azathioprine

Incorporation of 6-TG into the DNA of rapidly dividing precursor lymphocytes may contribute to the primary immunosuppressive effect of azathioprine. The subsequent methylation of a small fraction of DNA 6-TG bases to form 6-meTG possibly results in its toxic effect. Because less than 0.1% of 6-TG in DNA is methylated and converted into a lethal lesion, it follows that there is a threshold below which thioguanine bases remain in cellular DNA without overt toxicity. The existence of a toxic threshold is demonstrated by the fact that mismatch repair-proficient cells tolerate significant, albeit lower, levels of DNA 6-TG without being killed. The approximately 0.01% substitution of DNA guanines by 6-TG in circulating lymphocytes of patients undergoing thiopurine therapy for leukaemia [41] or Crohn's disease [42] suggests that there is a similar toxic threshold in vivo. Since patients often receive systemic azathioprine for many years, particularly organ transplant recipients, it is likely that cells in other tissues also accumulate significant steady-state levels of DNA 6-TG. In addition to this, azathioprine metabolites also inhibit de novo purine synthesis. The effect of 6-TG on dNTP synthesis may contribute to immunosuppression as the dNTP pool of T cells is normally increased upon their activation, a requirement for subsequent function [43].

More recently, the discovery that 6-TdGTP can alter signalling pathways in activated T cells resulted in the proposal of an alternative/additional mechanism for the primary immunosuppressive effect of azathioprine [44, 45]. Apoptosis of activated T cells is prevented by the Rac-initiated signalling pathway that activates the apoptosis inhibitor bcl-x_L. Activation of the Rho GTPases Rac1 and Rac2 is stimulated by the Vav protein. 6-TdGTP can bind to Rac proteins instead of GTP and is subsequently hydrolysed to 6-TdGDP. Vav, however, is unable to stimulate exchange of 6-TGDP for GTP or 6-TdGTP, thereby inactivating Rac; this results in inhibition of the downstream signalling pathway and failure to activate bcl-x_L, thus allowing apoptosis to occur. The subsequent removal of activated T cells means that foreign antigens from the allograft in organ transplant recipients are tolerated. This proposed mechanism may explain why T cells are specifically affected by azathioprine, 6-TG, and 6-MP.

Potential Enhancement of UV-Induced Skin Carcinogenesis by Azathioprine

UVA produces DNA damage via endogenous cellular UVA photosensitisers that remain largely unidentified. Azathioprine and/or its metabolites also act as photosensitisers and increase the oxidative DNA damage caused by UVA irradiation. Thiopurines possess distinct photochemical properties, absorbing light in the UVA region in vitro, with 6-TG absorbing maximally at 342 nm. 6-MP generates reactive oxygen species (ROS) when exposed to UVA [46], as does 6-TG [47]. Human cells grown in nontoxic concentrations of 6-TG are sensitised to killing and mutation by low UVA doses within the normal sunlight range [47, 48]. The skin of patients on azathioprine also contains DNA 6-TG and is selectively photosensitive to UVA wavelengths [47]. DNA 6-TG and UVA interact to generate DNA-damaging ROS in cell nuclei [47]. Because oxidative DNA damage to normal DNA bases is implicated in the development of human cancer [49], it is plausible that 6-TG-mediated photochemical oxidation of DNA may contribute to the development of transplant-related skin cancer. In addition, guanine-6-sulfonate, the photochemical oxidative product of UVA/6-TG interaction, is a strong replication block. Bypass of replication-blocking guanine sulfonate by error-prone Y-family DNA polymerases may represent another potential source of mutation and a carcinogenic hazard [47] (Fig. 1).

mTOR Inhibitors/Proliferation Signal Inhibitors (PSI)

The mTOR inhibitors, also known as proliferation signal inhibitors (PSI), are a more recent addition to the immunosuppressive regimes used to prevent allograft rejection in solid organ transplant recipients. The mTOR inhibitor rapamycin (sirolimus), a macrocyclic lactone isolated from a strain of *Streptomyces hygroscopicus*, inhibits the mammalian target of rapamycin (mTOR)-mediated signal transduction pathways, which results in arrest of the cell cycle of various cell types, including T- and B lymphocytes. The mechanism of immunosuppression is described in detail in Part I of this book. mTOR inhibitors are potentially useful for organ transplant recipients as, in addition to their immunosuppressive effect, they also possess anticancer properties (see Part I). The mTOR pathway controls various signalling pathways required by cancer cells, and so inhibition of this pathway may reduce the prevalence of cancer in this high-risk group of patients. Preliminary evidence suggests that conversion of transplant recipients to mTOR inhibitors such as rapamycin or treating patients with rapamycin from the time of transplantation may reduce development of nonmelanoma skin cancer [50]. One study reported remission of nonmelanoma skin cancers in 37 of 53 (70%) renal transplant recipients after converting to mTOR inhibitors [51], and another study concluded that mTOR inhibitors may be useful in the management of post-transplant cutaneous and extracutaneous tumours [52]. Mathew et al. found that transplant recipients receiving rapamycin

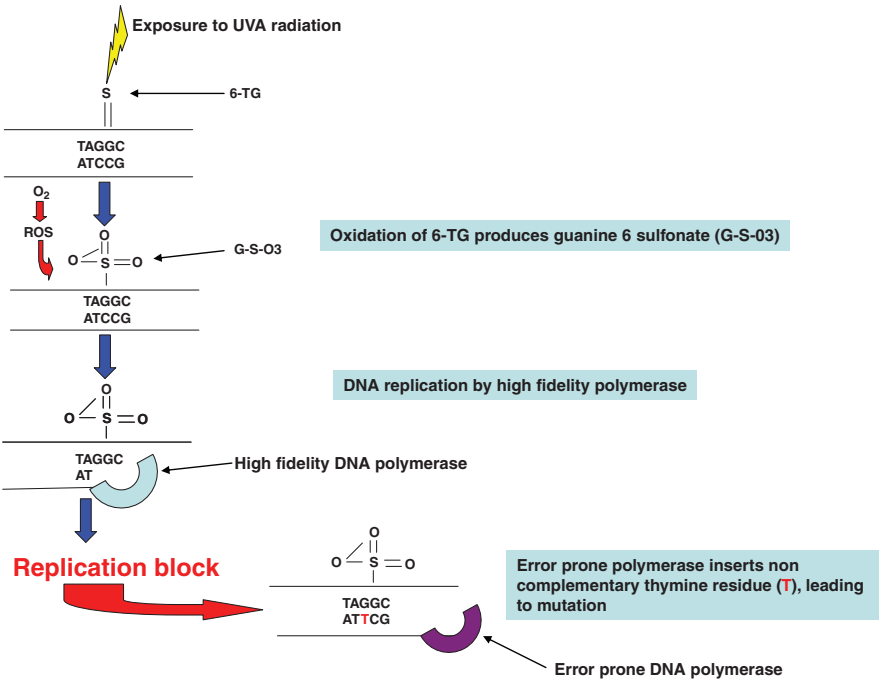

Fig. 1 Generation of mutagenic oxidative DNA damage by the interaction of 6-thioguanine (6-TG) and UVA. 6-TG, a metabolite of azathioprine, is incorporated into the DNA of skin cells of patients receiving azathioprine. UVA radiation photoactivates 6-TG to produce guanine-6-sulfonate (G-S-O3). DNA strands separate, and a high-fidelity DNA polymerase attempts to synthesise a new strand. However, G-S-O3 is a powerful block to high-fidelity replicative DNA polymerases, resulting in recruitment of low-fidelity error-prone polymerases, which facilitate the insertion of a noncomplementary residue leading to mutations [45]

without cyclosporin or rapamycin maintenance therapy after early cyclosporin withdrawal have a lower risk of malignancy in the first 2 years after renal transplantation [9]. Although these studies appear promising, further clarification of the potential benefits of mTOR inhibitors in this patient group is required.

Summary

This chapter outlines possible carcinogenic mechanisms of three immunosuppressive agents, namely cyclosporin, prednisolone, and azathioprine, all in routine use until recently, and their contribution to the development of post-transplant skin cancer. Many transplant units are now using other immunosuppressive regimens, comprising newer agents such as mycophenolate mofetil, tacrolimus, and sirolimus. The longer-term effects of these drugs on skin and other cancers in organ transplant recipients, whose life expectancy post transplant is now considerable, will become clearer in future.

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Oncogenic Viruses

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General Principles of Oncogenesis

Tissue homeostasis in the mature organism results from the net effects of cell proliferation and programmed cell death (apoptosis) and is guaranteed by highly complex extracellular and intracellular control of cell cycle and apoptosis. It is a hallmark of malignant cells to grow in the absence of appropriate extracellular signals such as growth factors or cytokines, which are either not sensed or not required as a consequence of disturbed intracellular control.

Extracellular signals are transmitted by signal transduction cascades involving tyrosine-specific and serine/threonine-specific kinases, GTP-binding GTPases, and several transcription factors (NFκB, AP1, c-*myc*) [1–3]. Many of these factors were originally identified as oncoproteins of acutely transforming animal retroviruses, which have acquired the respective genes from the cellular genome of their host, usually in a slightly mutated version, and which overexpress these genes under the control of the strong viral transcriptional promoters. It became thus very clear that physiological genes, involved in the control of cell proliferation, may turn into oncogenes when mutated, or overexpressed, or both. These genes are therefore referred to as proto-oncogenes. Their oncogenic activation by gene amplification, chromosomal translocation, or point mutation is frequently observed in various human cancers (glioblastoma, chronic myeloic leukemia, and colon, lung, and bladder carcinoma).

The cell cycle is mainly regulated by cyclins, cyclin-dependent kinases (CDK), CDK inhibitors (p21, p27), and so-called tumor suppressor proteins such as the retinoblastoma protein (pRb) and p53 [4–6]. Cyclin D1–3/CDK4 and CDK6 complexes and the cyclin E/CDK2 complex are important to overcome the G_1/S checkpoint and to enter the S phase of the cell cycle, where cellular DNA replication takes place. These CDKs phosphorylate pRb to release the transcription factor E2F from its complex with hypophosphorylated pRb and to allow transcriptional activation of the promoters of S phase-specific genes such as DNA polymerase-α by E2F. CDK inhibitors may prevent the cyclin–CDK assembly and the kinase activation

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and may thus lead to cell-cycle arrest. Numerous stress situations such as exposure to carcinogens or viral infection lead to activation of p53, which results in a transient cell-cycle arrest, for example, via activation of the CDK inhibitor p21, to allow DNA repair before entry into the S phase and to avoid irreversible mutations generated by replication of damaged DNA. In cases of extensive damage that cannot be repaired properly, p53 induces apoptosis to eliminate the severely affected cell; this is achieved by changing the relative amounts of pro- and anti-apoptotic members of the bcl2 protein family, and increased levels of pro-apoptotic bax and bak proteins finally lead to caspase activation and apoptosis. In lymphocytes, the fas receptor (CD95) is a key factor in extracellular induction of apoptosis [7,8]. When activated, it triggers an intracellular signal transduction cascade comprising the Fas-associated death domain-like interleukin-1β-converting enzyme (FLICE) [9] and leading to the activation of caspases.

Most of the key regulators of cell cycle and apoptosis can appear mutated alone or in various combinations in human cancers. Most notorious is p53, which is affected in about half of all human tumors, in 70% of colon cancers, and in most small cell lung cancers and skin cancers. It must be noted that dysregulation of cell cycle and apoptosis leads to an increased mutation rate and to genomic instability, implying further mutations in proto-oncogenes and tumor suppressor genes.

Tumor progression is accompanied by escape of the cancer cell from immunological surveillance and by acquisition of additional properties favorable for invasion, tumor growth, and metastasis such as increased angiogenesis.

Induction of Cancer by Viruses

It has to be acknowledged that viruses did not evolve to induce cancer, but rather to replicate efficiently and to be spread widely within their host species. Aggressive viruses lead to high titer replication, fulminant disease, and sometimes the rapid death of the patient. In contrast, those viruses that are adapted best to man follow the strategy of low-level replication without too much harm to their host, but not infrequently with lifelong persistence, guaranteeing viral spread over many years or even decades, as is true for papillomaviruses and herpesviruses such as Epstein–Barr virus or human herpesvirus 8. Viruses such as these are relevant human tumor viruses. They developed mechanisms to fight the innate and adaptive immunity of the host to enable a persistent infection in the presence of an intact immune system. They also developed anti-apoptotic mechanisms to prevent the early death of the host cell, which would exclude the late production of mature and infectious progeny viruses.

Particularly small DNA viruses such as papillomaviruses are furthermore endowed by mitogenic activities. Because of the small size of their genome with limited coding capacity, they have to rely on cellular enzymes such as DNA polymerase-α for replication of the viral DNA. These typical S-phase enzymes only become available in infected resting cells after cell-cycle progression has been induced by mitogenic viral proteins.

It is important to realize that the potentially oncogenic, mitogenic, and anti-apoptotic functions of viruses are originally supposed to serve the productive viral life cycle. As a matter of fact, usually no tumors arise in the vast majority of persistently infected people over many decades. Only when expressed in the wrong place and/or in unusual amounts do viral oncoproteins lead to host cell transformation and immortalization and to tumor progression [10].

Apart from this classical concept of direct viral oncogenesis, tumor viruses may also act by inserting viral transcription control elements (enhancers) in the vicinity of cellular proto-oncogenes. Viral DNA has to be detectable in these cases adjacent to relevant proto-oncogenes of cancer cells. A harmful integration is certainly a very rare event among many random insertions, which is selected for by tumor growth in vivo. There is indeed evidence for such a mechanism in rare cases of anogenital papillomavirus- and hepatitis B virus-associated tumors, but overall this seems to be exceptional.

Theoretically, viruses may contribute to tumor development at various stages of the multistep carcinogenesis progress and by quite different mechanisms. For bovine papillomavirus (BPV) 4, a "hit-and-run" mechanism has been proposed. Carcinomas grow in close association with BPV 4-induced esophageal papillomas, and transformation of papillomas to carcinomas has been observed, but usually no viral DNA is detectable in the cancers [11]. Malignant conversion occurs in animals feeding on bracken fern that contains mutagenic and immunosuppressive chemicals. It was confirmed in an experimental setting that the bracken diet is important for viral persistence and spread as well as for neoplastic conversion, but no malignancies were detected in animals not inoculated with BPV4 [12]. The virus may act as an additional mutagen in such a setting, inducing hits, and contributing to cell transformation. The final cancer cell phenotype would then be compatible with the loss of viral DNA (run).

If an increase of the risk of mutations is important, the virus may act even more indirectly via induction of cell proliferation in the course of chronic inflammatory reactions accompanied by continuous cell regeneration, as discussed for hepatitis B and C viruses.

Human skin carcinomas are distinguished by only a few cancer cells being positive for papillomavirus DNA by in situ hybridization, which persists overall at a very low copy number. Such a partial loss of viral DNA from cancer cells would be compatible with paracrine effects of the virus, inducing, for example, the secretion of pro-inflammatory, immunomodulatory, and/or pro-angiogenic factors and/or metalloproteases by virus-positive cells, which could affect growth and the invasive properties of the whole tumor.

The etiological significance of a virus is difficult to prove experimentally in these cases. Large-scale, long-term prospective epidemiological studies are the best way to define the risk of a given infection.

Basic research on the molecular biology of human viruses provided evidence for one or the other possibly oncogenic pathway just outlined. Based on these data together with results from epidemiological studies, several viral infections are firmly linked to, or associated with, various tumor types, as listed in Table 1 [10].

Table 1 Established human tumor viruses

Virus family	Virus type	Tumor types	Prevalence of virus in tumor
Papillomaviridae	Human papillomavirus types 16, 18, etc.	Cancer of the cervix and anus	~100%
		Vulvar, penile, and vaginal cancers	~50%
		Oropharyngeal and laryngeal cancers	~25%
	Human papillomavirus types 5, 8, 38, etc.	Nonmelanoma skin cancer	~50%
			~85% in IS
Herpesviridae	Epstein–Barr-virus	Nasopharyngeal cancer	~100%
		Burkitt's lymphoma	>95% in endemic areas 20% global
		B-cell lymphomas in IS	~50%
		Hodgkin's disease Subset of T-cell lymphomas	30–40%
		Gastric cancer	~10%
	Human herpesvirus 8	Kaposi's sarcoma	100%
		Body-cavity-based B-cell lymphoma	
Hepadnaviridae	Hepatitis B virus	Liver cell carcinoma	
Flaviviridae	Hepatitis C virus	Liver cell carcinoma	
Retroviridae	Human T cell Leukemia virus 1	Adult T-cell-leukemia/lymphoma	100%

IS, immunosuppressed patients.

Infections with human papillomaviruses (HPV), hepatitis B virus, and Epstein–Barr virus (EBV) contribute most to the global tumor burden [13–15]. Cutaneous tumors such as nonmelanoma skin cancer and Kaposi's sarcoma are linked to a subset of human papillomaviruses [15] and to Kaposi's sarcoma herpesvirus (KSHV)/human herpesvirus 8 (HHV8) [16], respectively. Iatrogenic immunosuppression following organ transplantation causes a partially dramatic increase in the incidence of papillomavirus-associated squamous cell carcinomas of the skin and the cervix uteri, of Epstein–Barr virus-associated immunoblastic B-cell lymphomas, and of Kaposi's sarcoma.

Persistence and Immune Modulation

It is an essential characteristic of all tumor viruses to establish a persistent infection because frankly cytolytic infections obviously exclude tumor development. Tumor viruses are able to maintain their genome within host cells over time and to avoid elimination by the host's immune system [17]. Following acute primary infection, the virus may apparently disappear into a latent state with minimal expression of

viral proteins, as best characterized for herpesviruses [18, 19]. Perfect latency over decades only persists in varicella-zoster virus, whereas continuous reactivation in a subset of latently infected cells occurs in all other systems, leading to persistent or recurrent production of infectious virus and the picture of a chronic infection. The best known chronic human tumor virus infections are hepatitis B and C with continuously active, nonlytic replication at high level [20, 21]. The molecular mechanisms and the cellular reservoir of persistent papillomavirus infections are still poorly understood, but there also seems to occur a continuous activation leading to clinically inapparent or apparent production of infectious virus, guaranteeing the wide distribution of anogenital and particularly cutaneous papillomavirus infections [22].

Viral strategies for evading the immune system comprise the following [17]:

1. Infection of immunologically privileged sites or sites not readily accessible to the immune system (papillomaviruses in epidermis)
2. Interference with antigen processing and presentation [papillomaviruses interfere with the transporter associated with antigen presentation (TAP), the EBV EBNA1 protein inhibits proteosome-mediated processing, papillomaviruses downregulate cell-surface presentation of MHC class I molecules, essential to dictate activation and cytolytic function of T lymphocytes]
3. Inhibition of cytokine function (anogenital papillomaviruses interfere with the activation of the interferon-β promoter and with type I interferon signaling)

The eminent role of the immune system in tumor cell surveillance, even if impaired by persistent viral infection, becomes obvious by the increased incidence of virus-induced tumors after further severe immunosuppression in the context of transplantation or human immunodeficiency virus infection.

Molecular Mechanisms of Viral Oncogenesis

Oncogenes could be identified in all tumor viruses listed in Table 1, which, when experimentally expressed in cell culture or transgenic animals, led to oncogenic transformation in vitro or tumor development in vivo [23]. Three basic molecular functions can be distinguished:

1. Protein–protein complex formation of viral oncoproteins and cellular regulator proteins, leading to inactivation or functional modification of the cellular factors
2. Viral oncoproteins, homologous to cellular proteins, competing with or replacing the cellular counterpart and revealing oncogenic activities
3. Transactivation of cellular transcription, either directly or via interaction with cellular transcription factors or co-activators

In one way or the other, different oncoproteins lead to deregulation of the cell cycle and an increased proliferation rate, to inhibition of apoptosis, and to fundamental changes in the cellular transcriptome, also affecting secretory activities. Many oncoproteins have pleiotropic effects, targeting several pathways.

The general picture is specified in the following sections for Epstein–Barr virus and papillomaviruses because of their outstanding role in transplantation. For HHV8, the reader is referred to another chapter.

Epstein–Barr Virus

Patients under immunosuppressive therapy after organ transplantation are at a 30- to 50-fold-increased risk for the development of EBV-positive B-cell lymphoproliferative disease [24]. The post-transplantation lymphomas are polyclonal or monoclonal tumors, which mostly arise during the first year after the start of severe immunosuppression. The EBV gene expression in immunoblastic lymphomas is the same as in lymphoblastoid B-cell lines, immortalized by EBV in vitro. It is generally referred to as latency pattern III and comprises EBV-specific nuclear antigens (EBNA)-1, -2, -3A, -3B, and -3C, EBNA-leader protein (LP), latent membrane proteins (LMP)-1, -2A, and -2B, as well as abundantly expressed but untranslated RNAs EBER-1, EBER-2, and BART [25]. This finding suggests that the post-transplantation lymphomas originate from virus-transformed cells, closely resembling in vitro transformed lymphocytes that grow in the absence of an effective T-cell surveillance.

EBNA2, -3A, -3C, and LMP1 emerged early on as essential viral genes for the immortalization of B lymphoblasts. LMP1 functionally mimics members of the superfamily of tumor necrosis factor receptors. Independent of a physiological ligand, it exerts its pleiotropic effects via several signaling pathways, including the NFκB transcription factor pathway, finally upregulating, among others, anti-apoptotic proteins such as Bcl2 and A20. EBNA2 acts as a transcriptional coactivator regulating the expression of a number of viral (LMP1) and cellular genes such as the B-cell activation antigen CD23 and c-*fgr* [14]. A major role of EBNA2 is to mimic the Notch signal transduction pathway. EBNA3C has been shown to overcome the pRB checkpoint in the G_1 phase of the cell cycle, to cooperate with the *ras* oncogene, to bind to Nm23-H1, a suppressor of cell migration and metastasis, and to upregulate the matrix metalloproteinase (MMP)-9 [26].

EBNA-LP has been reported to bind retinoblastoma and p53 proteins [27], which will be appreciated in more detail in the context of papillomaviruses. EBNA-LP contributes significantly to efficient transformation of B lymphocytes.

More recently, the important role of EBNA1 has been appreciated. EBNA1 is consistently expressed in all EBV-positive tumors. It enhances B-cell immortalization several thousandfold [28] and has been shown to be a survival factor in Burkitt's lymphomas [29]. It also interacts with and suppresses functions of the cell migration inhibitor Nm23-H1 [30].

EBER-RNAs finally confer resistance to interferon-α- and fas-induced apoptosis [31,32], and regulatory micro-RNAs were shown to originate from introns of BART-RNAs, which target regulators of cell proliferation and apoptosis [33].

Papillomavirus

The oncogenic activities of papillomaviruses have been studied in great detail in high-risk anogenital representatives of genus alpha [34] and are less well understood in cutaneous HPV of genus beta. In genus alpha HPV, the oncogenes E6 and E7 are necessary and sufficient for transformation and immortalization of keratinocytes, tumor progression, and finally maintenance of the malignant phenotype, particularly by interference with cell-cycle control and apoptosis. The E7 protein physically interacts with regulators of the cell cycle, transcription factors, and chromatin remodeling enzymes [35]. The E6 protein interacts with pro-apoptotic proteins, transcription factors, and coactivators, cellular proteins involved in cell architecture, polarity, and adhesion and in DNA replication and repair. In an organotypic keratinocyte culture system, HPV E6 and -E7 significantly altered the expression of more than 1,300 cellular genes, highlighting the far-reaching consequences on the cellular transcriptome [36]. A large increase was noted in transcripts associated with DNA and RNA metabolism, whereas multiple genes associated with protein translation were downregulated. Major alterations were observed in transcripts associated with the cell cycle and cell differentiation.

Most notorious are the interference of E7 with the retinoblastoma protein pRb and the pRb-related proteins p107 and p130 and the interference of E6 with p53. The tumor suppressor proteins pRb and p53 are also targeted by other small DNA tumor viruses such as SV40 (polyomaviridae) and adenovirus and by EBV [37].

E7 binds pRb and mediates its degradation through the ubiquitin proteasome pathway. It associates with cyclins A and E and CDK inhibitors p21 and p27 to further enhance CDK activity and pRb inactivation. Independent of pRb inactivation, E7 relieves repression of E2F-inducible promoters by binding to histone deacetylases. The E6 protein of high-risk alfa-HPV forms a ternary complex with p53 and the ubiquitin ligase E6AP, resulting in proteasomal degradation. It also downregulates p53 activity via association with p300, the transcriptional coactivator of p53 [38].

Important for keratinocyte immortalization is the interaction of E6 proteins with proteins of the PDZ family (hDLG, MUPP-1, hSCRIB, MAGI-1), which are localized at areas of cell–cell contact. E6 can target PDZ proteins for degradation. Another important function necessary for immortalization is the activation of expression of the catalytic subunit of the telomerase. Apart from p53, E6 also targets the pro-apoptotic Bak protein for ubiquitin-mediated degradation [39]. The expression of E6 and E7 in proliferating cells severely disturbs the mechanisms of chromosome duplication and segregation during mitosis [40]. In the context of interference with cell-cycle control and apoptosis, this leads to severe chromosomal instability, which favors further genetic changes in the persistently infected host cell, such as loss of telomerase suppressors and tumor suppressors as well as amplification of oncogenes [34]. The continuous selection of oncogenic alterations finally provides cells with a malignant phenotype.

Except for HPV38, the E7 proteins of β-papillomaviruses (HPV5, -8, -20) usually bind less strongly to pRB [41, 42], but HPV8 E7, for example, is nevertheless able to inactivate pRB and deregulate the G_1/S transition control [43]. Regarding tumor progression, it is interesting to note that HPV8 E7 can activate the expression of the MT1-matrix metalloproteinase at both mRNA and protein levels with an efficiency similar to HPV16 E7 [44], which may facilitate epidermal invasion.

Although E6 proteins of cutaneous β-HPV do not degrade p53 and show no binding motif for the tumor suppressor hDLG [45, 46], they are able to transform rodent cells [47] and to induce dysplasias and carcinomas of the skin in transgenic mice (Pfister, unpublished). This finding points to oncogenic activities so far underdetermined.

The anti-apoptotic activities of β-HPV oncoproteins and their interference with DNA repair result in chromosomal instability.

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Epidemiology of Cutaneous Human Papillomavirus Infections

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Introduction

Human papillomaviruses (HPV) are small epitheliotropic DNA viruses. HPV infect keratinocytes in the skin as well as the mucosa. More than 100 different human papillomaviruses have been sequenced so far. Some HPV are involved in cancer development. HPV infection is particularly associated with anogenital cancer, most notably cervical cancer, but likely also with nonmelanoma skin cancer (NMSC). The beta papillomaviruses (beta-PV) are the most likely candidates to be involved in skin carcinogenesis.

In this chapter, the role of beta-PV in the etiology of NMSC is discussed. The prevalence of beta-PV in lesions and unaffected skin from immunosuppressed organ transplant recipients (OTR) and immunocompetent individuals is outlined, as well as the epidemiological association between beta-PV infection and NMSC.

Papillomaviruses

Papillomaviruses are small DNA viruses that can induce a wide variety of hyperproliferative lesions (papillomas, warts, carcinomas) in the skin and mucosa of mammals (rabbit, horse, dog, sheep, deer, elk, cattle, primates, and humans) and birds.

In 1933, the etiological agent of cutaneous warts in cottontail rabbits was identified by Richard Shope [1]. It was identified as a transmissible virus and was later called the cottontail rabbit papillomavirus (CRPV). In 1949, the nature of the infectious agent of human warts was investigated by Strauss and Shaw [2]. They were the first to detect viral particles in human warts by electron microscopy. In total, almost 100 different full-length HPV genomes have been described [3, 4]. A new papillomavirus (PV) isolate is recognized as such if the complete genome has been cloned and the DNA sequence of the L1 open reading frame (ORF) differs by more than 10% from the closest known PV type [4].

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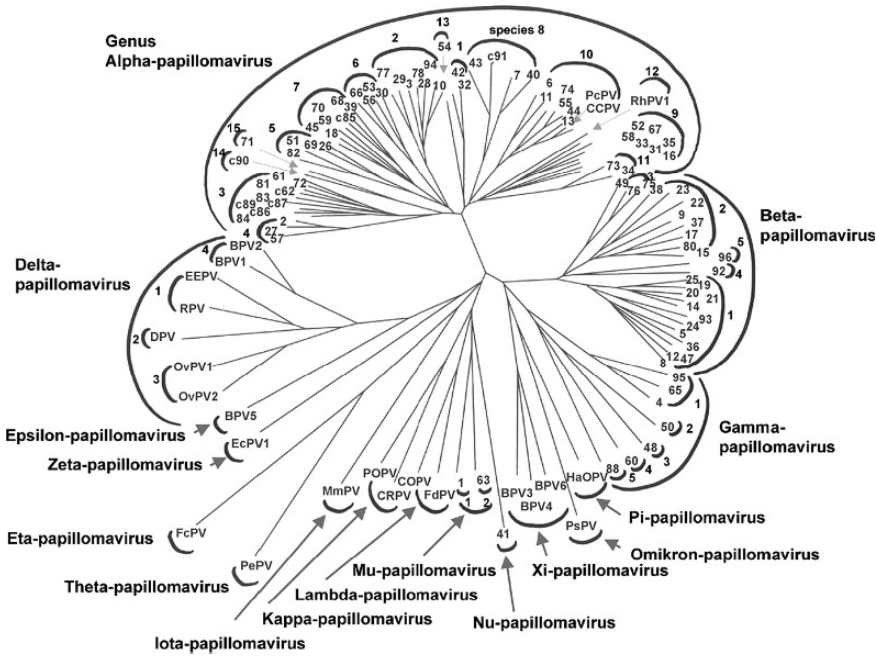

Fig. 1 Phylogenetic tree containing the sequences of 118 papillomavirus types. The numbers at the ends of each of the branches identify a human papillomavirus (HPV) type; "c" numbers refer to candidate HPV types. All other abbreviations refer to animal papillomavirus types. The *outermost semicircular symbols* identify papillomavirus genera, e.g., the genus alpha-papillomavirus. The number at the *inner semicircular symbol* refers to papillomavirus species. This figure is reprinted from reference 4 (De Villiers et al.) with permission from the publisher

Papillomaviruses are subdivided into different genera (Fig. 1). The alpha papillomaviruses (alpha-PV) are primarily mucosal types that include high-risk types such as HPV16 and HPV18, which are known to cause cervical cancer, and low-risk alpha-PV types such as HPV6 and HPV11, known to cause condylomata acuminata. The other HPV types belong to the genera beta, gamma, mu, and nu. The beta-PV types are exclusively cutaneous types, a subset of which probably plays a role in the development of cutaneous squamous cell carcinomas (SCC).

The replication cycle is divided into two stages. First, the virus is maintained at low copy numbers within the initially infected, but still replicating, cells. The viral proteins E1 and E2 are essential for this basal DNA replication. When the basal cells are pushed to the suprabasal compartment, they lose their ability to divide and instead initiate the terminal differentiation program. Papillomaviruses replicate in this compartment, and for their release into the environment, take advantage of the disintegration of the epithelial cells that occurs as a consequence of their natural turnover at the superficial layers [reviewed in reference [5].

The carcinogenic role of high-risk mucosal HPV in cervical cancer is well established and was first proposed in 1976 [6]. After initial infection, viral particles reach the basal layer of the epithelium, where they bind to and enter into cells [5].

It has been suggested that for maintenance of the infection the virus has to infect an epithelial stem cell [7]. It is known that persistence of the virus is necessary for development of cervical cancer [8]. Integration of the HPV genome into the DNA of the host cell is an important event of alpha-PV-induced carcinogenesis. Integration appears to be a random event, where expression of parts of the genome is usually lost, whereas expression of the viral genes E6 and E7 is maintained. As a result of uncontrolled expression the viral proteins E6 and E7, which normally secure viral replication in the differentiated keratinocyte compartment, can turn into oncoproteins by neutralizing the action of the tumor suppressor proteins p53 and pRb, respectively. The affinity of E6 and E7 for p53 and pRb, respectively, correlates in general with the oncogenicity (high-risk/low-risk) of the HPV type in question.

Cutaneous HPV

Warts are very common in the healthy population, with a prevalence of about 20% [9]. Most persons develop warts at some time in life, but they occur most frequently in children and adolescents 12 to 16 years of age [10]. HPV types associated with warts are HPV1, -2, -3, -4, -7, -10, -26, -27, -28, -29, -41, -57, -60, -63, and -65, belonging to the genera alpha and gamma [4]. HPV1 is believed to cause myrmecia warts, mainly on the plantar site of the foot. A study showed HPV1 in 19.5% of plantar warts. HPV2 was detected in 21.5% and HPV4 in 7.8% of the plantar warts [11]. Verrucae vulgaris, localized mainly on the hands, are caused by HPV2, -4, -7, and -57. HPV2 is the most often detected HPV type in Europe and the United States [12]. In a Taiwanese population, however, in only 7% of verrucae vulgares was HPV2 or -3 found [13]. HPV types 1 and 4 were the most frequent types, in 13% and 16% of the warts, respectively. HPV7 is associated with butcher's warts, which are verrucae vulgares on the hands of slaughterhouse workers or meat handlers. HPV7 was found in 30% of butchers [14]. Verrucae planae are associated with HPV3, -10, and -41. They are located on the dorsum of the hands and in the face. Intermediate warts have clinical characteristics of verrucae vulgares, but also resemble verrucae planae. They are located mostly on the dorsa of the hands, but in immunosuppressed patients, they may be widely disseminated. The HPV types mainly found are HPV10, -26, -27, -28, and -29. Cystic warts are plantar warts, with different characteristics when viewed under the microscope, and are associated with HPV types 60, 63, and 65 [15].

It should be noted that HPV in some of these studies was typed with polymerase chain reaction (PCR). It cannot be excluded that the distribution and frequency of HPV infections in verrucae vulgares and planae will change when more sensitive techniques are applied.

Betapapillomaviruses (Beta-PV)

There is increasing evidence for a carcinogenic role of beta-PV in NMSC, especially in SCC of the skin. At present, 25 beta-PV types are fully sequenced (HPV5, -8, -9,

-12, -14, -15, -17, -19, -20, -21, -22, -23, -24, -25, -36, -37, -38, -47, -49, -75, -76, -80, and cand92, -93, and cand96). Based on partial sequences, probably more than 35 new types have to be added to this list of beta-PV types [4, 16] (see Fig. 1). Because of the relationship between the rare genetic disease epidermodysplasia verruciformis (EV) and HPV, beta-PV types were formerly called EV-associated HPV types. Since the new taxonomic classification of papillomaviruses, these types are called beta-PV types [4].

Not much is known yet about the natural history of beta-PV infections. Beta-PV infections are very common in most people [17], but the clinical picture of cutaneous beta-PV infection is not clear. OTR often develop extensive warts and other hyperkeratotic lesions, which have been linked to beta-PV infection [18]. In contrast, infection with beta-PV in immunocompetent individuals probably remains subclinical. It is likely that beta-PV infection is transmitted through skin and hair derivates, as proposed in a study describing that children are infected with the same cutaneous HPV types as their parents within months after birth [19]. Beta-PV DNA is found in skin swabs of the forehead [20], the arms and legs, and in hairs from eyebrows, arms, and legs [17] of both healthy individuals and OTR.

The HPV life cycle is closely linked to the biology of the specific host cells, the keratinocytes, which are responsible for the renewal, cohesion, and barrier function of pluristratifying epithelia [21]. It is thought that beta-PV target stem cells are located in the basal layer of the epidermis and in the bulge of the hair follicles [17, 22]. The hair bulge is considered as an immune privileged region [17].

Persistence is considered an important aspect of mucosal infections in relation to cervical carcinogenesis. Recent studies indicate that beta-PV infections also persist. In a small cohort of healthy adults it was demonstrated that the majority of detected beta-PV infections persisted [23]. Another recent study showed persistent beta-PV DNA positivity in 48% of the healthy individuals and 33% of the OTR after 7 years [24]. These data are in contradiction with the study by Berkhout et al., which shows more beta-PV DNA persistence in OTR than in healthy individuals during up to 5.6 years in 2 to 5 time points [25]. In the study by Berkhout, 77.5% of the hyperkeratotic papillomas were positive for a beta type or a selected population of alpha-PV types: 77.8% of the SCC, 67.9% of the actinic keratoses (AK), only 35.7% of the basal cell carcinomas (BCC), 38.5% of benign lesions, and 32.3% of clinically normal skin were positive. Whether beta-PV persistence is a factor in skin cancer carcinogenesis needs further research.

Beta-PV can be found on different parts of the skin. The reservoir of the virus is possibly within epidermal stem cells of the hair bulge. Beta-PV DNA can easily be isolated from plucked eyebrow hairs. The presence of beta-PV in plucked eyebrow hairs has frequently been used as a measure of beta-PV infection in several epidemiological studies. Detection of beta-PV DNA is usually performed with polymerase chain reaction (PCR) on DNA extracted from hairs, skin swabs, or biopsies by which preferential areas of the genome can be amplified.

Over the years, several broad-spectrum PCR methods have been developed suitable to detect cutaneous HPV types, species, or genera: CPI/IIs [26], FAP59/64 [20],

F/G [27], modified F/G (M^aH^a) [28], HPV type-specific PCR [29], degenerate and nested PCR [30], and PM-PCR RHA [31].

Using virus-like particle (VLP) enzyme-linked immunoassay (ELISA) or multiplex technology (Luminex), antibodies against beta-PV viral proteins can be detected, to determine a person's beta-PV serological status. Antibodies can be detected against the major capsid protein L1 and the nonstructural protein E6 using HPV virus-like particle (VLP) or GST-HPV fusion proteins in ELISA [32, 33] or with multiplex serology using GST-L1 fusion proteins, respectively [34]. The latter method (Luminex) is a new method based on fluorescent bead technology that allows simultaneous detection of antibodies against up to 100 different in situ affinity-purified recombinant HPV proteins [35].

Human Papillomavirus, Epidermodysplasia Verruciformis, and Skin Cancer

Epidermodysplasia Verruciformis

The association between HPV and SCC originated from patients with the rare hereditary disease epidermodysplasia verruciformis (EV). EV patients have an abnormal susceptibility to widespread beta-PV infections of the skin [36, 37]. EV patients develop pityriasis versicolor-like lesions and flat warts and get numerous SCC on sun-exposed sites. In the SCC of EV patients, mainly beta-PV types 5 and 8 are found [38]. Recent studies have shown two genes, EVER 1 and -2, to be related with EV. EVER mutations have been described in EV patients worldwide. EVER genes are members of a transmembrane channel-like (TMC) gene family. The function of TMC is still unknown. It has been proposed that TMC proteins could constitute a novel group of ion transporters, or channels or modifiers of such activities, and could be involved in signal transduction [reviewed in reference [21]].

Also, OTR have an increased risk of developing SCC on sun-exposed sites, often preceded by hyperkeratotic lesions (actinic keratosis) [39–42]. Because the situation in OTR to some extent resembles that of EV patients, it was investigated whether HPV was present in these patients [43, 44].

Prevalence of Papillomavirus in Nonmelanoma Skin Cancer

HPV DNA is found in NMSC and precursors of both immunocompetent and immunosuppressed individuals. The percentage of HPV DNA positivity varies with populations tested, immunocompetent or OTR, as well as the detection method.

Extensive research has been carried out in OTR to discover the association between warts, hyperkeratotic skin lesions, NMSC, and HPV infection in immunosuppressed individuals [17,18,25,39,40,44–46]. OTR are at a high risk of developing

NMSC in the years following transplantation and on immunosuppressive drugs. The risk of developing skin cancer increases from 10% in the first 10 years after transplantation to 40% 20 years after the transplantation [41]. In this patient group, several studies have been performed to determine the prevalence of HPV DNA in healthy skin, benign keratotic lesions, AK, BCC, and SCC (Table 1). Already in 1989, Barr et al. found HPV5/8 DNA in 60% of SCC from OTR [40]. de Jong-Tieben et al. found a prevalence of HPV DNA in 80% of SCC biopsies, 50% of BCC, and 93% of AK [47]. In warts of OTR patients, HPV DNA was detected in 66% [43] and 91% [46], respectively. In most warts, HPV1, -2, or -4 was found. In benign keratotic skin lesions of OTR, in 55% of the lesions HPV DNA was found. Meyer et al. found HPV DNA in 75% of the SCC from OTR, 38% in AK and 17% in healthy skin [46]. Berkhout et al. found HPV DNA in 77.8% of SCC, 77.5% of hyperkeratotic papillomas, 67.9% of AK, 35.7% of BCC, 38.5% of benign lesions, and 32.3% of healthy skin [25]. They also found more persistence of HPV DNA in OTR than in healthy individuals [25]. Also, in nonlesional skin the prevalence of HPV DNA infections measured in plucked hairs was very high in OTR, up to 92% [17], with predominantly beta-PV types present. Forehead skin swabs also are frequently positive for HPV DNA, in 71% to 90% of OTR [24]. In Table 1, the different HPV prevalence studies in NMSC and precursor lesions are listed, and the predominant HPV types and genera found are shown.

HPV prevalence in cutaneous lesions from immunocompetent individuals has been less studied and is generally lower than in OTR. HPV DNA was found in 47% of SCC biopsies, 36% of AK, and 16% in healthy skin [46]. In an English study in immunocompetent patients, HPV DNA was found in 35% of the normal skin biopsies [48]. Of the SCC cases in a Dutch study, 71% was positive for HPV DNA, and of the healthy controls, 55.2% [49]. In plucked hairs from different sites of the body of healthy individuals, HPV DNA was present in 45% of the samples [17]. HPV DNA, isolated from plucked eyebrow hairs of Australian individuals, was present in 54% of AK cases. In SCC cases and tumor-free controls, the percentage was 44% and 40%, respectively [50]. A study in AK in immunocompetent individuals showed a prevalence of beta-PV of 85% in frozen biopsies and 67% in formalin-fixed biopsies [51]. No difference was found in the prevalence of beta-PV DNA between high-risk or low-risk AK. In BCC of immunocompetent persons, HPV DNA is found in approximately 43.5% [48, 52].

Concluding on the basis of these studies, HPV DNA is often found in biopsies of SCC, in both immunosuppressed and immunocompetent patients. Also in other NMSC biopsies and normal skin, as well as plucked hairs and skin swabs, HPV DNA is often found. Most frequently beta-PV types are found, but so far, no high-risk types were identified based on these HPV prevalence studies, possibly because the different PCR methods used in the different studies make it hard to compare results. Also, a study showed that stripping of the stratum corneum of SCC reduced the level of HPV DNA found in the tumor [53]. This finding might show that part of the HPV DNA found in tumor (biopsies) is contamination, or that HPV DNA is more present in the superficial layers and is not evenly distributed throughout the tumors.

Table 1 List of human papillomavirus (HPV) prevalence studies in nonmelanoma skin cancer (NMSC) and precursor lesions, and the predominant HPV types and genera present

Author (reference)	Patients	Lesions	Method	HPV types	Prevalence
Barr [40]	RTR	SCC	Southern blot	5, 8	60%
de Jong-Tieben [47]	RTR	SCC/AK/ BCC	Broad-spectrum PCR	Beta PV	80%/93%/50%
Meyer [46]	RTR	SCC/AK/normal skin	Degenerate PCR	Beta PV	75%/38%/17%
Berkhout [25]	RTR	SCC/papillomas/ AK/BCC/benign lesions/normal skin	MaHa PCR	Beta PV	77.8%/77.5%/ 67.9%/35.7%/ 38.5%/32.3%
Rudlinger [43]	RTR	Verrucae	PCR	1, 2, 4	66%
Meyer [46]	RTR	Verrucae	Degenerate PCR	1, 2, 4	91%
Harwood [54]	RTR	Normal skin	Degenerate/nested PCR	Beta PV	87%
Boxman [17]	RTR	Eyebrow/arm/leg hairs	Degenerate PCR	Beta PV	92%
Hazard [24]	RTR	Skin swabs (healthy skin)	FAP PCR	Beta PV/ gamma PV	71%–92%
Antonsson [65]	RTR	Skin swabs (healthy skin)	FAP PCR	Alpha/ beta/ gamma PV	94%
Meyer [46]	IC	SCC/AK/normal skin	Degenerate PCR	Beta PV	47%/36%/16%
Harwood [54]	IC	Normal skin	Degenerate/nested PCR	Beta PV	35%
Pfister [51]	IC	SCC/AK	Nested PCR	Beta PV	67% (formalin)/ 85% (frozen)
Struijk/Hall [33]	IC	AK/SCC/healthy skin	Type-specific PCR	5, 8, 15, 20, 24, 38	54%/44%/40%
Shamanim [66]	IC	SCC	Degenerate	HPV	
Wieland [52]	IC	BCC	Nested PCR	6, 8, 19, 20, 23, 24, 28, 34, 36, 37, 38	43.5%
Boxman [28]	IC	NMSC/eyebrow hairs	Nested PCR	Beta PV	39%/66%
Boxman [60]	IC	Eyebrow hairs (men/women)	Nnested PCR	Beta PV	49%/44%
Boxman [17]	IC	Eyebrow/arm/leg hairs	degenerate PCR	Beta PV	45%
Struijk [49]	IC	Eyebrow hairs	Type-specific PCR	2, 5, 8, 15, 16, 20, 24, 38	63.1%
de Koning [23]	IC	Eyebrow hairs	PM-PCR/RHA	Beta PV	96%
Termorshuizen [61]	IC	Eyebrow hairs (healthy/SCC)	Type-specific PCR	5, 8, 15, 20, 24, 38	44.8%/60.4%
Antonsson [65]	IC	Skin swabs (healthy skin)	FAP PCR	Alpha/ beta/ gamma PV	80%

RTR, renal transplant recipients; IC, immunocompetent; SCC, squamous cell carcinoma; AK, actinic keratosis; BCC, basal cell carcinoma; PCR, polymerase chain reaction.

Association Between HPV Infection and Skin Cancer

Case-control studies were initiated to investigate the association between markers of HPV infection, in particular, beta-PV infection and NMSC, in immunocompetent individuals.

Molecular Studies (Beta-PV DNA Detection)

In a case-control study performed with 155 immunocompetent individuals with a history of SCC and 371 controls without skin cancer, a statistically significant association was found between the overall prevalence of beta-PV DNA in plucked eyebrow hairs (types 2, 5, 8, 15, 16, 20, 24, and 38) and SCC [49]. The presence of beta-PV DNA was also significantly associated with increasing age and male sex. The odds ratio, adjusted for age and sex, to develop SCC in the presence of HPV DNA in eyebrow hairs was 1.7 [95% confidence interval (CI), 1.1–2.7]. HPV2 and HPV16 were only rarely present in eyebrow hairs in this study and were not associated with SCC. In an English study a significant association was found between the presence of beta-PV DNA in normal skin and NMSC status [odds ratio (OR), 6.41; 95% CI, 1.79–22.9] [54]. Both studies [49,54] showed that the presence of beta-PV DNA is associated with SCC and/or AK in immunocompetent individuals. Another study, also using plucked eyebrow hairs, found a significant association between beta-PV DNA and AK in males, with an odds ratio of 3.4 (95% CI, 1.8–6.5) [33]. For females, no such association was found in this study. Presence of beta-PV DNA was again associated with increasing age [33].

Serological Studies (Beta-PV Antibody Detection)

In a study among 540 immunocompetent cases with a history of skin cancer and 333 controls, seroreactivity to L1 virus-like particles (VLP) of beta-PV types 5, 8, 15, 20, 24, and 38 was measured [32]. Seroreactivity to HPV8 and HPV38 was significantly associated with SCC. The odds ratio (OR) for SCC adjusted for age and sex for HPV8 was 14.7 (95% CI, 1.6–135) and for HPV38, 3.0 (95% CI, 1.1–8.4) [32]. In another large study in immunocompetents, with 252 SCC cases, 525 BCC cases, and 461 controls [34], seroreactivity against L1 of HPV1, -2, -3, -5, -6, -8, -10, -15, -16, -20, -24, -32, -36, -38, and -57 was measured with multiplex Luminex technology [35]. Overall, HPV antibodies were statistically significantly associated with SCC (OR, 1.6; 95% CI, 1.2–2.3). Especially, beta-PV seropositivity was associated with an increased risk of SCC (OR, 1.5; 95% CI, 1.0–2.1) and particularly HPV5 (OR, 1.8; 95% CI, 1.0–3.1). The highest SCC risk was associated with positivity for multiple HPV types [34]. A third case-control study of immunocompetent individuals from Australia correlated HPV seroreactivity to L1 and E6 (HPV5, -8, -15, -16, -20, -24, and -38) and HPV DNA positivity with current AK and SCC [33].

The presence of seroreactivity to betaPV L1 was associated with AK (OR, 2.3; 95% CI, 0.85–4.9) and SCC (OR, 3.9; 95% CI, 1.4–10.7), and the presence of AK was inversely associated with seroreactivity to beta-PV E6 (AK: OR, 0.6; 95% CI, 0.29–3.0; SCC: OR, 0.45; 95% CI, 0.19–1.1). E6 and L1 antibodies were hardly ever found concomitantly, suggesting that antibody responses to the early (nonstructural, intracellular) and late (structural, also extracellular) beta-PV proteins take place at different times and phases during HPV infection or HPV-associated tumor development [33]. HPV DNA positivity and L1 seropositivity were correlated, and E6 seropositivity was inversely correlated with HPV DNA positivity, which might be in line with the hypothesis that E6 antibodies to some extent protect against SCC or that SCC patients have difficulties inducing immune responses to cutaneous HPV E6 proteins [33, 49].

A summary of the retrospective case-control studies available to date has been published recently (Fig. 2). This serological pilot study reported results on the first prospective data looking at the association between the L1 antigens of 38 HPV types and the development of SCC in immunocompetent patients with blood taken before diagnosis. Based on 39 patients with SCC and 80 controls, there was no statistically significant difference in seroprevalence of antibodies against any of the HPV types examined between cases and controls. Seroprevalence for many beta-PV types was higher among cases for whom blood was collected within 1.5 years of diagnosis than in those whom SCC was diagnosed more than 1.5 years after blood collection [55].

Although basal cell carcinomas (BCC) are the most common NMSC, its association with HPV infections in immunosuppressed individuals remains controversial [25, 47, 48]. The relative risk for superficial multifocal and nodular BCC was increased in persons positive for HPV8 (OR, 17.3; 95% CI, 2.1–143; and OR, 9.2; 95% CI, 1.1–78.2) and HPV20 (OR, 3.4; 95% CI, 1.2–9.5; and OR, 3.2; 95% CI, 1.3–7.9) in a study among immunocompetent individuals [32]. In another study, however, HPV seropositivity was not associated with BCC (OR, 0.8; 95% CI, 0.6–1.1) [34]. A third study found no significant association between HPV antibody prevalence of BCC patients and healthy individuals; as well, the serological data did not correlate with special types found in the tumors [52].

Association Between HPV Infection, Skin Cancer, and Sun Exposure

Individuals in subtropical areas have an increased risk of AK and NMSC because the principal causal factor is excessive exposure to solar UV radiation [56–58]. Because HPV is a possible cofactor in the development of AK and SCC, in Queensland, Australia, where reported incidence rates are the highest in the world [59], a number of studies have been performed to investigate the role of HPV in the development of NMSC [28, 33, 60]. A nested case-control study in patients with NMSC (not specified), SCC, or BCC showed a nonsignificant negative association between beta-PV and NMSC (OR, 0.77; 95% CI, 0.34–1.8) and BCC (OR, 0.58; 95% CI, 0.23–1.50) and a nonsignificant positive association between beta-PV and

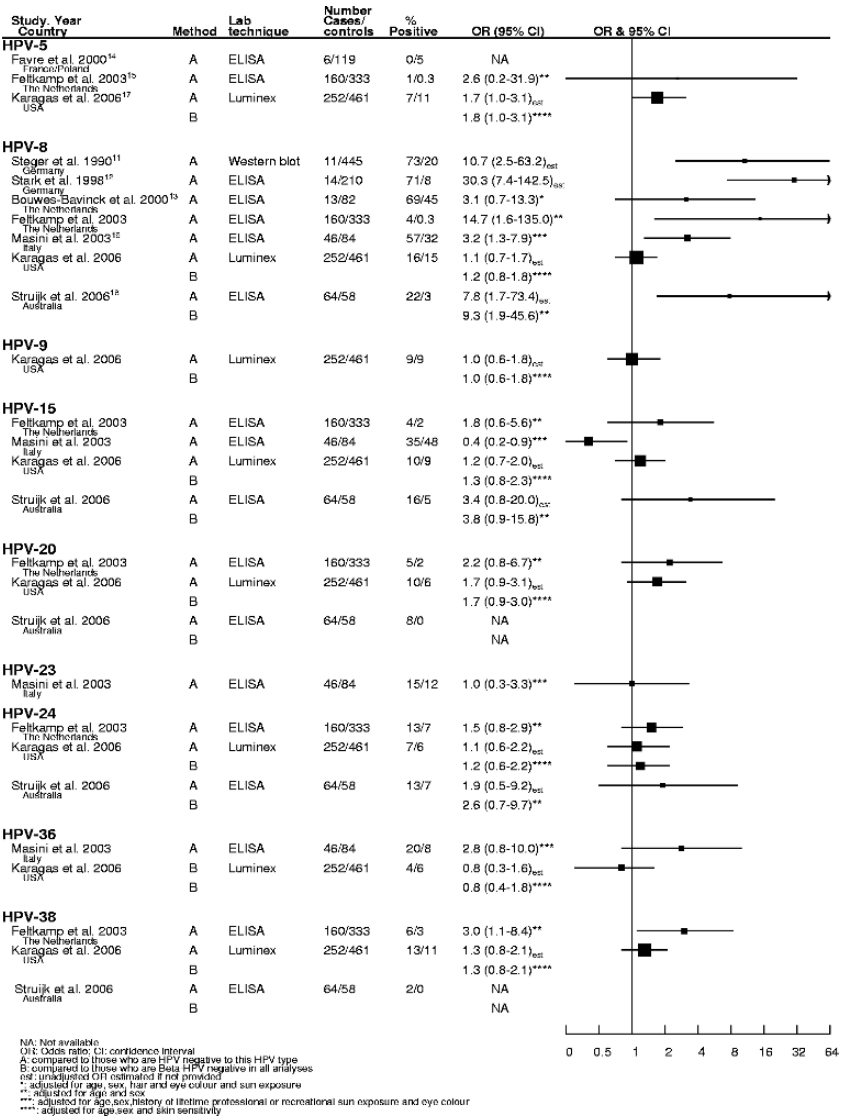

Fig. 2 Studies of cutaneous squamous cell carcinoma in relation to the detection of antibodies against capsid L1 protein of betaPV types. Odds ratio are presented by *squares*, the area of each square being proportional to the amount of statistical information for each study, and 95% CI are indicated by *lines*. Figure reprinted with permission from the publisher from Casabonne et al., Int J Cancer (2007) 121: 1862–68

SCC (OR, 2.00; 95% CI, 0.5–8.0) [28]. In the same population, a cross-sectional study was performed of the eyebrow hairs of individuals with AK [60]. There was a strong association between the presence of beta-PV and higher age and also between AK and higher age. Also, an association was found between male sex and beta-PV

(OR, 3.4; 95% CI, 1.77–6.53). A Dutch case-control study showed an association between sunburn in the past, especially at age 13–20 years, and higher beta-PV positivity [61]. A higher lifetime sun exposure, however, was associated with a lower risk of HPV infection. In the United States, a case-control study showed no significant relationship between HPV seropositivity and age, skin sensitivity, and number of sunburns [34]. However, SCC risk was elevated in beta-PV-positive individuals with a more sun-sensitive phenotype. SCC risk was also increased among those who reported 10 or more sunburns. In addition, an analysis was done to elaborate the possible joint effect of HPV and UV. The analysis, nested in an SCC case-control study in Queensland [33], showed that the combined effect of beta-PV seropositivity and presence of a susceptible phenotype or high lifetime sun exposure resulted in a greater risk of SCC than either risk factor alone [50].

Discussion

A role of beta-PV in the development of skin cancer has been proposed, and epidemiological evidence, as summarized in this chapter, was found in both immunocompetent and immunosuppressed individuals. The role that beta-PV potentially play in skin carcinogenesis, however, is far from clear, and seems to differ from known high-risk mucosal HPV types on essential points. So far, the beta-PV prevalence studies have not identified potentially high-risk beta-PV types in analogy to HPV16 and -18 in cervical cancer, although SCC case-control studies have suggested that HPV5, -8, and -38 are high-risk beta-PV types.

Further discrepancy between mucosal and potentially cutaneous high-risk HPV types lies in the viral load present in the tumors. In SCC, beta-PV DNA is found in approximately 1:100 cells [62]. This finding contrasts with the situation in cervical cancer, where high-risk HPV DNA is generally found integrated in the genome of every cancer cell. Apparently beta-PV are dispensable when it comes to maintenance of the transformed state of the cancer cells, and more likely play a role early in the carcinogenesis; this was also suggested by observations demonstrating that both the prevalence and the load of beta-PV are higher in early premalignant lesions such as actinic keratosis compared to malignant lesions [51, 62, 63]. Taken together, the role of HPV in skin carcinogenesis could be more important for tumor initiation than for tumor progression. Most experimental studies that investigated beta-PV-mediated cell transformation seem to support this notion. They indicated the ability of some beta-PV types to inhibit UVB apoptosis, which could be considered an early event in carcinogenesis, and the inability to inactivate the tumor suppressor proteins p53 and pRb, which can be considered important for transformation maintenance.

To summarize, the role of beta-PV in skin carcinogenesis is not understood yet. Exposure to UV radiation is the most important risk factor for the development of NMSC, and HPV infection might act as a cofactor in this regard. Recent epidemiological [34,50] as well as experimental studies [64] argue in favor of this hypothesis,

suggesting a possible synergetic effect between HPV infection and UV radiation in carcinogenesis of the skin.

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Interaction Between Ultraviolet Radiation and Human Papillomavirus

Alan Storey and Mark Simmonds

Introduction

Human papillomaviruses (HPV) are small double-stranded DNA viruses that predominantly form hyperproliferative lesions in mucosal and cutaneous epithelial tissues and are strictly epitheliotropic. More than 120 different types have been characterised based on DNA sequence homology [1]. The association between HPV and cervical cancer has been well documented, with 99% of cervical tumours containing HPV DNA [2]. The expression of E6 and E7 proteins of high-risk anogenital types, such as HPV 16 and 18, has been linked with the subsequent degradation of both p53 and pRb, respectively, and the constitutive expression of both viral proteins in anogenital tumours is required to maintain this transformed state [3].

The association of HPV with nonmelanoma skin cancer (NMSC) is less well understood, because only those individuals who are unable to effectively clear the virus, and therefore suffer from extensive wart infection, develop the majority of viral-associated skin tumours on sun-exposed sites [4]. These cases include epidermodysplasia verruciformis (EV) patients, where the DNA of associated HPV types 5 and 8 of the β-group is detected in squamous cell carcinomas (SCCs), and renal transplant patients, who have a 200-fold and 10-fold risk of SCCs and basal cell carcinomas (BCCs), respectively [5, 6].

HPV and Inhibition of UV-Induced Apoptosis

UV Irradiation

Ultraviolet (UV) radiation is an important environmental carcinogen that increases the risk of developing NMSC upon persistent exposure. An association between UV

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exposure and HPV has been proposed in NMSC development, where the virus acts as a cofactor in tumour formation in EV and immunosuppressed patients.

UV light is categorised into three forms: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). High-energy UVB has been identified as the most important component in skin tumour formation as it induces DNA damage in the upper layers of the skin, of which the formation of cyclobutyl rings between adjacent pyrimidines is an important adduct. The resultant pyrimidine dimers are mutagenic, although they can be removed by nucleotide excision repair (NER) before DNA replication.

The importance of NER in the prevention of skin cancer has been previously reported, since mutations in genes actively involved in the DNA-repair pathway cause an increase in the incidence of NMSC and keratoses [7]. The failure of these DNA-repair mechanisms can allow the clonal expansion of a cell having mutations in both oncogenes and tumour suppressor genes [8]. These genes have a critical role in cell-cycle control, maintenance of genome integrity, and cell proliferation and differentiation. They are classified into two groups: gatekeeper and caretaker genes. The former control proliferation and apoptosis (e.g., pRb and p53), and the latter maintain genome integrity (e.g., ATM, BRCA-1, and BRCA-2) [9].

UVB irradiation also causes local and systemic damage to the immune system, which suppresses the detection and therefore the removal of damaged cells. In the epidermis, Langerhans' cells serve as antigen-presenting cells, which interact with T lymphocytes. UVB induces immunosuppression by affecting this system through contact hypersensitivity and delayed-type sensitivity and by stimulating cytokine release from keratinocytes, which enhances this immunosuppressive effect [10].

Cell-Cycle Regulation

The risk of developing NMSC can increase as the presence of HPV in the skin may allow the survival and proliferation of DNA-damaged cells through the expression of the E6 and E7 viral proteins. These proteins effectively enhance the cellular environment for the production of virions in terminally differentiated keratinocytes that have exited the cell cycle. HPVs rely on the host cell DNA replication machinery to copy their genomes, and therefore require the host cell to be in an S-phase-like state, so that this machinery is available. The expression of E7 by high-risk anogenital HPV types allows maintenance of the viral episome by inducing the production of S-phase proteins in the absence of growth factors; this is achieved through the degradation of pRb, with the release of E2F-1 and the binding of cyclins, cdks, and cdk inhibitors, which then stimulate the production of components for DNA replication, by enabling a G_1–S-phase transition in the absence of growth signals [11]. Few studies have been performed on the molecular interactions of cutaneous HPV E7 proteins with cellular targets, although HPV type 38 is able to target pRb for ubiquitin-mediated degradation [12]. The possible interaction of β-HPV E7 proteins with cell-cycle regulatory proteins remains to be determined.

p53 and HPV Interactions

The tumour suppressor protein p53 becomes activated and stabilised upon exposure to DNA damage-inducing UV light in the epidermis. This process either causes growth arrest, so that cells may attempt to repair the DNA, or, if the damage is severe, allows induction of apoptosis [13]. To combat the antiproliferative effects of p53 activation, the high-risk anogenital HPVs, such as types 16 and 18, express the E6 protein that is able to degrade p53 by the ubiquitin-proteosome pathway. The targeted degradation of p53 by HPV E6 removes a major obstacle to viral replication and virion production.

Cutaneous HPVs, however, encode E6 proteins that are unable to target p53 for ubiquitin-mediated degradation, but these can overcome p53-mediated stress responses by other mechanisms. HPV 38 E6 and E7 expression in keratinocytes induces stabilisation of p53, which promotes ΔNp73 upregulation. This isoform of p73 causes transcriptional repression of p53, resulting in the inhibition of p53-responsive gene expression. The downregulation of ΔNp73 allows the recovery of p53-responsive gene expression, resulting in induction of apoptosis [14]. HPV 77 E6 selectively attenuates UV-induced transactivation of the p53-regulated pro-apoptotic genes Fas, *PUMA*, Apaf-1, and *PIG*3, leaving other p53 target genes, such as those involved in cell-cycle regulation, unaffected [15]. There is also increasing evidence of the involvement of p53-independent apoptotic pathways in the skin. It has been discovered that both Li-Fraumeni patients, who only have one functional copy of p53, and patients with patches of mutated p53 on sun-exposed sites, are not predisposed to NMSC development [16, 17]. Analysis of NMSC biopsies, however, shows that UV-related p53 gene mutations are present in both BCCs and SCCs and that these occur early in carcinogenesis and are non-rate limiting. Keratinocytes expressing mutated p53 may still undergo apoptosis upon UV exposure, although this is less efficient than wild-type cells [18].

Bak/Bax and HPV Interactions

Bak is an apoptogenic member of the Bcl-2 family that is located on the outer mitochondrial membrane. In response to genotoxic stress, Bak is stabilised and multimerises to allow pore formation through the mitochondrial membrane. This formation facilitates the movement of mitochondrial cytochrome *c* and other pro-apoptotic factors into the cytoplasm, where an apoptotic cascade is initiated [19]. Bak has been identified as a key apoptotic regulator, which is targeted by both anogenital and cutaneous HPV E6 proteins for ubiquitin-mediated degradation [20].

The discovery that cutaneous HPV E6 proteins do not target p53 for degradation suggests that they can efficiently prevent UV-induced apoptosis by targeting Bak only. Furthermore, the use of Bak silenced cells has highlighted its critical role in UV-induced release of mitochondrial factors, such as AIF (apoptosis-inducing factor) and Omi, and that the expression of E6 prevents the release of these factors by targeting Bak for proteolytic degradation [21].

Recently, a further apoptogenic member of the Bcl-2 family, Bax, has also been identified as a target for the high-risk anogenital type HPV 16 E6. These studies indicated that the stable expression of E6 in differentiating keratinocytes was associated with sustained Bcl-2 levels and reduced Bax mRNA expression and protein stability; however, no physical interaction between E6 and Bax was demonstrated [22]. Further work has indicated that RNAi inhibition of E6 expression in cervical tumour cells leads to transcriptional stimulation of the *PUMA* promoter in a p53-independent manner; this has been linked to the activation and translocation of Bax to the mitochondria and subsequent release of cytochrome *c* and caspase 3 activation in a *PUMA*-dependent manner [23]. Analysis of NMSC biopsies has revealed that HPV-positive tumours have a low apoptotic and high proliferation rate, compared with HPV-negative tumours that have both a high apoptotic and proliferation rate.

This result suggests that the presence of HPV in NMSC is favouring tumour expansion through a disruption in the balance between proliferation and apoptosis [24]. There is increasing evidence that the targeted degradation of Bak by cutaneous HPV E6 proteins is able to negate the tumour suppressive properties of

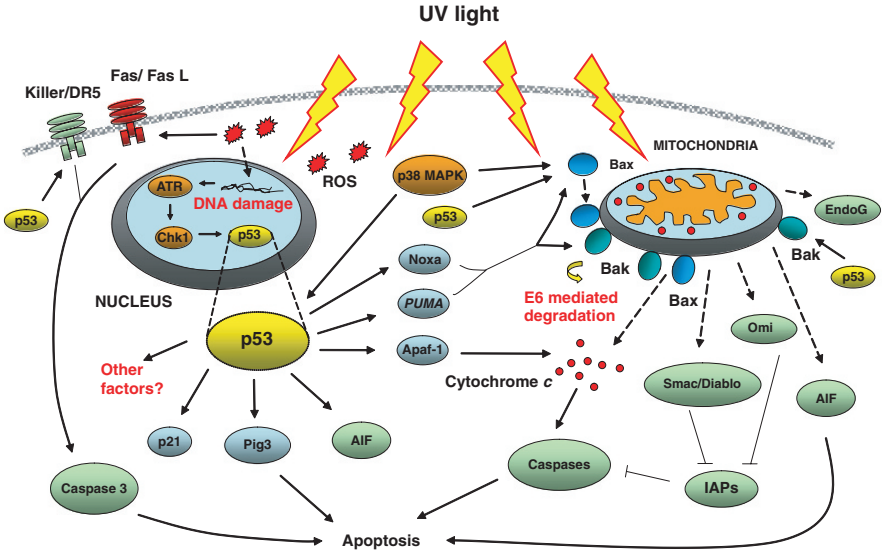

Fig. 1 Apoptotic pathways in epidermal cells. UV light induces DNA damage through direct absorption and reactive oxygen species (ROS), activating p53 through Chk1 and p38 MAPK, which stimulates apoptosis through the initiation of downstream targets. These include Noxa and PUMA, which cause cytochrome *c* release, and Apaf-1, which complexes with cytochrome *c* and caspases to form the apoptosome. UV light also directly initiates apoptosis through extrinsic pathways such as the Fas ligand. The activation of Bax and Bak are key to cytochrome *c* release from the mitochondria, as well as the release of other proapoptotic mitochondrial factors, such as Smac/Diablo, Omi, and AIF. p53 also activates Bax and facilitates Bak multimerisation at the mitochondria, as well as regulating apoptosis-inducing factor (AIF) expression. HPV E6 can prevent UV-induced apoptosis through the degradation of Bak and may target other apoptotic pathways [25, 26]. How and where E6 interferes with many of these apoptotic pathways remains to be determined

p53 by affecting p53-mediated downstream targets (see Fig. 1). p53 interacts with and activates Bak at the mitochondria; therefore, E6 targeting Bak can inhibit at least part of the apoptotic activity of p53.

Inhibition of DNA Repair

HPV and Interference with DNA-Repair Pathways

A key component in the cofactorial role of HPV in the development of NMSC is the ability to induce genomic instability. The expression of E6 in the differentiating epidermis allows keratinocytes to bypass the G_1–S checkpoint where DNA repair would normally occur before replication. Subsequent UV exposure, at doses that are insufficient to stimulate apoptosis, can then lead to mutagenic accumulation. This pathway has been demonstrated for the HPV 5 E6 protein, which is able to compromise the repair of UVB-induced cyclobutane pyrimidine dimers (CPDs) in epidermal cells [27].

Additionally, HPV 8 E6 binds the XRCC1 protein, which is involved in the repair of DNA single-strand breaks (SSBs), thereby reducing its efficiency. If these SSBs are not repaired, they can be converted into double-strand breaks during DNA replication, which may result in chromosomal instability [28]. Additionally, transgenic mice ubiquitously expressing CPD-photolyase and 6–4 pyrimidone photoproducts (PP) photolyase show rapid light-dependent repair of these UV-induced photoproducts and do not develop NMSC [29], which suggests that the removal of CPDs is critical in NMSC prevention and that the partial inhibition of CPD or SSB repair, by HPV 5 and −8, coupled with the prevention of apoptosis through the degradation of Bak, allows the persistence of damaged cells.

Additional HPV Interactions

Mouse Models

Studies on the transforming potential of cutaneous HPV E6 and E7 proteins suggest that they are only weakly transforming in vitro, because, in contrast to the high-risk anogenital HPV E6 and E7 proteins, coexpression does not lead to human foreskin keratinocyte immortalisation [30, 31]. Interestingly, the expression of HPV 38 E6 and E7 in primary human keratinocytes resulted in an extended lifespan [12]. HPV E6 from EV-associated β-types induces morphological transformation and anchorage-independent growth in rodent cells but does not cause tumour formation in nude mice. Additionally, the E7 protein of HPV types 5 and 8 can only transform rodent cells in the presence of an activated H-*ras* gene [32].

Recently, the production of various transgenic mouse models has given insights into the transforming properties of E6 and E7 in vivo. Mice expressing the complete early region of HPV 8 in basal epidermal keratinocytes developed benign tumours in 90% of cases [33]. A significant proportion of these lesions progressed

to SCCs without exposure to any further physical or chemical carcinogens, and it is possible that subsequent UV exposure of these mice would further enhance SCC development. The expression of HPV 38 E6 and E7 in mice induced epidermal cell proliferation, dysplasia, and hyperplasia, and spontaneous tumours only arose upon exposure to further carcinogens.

Interestingly, UV exposure did not cause p21 accumulation in mice expressing E6 and E7 compared to controls, suggesting that cell-cycle arrest is impaired [34]. Mouse models expressing HPV 20 and 27 E6 and E7, which were exposed to chronic UV irradiation, showed enhanced proliferation and papilloma formation in epidermal layers, which progressed to SCCs [35].

Epidermal Invasion in Organotypic Cultures

The development of organotypic cultures using either collagen or de-epidermalised dermis has aided our understanding of HPV gene function. Raft cultures using de-epidermalised dermis more closely mimic in vivo conditions, since they maintain the structure of skin and contain extracellular matrix (ECM) proteins. Keratinocytes expressing the HPV 8 E7 gene, when seeded onto de-epidermalised dermis, display a tumourigenic phenotype, in that they cause epidermal invasion; this is facilitated by the overexpression of the extracellular metalloproteinases MMP-1, MMP-8, and MT1-MMP, which disrupt basement membrane (BM) and ECM integrity through the degradation of the collagen types IV, VII, and laminin V [36]. These data suggest that HPV 8 may actively promote tumour invasion in EV patients, although the molecular interactions of E7 in this process have not been determined. The effects of UV on this process remain to be investigated.

UVB-Induced Clonal Expansion

It is clear that for HPV to establish a persistent infection in the epidermis, it must reside in cells that are maintained rather than shed, such as those present in the stem cell population. This theory is supported by the detection of high-risk cutaneous HPV DNA from hair follicle cells present in plucked eyebrow samples, although the target cell types are unknown [37]. The persistence of HPV within stem cells, which are normally thought to be particularly sensitive to DNA damage, may affect their apoptotic properties and allow the gradual accumulation of genetic mutation, and it is possible that expansion of these cells is facilitated by apoptotic clearance of normal DNA-damaged keratinocytes [38]. Therefore, it may be possible to consider these HPV-infected cells as “cancer stem cells” that could efficiently support a larger population of cells within a tumour as they persist within the epidermis.

The presence of E6 HPV DNA of various types has been detected in SCCs, but the link between the presence of the virus and active involvement in tumourigenesis through expression of the E6 and E7 proteins remains unclear because HPV persists on healthy skin. PCR analysis has detected higher viral loads in warts and actinic

keratoses (AK) than in SCCs [39], which suggests that HPV may be involved in the early stages of tumourigenesis and that perhaps initial cellular damage caused by infection of the virus, coupled with either UV exposure or immunosuppression, is sufficient to instigate a progression to tumour formation.

Summary

Current evidence suggests that skin tumour development requires both UV-induced mutations to occur and is enhanced by immunosuppression and that cutaneous HPVs have a cofactorial role in this process. It is clear that both renal transplant and EV patients, who are more susceptible to HPV infection, are at a greater risk of NMSC than immunocompetent patients. However, the viral mechanisms involved in this process are poorly understood, as there are other potential cellular targets of E6 and E7 that remain to be determined. Studies on the molecular basis of cancer have revealed a series of complex environmental and genetic events that lead to specific phenotypes. The ability of HPV to allow the clonal expansion of damaged cells appears to increase the likelihood of tumour development in addition to these factors. Moreover, the specific tropism of cutaneous and anogenital HPV strains suggests that the life cycle is adapted to particular epithelial cell types, which implies that cancer models for cutaneous HPVs cannot be based on those for anogenital types and that the involvement of HPV in skin cancer represents a new paradigm for how the virus is involved in cancer development. Furthermore, the difficulties associated with producing differentiating epithelia in vitro have hampered molecular studies involving cutaneous types, as HPV genes are expressed in vectors rather than by a productive infection in the epidermis. Further studies are required on the molecular basis of HPV persistence in cutaneous epithelia to understand the role of HPV in NMSC.

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Human Herpesvirus 8

Celeste Lebbé and Camille Francès

Abbreviations marked with a star are explained in the appended lexicon when they appear in the text for the first time.

Introduction

Herpesviruses are double-stranded DNA viruses that can remain latent in vertebrate hosts. Eight human herpesviruses have been discovered to date. Herpesviruses are classified into three subfamilies, α, β, and γ, on the basis of their genome structure and biological properties. Kaposi's sarcoma-associated virus (KSHV), also known as human herpesvirus 8 (HHV8), is a gamma herpesvirus (rhadinovirus) closely related to Epstein–Barr virus (EBV) and in the lineage of various rhadinoviruses infecting macaques, African green monkeys (Fig. 1). It was discovered in 1994 by Chang et al. [1] in a Kaposi's sarcoma (KS) specimen from an human immunodeficiency virus (HIV)-infected patient using representational difference analysis and was shown to be the long-sought infectious agent responsible for KS. Since then, its association has been confirmed in all clinical settings of KS and was extended to a limited number of lymphoproliferations including multicentric Castleman's disease and primary effusion lymphoma (PEL) [2].

In contrast with other widely distributed human herpesviruses, HHV8 is not ubiquitous, and its distribution is heterogeneous around the world. A common property of gamma herpesviruses is their capacity to induce neoplasia in their hosts. Indeed, even more than other herpesviruses, KSHV has extensively pirated human cellular genes during evolution, some of these genes being involved in transformation, and HHV8 has been classified as a grade 1 carcinogen by the International Agency for Research Against Cancer (IARC). In line with other viruses, HHV8 is able to evade innate and adaptive immunity to maintain long-term infection.

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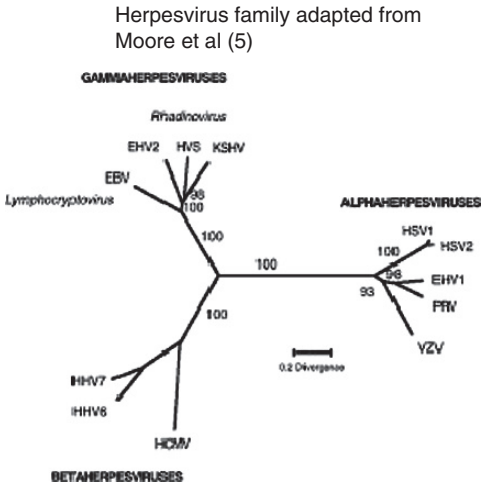

Fig. 1 Herpesvirus family. [Adapted from Moore et al. [5].] Phylogenetic trees of Kaposi's sarcoma-associated virus (KSHV) based on comparison of aligned amino acid sequences between herpesviruses for the MCP gene and for a concatenated nine-gene set

HHV8 Structure

HHV8 has a typical herpesvirus morphology. Using electronic microscopy on cellular cultures as well as KS biopsies, HHV8 presents as 100- to 150-nm particles with a lipid envelope and electron-dense central core [3, 4]. The HHV8 genome is 170 kilobases (kb) while some cell lines harbour forms up to 270 kb [3,5]. It is characterized by a long unique coding region, with at least 87 genes (open reading frame, or ORF) flanked by terminal repeats (Fig. 2). The long unique coding region includes 68 genes shared with other gamma herpesviruses as well as genes apparently unique to HHV8 (K1 to K15). The former genes intervene in DNA replication, packaging, viral entry, and capsid envelopment, while many of the K genes are pirated genes involved in growth control [6].

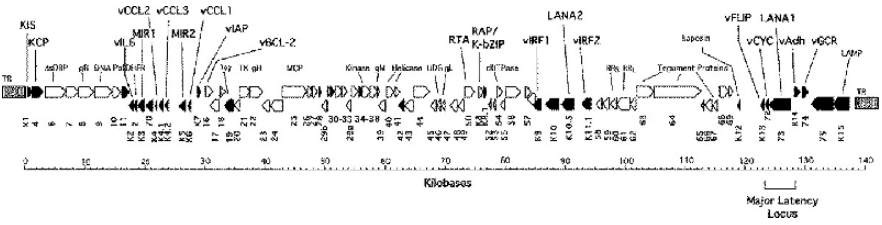

Fig. 2 Human herpesvirus 8 (HHV8) genome organization. [Adapted from Moore and Chang [45]]

According to the analysis of two polymorphic genes at the left and right extremity of the genome, K1 and ORF-15, six HHV8 variants (A to F) have been defined. Variant A and C predominate in Europe and North America and variant B in Africa, D and E have been identified in Pacific and in Amerindian populations, and variant F has been recently identified in Uganda [2, 7]. Such variants probably emerged following population migrations over the past 40,000 years [2]. They may have functional implications, although no association between variants and pathologies have been identified so far [8, 9].

HHV8 Biology

Several human cell lines have been shown to support HHV8 culture in vitro; these include cells from the lymphoid, endothelial, fibroblastoid, and keratinocyte lineage and various tumour cell lines [10, 11]. In vivo, the major natural reservoir for HHV8 is CD19B cells, but infection has also been shown in endothelial cells and spindle cells from KS tumours, which are currently considered as lymphatic endothelial-derived cells. HHV8 reprogramming of endothelial cells to lymphatic differentiation is probably a crucial event in KS pathogenesis [12, 13]. HHV8 can also be detected in vivo in monocytes, prostate epithelial cells, oral epithelial cells, renal tubular epithelial cells, and dorsal root sensory ganglion cells [14–17].

Little is known about the characteristics of HHV8 persistence in vivo. HHV8 enters human fibroblasts, B cells, and epithelial cells via endocytosis [18]. It interacts with heparin sulfate-like molecules via two viral envelope proteins, glycoprotein (gp) B and gpK8.1A [18–20]. Then, gB interacts with the $\alpha 3\beta 1$ integrin on the cell surface (endothelial and fibroblasts) and the transporter xCT* [19, 21]. Recently Veettil et al. [22] showed that RhoA* signalling and the associated SRC* activation were essential for HHV8 entry in adherent cells. In most cell types, however, no active infection occurs, at least from in vitro data. There is no detection of viral DNA after several passages, and viral transmission in vitro appears to be limited [23, 24].Viruses detected in some primary effusion lymphoma (PEL) cell lines such as JSC-1 appear more infective and are able to induce a spindle shaped pattern of dermal microvascular endothelial cells in vitro [25]. In vitro derived cells from primary effusion lymphoma (PEL) do exhibit persistent viral infection. These cell lines and, more recently, the availability of high-titre infective recombinant HHV8 virus has led to further insights into HHV8 biology [26].

As with other herpesviruses, HHV8 has two distinct phases in its life cycle: viral latency and lytic replication. In vitro and in vivo viral infection persists predominantly in a latent form. In latent infection, the virus genome is present as a multicopy circular episomal form in the nucleus. Viral DNA is copied by the host cell machinery during each division, in a cell cycle-dependent manner, and the same copy number is maintained. During cell mitosis, viral DNA remains tethered to host

chromatin via the latent nuclear antigen (LANA). Terminal repeats and LANA are sufficient in vitro for viral replication in stable latency, but establishment of latency is inefficient without in vitro drug selection.

Establishing latency in vivo is likely to be a multistep process involving one or more epigenetic modifications of the latent viral genome [27]. In latently infected cells, a restricted number of HHV8 genes are expressed. These latent genes include LANA, v-cyclin, a homologue of cellular cyclin encoded by ORF 72, and viral Fas*-associated death domain (FADD)* interleukin-1β-converting enzyme (FLICE)* inhibitory protein (v-FLIP) (ORF 71) [28, 29]. Other HHV8 genes can be expressed during latency such as K15, which has tumor necrosis factor receptor (TNFR)-associated factor binding domains and is susceptible to induced nuclear factor κB transcriptional activation and kaposin [30, 31]. Because HHV8 remains latent in the majority of infected cells, these latent genes probably play a major role in transformation (see below).

Latent virus can reactivate to enter lytic replication. HHV8 Rta (reactivation transcriptional activator), encoded by ORF 50, is required to activate the lytic cycle, like Zta* (ZEBRA protein from EBV) and Rta EBV proteins [32, 33]. Although the lytic cycle can sometimes be abortive, it will ultimately result in the production of mature virions and cell lysis. While a minority of cells harbour lytic infection, HHV8 reactivation probably plays a critical role for efficient viral transmission, spread, and possibly pathogenesis through paracrine mechanisms or abortive reactivation. Bearing in mind the inefficient establishment of KSHV latency in vitro, HHV8 reactivation is probably mandatory at early phases of HHV8-associated proliferation, particularly in KS. Indeed, continuous infection of HHV8-negative cells by infectious virus produced by lytic replication is probably required to maintain viral latency in the tumour [27]. It is likely that various cellular signals regulate the switch from latency to lytic replication such as the Ras*/Raf*/Mek*/Erk*/Ets-1* pathway activated by various growth factors and cytokines [34].

Disease Associations

HHV8 has been consistently associated with all KS clinical settings. It is also associated with two varieties of B-cell lymphoproliferative disorders, PEL and its related solid variants, multicentric Castleman's disease, and HHV8-positive plasmablastic lymphomas arising from multicentric Castleman's disease. More recently, HHV8 has also been associated with a spectrum of post-transplantation plasmacytic proliferations [35]. It has also been detected in three immunocompetent patients with germinotropic lymphomas consisting of the proliferation of plasmablasts coinfected by KSHV and EBV and preferentially involving the germinal centers of B-cell follicles [36]. Finally, it has anecdotally been reported in the context of bone marrow hypoplasia and haemophagocytic syndromes mostly occurring in immunosuppressed patients [37].

Insight in Functions of KSHV Homologous Genes, Focusing on KS Pathogenesis

Cell-Cycle Regulation and Apoptosis

HHV8 encodes a v-cyclin, a homologue of D-type cellular cyclins, which binds cyclin-dependent kinases*. K-cyclin/CDK phosphorylates a subset of substrates normally targeted by cyclins D, E, and A. Human cyclin D binds to and activates CDK4 and CDK6, which phosphorylate Rb*, releasing the repression on the transcription factor E2F* (Fig. 3). In normal uninfected cells, G_1/S progression is nega-

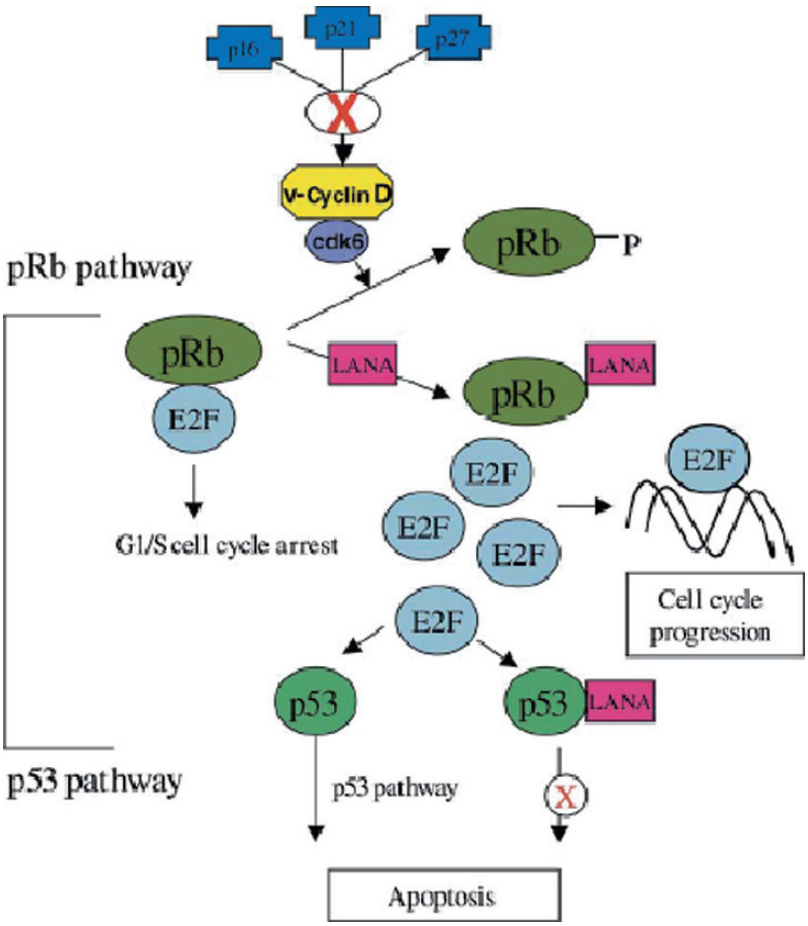

Fig. 3 Schematic model showing the role of LANA and v-cyclin in cell cycle progression. [Adapted from Moore and Chang [45].] In normal uninfected cells, G_1/S progression is negatively regulated by binding of pRb to E2F transcription factor. In infected cells, LANA competes with E2F for binding of hypophosphorylated pRbi thus, E2F is freed to activate the transcription of S-phase genes

tively regulated by binding of Rb to E2F transcription factor. In infected cells, E2F is freed to activate the transcription of S-phase genes. By contrast with D-type cellular cyclins/CDK complexes, the v-cyclin/CDK complexes are insensitive to CDK inhibitors (p16INK4a, p21CIP1, and p27KIP1)*. Moreover, v-cyclin has a higher range of substrates than its cellular homologues, including p27KIP1, which is then targeted for ubiquitin-mediated degradation and cannot induce cell-cycle arrest.

In vivo, V-cyclin is expressed in most KS spindle cells and probably drives cells to uncontrolled progression from the G_1 to S phase of the cell cycle; it also mimics S-phase mitotic cyclins [38, 39]. LANA or LANA1 is involved in cell-cycle regulation. It competes with E2F for binding hypophosphorylated pRb. E2F is then able to activate the transcription of genes involved in cell-cycle progression. LANA also binds p53 and blocks the p53-mediated apoptosis. LANA interacts with other cellular factors involved in transcription. LANA associates with GSK-3beta*, an important modulator of the Wnt* signaling pathway leading to the accumulation of cytoplasmic beta-catenin, and subsequent activation of the transcription factor Tcf/Lef*. LANA also blocks the expression of Rta, the reactivation transcriptional activator, and helps in maintaining viral latency [40] The Bcl2* family proteins regulate programmed cell death; vBcl-2, encoded by ORF16, inhibits apoptosis. vBcl-2 is not expressed during latency, and its relevance for KS in vivo is yet to be clearly demonstrated. However, its expression in advanced KS tumours has been shown using immunohistochemistry [41]. v-FLIP is a homologue of the cellular protein FLIP. FLIPs contain death-effector domains that interact with the adapter protein FADD, inhibiting the recruitment and subsequent activation of the protease FLICE by the CD95 (Fas) death receptor. HHV8 v-FLIP is able to protect cells from Fas-mediated apoptosis and permits clonal growth in the presence of Fas ligand [42]. v-FLIP is probably implicated in the pathogenesis of KS because expression of v-FLIP transcripts is increased in late-stage KS lesions in parallel with reduced apoptosis [43]. Nuclear factor κB induction by vFLIP is required for KS cells spindling. Nuclear factor κB induction also upregulates proinflammatory cytokines by endothelial cells [44]. Therefore, in addition to its anti-apoptotic function, vFLIP probably contributes to the inflammatory microenvironment of KS.

PR Angiogenic Genes

HHV8 encodes three chemokines named viral macrophage inflammatory proteins (vMIPs): vMIP-I (encoded by K6), vMIP-II (K4), and vMIP-III (K4.1). All three vMIPs, also called vCCL1, vCCL2, and vCCL3 [chemokine (C-C motif) ligand 1 protein], are angiogenic in the chick chorioallantoic assay [45].The vCCL3 is found in KS tumours and is thought to contribute to its pathogenesis [46]. The viral protein interleukin 6 (vIL-6, K-2) is expressed in multicentric Castleman's disease plasmablasts, in a minority of PEL cells, and rarely in KS lesions. vIL-6 shares 24% identical amino acid sequence with human IL-6 and binds directly to the gp130 subunit of the IL-6 receptor without need for the IL-6 receptor alpha chain (gp80).

It activates the Janus kinases/signal transducers. It is able to prevent plasmacytoma cell apoptosis and promotes proliferation of myeloma cells [45]. Mice inoculated with vIL-6-transfected (NIH3T3) cells develop more highly vascularised tumours than control mice, and the tumours also express higher levels of vascular endothelial growth factor (VEGF) [47].

HHV8-GPCR (G protein-coupled receptor) is encoded by ORF 74 of HHV8 [48, 49]. It belongs to the rhodopsin/β-adrenergic subfamily of G protein-coupled receptors and shows homology to human receptors including C-X-C* chemokine receptor type 1 (CXCR1). It is constitutively active but is also capable of interacting with a broad array of chemokines. HHV8-GPCR uses a variety of signaling pathways, including activation of phospholipase C and PKC, inhibition of adenyl cyclase, activation of nuclear factor-κB, activation of PI kinase, p42/44 MAPK*, and Akt*, and activation of SAPK/JNK (stress-activated protein kinase/c-jun kinase) pathway, p38 MAPK, and RAFK (RAF kinase)*. HHV8-GPCR stimulates secretion of VEGF, inflammatory cytokines, and adhesion molecules in vitro and induces the development of angioproliferative, KS-like tumours in transgenic mice. Notably, HHV8-GPCR is expressed in only a few spindle cells within KS lesions in human and in transgenic mice KS-like tumours. Therefore, HHV8-GPCR might have a role in KS pathogenesis in a paracrine manner, by inducing secretion of pro-inflammatory/proangiogenic factors [50, 51].

Viral antigen processing and presentation through major histocompatibility complex (MHC) I is critical for effective antiviral cell-mediated immune response. Two transmembrane proteins, MIR1 and MIR2 (modulator of immune recognition), encoded by ORFs K3 and K5, respectively, inhibit major histocompatibility complex (MHC) I surface expression. They remove MHC I from the plasma membrane via enhanced endocytosis, lysosomal targeting, and degradation of MHC molecules. Downregulation of MHC I and its accessory immune receptors stimulates natural killer (NK) cell response [52–54]. However, HHV8 inhibits NK-mediated killing through expression of v-FLICE-inhibitory proteins (vFLIPs) and viral chemokines vMIP. Such viral chemokines, as well as neural cell adhesion molecules such as adhesin (ORF K14, vOX-2/vAdh), homologous to CD200 (OX-2), are also capable of polarizing immune responses toward an antibody-predominant Th2 immune reaction [45]. HHV8 can interfere with innate immune responses in other ways. The KSHV ORF4 encodes a protein designated KSHV complement control protein (KCP), which has homology to human complement regulators and is able to inhibit C3 deposition on the cell surface [45, 52].

ORF K1 encodes a small transmembrane, immunoglobulin-like glycoprotein called KIS (K ITAM-signaling), which possesses a cytoplasmic immunoreceptor tyrosine activation motif (ITAM) similar to that of the B-cell receptor (BCR). KIS downregulates the expression of BCR by blocking the intracellular transport of BCR complexes to the cell surface [45, 52].

Interferons (IFNs) are important for antiviral innate immunity. KSHV has developed multiple ways to subvert IFN signalling, from inhibition of IFN-α signalling, antagonism of IFN-initiated gene transcription, and inhibition of interferon regulatory factors (IRF). Normally, IFN-α induces cell arrest through induction of

the p21CIP cyclin-dependent kinase inhibitor. However, vIL-6 inhibits Tyk2* and STAT2* phosphorylation by IFN-αR and blocks downstream signaling of interferon. Other viral proteins act by direct inhibition of IFN-induced transcription. For instance, vIRF1 sequesters the p300 and CBP* (CREB-binding protein) histone acetyltransferase coactivators and prevents them from entering the IFN transcriptional complex [45, 52].

Animal Models to Study HHV8 Infection and Pathogenesis

Recently, NOD (non-obese diabetic)/SCID (severe combined immunodeficiency) mice have been infected intravenously with purified HHV8. Latent and lytic viral transcripts could be measured in mice organs during 4 months. The virus was shown to establish infection in B cells, macrophages, NK cells, and, to a lesser extent, in dendritic cells. This model could be useful for assessing the benefit of antiviral drugs early after HHV8 infection [55]. Various PEL-derived cell lines are tumourigenic in immunosuppressed mice, offering good models for pathogenesis studies.

By contrast, no satisfying KS model has been reported so far. In vitro HHV8 infection of endothelial cells leads to a spindle phenotype induction and sometimes signs of transformation but no angiogenicity. Progenitor endothelial cells might be more suitable than terminally differentiated endothelial cells to induce KS lesions after HHV8 infection. Indeed, Mutlu et al. [56] recently transfected mouse bone marrow progenitor endothelial cells with a HHV8 bacterial artificial chromosome. Mice subsequently developed a KS-like proliferative disease characterized by the presence of HHV8-infected spindle cells and an angiogenic phenotype. In this model, the whole virus is present and not only v-GPCR as reported previously by various authors [57].

Clinical Detection, Viral Epidemiology, and Transmission

Various tests have been developed based on immunofluorescence, Western blot, and enzyme-linked immunosorbent assays to detect antibodies against latent and lytic genes. Currently serological assays are mainly used to study the prevalence of the viral infection.

Immunofluorescence Essays

The latent immunofluorescence assay (IFA) was the first test to be used for the detection of HHV8 antibodies. Cell lines derived from PEL are treated with patients sera at various dilutions. These cell lines harbour chronic HHV8 infection. They express latent and, to a lesser extent, lytic antigens. Latent reactivity is characterized

by a rather specific punctuate nuclear staining. Viral reactivation can be induced in vitro after stimulation of PEL cell lines with tumour-promoting antigens (tetradecanoyl phorbol ester acetate or butyrate) or histone deacetylase. PEL-treated cells express increased amounts of lytic viral antigens. Positivity is characterized by a homogeneous cytoplasmic staining. In general, lytic IFA tests are characterized by a higher sensitivity but lower specificity as compared to latent IFA tests [2].

Enzyme-Linked Immunosorbent Assays (ELISA)

Various ELISAs have been developed. Some of these use specific HHV8 antigens, such as single products of ORFs26, 65,73 or K8.1, whereas others use the whole virus, obtained from the virus lysates. There are two ELISA tests commercially available: the ABI HHV8 immunoglobulin G (IgG) antibody (Advanced Biotechnologies, Columbia, MD, USA) and the DIAVIR HHV-8 IgG ELISA (Diavir Ltd, Altomuenster, Germany) assays. The first uses an extract from sucrose gradient-purified HHV8 whole virions; the second one uses synthetic peptide antigens. Both tests detect HHV8 antibodies in more than 97% of patients with KS, but consistency with other tests remains problematic, limiting their use in clinical practice [58–60]. Recently, a novel serological approach has been published, using a multiantigenic ELISA with a combination of ORF K8.1, ORF 65, and modified LANA1 recombinant peptides. This multiantigen detection algorithm is claimed to be highly sensitive and specific for defining HHV8 seropositivity [61].

Immunoblotting Techniques

HHV8 Western blot analysis has also been utilized in serological studies, mostly for research purposes or to confirm ELISA results. The appearance of antibodies against lytic antigens precedes the appearance of antibodies against latent antigens, which may explain the lower sensitivity of assays based on latent HHV8 antigens. PEL-based lytic IFAs have been found to give higher seroprevalence results than other assays.

In most epidemiological studies, lytic PEL-based IFAs and lytic antigen-based assays appear concordant, which has led to major insight on HHV8 seroepidemiology all over the world. However, caution is mandatory in some situations where low specificity may account for seropositivity. Therefore, for such studies it is generally recommended to combine lytic PEL-based IFA with a second assay to confirm positivity [58] The lack of an international gold standard in the field of HHV-8 serology limits its general use in clinical practice, particularly for precise determination of HHV8 status in blood donors or organ transplant recipients [58–60]. However, these factors such as HHV8 status pre- and post-organ transplant are being addressed within the context of clinical research studies.

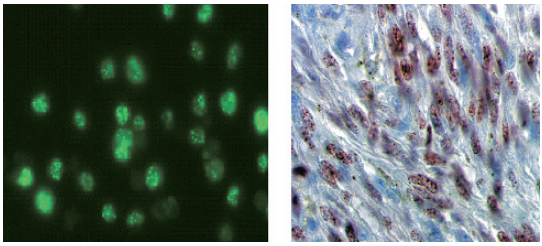

Fig. 4 Latent nuclear antigen (LANA) detection using immunofluorescence (IF) (*left*) and immunohistochemistry (IHC) (*right*)

PCR-based methods may be successfully used to detect HHV8 viral sequences in various specimens, for instance, in KS lesions, with a very high specificity and sensitivity. HHV8 sequences can also be detected in the plasma and in peripheral blood mononuclear cells. Although HHV8 viral load in peripheral blood mononuclear cells of KS individuals correlates with tumour burden, because of low interval variations this test cannot be used in clinical practice to monitor KS patients or to predict the occurrence of KS in transplant recipients [62–64].

Immunohistochemistry using a monoclonal antibody against LANA on paraffin-embedded sections, although less sensitive than PCR, is useful for pathological diagnosis of difficult angiogenic proliferations (Fig. 4) [65].

HHV8 Seroepidemiology

The geographical distribution of HHV8 infection in the general population is comparable to that of KS. There are high prevalence areas in sub-Saharan Africa (HHV8 seropositivity >50% in East and Central Africa), with relatively high or intermediate prevalence in Southern Italy and other Mediterranean areas, as well as in South America and West Africa (10% to 20% seropositivity), and very low prevalence areas in Northern Europe and the United States with seropositivity less than 5%. The HHV-8 prevalence is low in many countries of Southeast Asia and in Japan, where KS is very uncommon. A high prevalence has been observed among indigenous populations living in remote tribes of Amazonia and Papua New Guinea (Fig. 5) [66,67].

HHV8 Transmission

HHV8 transmission modalities appear to differ between highly endemic and subendemic areas. HHV8 can be transmitted by blood or blood products, and a recent study from Uganda found that 4.1% of seronegative individuals who received HHV8-positive blood seroconverted [68]. However, the risk appears low as far as deleukocyted blood units are concerned. In the same line, HHV8 prevalence is higher among intravenous drug users compared with the general population [68,69].

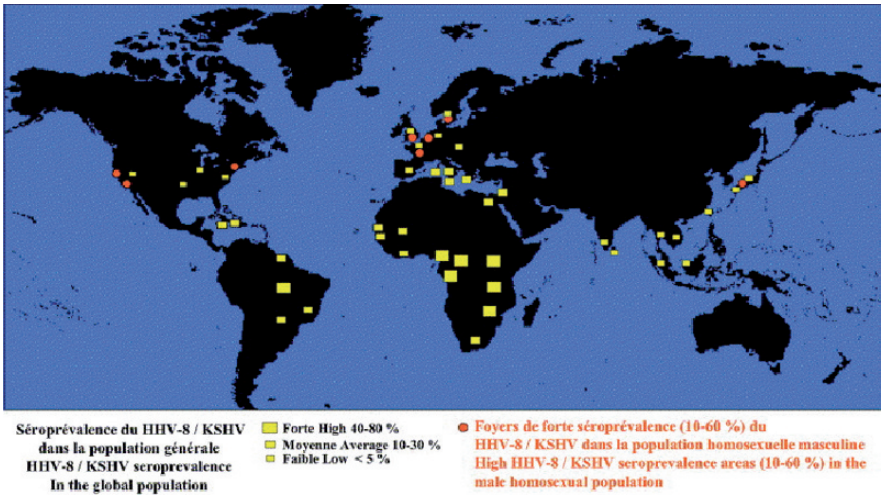

Fig. 5 HHV8 seroprevalence. [Adapted from Plancoulaine and Gessain [67]]

Anecdotal reports have shown that HHV8 can be transmitted by transplanted organs [70–72]. In an ongoing French national cohort survey, we will be able to prospectively estimate the risk of HHV8 transmission and morbidity in renal transplant recipients. In countries endemic for HHV8 infection, the virus can be transmitted among family members through close contact and promiscuity, particularly during childhood. Mother-to-child transmission mostly relies on close contact during childhood through the saliva, possibly through the habit of blood sucking of arthropod bites [67,73]. Transmission would depend on the close contact of the child with a seropositive mother (or relatives) whose infective saliva is used to relieve itching and scratching at the arthropod bite sites [74]. For unknown reasons, this type of transmission appears to be uncommon in low endemic areas. Mother-to-child vertical transmission can occur, although very rarely. As viral load is low in maternal milk, transmission during lactation is probably rare [73]. Among heterosexuals, sexual transmission is possible [75] but has not been consistently established. In contrast, HHV8 is sexually transmitted among homosexual men, oral-genital sex being a strong risk factor. Interestingly, in a low HHV8 endemic area the majority of infected individuals are homosexual men. Moreover, HHV8 prevalence is estimated to be as much as 70% in HIV-infected homosexual patients [66, 67].

Therapeutic Perspectives Driven by HHV8 Infection

Antiherpes (viral) drugs such as cidofovir, foscarnet, and ganciclovir can in vitro inhibit HHV8 replication in PEL-derived cells. However, their therapeutic value in KS remains to be demonstrated. They may prevent KS development, as suggested from a study on acquired immunodeficiency syndrome (AIDS) patients with cytomegalovirus retinitis [76]. In addition, foscarnet was shown to induce remission

in a few patients with AIDS-related KS [76–78]. In PEL, cidofovir and valganciclovir can be useful after remission is induced by chemotherapy [79]. The action of such drugs could be improved theoretically by inducing HHV8 to enter a lytic cycle, for instance, with pharmacological agents such as inhibitors of histone deacetylase such as valproate sodium. Such an approach, already investigated in patients with refractory post-transplant lymphomas, must be carefully monitored in clinical trials because there exists the risk of a deleterious effect from non-fully blocked HHV8 proliferation [77].

Recent in vitro data have shown that glycyrrhizic acid, contained in the licorice root, induces apoptosis of PEL cells by downregulating the synthesis of the HHV8 LANA1 [80]. Other therapeutic strategies could rely on targeting signaling pathways important for HHV8 de novo infection, reactivation, cell persistence, or cellular pathways activated by viral pirated genes such as the MAP kinase or the PI3 kinase pathway. Such kinase inhibitors are currently evaluated in clinical trials and include STI 571 (Gleevec), a small molecule inhibitor of the receptor tyrosine kinase, c-*kit*, which inhibits the morphological changes induced by HHV8 in dermal microvascular endothelial cells [81]. Rapamycin, an mTOR inhibitor located downstream from the PI3 kinase, has already proven of benefit in post-transplant KS and in anecdotal reports of classic KS [82, 83]. We and others have shown impairment of HHV8-specific immune responses in HIV- and non-HIV-associated KS cases as compared with tumour-free HHV8-infected patients [84, 85]. Such results support the use of vaccines or adoptive strategies to boost HHV8-specific cytotoxic T-lymphocyte responses in patients with KS.

Abbreviations

xCT: SYSTEM Xc(-) TRANSPORTER-RELATED PROTEIN

RhoA: RAS HOMOLOG GENE FAMILY, MEMBER A; RHOA

V-SRC AVIAN SARCOMA (SCHMIDT-RUPPIN A-2) VIRAL ONCOGENE; SRC

FAS: TUMOR NECROSIS FACTOR RECEPTOR SUPERFAMILY, MEMBER 6

FADD Fas-Associated Death Domain Protein – A signal-transducing adaptor protein that associates with TNF RECEPTOR complexes. It contains a death effector domain that can interact with death effector domains found on INITIATOR CASPASES such as CASPASE 8 (FLICE) and CASPASE 10. Activation of CASPASES via interaction with this protein plays a role in the signaling cascade that leads to APOPTOSIS

FLICE: FADD-HOMOLOGOUS ICE/CED3-LIKE PROTEASE, CASPASE 8 – A long pro-domain caspase that contains a death effector domain in its pro-domain region. It plays a role in APOPTOSIS by cleaving and activating EFFECTOR CASPASES. Activation of this enzyme can occur via the interaction of its N-terminal death effector domain with DEATH DOMAIN RECEPTOR SIGNALING ADAPTOR PROTEINS

Zta Epstein–Barr virus= ZEBRA protein from EBV, acts as a key regulatory switch in the transition between the latent and the lytic viral life cycle;

RAS – Family of retrovirus-associated DNA sequences (ras) originally isolated from Harvey (H-ras, Ha-ras, rasH) and Kirsten (K-ras, Ki-ras, rasK) murine sarcoma viruses. Ras genes are widely conserved among animal species and sequences corresponding to both H-ras and K-ras genes have been detected in human, avian, murine, and non-vertebrate genomes.

MAPK Mitogen-Activated Protein Kinases – A superfamily of PROTEIN–SERINE–THREONINE KINASES that are activated by diverse stimuli via protein kinase cascades. They are the final components of the cascades, activated by phosphorylation by MITOGEN-ACTIVATED PROTEIN KINASE KINASES which in turn are activated by mitogen-activated protein kinase kinase kinases (MAP KINASE KINASE KINASES).

RAF kinase – A family of closely related serine–threonine kinases that were originally identified as the cellular homologs of the retrovirus-derived V-RAF KINASES. They are MAP kinase kinase kinases that play important roles in SIGNAL TRANSDUCTION.

MEK, ERK – Signal transduction is controlled both by regulation of enzyme activation and by organization of enzymatic complexes with nonenzymatic adaptors, scaffolds, and anchor proteins. The extracellular signal-regulated kinase (ERK) cascade is one of several evolutionarily conserved mitogen-activated protein (MAP) kinase cascades important in the regulation of growth, apoptosis, and differentiation. The MAP kinases ERK1 and ERK2 are activated by the MAP kinase kinases MEK1 or MEK2. MEKs, in turn, are activated by members of the Raf family

Proto-Oncogene Proteins c-ETS – A family of transcription factors that share a unique DNA-binding domain. The name derives from viral oncogene-derived protein ONCOGENE PROTEIN V-ETS of the AVIAN ERYTHROBLASTOSIS VIRUS.

Rb: Retinoblastoma Protein – Product of the retinoblastoma tumor suppressor gene. It is a nuclear phosphoprotein hypothesized to normally act as an inhibitor of cell proliferation. Rb protein is absent in retinoblastoma cell lines. It also has been shown to form complexes with the adenovirus E1A protein, the SV40 T antigen, and the human papilloma virus E7 protein.

E2F Transcription Factors – A family of basic helix-loop-helix transcription factors that control expression of a variety of GENES involved in CELL CYCLE regulation. E2F transcription factors typically form heterodimeric complexes with TRANSCRIPTION FACTOR DP1 or transcription factor DP2, and they have N-terminal DNA-binding and dimerization domains. E2F transcription factors can act as mediators of transcriptional repression or transcriptional activation.

Cyclin-Dependent Kinase Inhibitor p16 – A product of the p16 tumor suppressor gene (GENES, P16). It is also called INK4 or INK4A because it is the prototype

member of the INK4 CYCLIN-DEPENDENT KINASE INHIBITORS. This protein is produced from the alpha mRNA transcript of the *p16* gene. The other gene product, produced from the alternatively spliced beta transcript, is TUMOR SUPPRESSOR PROTEIN P14ARF. Both p16 gene products have tumor suppressor functions.

p21CIP1 Cyclin-Dependent Kinase Inhibitor p21 – A cyclin-dependent kinase inhibitor that mediates TUMOR SUPRESSOR PROTEIN P53-dependent CELL CYCLE arrest. p21 interacts with a range of CYCLIN-DEPENDENT KINASES and associates with PROLIFERATING CELL NUCLEAR ANTIGEN and CASPASE 3.

p27KIP1Cyclin-Dependent Kinase Inhibitor p27 – A cyclin-dependent kinase inhibitor that coordinates the activation of CYCLIN and CYCLIN-DEPENDENT KINASES during the CELL CYCLE. It interacts with active CYCLIN D complexed to CYCLIN-DEPENDENT KINASE 4 in proliferating cells, while in arrested cells it binds and inhibits CYCLIN E complexed to CYCLIN-DEPENDENT KINASE.

GSK3: Glycogen Synthase Kinase 3 – A glycogen synthase kinase that was originally described as a key enzyme involved in glycogen metabolism. It regulates a diverse array of functions such as CELL DIVISION, microtubule function, and APOPTOSIS.

Wnt Proteins – Wnt proteins are a large family of secreted glycoproteins that play essential roles in EMBRYONIC AND FETAL DEVELOPMENT, and tissue maintenance. They bind to FRIZZLED RECEPTORS and act as PARACRINE PROTEIN FACTORS to initiate a variety of SIGNAL TRANSDUCTION PATHWAYS. The canonical Wnt signaling pathway stabilizes the transcriptional coactivator BETA CATENIN.

TCF Transcription Factors – A family of DNA-binding proteins that are primarily expressed in T-LYMPHOCYTES. They interact with BETA CATENIN and serve as transcriptional activators and repressors in a variety of developmental processes.

bcl-2 genes – The B-cell leukemia/lymphoma-2 genes, responsible for blocking apoptosis in normal cells, and associated with follicular lymphoma when overexpressed. Overexpression results from the t(14;18) translocation. The human *c-bcl-2* gene is located at 18q24 on the long arm of chromosome 18.

CXC, chemokine CXC motif identifies a family of small (approximately 8–14 kD) chemotactic cytokines that direct the migration of leukocytes during inflammation and may be involved in the constitutive homing of lymphocytes into follicles and T-cell zones. They act through interactions with a subset of 7-transmembrane, G-protein-coupled receptors. Chemokines are divided into two major subfamilies, CXC and CC, based on the arrangement of the first 2 of the 4 conserved cysteine residues; the two cysteines are separated by a single amino acid in CXC chemokines and are adjacent in CC chemokines.

MAP Kinase Signaling System – An intracellular signaling system involving the MAP kinase cascades (three-membered protein kinase cascades). Various upstream

activators, which act in response to extracellular stimuli, trigger the cascades by activating the first member of a cascade, MAP KINASE KINASE KINASES (MAPKKKs). Activated MAPKKKs phosphorylate MITOGEN-ACTIVATED PROTEIN KINASE KINASES, which in turn phosphorylate the MITOGEN-ACTIVATED PROTEIN KINASES (MAPKs). The MAPKs then act on various downstream targets to affect gene expression. In mammals, there are several distinct MAP kinase pathways including the ERK (extracellular signal-regulated kinase) pathway, the SAPK/JNK (stress-activated protein kinase/c-jun kinase) pathway, and the p38 kinase pathway. There is some sharing of components among the pathways depending on which stimulus originates activation of the cascade.

AKT – A protein–serine–threonine kinase that is activated by PHOSPHORYLATION in response to GROWTH FACTORS or INSULIN. It plays a major role in cell metabolism, growth, and survival as a core component of SIGNAL TRANSDUCTION. Three isoforms have been described in mammalian cells.

JNK Mitogen-Activated Protein Kinases – A subgroup of mitogen-activated protein kinases that activate TRANSCRIPTION FACTOR AP-1 via the phosphorylation of C-JUN PROTEINS. They are components of intracellular signaling pathways that regulate CELL PROLIFERATION; APOPTOSIS; and CELL DIFFERENTIATION.

TYK2 Kinase – A Janus kinase subtype that is involved in signaling from a broad variety of CYTOKINE RECEPTORS. The TYK2 kinase is considered the founding member of the Janus Kinase family and was initially discovered as a signaling partner for the INTERFERON ALPHA-BETA RECEPTOR. The kinase has since been shown to signal from several INTERLEUKIN RECEPTORS.

STAT2 Transcription Factor – A signal transducer and activator of transcription that mediates cellular responses to TYPE I INTERFERONS. Stat2 protein is associated constitutively with INTERFERON REGULATORY FACTOR-9. After PHOSPHORYLATION Stat2 forms the IFN-STIMULATED GENE FACTOR 3 COMPLEX to regulate expression of target GENES.

CBP p300-CBP Transcription Factors – A family of histone acetyltransferases that is structurally related to CREB-BINDING PROTEIN and to E1A-ASSOCIATED P300 PROTEIN. They function as transcriptional coactivators by bridging between DNA-binding TRANSCRIPTION FACTORS and the basal transcription machinery. They also modify transcription factors and CHROMATIN through ACETYLATION.

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Molecular Events in Skin Cancer

Thomas Meyer

Skin cancer represents the most frequent cancer in humans and includes different entities, based on the cell types and tissues affected. Next to epithelial tumors, such as keratinocyte-derived basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), and neuroendocrine Merkel cell carcinoma (MCC), skin malignancies also include neuroectodermal malignant melanoma (MM), as well as tumors of skin-associated tissues, lipomas, angiosarcomas, tumors of connective tissue, and cutaneous lymphomas. Ultraviolet (UV) radiation is an important risk factor for epithelial tumors and MM, because most of the tumor lesions occur in sun-exposed skin areas and contain UV signature mutations [1, 2]. However, some tumors are located in sun-protected body areas, indicating other factors involved in carcinogenesis. These factors include immunosuppression, chemical carcinogens, ionizing radiation, and physical factors such as fair complexion [3]. At least for SCC, human papillomavirus (HPV) may also be involved in pathogenesis [4]. In addition, predisposition to skin cancer is mediated by genetic factors including both germinal and somatic mutations.

The role of germinal (inherited) mutations is well known for patients with xeroderma pigmentosum (XP), an autosomal recessive disorder affecting DNA repair [5]. Several polymorphisms of genes involved in DNA repair were described to be associated with the development of skin cancer. An increased risk of skin cancer at younger ages, including MM, SCC, and BCC, is seen in patients with mutations in XPD [6].

In patients with the nevoid basal cell carcinoma syndrome (NBCCS, Gorlin syndrome), as well as in many cases of sporadic BCC, tumorigenesis is associated with abrogation of the sonic hedgehog pathway (SHH) [7]. Germinal mutations detected in NBCCS include both loss-of-function mutations of the PTCH gene encoding a suppressor of the hedgehog pathway and activating mutations of the gene SMOH encoding a signal transducer of the SHH pathway [8, 9].

Susceptibility genes for the development of MM were identified in genetic studies of families with a high incidence of melanoma. These genes were represented

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by CDKN2A encoding the cyclin-dependent kinase inhibitor $p16^{INK4a}$ and tumor suppressor $p14^{ARF}$, as well as CDK4 and CDK6 encoding cyclin-dependent kinases 4 and 6 [10]. Variants of melanocortin-1 receptor (MC1R) are also associated with increased risk of both MM and BCC [11,12]. MC1R is involved in regulation of skin pigmentation. The MC1R gene is highly polymorphic, and variant alleles, mainly those associated with red hair colour, were described to be associated with fair pigmentation, more frequent development of nevi, and increased melanoma risk [11].

The development of MM and SCC is generally considered a multistep process that requires additional genetic changes (somatic mutations) affecting cell proliferation, induction of apoptosis, angiogenesis, and invasion of the basal membrane [13]. For MM, mutations in N-*ras* and BRAF were reported to lead from benign precursors to dysplastic nevi and superficial melanoma [14]. The progression to nodular melanomas, which invade into the dermis and are capable of metastasis, is associated with additional mutations, reflected by cytogenetic changes such as gains of chromosomes 7q and 8q and losses of chromosomes 1p, 3, 6q, and 10q. Loss of chromosomes 3 and 10q results in abrogation of growth suppression. Loss of heterozygosity (LOH) of 10q affects expression of phosphatase PTEN, a negative regulator of PI3K pathway, promoting proliferation and cell survival [15]. c-*myc* overexpression, as a result of gain of chromosome 8, also induces cell proliferation [16]. Gain of 7q corresponds to increased expression of c-MET, a tyrosine-kinase receptor for HGF, which after stimulation induces cell growth and disruption of cell adhesion by downregulation of E-cadherin and desmoglein 1 [17].

Early molecular events in the development of SCC include mutations of p53. Most of these represent UV signature mutations underlining the importance of cumulative UV exposure as a risk factor. p53 mutations have been detected in patches of both normal skin and in precancerous lesions (actinic keratosis, AK). The progression towards invasive SCC is associated with additional cytogenetic changes. In AKs, gains of chromosomes 7, 9, and 18 have been detected [18]; in SCC, cytogenetic changes were reported for several other chromosomes. Losses of chromosomal regions mainly relate to 3p, 4q, and 18, whereas gains were most frequently observed for 3q, 17q, 4p, Xq, 14q, 8q, and 9q [19].

Cell culture studies using the HaCaT in vitro skin cancer progression model provided some clues of the genes that might be affected by these changes. HaCaT cells are spontaneously immortalized keratinocytes with UV-specific p53 mutations and some chromosomal aberrations, such as loss of 3p and 9p and gain of 3q [20]. These cells are not tumorigenic in immunocompetent mice but require additional mutations for tumorigenic conversion, such as Ha-ras expression [21]. Gain of 11q, which correlates with amplification of the cyclin D1 locus and overexpression of the protein, was shown recently to be an essential early step in skin carcinogenesis [22]. The shift from benign to malignant phenotype was found to be associated with the expression of granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as loss of chromosome 15 [23–25]. The latter results in a loss of thrombospodin (TSP-1), a matrix protein with antiangiogenetic properties, and thus would promote vascularization of tumor tissue. Furthermore, the metastatic potential of HaCaT cells, as determined by in

vivo passages of the cells, is associated with increased Ha-*ras* oncogene expression, gains of parts of 11q, and loss of chromosome 2p [26]. The relevant genes of 2p and 11q are still not identified, but gain of 11q may correspond to upregulation of matrix metalloproteinase 1 (MMP1), located on 11q22.3, as recently identified by microarray expression profiling [27].

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Molecular Pathogenesis of Basal Cell Carcinoma

T. Meyer

Basal cell carcinoma (BCC) is the most frequent cancer among the white population, representing 75% of all skin cancers [1]. The incidence of BCC cases is increasing, probably because of changes of leisure activities and migration to regions with higher solar radiation. BCCs rarely metastasize ($< 0.1\%$), and mortality rates are low; however, some tumors grow aggressively and may cause extensive tissue damage. Aggressive growth of BCC correlates with histological subtypes. Nodular and superficial BCC, representing 60% and 25% of all BCC, respectively, are usually considered less aggressive than morpheaform, infiltrative, micronodular, and metatypic BCC, which are associated with a higher rate of local recurrences [2, 3]. Several risk factors for the development of BCC have been described, which include physical characteristics, exposures to environmental carcinogens, immunosuppression, and genetic predisposition. Other genetic changes, acquired subsequently and affecting cell proliferation and apoptosis, may also be involved in tumorigenesis. In the following sections, some recently identified molecular mechanisms are described that are involved in BCC development and which potentially represent targets of new pharmacologic treatment modalities.

Risk Factors

Exposure to ultraviolet (UV) radiation is generally considered the most important risk factor of basal cell carcinoma (BCC) [4]. Although squamous cell carcinomas (SCC) appear to be related to cumulative sun exposure, the correlation of BCC development and UV radiation seems to be more complex. In addition to the amount of UV radiation, timing and pattern are also important, as reflected by the significantly increased risk of BCC by intermittent UV exposure during childhood and adolescence.

About 20% of BCC occur in relatively sun-protected regions, such as trunk, armpits, or anogenital regions [5], indicating other factors are also involved in

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tumorigenesis; these include physical characteristics such as fair complexion, red or blond hair or light eye colour, and exposure to ionizing radiation, coal tar, arsenic, psoralen UV-A radiation (PUVA), and smoking [5]. Immunosuppression is also an important risk factor for BCC, as indicated by the increased incidence in transplant recipients compared to the general population [6]. Furthermore, several types of human papillomavirus (HPV) have been detected in BCC [7–9], but the pathogenic role of HPV infection for BCC formation is questionable. Although cutaneous HPV types were shown to be able to induce loss of cell-cycle control by inactivation pRB [10] and to abrogate UV-induced apoptosis [11], which may represent pathogenic mechanisms in skin carcinogenesis, the low number of viral genome copies per tumor cell argues against a direct activity of HPV in BCC development [9]. Our current knowledge of HPV and skin carcinogenesis indicates a possible pathogenic role of particular HPV types in the development of SCC but probably not of BCC.

Genetic Predisposition

It is likely that any kind of genetic changes resulting in impairment of protection and repair of damages caused by UV radiation can increase susceptibility to BCC, such as factors involved in skin pigmentation, including melanocortin-1 receptor (MC1R). The MC1R gene is highly polymorphic, and specific variants of MC1R gene have been shown to be associated with fair pigmentation, red hair, low tanning ability, and increased risk of skin cancers, including both melanoma and BCC [12–14].

Polymorphisms of glutathione *S*-transferase (GST) have also been described to be associated with an increased risk of BCC. GST represents an important cellular protein involved in detoxification processes. It mediates disposal of potential mutagenic compounds, such as lipid peroxides and DNA hydroperoxides, generated by UV irradiation [15]. Five isozymes of GST have been described in humans, with the π-isozmye being the most prevalent in skin tissues, expressed predominantly in sebaceous glands and hair follicles [16]. The increased risk of BCC associated with polymorphisms of some GST members probably relates to reduced defences of UV-induced oxidative stress [17–19]. Similarly, risk of BCC development is also associated with polymorphisms of CYP2D6, the gene encoding cytochrome P450, which is also involved in removal of photosensitizing agents [19, 20].

In addition to proteins involved in defences of UV-induced oxidative stress, factors involved in repair of UV-induced DNA damage are also associated with susceptibility of BCC. Xeroderma pigmentosum (XP) is a genetic disorder, characterized by defects in DNA repair, resulting in severe photosensitivity and increased risk of skin cancers including malignant melanoma, SCC, and BCC [21, 22]. In epidemiological studies, polymorphisms of different DNA repair genes have been identified to be associated with a risk of different cancers [23]. Increased risk of BCC was reported to be associated with specific polymorphisms of XPD [24–26]. Because

the XPD gene product represents a component of the transcription complex TFIIH, mutations of XPD not only mediate increased UV sensitivity but sometimes are also associated with developmental disorders such as growth disorders and neurological defects [27].

The characterization of the most important genetic determinants for BCC development came from studies on patients with nevoid basal cell carcinoma syndrome (NBCCS), an autosomal dominant hereditary disease, also called Gorlin syndrome. Patients with NBCCS develop multiple BCCs early in life, sometimes before the age of 10. Additional characteristics of the disease include skeletal abnormalities, such as jaw cysts, intracranial calcification, and pits on palms and soles [28]. Based on the frequent loss of chromosome 9q in patients with NBCCS, the responsible gene defect was mapped to 9q [29, 30], and subsequently was identified as a mutation of the human homologue to the Drosophila segment polarity gene patched, PTCH1, located at 9q22-32 and encoding a receptor of the hedgehog signalling pathway [31, 32].

Sonic Hedgehog Pathway

The hedgehog (HH) signalling pathway was originally identified in *Drosophila*, where it is involved in embryonic development [33]. In vertebrates hedgehog signalling is also associated with developmental processes. An important function of the pathway is to trigger proliferation of distinct target cells during organogenesis of the forebrain, neural tube, eye, and limb [34]. In skin, HH signalling is involved in growth and morphogenesis of hair follicles [35]. Aberrant signalling of the HH pathway can result in severe developmental abnormalities and has been shown to be an important mechanism in tumor development [36, 37], including BCC, which are generally considered hair follicle-derived tumors [38, 39].

The hedgehog pathway is activated by binding of secreted hedgehog molecules (such as sonic hedgehog, SHH) to PTCH1, a 12-pass transmembrane receptor protein. PTCH1 represents a tumor suppressor that in the absence of SHH binds to and inhibits smoothened (SMOH), another 7-pass transmembrane protein. Binding of sonic hedgehog (SHH) to PTCH results in release of smoothened (SMOH) and activation of downstream signalling of the HH pathway (Fig. 1), which finally leads to transduction of the signal to the nucleus by activation and translocation of the glioma transcription factor (GLI) proteins. Regulation of GLI activity is a complex mechanism that is still not completely understood in vertebrates [40, 41], but it is known to involve activation and modification of a large number of cytoplasmic proteins including glycogen synthase kinase (GSK)-3β, suppressor of fused (SuFuH), and the actin-binding protein MIM (missed in metastasis) [42, 43]. After translocation to the nucleus, GLIs bind to DNA at a consensus GLI-binding site through a highly conserved zinc-finger DNA-binding domain [44]. In vertebrates three distinct GLI genes have been identified (GLI-1, GLI-2, and GLI-3) that all contain a C-terminal transactivation domain, but only GLI-2 and GLI-3 were shown

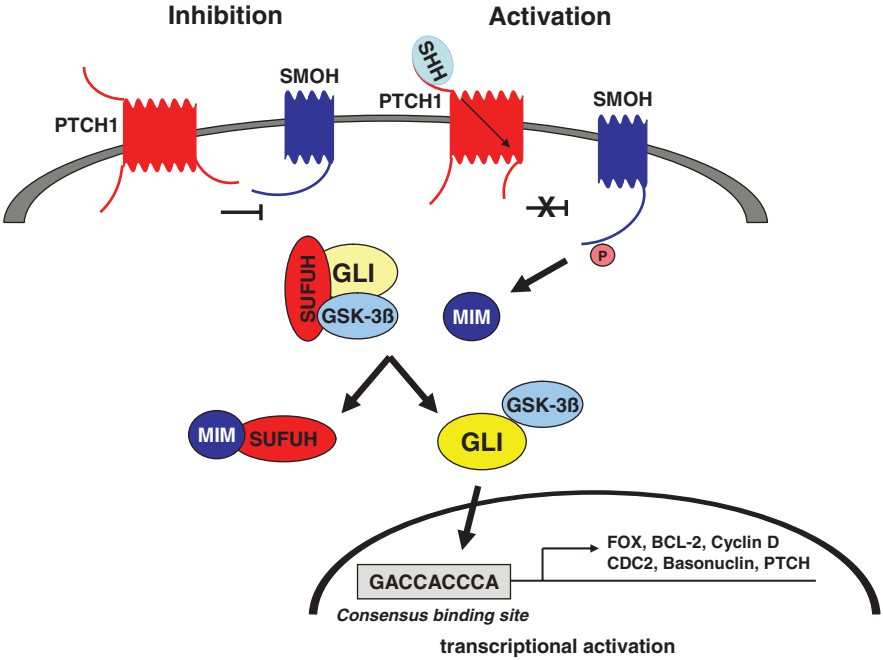

Fig. 1 Hedgehog pathway: simplified model of activation. In the absence of ligands of patched1 (*PTCH1*) hedgehog signalling is inhibited by blocking of smoothened (*SMOH*) by PTCH1. Binding of sonic hedgehog (*SHH*) to PTCH1 abrogates inhibition of SMOH, which, after phosphorylation of its cytoplasmic domain, leads to activation of transcription factors GLI1-3. The mechanism of *GLI* activation in vertebrates is still not fully understood. It probably involves modification of several cytoplasmic proteins, including both GLI-suppressing and GLI-activating proteins such as *SUFUH* (human homologue of suppressor of fused) and *MIM* (missed in metastasis). After activation and translocation, GLIs bind to consensus binding sites that are present in promoters of many genes encoding proteins involved in cell proliferation, epidermal differentiation, and apoptosis

to have an N-terminal repression domain [45]. Therefore, GLI-1 represents the main activator of gene transcription whereas GLI-3 acts mainly as a repressor [46].

A number of target genes, containing the GLI-binding site, have been identified. In vertebrates, these include transforming growth factor (TGF)-β, BCL-2, basonuclin, members of the forkhead box (FOX) proteins, and PTCH itself [47–49]. In addition, GLI-2 has been shown to induce G_1–S cell-cycle progression in contact-inhibited keratinocytes through activation of transcription factor E2F1 [50]. Other genes involved in cell-cycle progression (cyclin D, CDC2, CCND1, CDC45L) were also activated, while genes associated with epidermal differentiation were repressed [50, 51]. The number of genes upregulated and downregulated by GLIs is probably much greater, as indicated by a recent study by Eichberger et al. [52], who identified more than 400 genes with a more than twofold change of expression level by GLI-1 and GLI-2 using genome expression profiling in an HaCaT keratinocyte expression system.

FOX proteins or winged helix domain proteins constitute a large number of transcription factors involved in both embryonic development and adult tissue homeostasis by regulating cell proliferation, differentiation, longevity, and transformation [53]. In mice, some FOX genes (foxa2, foxd2, foxf1) have been shown to be direct targets of SHH signalling during embryogenic development [54, 55]. FOXM1 expression was also activated in murine hepatocytes in response to hepatectomy and toxin-induced liver damage [56,57]. In addition, FOXM1 was detected in human skin and cultured keratinocytes and was found to be significantly upregulated in primary keratinocytes after constitutive expression of GLI-1 [58]. Another FOX protein, FOXE1, can be induced by GLI-2. FOXE1 was shown to be expressed in basal keratinocytes of human epidermis and also in the outer root sheath of the hair follicle, indicating an effector role of FOXE1 in SHH-induced morphogenesis [49].

TGF-β is induced by GLI-1 and can induce upregulation of metalloproteinases in fibroblasts, which may cause dissolution of basement membranes, an important mechanism involved in aggressive growth of BCCs as well as for metastasis of cancer cells [59]. Another mechanism associated with invasiveness and metastasis activated by GLI-1 represents the upregulation of Snail, a transcription factor involved in transition from epithelial to mesenchymal character of cells [60]. BCL-2, predominately induced by GLI-2, is a key anti-apoptotic factor that promotes cell survival by prevention of apoptosis via the mitochondrial pathway. Upregulated expression of these factors by GLIs is of fundamental importance for development and maintenance of tumor cells.

Induction of basonuclin, a cell type-specific transcription factor for rRNA genes (rDNA), represents another important function of GLIs. High levels of ribosomes, containing rRNA, are required in proliferating cells with enhanced protein synthesis. Basonuclin was detected mainly in basal keratinocytes of squamous epithelia of the skin, oral epithelium, and vagina. In humans the highest concentrations of basonuclin were found in the outer root sheath of the hair follicle [61], probably induced by GLIs, because activation of the HH pathway is essential for hair follicle development [62].

Induction of PTCH by GLI proteins represents a negative regulatory feedback mechanism of HH signalling, which, however, would be inefficient in case of mutated PTCH or activating mutations of the HH pathway.

Pathogenic Role of Hedgehog Signalling in BCC Development

Aberrant signalling of the hedgehog pathway has been shown to be involved in the development of different malignancies such as BCC, medulloblastoma, and rhabdomyosarcoma and also cancers of the lung, prostate, and gastrointestinal tract [63–67]. Germline mutations of PTCH1 resulting in loss of function of the encoded protein are present in patients with NBCCS [31, 32]. Usually, NBCCS patients inherit only one mutated copy of the gene. Thus, inactivation of the other copy is required for tumor development [68].

PTCH1 mutations were also detected in 30% to 40% of sporadic BCC [69,70]; in some other studies an even higher percentage was reported [71]. In addition to mutational inactivation of PTCH1, activating mutations of SMOH and SHH or upregulation of GLI proteins were also described to play a role in development of sporadic BCC [72–77]. Mutations of SuFuH were also detected recently in a small number of cases (10%) [71]. Taken together, dysregulation of HH signalling by mutation of any of these factors are present in more than 70% of sporadic BCCs. As a consequence, these mutations may lead to constitutive HH signalling as reflected by upregulated expression of GLI-1 and -2, BCL2, basonuclin, FOXE1, and FOXM1 in BCC lesions [48,49,58,78]. Enhanced expression of basonuclin correlates with increased transcription of rRNA genes indicating augmented ribosome biogenesis [78]. The importance of the increased amount of ribosomes for tumor pathogenesis probably relates to the higher demand of protein synthesis in neoplastic cells, resulting from increased metabolic activity and cell proliferation [79]. Upregulation of FOXM1 expression may be characteristic for sporadic BCC as it was not detected in normal skin and SCC [58].

The great number of GLI target genes identified during past years indicates that the oncogenic activity of aberrant signalling of the HH pathway in development of BCC as well as other tumors relies on multiple cellular processes; these include stimulation of cell proliferation, repression of epidermal differentiation, enhancement of cell survival by induction of anti-apoptotic factors, and increase of invasiveness and metastatic potential.

Other Molecular Changes in BCC

The findings of aberrant hedgehog signalling in NBCCS and in more than 70% of sporadic BCCs indicate the central role of this pathway in BCC development, which in principle should be sufficient for tumorigenesis. This idea is supported by cell culture analyses demonstrating that GLI-1-induced transcription is able to convert normal keratinocytes into tumor cells without other genetic aberrations [80] and also by transgenic mouse models showing overexpression of GLI-1 to be sufficient for tumor development [76, 81].

However, the 20% to 30% of sporadic BCC without detected perturbation of HH signalling indicates other mechanisms and pathways of tumorigenesis to be involved. Next to the mutations of the HH pathway, the most common genetic changes detected in BCC were found in the p53 gene, which is mutated in about 50% of BCCs [71, 82]. Mutations in p53 predominantly represent UV signature mutations [82], underlining the importance of UV radiation as a risk factor for BCC development. Mutations are not randomly distributed over the p53 gene, but appear in hotspots, which are different from p53 mutations in internal cancers [82]. By analyzing p53 mutations in nonmelanoma skin cancers, a mutation at codon 177 has been identified to be specific for BCC, whereas mutations at codon 278 seem to be specific for SCC of the skin [83].

Other proteins involved in cell-cycle regulation, such as the p53-controlled 14-3-3-sigma protein, may also be impaired in BCCs. 14-3-3-sigma prevents mitotic death of cells with DNA damage by maintaining them at the G_2 checkpoint of the cell cycle [84]. In BCC, loss of 14-3-3-sigma expression is caused by CpG hypermethylation of the 14-3-3-sigma promoter [85]. Mutations in *ras* oncogenes were detected rarely or not at all in BCC [71, 86], indicating that these factors are unlikely to contribute to BCC development. $P16^{INK4a}$, a tumor suppressor that induces cell-cycle arrest by inhibiting CDK4 and by activating p53 through binding to MDM2, the negative regulator of p53, is also rarely affected by point mutations in BCC [87,88], although allelic loss of one or more loci on chromosome 9 has been reported [88]. Moreover, $p16^{INK4a}$ was shown to be upregulated at the front of tumor cells of BCC with infiltrative growth patterns and may be associated with the change from proliferative to invasive phenotype in invasive BCC [89]. These findings are in contrast to SCC, where function of $p16^{INK4a}$ is lost during carcinogenesis in a subset of cases, although function of 14-3-3-sigma is usually not impaired [90, 91]. Thus, expression of 14-3-3-sigma and $p16^{INK4a}$ could be targets for specific molecular changes of the two types of nonmelanoma skin cancer.

Role of p53 in BCC Development

The p53 phosphoprotein is an important regulator of cell-cycle progression [92]. Upon DNA damage, p53 is activated by phosphorylation, which leads to stabilization of the protein and detachment from the regulatory protein MDM2 [93]. To repair DNA damage, p53 causes cell-cycle arrest through activation of CDK inhibitor p21. In cells with irreparable damage, p53 induces apoptosis by inducing expression of proapoptotic protein bax [94]. Mutant p53 accumulates in cells and is not able to arrest the cell cycle or to induce apoptosis, thus enabling these cells to replicate in the presence of acquired genetic instabilities, which potentially may increase their malignant potential.

At present, the importance of p53 mutations in BCC development is unclear. As the incidence of BCC is not increased in patients with Li-Fraumeni syndrome, and because of the limited amount of DNA damage and chromosome instability, as reflected by the low number of aberrations in cytogenetic studies, p53 mutations probably represent secondary events in BCC and are not primarily involved in tumor initiation [38]. However, inactivation of p53 may correlate with the severity of tumor lesions, because p53 accumulation, probably representing mutated forms, is preferentially associated with aggressive growth variants of sporadic BCCs [95,96]. Moreover, aggressive forms of BCC were described to appear more frequently in UV-exposed skin areas [97].

Formation of BCC may be described as a result of dysregulation of factors and mechanisms involved in cell proliferation and cell death by apoptosis. In general, BCCs are characterized by a relatively high number of proliferating cells [98, 99], but nevertheless BCC are usually slow-growing tumors; this indicates a significant

degree of cell loss, and indeed, a high apoptotic rate has been described for BCCs [100]. Lack of inducing apoptosis by mutant p53 may shift the balance of cell proliferation and cell death, leading to an increase of tumor growth. As impaired apoptosis allows cells with genetic changes to replicate, p53 mutations potentially promote genetic instabilities during tumor development, which eventually may also lead to increased malignancy.

Future Directions

The discovery of signal transduction pathways, namely the sonic hedgehog pathway, involved in BCC development represents important achievements in understanding the biology of BCC and other tumors. In addition, the unravelling of mechanisms of HH signalling represents an example of how basic research in developmental processes in flies and vertebrates provides an important basis for development of pharmacologic treatments of BCC and other malignancies.

Regression of BCCs by topical application of the steroidal alkaloid cyclopamine results in selective and efficient induction of differentiation and apoptosis in tumors by inhibition of hedgehog signalling [101]. Cyclopamine has been shown to mediate inhibition by direct binding to SMOH [102]. Other studies have identified activity of GLIs as a promising target for future therapeutic intervention. Studies on transgenic mice have shown that inactivation of GLI-2 leads to BCC regression, accompanied by reduced tumor cell proliferation and increased apoptosis [103].

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Molecular Pathogenesis of Squamous Cell Carcinoma

Ingo Nindl and Frank Rösl

Nonmelanoma Skin Cancer in Humans: Clinical and Molecular Aspects

Nonmelanoma skin cancer (NMSC), comprising basal cell carcinoma (BCC), Bowen's disease, cutaneous squamous cell carcinoma (SCC), and its early-stage actinic keratosis (AK), is the most frequent malignancy among populations of European origin [1,2]. Cutaneous SCC accounts for 10% to 20% of all skin malignancies and is the second most common skin cancer after BCC. Epidermal keratinocytes from the suprabasal layer are the origin of this cancer. The major risk factor is ultraviolet radiation (UV), and multiple factors result in the development of this disease. SCC are invasive tumours whose cells histologically appear like differentiated suprabasal keratinocytes, and approximately 3% metastasise. It grows faster than BCC and produces a more indurated, hyperkeratotic lesion with ulceration. Cutaneous SCC frequently occurs as multiple primary tumours in the same skin area ("field") in proximity to each other and is termed field cancerisation [3].

Cutaneous SCC is extremely common in light-skinned and sun-sensitive individuals, and the highest frequencies of this disease are found on sun-exposed sites of the body [4]. Furthermore, skin cancer risk is relatively increased in Caucasians who have been born in countries close to the equator, compared to those who have migrated to them after birth, indicating that the exposure to solar radiation early in life has importance for tumourigenesis. This finding highlights the role of UV rays as a major environmental risk factor for the development of cutaneous SCC. The main physical adaptation to UV radiation, which is the most ubiquitous human carcinogen, is pigmentation of the skin, the degree of which is largely based on genetic inheritance.

UV radiation is a component of sunlight and comprises three wavelength classes with decreasing solar energy: UV-C (ranging from 185 to 280 nm), UV-B (280–320 nm), and UV-A (320–400 nm). The majority of UV radiation on Earth, 90% to

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99%, is UV-A. The nonionising radiation cumulatively induces SCC, causing gene mutations and locally immunosuppression, which may prevent local immunological recognition of dysplastic cells. UV-A has higher cutaneous penetration than UV-B, but it is less effective in producing genetic mutations. In animal models, UV-B is approximately 1,000 times more effective than UV-A in the initiation and promotion of cutaneous SCC. UV-B can be absorbed by DNA, results directly in the formation of cyclobutane (thymidine) dimers and transitions in DNA, and is considered an initiator and promoter of SCC. UV-A induces photo-oxidative stress and suppresses the cutaneous immune system. UV-A is a tumour promoter, but not an initiator, and in addition with UV-B it is important in the development of cutaneous SCC.

Tumour promotion via UV-B-induced gene mutations may lead to an uncontrolled DNA replication and a changed transcription, most effective when oncogenes and tumour suppressor genes are affected. Xeroderma pigmentosum (XP) patients, who have a defect in the DNA-repair mechanisms, have an increased risk to develop cutaneous SCC. Thus, genetic changes may lead to permanent mutations in keratinocytes because of the absence of effective repair mechanisms, which subsequently have the potential to progress into SCC [5].

Cutaneous SCC is usually not associated with any hereditary disease despite exceptions (see earlier paragraph on XP), but aneuploidy is frequent, occurring in between 20% and 80% of patients. The development of SCC clearly results from the mutual interaction of multiple factors with the major risk factor, solar radiation. UV-B fingerprint type of mutations in NMSC, comprising characteristic C–T and CC–TT substitutions, have been particularly identified in the target genes *p53* (chromosome 17p13.1) [6], $p16^{\mathrm{INK4a}}$ (chromosome 9p21) [7–10], and Ha-*ras* (chromosome 11p15.5) [11–13].

Moreover, the existence of multiple factors during carcinogenesis is also reflected by distinct loss of heterozygosity (LOH) patterns of both various chromosomes and many genes in this disease [14]. LOH has been specifically identified on chromosome 3 (25%), 9 (40%), and 17 (40%), with an overall fractional allelic loss of 30% [13]. Interestingly, both tumour suppressor genes *p53* and $p16^{\mathrm{INK4a}}$ are located on chromosomes that showed the highest rate of LOH.

p53 is organised into five structural and functional regions comprising 11 exons, and mutations of the gene have been considered early, if not initial, events in cancer development. The precise role of p53 during skin carcinogenesis is elusive at present. Up to 90% of cutaneous SCC specimens have been reported to contain p53 mutations that were therefore highly indicative for skin cancer. However, the precise mutation rate of p53 in SCC has still to be determined in large epidemiological cohort studies. The mutation rate in dysplastic cells differs between various exons of p53. Several hotspot regions (i.e., gene regions with a high mutation frequency) have been identified, particularly in exon 7 and exon 8. In AK lesions, which are earlier stages of SCC, the p53 mutation rate of these two exons seems to be lower and ranges between 11% and 49%.

The cyclin-dependent kinase inhibitor 2a (CDKN2A) uniquely encodes for two candidate tumour suppressor genes, $p16^{INK4a}$ and $p14^{ARF}$. They share common exons 2 and 3, but have alternatively spliced first exons: exon 1α for $p16^{INK4a}$

and exon 1β for $p14^{ARF}$. Of the two tumour suppressor genes at the CDKN2A locus, $p16^{INK4a}$ has been studied more intensely and is inactivated predominantly by homozygous deletion in human cancers including SCC. Furthermore, intragenic mutations occur in a smaller proportion of tumours and are considered rather late events during skin carcinogenesis; exon 2 has been identified as the hotspot region of $p16^{INK4a}$.

In contrast to tumour suppressor genes, oncogenes are expressed in dysplastic cells and promote the development of cancer. The *ras* genes comprising three p21 proteins, Ha-(Harvey)-ras, N-(neuroblastoma)-ras, and the two splice variants of K-(Kirsten)-ras, are related GTP-binding enzymes with transforming potential. Mutational activation of ras proteins promotes cancer development by interfering with a wide range of cellular processes, including the regulation of cell-cycle progression. More than 2,000 human tumour specimens in a variety of tumour types have been analysed for the presence of *ras* mutations, and approximately 20% of all tumours are considered to harbour at least one mutation.

The identification of tumour-specific mutations of the Ha-ras gene during skin carcinogenesis of mice has attracted considerable interest in studying human NMSC from this aspect (see below). Activation of the oncogene by genetic alteration takes place at initiation and not during progression of tumour development. The mutational hotspot regions that are responsible for gene activation are located in codons 12 and 13 of exon 1 and in codon 61 of exon 2. Conflicting results have been reported in different studies analysing the prevalence of *ras* mutations in NMSC. The majority of studies have not detected any genetic alteration of Ha-*ras* in cutaneous SCC. To the contrary, a high frequency (46%) of G–T transversions in the second position of codon 12 has been found in cutaneous SCC and at least one mutation of codons 12, 13, or 61 in 9% to 12% of cutaneous SCC specimens [15–17]. Overall, the numbers of patients analysed are low, indicating that epidemiological studies have to be performed to determine the mutation rate of all three genes – *p53*, $p16^{INK4a}$, and Ha-*ras* – in SCC, which may differ in different countries and/or patients with different skin types adjusted for sun exposure, sex, and age.

Moreover, Dooley and colleagues [18] examined the expression profile in human biopsies of NMSC and skin cancer cell lines by microarray, analyzing approximately 7,400 genes. Although there was only a minimal overlap between human tissue and cell lines, 5 genes were differentially expressed both in vivo and in vitro, namely, *fibronectin 1*, *annexin A5*, *glyceraldehyde 3-phosphate dehydrogenase*, *zinc-finger protein 254*, and *huntingtin-associated protein interacting protein*. Of these genes, the calcium- and phospholipid-binding protein *annexin 5* was overexpressed (ratio 2.1), and *annexin 1* showed slight overexpression in cutaneous SCC. In the same year, Tilli et al. [19] found that *Lamin A* and *C* were significantly higher expressed in AK and SCC compared with normal skin as analysed by immunohistochemistry. Nindl and colleagues [20] identified 118 differentially expressed genes by microarray technology containing 22,283 human genes in normal human skin biopsies compared with tissue of AK and SCC patients. The majority of identified differentially expressed genes in cutaneous SCC were previously not described. The

same group [21] showed in another study that the expression of 1 of these 118 genes, namely Tenascin-C, is increased during skin cancer development and may prove useful for diagnostic approaches in cutaneous SCC.

In conclusion, genomic instability is a driving force in skin cancer development in general, and *p53* mutations rather than $p16^{INK4a}$ and/or Ha-*ras* mutations seem to be early events during the development of cutaneous SCC. The overall function of additional unknown genes, as well as the role of epigenetic phenomena, the immune status, and infectious agents, have yet to be determined.

Multistage Mouse Skin Carcinogenesis

It is generally accepted that the development of cancer is a multistep process, generating a homogeneous accumulation of descendant cells, initially deriving from a single progenitor [22]. This process can be regarded as extremely complex and therefore requires simplification in the form of appropriate model systems. In the case of skin cancer, which represents the most prevalent form of human cancer, inbred mouse strains have helped considerably to understand the various molecular processes involved in the formation of SCC [23]. Although rodent systems have quite obvious limitations in mimicking certain human forms of cancer (e.g., differences in tissue organisation and composition with specialised cells, frequency and probability of immortalization, telomere length, etc.), it was still possible to identify and to dissect relevant pathways playing a pivotal role in appearance and progression to cancer [for review, see reference [24]]. The possibility to manipulate the mouse genome, either by overexpressing an oncogene or by conditionally inactivating putative tumour suppressor genes in a cell- and tissue-specific manner, paved the way to establish a functional link between different regulatory networks finally leading to tumour development [25].

To avoid reiterations in summarising the biochemical and molecular biological properties of mouse skin carcinogenesis, the present section describes some regulatory aspects of the transcription factor AP-1 and its involvement in the formation of this kind of tumour.

Some Introductory Remarks

Several approaches have been developed to study skin carcinogenesis in an animal system. The painless accessibility of the skin to physical and chemical carcinogens produces a model of SCC that approximately recapitulates many characteristics of the corresponding human disease. Exposure to ultraviolet light, an important risk factor for melanomas, SCC, and BCC, has been used to provoke tumours in mice [for review, see reference [26]]. Moreover, it has been known for several decades that skin tumours can be also induced by a two-stage carcinogenesis protocol that comprises processes operationally and mechanistically defined as initiation and promotion [for review, see reference [27]]. The availability of such protocols allows a

simple and highly reproducible way to analyse and to quantify tumour formation in an animal system.

Initiation is generally achieved by the topical application to the dorsal skin of a single subcarcinogenic dosage of the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[*a*]anthracene (DMBA). Because of its mutagenic activity on DNA (inducing a A→T transversion), treatment with DMBA results in an activation of the Ha-*ras* oncogene. In fact, Ha-*ras* mutations are causally involved in the initial stage, as the transgenic animal, carrying already activated Ha-*ras* alleles, can substitute for DMBA application [28].

However, an initiating dose of the carcinogen per se will not produce tumours. Obviously, tumour formation will only take place after subsequent prolonged and repeated topical applications of a tumour promoter, such as croton oil or its most active constituent, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) to the initiated skin. This procedure initially gives rise to papillomas, which are histologically considered as benign tumours. However, a small proportion of benign papillomas can progress to locally invasive SCC [for review, see reference [29]].

The Role of AP-1 in the Development of SCC

Comprehensive studies in the past as well as recent microarray technologies have demonstrated that activation of Ha-*ras* and chronic treatment with the phorbol ester TPA have pleiotropic effects on the respective target cells, ranging from changes in the metabolism to alterations in the epigenetic profile, the intracellular redox status, and signal transduction [for review, see references [29, 30]].

An important pathway that apparently plays a fundamental function in skin carcinogenesis is the mitogene-activated protein (MAP) kinase pathway. Mammalian MAP kinases consist of three groups: the extracellular signal-regulated kinases (ERK), the c-JUN NH_2-terminal kinases (JNK), and the p38 MAP kinases [31]. Both oncogenic *Ras* proteins and TPA can affect the transcription factor AP-1 on the transcriptional as well as on the translational level by activation of JNK and ERK.

AP-1 represents a dimeric protein, consisting either of homodimers between c-Jun, JunB, and JunD or of heterodimers with members of the Fos family (c-Fos, FosB, Fra-1, Fra-2, ATF) by physically interacting via an intramolecular "leucine zipper" region [for review, see reference [32]]. Considering normal mouse skin, for example, AP-1 family members are tightly regulated in the course of cell differentiation. Differentiation-inducing agents such as calcium repress transcription of certain marker genes by excluding c-*fos* in favor of *fra-1* and *fra-2*, while TPA is exerting just the opposite effect [33]. Thus, alterations in AP-1 composition represent a mode to regulate the same gene in a diverse fashion.

Using transgenic or knockout mice, the importance of AP-1 in skin carcinogenesis was demonstrated. For instance, c-Jun and c-Fos seem to be two key players, as germline deletion of the latter did not result in malignant conversion in response to

Ha-*ras* activation and subsequent TPA treatment [34]. Hence, c-*fos* is critical in the formation of SCC as AP-1 is also functionally linked with the expression of matrix metalloproteinases (MMP), necessary for the degradation of the extracellular matrix and basement membrane. Furthermore, ectopic expression of a *trans*-dominant negative form of c-Jun can also block tumor formation [35], further substantiating the absolute requirement for functional AP-1 in a Jun/fos dimer constellation [36].

The consequent dissection of such central regulatory pathway is a powerful strategy and will be helpful in the development of novel therapeutic approaches against cutaneous SCC.

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New Trends in the Susceptibility to Melanoma

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In contrast with cutaneous squamous cell carcinomas, the risk for development of melanoma does not appear to be greatly increased after solid organ transplantation, except for the rare case of donor-derived melanoma. The clinical aspects of melanoma in organ transplant recipients are discussed elsewhere, but it would seem that genetic susceptibility to melanoma is likely to be of similar relevance to the immunosuppressed individual as to the immunocompetent individual. Because the outcome of melanoma, particularly thicker melanomas, is worse after transplantation (Matin et al., in press), patients who come from melanoma-prone families or who have a history of multiple melanomas must be carefully counselled before transplantation. Extremely close skin surveillance and a low threshold for biopsy of melanocytic lesions would be advisable.

Introduction

In the past decade, considerable progress has been made in our understanding of the genetic predisposition to cutaneous melanoma (CMM). A positive family history of the disease is one of the most established risk factors for CMM, and it is estimated that 10% of MM cases result from an inherited predisposition [1]. Two highly penetrant melanoma-predisposing genes have been identified to date, *CDKN2A* and *CDK4* [2–4]. The *CDKN2A* gene on chromosome 9p21 encodes two structurally distinct tumour suppressor proteins by virtue of different first exons spliced in different reading frames to common exons 2 and 3. Exons 1α, 2, and 3 encode p16INK4a, while exon 1β is transcribed using a different promoter spliced to exons 2 and 3 in a different reading frame and encodes p14ARF protein (ARF, also called p19ARF in mice) [5]. P16INK4a is part of the G_1–S cell-cycle checkpoint mechanism that involves the retinoblastoma susceptibility tumour suppressor protein (pRB). The other product of the *CDKN2A* locus, p14ARF, also acts as a tumour suppressor. ARF mediates G_1 and G_2 arrest at least partly by its interaction with MDM2, resulting

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in stabilization and accumulation of TP53 protein and also of its downstream target p21, an inhibitor not only of CDK4 and 6 but also of other CDKs [5].

High-Penetrance Susceptibility Genes

CDKN2A

Since the *CDKN2A* gene was identified as the first high-penetrance melanoma susceptibility gene in 1994, there have been numerous reports worldwide of germline mutations occurring in melanoma-prone families. Germline mutations in the *CDKN2A* gene are found on average in approximately 20% of melanoma-prone families [reviewed in references [6, 7]]. The frequency of *CDKN2A* mutations in melanoma probands increases with (i) the number of affected relatives, (ii) the presence of multiple melanomas in the same patient, [8–10], and (iii) a history of pancreatic cancer cases in the family [10,11]. Most are missense mutations localized in exon 1α or in exon 2. GenoMEL, comprising major familial melanoma research groups from North America, Europe, Asia, and Australia, has created the largest familial melanoma sample yet available to characterize mutations in the high-risk melanoma susceptibility genes. Single-founder CDKN2A mutations were predominant in Sweden (p.R112_L113insR, 92% of family's mutations) and the Netherlands (c.225_243del19, 90% of family's mutations). France, Spain, and Italy had the same most frequent mutation (p.G101W). Similarly, Australia and United Kingdom had the same most common mutations (p.M53I, c.IVS2-105A>G, p.R24P, and p.L32P) [10].

The second group in whom *CDKN2A* mutations are common are patients affected by multiple sporadic melanomas. The results from four major studies show a mutational rate ranging from 9% to 15%, although many of the mutation-positive cases had a documented family history of melanoma [12–15].

Apart from familial melanomas or multiple sporadic melanomas, the prevalence of germline *CDKN2A* mutations in the general population is exceedingly low. In a population-based study of melanoma cases from Queensland, Australia, Aitken et al. [16] estimated that just 0.2% of all melanoma cases are caused by *CDKN2A* mutations [16].

Increased Risk of Nonmelanoma Cancers in CDKN2A Mutation-Positive Families

There is clearly an increased risk of pancreatic cancer in families with *CDKN2A* mutations, with an estimated 21.8-fold relative risk of this tumour type in individuals with a germline *CDKN2A* mutation [11]. There have also been suggestions of an increased risk of breast, prostate, colon, and lung cancers and oral epidermoid

carcinomas (SCCs), but these are the most common of all cancers and therefore their occurrence in melanoma families may be coincidental.

CDK4

Worldwide, only six families have been reported to carry mutations of CDK4. Interestingly, all mutations occur in codon 24, with two families carrying an R24C change [4] and four others having an R24H substitution [8, 17, 18]. It has recently been shown that multiple mutational events arise in these *CDK4*-positive melanoma families, as no ancestral allele could be demonstrated [17]. The R24 residue is critical in the p16INK4A–CDK4 interaction, and when substituted, p16INK4A can no longer bind to and inactivate the CDK4 protein, resulting in "gain-of-function" mutations [19, 20].

ARF

There is now evidence that p14ARF, the product of the alternative transcript arising from the *CDKN2A* locus, is also involved in melanoma predisposition together with nervous system tumours (NSTs). Similar to p16INK4A, p14ARF is a tumour suppressor protein, but acts through a different pathway by stabilization of p53 through abrogation of MDM2-induced p53 degradation [21]. There have been four reports of a germline deletion of the exon 1β from ARF, together with exon 1α deletions resulting in melanoma and a range of neural tumours including astrocytoma, meningioma, schwanomma, and neurofibroma [22, 23]. However, the first unequivocal report of a germline deletion of ARF in the absence of a concomitant loss of either *CDKN2A* or *CDKN2B* was found in a similar family segregating melanomas and NSTs [23]. More recently, ARF-specific deletions or mutations affecting splicing of exon 1β have been described in melanoma families [24, 25]. These studies and others where CDKN2A germline mutations found in melanoma kindreds are shown to affect p14ARF function [26] clearly implicate p14ARF as a melanoma susceptibility gene.

Penetrance and Factors Modifying the Penetrance of *CDKN2A* Mutations

Genetic epidemiological studies suggest that penetrance of each CDKN2A mutation is modified by other factors, either genetic or environmental. Epidemiological studies have also identified other major host factors important in the development of melanoma.

The penetrance of CDKN2A mutations has been estimated from 80 melanoma families included in an international consortium (GENOMEL) by the means of a

statistical logistic regression analysis. The penetrance of *CDKN2A* mutations doubles with increasing age and shows a significant geographical variation, with a melanoma risk by the age of 80 years of 58% in European countries versus 91% in Australia [27], pointing out a potential interaction between ultraviolet radiation (UVR) and *CDKN2A*.

In addition, it has been shown that the presence of clinically identified dysplastic nevi, the total nevi count, and history of sunburn greatly increased the risk of melanoma in European, North American, and Australian populations [28,29]. More recently, it has been shown that functional variants of MC1R also increase the penetrance of CDKN2A mutations (see below) [30–32].

Existence of Other Potential High-Penetrance Genes

The *CDKN2A* and CDK4 genes account for predisposition in only 20% to 30% of all multiplex melanoma families. Clearly, additional cutaneous melanoma major predisposition genes are likely to exist. One of these may be BRCA2 with an excess of melanomas, especially ocular melanoma cases, found in BRCA2 breast cancer families. Further melanoma susceptibility loci are proposed on 9p21 and 1p22. Genome-wide scans being carried out by the International Melanoma Genetics Consortium may prove successful in localizing further melanoma susceptibility genes at these or other loci.

Polygenic Inheritance to Melanoma: Genes Belonging to the Pigmentation Pathway

MC1R and Melanoma

MC1R is the best known and most studied low-penetrance melanoma susceptibility locus.

The *MC1R* gene, localized at 16q, encodes a seven-pass transmembrane G protein-coupled receptor consisting of 317 amino acids that is expressed in several cell types, including melanocytes and keratinocytes [33]. Stimulation of MC1R by the α-melanocyte-stimulating hormone (α-MSH) leads to enhanced adenylate cyclase and cAMP activity, resulting in the synthesis of the black photoprotective pigment eumelanin pigment instead rather than of the nonprotective red pigment phaeomelanin. *MC1R* is highly polymorphic in Caucasian populations, and numerous *MC1R* variants have been demonstrated that lead to a loss of function, decreasing either cAMP production or α-MSH-binding affinity [34–39].

Four *MC1R* variants alleles (R142H, R151C, R160W, and D294H) have been shown to be associated with the red hair and fair skin phenotype (RHC, for red hair color) that is characterized by fair skin, red hair and freckles, and sun sensitivity. In addition, seven other alleles (V60L, 86insA, D84E, R142H, I155T, 537insC,

and H260P) may be considered as full or partial RHC-causing alleles, as shown by genetic associations in populations or through inheritance of the phenotype in families [40, 41].

Loss-of-function variants of *MC1R* have been shown to play an important role in determining melanoma and nonmelanoma skin cancer (NMSC) risks [reviewed in reference [42]]. Concerning melanoma risk, six case-control studies have been performed worldwide (Australia, The Netherlands, France, Greece, Italia, Poland). Results of these studies are concordant, showing an increase in melanoma risk (OR > 2) in individuals carrying one *MC1R* nonsynonymous variant, the risk doubling when two *MC1R* alleles variant are present [43–47]. However, several differences exist across the different studies. First, RHC variants are the most frequent variants implicated in melanoma risk in populations of Celtic origin (Australia or The Netherlands), whereas in populations from the south of Europe, other *MC1R* variants also seem to play an important role in determining melanoma risk [45, 46]. Importantly, the *MC1R* melanoma-predisposing effect seems to involve, at least partly, mechanisms independent from its effect on pigmentation in European populations [44–46].

Somatic BRAF Mutations and *MC1R* Status

The majority of melanomas that occur on skin with little evidence of chronic sun-induced damage have mutations in the BRAF oncogene, whereas in melanomas on skin with marked chronic sun-induced damage, BRAF mutations are less frequent. In two independent Caucasian populations, *MC1R* variants were strongly associated with BRAF mutations in nonchronic sun-induced damage melanomas [48].

OCA2 and Melanoma

Tyrosinase-positive oculocutaneous albinism (OCA, type II), the most prevalent type of albinism [49], is an inherited autosomal recessive disorder caused by germline mutations of the *P* gene (*OCA2*). Albino patients have fair pigmentation and are highly predisposed to sun-induced skin cancers, mainly nonmelanoma skin cancers (NMSC), but also less frequently melanoma. A recent study examining intragenic SNPs within OCA2 in melanoma patients and controls found an association between melanoma and OCA2 ($P = 0.03$ after correction for multiple testing), suggesting that a second pigmentation gene, in addition to *MC1R*, seems to be involved in genetic susceptibility to melanoma, but replication studies are needed to confirm these data. In a multiple logistic regression analysis, a similar strength of association was seen with endothelin receptor-B variants and melanoma (manuscript in preparation? unpublished data?). These findings need to be confirmed in larger melanoma cohorts.

Polymorphic Variants of Genes Involved in DNA Repair

Polymorphic variants of genes involved with base excision repair (BER) and nucleotide excision repair (NER) have been associated with both increased and decreased risk of melanoma; this includes a significantly reduced melanoma risk associated with the 148 Glu allele of the APE1 gene, which plays a key role in base excision repair of oxidative lesions [50]. Larger studies on BER genes are needed to verify these findings. Individuals with the rare inherited nucleotide excision repair deficiency disease xeroderma pigmentosum have a 1000-fold-increased incidence of skin cancer, including an increased risk of melanoma. Several case-control studies have been performed, concerning mainly XPD (ERCC2) and XPC polymorphisms. Baccarelli et al. analyzed the two common ERCC2 nonsynonymous variants (Asp312Asn and Lys751Gln) and found a direct association of each variant with MM risk only in older (>50 years) subjects [odds ratio (OR) = 3.4, 95% confidence interval (CI) = 1.6–7.3 for 312Asn; OR = 2.3, 95% CI = 1.1–4.9 for 751Gln] [51].

In a nested case-control study within the Nurses' Health Study (219 MM and 874 controls), the Lys751Gln and Asp312Asn polymorphisms were not shown to be associated with melanoma risk. However, they were shown to interact with environmental factors (lifetime severe sunburns, cumulative sun exposure) [52].

In a multiethnic study from nine geographic regions in Australia, Canada, Italy, and the United States comparing patients with multiple primary melanomas (1,238) to those with a single melanoma (2,485), weak positive associations were observed for XPD 312 Asn/Asn versus Asp/Asp (OR = 1.5, 95% CI = 1.2–1.9) and XPD 751 Gln/Gln versus Lys/Lys (OR = 1.4, 95% CI = 1.1–1.7) genotypes and melanoma [53]. In conclusion, several studies have suggested that XPD variants may directly increase melanoma risk and/or interact with specific host characteristics or UV exposure [51, 52] There have been fewer studies examining XPC variants, but an alternatively spliced XPC mRNA with reduced DNA repair shows a similar association with melanoma risk [54]. Further studies are needed to precisely understand the role of the *XPD* and *XPC* genes.

Other DNA-Repair Polymorphisms

Variants in DNA double-strand break repair genes (XRCC2, XRCC3, and ligase IV) genes were studied in the same nested case-control study within the Nurses' Health Study. Although variants in XRCC2 and XRCC3 were associated with basal cell carcinoma risk, no association was found with MM risk [55].

Genes Belonging to the Immune System

Vitamin D Receptor Polymorphism

Hutchinson et al. [56] proposed that the vitamin D receptor gene (VDR) might contribute to melanoma risk since the VDR ligand, calcitriol, has antiproliferative and

prodifferentiation effects on melanocytes and melanoma cells, and polymorphisms of this gene have been associated both with serum calcitriol levels and risk of several other types of cancer [56] . In a case-control study comprising 316 melanoma cases and 108 controls, all of Northern European Caucasian decent, the rarer allele of a Fok I polymorphism, which results in a novel VDR translation start site, was significantly more common in melanoma cases than controls (39.9 versus 31.5%; $P = 0.029$) [56].

Polymorphisms of FAS and FAS Ligand Genes

Sunlight causes damage to DNA, and cells with excessive DNA damage undergo programmed cell death, or apoptosis, which prevents the cell from abnormal growth. FAS, also known as TNFRSF6/CD95/APO-1, is a cell-surface receptor that is involved in apoptotic signaling in many types of cells. FAS ligand (FASLG), also known as TNFSF6/CD95 l, is a member of the tumor necrosis factor superfamily and can trigger an apoptotic cascade by cross-linking with its receptor, FAS. The FAS/FASLG system play a crucial role in regulating UV-induced DNA damage-triggered apoptosis and maintaining cellular homeostasis. In a hospital-based case-control study of 602 non-Hispanic white MM patients and 603 cancer-free age- and sex-matched control subjects, two FAS SNPs (1377G>A, 670A>G) and two FASLG SNPs (-844T>C and IVS2nt-124G>A) were genotyped. An increased risk of MM was associated with the FAS-1377GG and -670AA genotypes [adjusted odds ratio (OR) 1.32, 95% CI 1–1.75; and OR = 1.28, 95% CI = 1–1.65], and with the FASLG-IVS2nt-124 (AG + GG) genotypes (OR = 1.54, 95% CI = 1.18–2.01) [57].

Conclusion

Two well-characterized highly predisposing genes, *CDKN2A* and *CDK4*, are involved in genetic predisposition to melanoma. Other major melanoma loci have been characterized, but the relevant genes have not yet been identified.

A key gene involved in pigmentation, *MC1R*, has been clearly shown to be a low-penetrance melanoma-predisposing gene across multiple different populations. In addition, a growing list of loci involved in polygenic predisposition to melanoma is now appearing. Further works including replication studies across different populations is clearly needed, and whether there is an interaction between all these genetic factors or with environmental factors is currently under investigation.

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Part III
Specific Skin Cancers

Actinic Keratoses

Eggert Stockfleth

Definition and Pathogenesis

Actinic keratoses (AKs) are defined as keratotic macules, papules, or plaques with superficial scale on a red base, occurring on areas extensively damaged by sunlight. They should be classified as in situ squamous cell carcinomas (SCC) [1, 2]. Histopathologically, an intraepidermal proliferation of atypical keratinocytes can be observed.

AKs are mainly caused by nonionising radiation, especially through ultraviolet (UV) light associated with chronic sun exposure. While UV-A (320–400 nm) induced photooxidative stress indirectly induces characteristic DNA mutations, the spectrum of UV-B (290–320 nm) irradiation directly results in the formation of cyclobutane (thymidine) dimer formation and C → T or CC → TT transitions in DNA and RNA. In the absence of appropriate repair mechanisms, these DNA changes represent the initiation of keratinocyte mutations, which can progress into the development of AKs [3]. Other factors such as repeated iatrogenic exposure to UV-A with or without combination with psoralenes, X-rays, or radioisotopes are known to induce AKs. Human papilloma viruses (HPV) play a role as a co-carcinogen in the ethiopathogenesis of AKs [4, 5]. The association between cutaneous HPV types and skin carcinogenesis has been well known since 1978 in patients with epidermodysplasia verruciformis [6]. In AKs, often cutaneous HPV types and rarely genital types have been detected [7]. Tumour-inducing effects have also been shown for viral E6 protein of cutaneous HPVs. E6 interacts with pro-apoptotic Bak-protein and therefore inhibits apoptosis [8, 9]. Other factors include the skin phototype of the individual, genetic factors, chronic immunosuppression, and history of arsenic exposure.

AKs can occur as a single lesion or affect an entire field such as sun-exposed areas on the forehead or the back of the hand (syn., field cancerisation) [10]. Cancer-related molecular alterations are found in both AK and SCC. This genetic

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link supports the malignant nature of AKs from its inception. The transformed keratinocytes show a high mutation rate of the tumour suppressor gene p53 and expression of telomerase [11, 12]. Additionally, similar chromosomal aberrations have been described for invasive typical SCC and AK [13].

Epidemiology

Epidemiological data show a high occurrence rate of AKs in populations with skin phototype I–III and an increase of AKs in the past decades worldwide. Regions with higher UV exposure have a higher prevalence of AKs. In Europe, a prevalence of 15% in men and 6% in women has been documented in a recent report from the U.K. [14]. Over the age of 70 years, 34% of males and 18% of females were found to have AKs. The United States show prevalences between 11% and 26% [15], and in Australia (Queensland) a very high prevalence of AKs (55% of men aged 30–70 years showed AKs, as opposed to 37% of women) has been reported [16].

In addition to sex, gender, and age, other risk factors are known. Geographical factors such as altitude and latitude, increased vacation and recreational sun exposure, a history of severe sunburns in childhood, genetic disorders (xeroderma pigmentosum), and immunodeficiency contribute to the development of AKs. Clinically, the affected individual often presents with the characteristic signs of dermatoheliosis such as freckles, solar lentigines, and rhytides. High-risk AKs occur mainly in immunosuppressed patients [17]. Organ-transplanted patients have a 250-fold-higher risk to develop AKs and a 100-fold-higher risk to develop invasive SCCs [18, 19]. Although about 40% of immunosuppressed patients develop an invasive SCC, only approximately 10% (6%–16%) of immunocompetent patients with AKs show this progression [18, 20].

In conclusion, the incidence of AKs is increasing such that millions of patients are affected worldwide, making actinic keratoses the most frequent carcinoma in situ in humans.

Clinical Aspects

Typical AKs are skin-coloured to reddish-brown scaly macules, papules, or plaques occurring in areas of chronic sun exposure, especially on face, forehead, scalp, ears, neck, décolleté, arms, dorsum of hands, and lower lips.

Lesion size ranges from a few millimetres up to 2 cm or more in diameter. AKs rarely develop as solitary lesions; in fact, multiple lesions are commonly present (field cancerisation) (Fig. 1). A clinical classification is illustrated in Table 1.

No distinct clinical boundaries exist between AKs and SCC. It has been reported that before AKs progress to invasive SCC, they may become inflamed and painful [21].

Diagnosis of AKs is based upon the typical clinical aspects. Histological confirmation is necessary when clinical doubts exist or when special forms of treatment

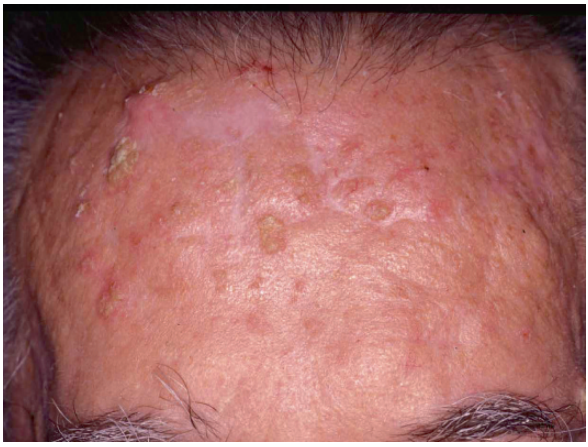

Fig. 1 Typical case of multiple Ak's - field cancerisation in an elderly patient

Table 1 Clinical features

Clinical classification[a]
– Keratotic
– Atrophic
– Cornu cutaneum
– Verrucous
– Pigmented
– Lichenoid

[a]Overlapping between subtypes may be observed.

are considered. A biopsy that includes the dermis is required if deeper involvement needs to be excluded.

Dermoscopy can be helpful in the differential diagnosis of pigmented actinic keratosis versus lentigo maligna melanoma and superficial basal cell carcinoma. Other techniques, including confocal scanning laser microscopy, have been utilised in serial clinical investigations [22].

Histopathology

The histological criteria of AKs are summarized in Table 2.

The lichenoid subtype of AK is accompanied by dense bandlike infiltrates of lymphocytes in the stratum papillare. Acantholytic dyskeratotic cells above suprabasal clefts are found in acantholytic AKs. The degree of intraepidermal involvement by keratinocytic atypia is graded as mild (AK I), moderate (AK II), or severe (AK III) [23].

Table 2 Histopathological features

Early lesions
– Focally atypical keratinocytes (large pleomorphic nuclei, hyperchromatic nuclei) in the basal layer of the epidermis
– Crowding of nuclei
– Alternation of ortho- and parakeratosis
– Actinic elastosis
Fully developed lesions
– Hyperplasia (or sometimes atrophy) of the epidermis
– Rete ridges arranged in buds or columns
– Alternation of ortho- and parakeratosis
– Neoplastic cells spare both acrosyringia and acrotrichia
– Atypical epidermal keratinocytes involve mostly the lower half of the epidermis, sometimes with focal involvement of the entire thickness of the epidermis
– Atypical keratinocytes extend along adnexal epithelia
– Dyskeratotic cells and mitotic figures
– Actinic elastosis
– Lymphocytic infiltrate of variable density

The classification of AKs takes into consideration that AK is an early stage of cancer and that both AKs and SCC are stages in the evolution of a continuous process characterised by the proliferation of atypical keratinocytes. On histopathological grounds alone, AK and SCC are indistinguishable in the epidermal layer, and AKs fulfill all criteria for SCC (Fig. 2). Both contain atypical keratinocytes with loss of polarity, nuclear pleomorphism, disordered maturation, and increased numbers of

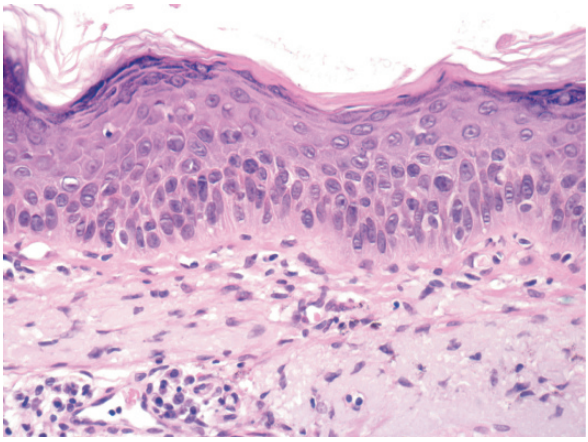

Fig. 2 Histology of a patient with Ak's

mitotic figures [1]. AK and SCC are frequently contiguous with one another. It is important to emphasize that, in a study of more than 1,000 SCC on sun-damaged skin, nearly 100% of these lesions contained histopathological changes of AK at the periphery [24].

Treatment Options

Overview

It is impossible to predict which AK will become thicker or more invasive with a potential for destructive growth and risk for metastases, that is, develop into metastatic SCCs. AKs should therefore be treated.

In deciding which therapy should be chosen, the following factors play a major role: duration and course of lesions, localisation and extent of disease, solitary or multiple AKs, the age, comorbidity, mental condition, and compliance of the patient, preexisting (skin) cancer, and as well as the presence of other risk factors (especially immunosuppression) [25].

When considering treatment options for AKs, there is a great variety of accepted methods. When using nonsurgical treatment modalities, an exemplary probe biopsy for histological diagnosis may be indicated before therapy.

Surgical Excision, Dermabrasion, and Curettage

Excision of AKs is not routinely used and is only chosen if invasive SCC is suspected or recurrent lesions are present. Shave excision is frequently used for AK. Sutures are not necessary, and a histological diagnosis can be provided [26]. Similarly, curettage may be used alone or in conjunction with electrosurgery or cryotherapy with excellent cure rates [27]. Dermabrasion is especially useful for larger areas of AK on the scalp [28]. All these methods require local anesthesia and may leave epidermal changes or scarring.

Cryotherapy

Cryotherapy is the most common treatment for AKs, especially for the management of multiple AKs [27, 29, 30]. Cryotherapy is available in techniques using liquid nitrogen or as spray or contact cryotherapy. Field cancerisation, which describes a chronically sun-damaged region, can be treated by cryo-peeling [31]. Cryotherapy is not standardised concerning frequency, duration, intensity, and definitive specification of temperature in the frozen tissue. As a nonspecific technique, cryotherapy destroys atypical, but also normal, cells by disruption and separation of the epidermis from the dermis. Pain, redness, edema, and blistering can occur during

and after treatment. Scarring and hypo- or hyperpigmentation are commonly observed.

Although cryotherapy is often used, controlled studies are lacking. Complete responses vary from 75% to 98% [32, 33], and the recurrence rates of AKs have been estimated from 1.2% to 12% within a 1-year follow-up period [31, 34].

Chemical Peeling

Chemical peeling is a destructive method through the application of caustic agents such as trichloroacetic acid, alpha-hydroxy acids, zinc chloride, or phenolic acid. Chemical peeling can be a useful alternative for treatment of extensive facial AK [35]. The efficacy of chemical peelings depends on the agent used and is quoted to be about 75%; recurrence rates are from 25% to 35% within 1 year after therapy. Side effects of chemical peelings include pain, inflammation, pigmentary alterations, and the risk of scarring [36–38].

Laser

Near-infrared laser systems such as carbon dioxide (CO_2) or erbium-YAG lasers are indicated in special cases for AKs. Both are ablative laser systems and can be used for single lesions as well as full-face resurfacing. Full-face laser resurfacing provides long-term effective prophylaxis against AKs and may reduce the incidence of AK-related SCC [39]. Adverse events are pain, inflammation, pigmentary changes, and scarring as well as delayed healing and postinflammatory erythema. Although complete remission is documented, from 90% to 91%, recurrence rates for single lesions range from 10% to 15% within 3 to 6 months [40, 41]. Disappointing results reported in earlier literature may be related to technical aspects, as the outcomes of full-face resurfacing are strongly user dependent. [42]. The expert opinion is that in skilled hands there is a place for CO_2/erbium-YAG laser in the management for AKs and the treatment of actinic cheilitis.

The treatment of AKs with X-rays is considered obsolete because of the co-carcinogenic effect of X-rays.

Photodynamic Therapy

Topical photodynamic therapy (PDT) acts through the selective destruction of atypical keratinocytes (depth of penetration, 3–4 mm) through light activation of a photosensitiser in the presence of oxygen. The neoplastic cells accumulate more photosensitiser than normal cells. The photosensitiser generates reactive oxygen species upon illumination, which results in selective photochemical and photothermal effects on the irradiated tissue. The most commonly used precursors of

protoporphyrin IX are 5-aminolevulinic acid (ALA) and its derivatives such as the new lipophilic agent methyl aminolevulinate (MAL). MAL-PDT is applied as a cream under occlusion for 3 h before illumination with high-intensity red light. For Europe, the European Medical Evaluation Agency (EMEA) labelled MAL as an indication for AK. The clinical experience in AK patients receiving MAL-PDT shows a complete response rate of 70% to 78% after a single treatment session and 90% after two treatment sessions 1 week apart. Negative effects of PDT are local pain, risk of photosensitivity (mainly for ALA), and time delay between application of cream and treatment. Photodynamic therapy in comparison to cryotherapy shows significantly better cosmetic results (as evaluated by patients and doctors) [17, 32, 43–45]. Advantages of PDT include the selective absorption and treatment of subclinical lesions, and the fluorescence of the photosensitiser can be visualised using Wood's light before the initiation of therapy [46]. On the other hand, the costs of treatment are considerably higher compared to cryotherapy. In a paper described by Bavinck et al., it was shown that the use of ALA-creme and PDT does not prevent skin cancer in organ-transplanted patients.

Imiquimod

Imiquimod 5%, a member of a novel class of immune response modifiers (IRMs), is a toll-like receptor (TLR)-7 agonist and stimulates the immune response by induction, synthesis, and release of cytokines. These cytokines increase cellular immunity. Therefore, imiquimod has an indirect antiviral and antitumoral potency [47,48]. Topically applied imiquimod causes a local skin reaction, including erythema, itching, and burning, that is generally mild to moderate in intensity. Apart from the capability of imiquimod to "light up" subclinical AKs, imiquimod is effective and safe in patients with AKs. Response rates show complete remission in 84%, and a recurrence rate of 10% within 1-year follow-up and 20% within 2-year follow-up [49,50]. A randomised, double-blind, placebo-controlled study in 436 patients with actinic keratoses showed a complete resolution of all lesions in 45.1% (vs. 3.2% placebo) and a partial reduction of actinic keratoses in 59.1% (vs. 11.8% placebo) after a treatment period of 16 weeks (twice per week) [51]. A label for the indication of AK through the EMEA is in process. Imiquimod is labelled for the indication of superficial basal cell carcinoma in the United States, Australia, and Europe. In a study by Ulrich et al. [25], a total of 43 patients in six European transplant centers applied two sachets of topical imiquimod or vehicle cream three times per week to a 100-cm^2 field. Dosing continued for 16 weeks regardless of lesion clearance. Patients were assessed for safety variables that included adverse events, local skin reactions, laboratory results, vital signs, dosage of immunosuppressive medication, and indication of graft rejection. A blind independent expert committee was responsible for safety monitoring and final safety assessment.

No graft rejections or trends for a deterioration of graft function were detected. No meaningful trends were observed in laboratory results. Among patients randomized to imiquimod, the complete clearance rate was 62.1% (18 of 29); for vehicle

patients, the complete clearance rate was 0% (0 of 14). Clinical clearance was confirmed histologically in all cases.

Imiquimod appears to be a safe alternative for the treatment of multiple AKs in patients with solid organ transplants. Efficacy was within the range previously observed in nontransplanted populations [25].

Topical 5-Fluorouracil

5-Fluorouracil (5-FU) is a topical chemotherapeutic antimetabolite that destroys clinical foci via interference with DNA and RNA by blocking the methylation reaction of deoxyuridylic acid to thymidylic acid. Thus, 5-FU interferes with the synthesis of DNA and, to a lesser extent, inhibits the formation of RNA. 5-FU can be used for the treatment of multiple lesions and is applied twice a day (for 2–4 weeks). These effects may cause life-risk complications if dihydropyrimidine-dehydrogenase deficiency exists [52]. Topical 5-FU can result in severe dermatitis with wound infections, pruritus, pain, and ulceration with scarring, and the application is of limited help in the therapy of extensive AKs. For localised disease, clearance rates of approximately 50% and recurrence rates up to 55% have been reported with 5-FU [36, 53, 54]. Meanwhile, new formulations with different concentrations and galenics of 5-FU are under clinical investigations [54–57].

Retinoids

Retinaldehyde is a natural derivative of vitamin A; it has effects similar to retinoic acid [58]. Besides counteracting the UV-induced vitamin A deficiency of the epidermis, topical retinaldehyde may have an antioxidant effect [59, 60] and decreases the number of sunburn cells. A placebo-controlled randomised study documents that systemic administered etretinate reduces AKs in 85% [61]. Some publications show that the epidemiological characteristics of AKs were not modified by the application of retinaldehyde and that retinaldehyde has no prophylactic effects on the development of AKs [62, 63]. Side effects of topically applied retinoids are increased sensitivity to sunlight, erythema, erosions, pruritus, and pain.

Retinoids can be also administered orally, especially in patients who develop large numbers of skin cancers. Systemic therapy can be considered for high-risk patients, for example, patients with inherited disorders such as xeroderma pigmentosum (abnormal repair of UV-induced DNA damage), nevoid basal cell carcinoma syndrome (tumour suppressor gene abnormality), or in organ transplant recipients with chronic immunosuppression [64, 65].

Diclofenac in Hyaluronic Acid Gel

Within past years, the antineoplastic and apoptopic properties of selective inhibitors of cyclo-oxygenase 2 (COX-2) have increasingly been investigated [66]. These

new agents inhibit prostaglandin E_2 (PGE_2) synthesis, which is known to suppress the production of immunoregulatory lymphocytes, T- and B-cell proliferation, and the cytotoxic activity of natural killer cells. Furthermore, activation of COX-2 has implications for tumour angiogenesis through upregulation of vascular endothelial growth factor (VEGF), which is a potent angiogenic factor required for tumor growth and metastases [67]. Apart from its affinity to the inducible COX-2, nonsteroidal anti-inflammatory drugs (NSAIDs) have been demonstrated to activate peroxisome proliferator-activated receptor-gamma (PPAR-γ), which decreases cancer cell proliferation. Topical diclofenac is applied in hyaluronic acid (HA).

Several randomised, double-blind, HA gel vehicle-controlled clinical studies have evaluated the efficacy of topical diclofenac HA gel in patients with AK. The 30-day interval between the end of treatment and the evaluation of efficacy was caused by earlier findings stating a significant advantage for diclofenac HA gel over placebo when efficacy was evaluated 4 weeks after the end of treatment. The product significantly reduced lesions when applied for 60 or 90 days. A double-blind, randomised, placebo-controlled multicenter study showed responding rates of 79% (verum group) versus 45% in the placebo group; a complete healing was seen in 50% (verum group) versus 20% in the control group ($P < 0.001\%$) [68]. Other controlled studies showed similar effects [69, 70]. Adverse effects were skin related and mild to moderate in severity (pruritus, erythema, dry skin, hyperparesthesia, and paraesthesia). Systemic bioavailability of diclofenac was demonstrated to be considerably lower after topical application than after systemic administration, and the drug demonstrated a good safety profile. Ulrich et al. described the use of diclofenac in hyaluronic acid in six organ transplant recipients using it twice per day over 16 weeks. They reported a total clinical and histology clearance rate of more than 50% in all patients; safety was not an issue [25].

Prevention

Prevention of AKs is an important part of AK management [71, 72]. Education of patients (UV protection, self-examination, and detection of early lesions) is particularly important. AK is an ongoing disease that requires frequent follow-up and long-term management. In a study conducted by Ulrich et al., the group described that the regular use of a liposome sunscreen (daylong Actinica) reduced the incidence of AKs and invasive SCC as well in organ transplant recipients [25].

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Squamous Cell Carcinoma

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During the past years, solid organ transplantations have been performed with increasing success. The long-term survival of organ transplantation is related particularly on the prevention of allograft rejection. Various regimens have been utilised to suppress the host immune response to the transplanted organs. It has been well demonstrated that a relationship exists between immunosuppression and the risk of different malignancies in organ transplant recipients (OTRs). Moreover, patients who have received solid organ transplants are known to have increased susceptibility of developing skin cancer as a result of the intense immunosuppressive regimens. In particular, transplant recipients have a significantly increased risk of developing squamous cell carcinoma (SCC). The cumulative incidence varies from 1% to 6.5% and from 6% to 35% at 5 and 10 years post transplantation, according to different studies [1–5]. In contrast to the normal population, where basal cell carcinoma (BCC) outnumbers SCC, the ratio of BCC to SCC is reversed, so that SCCs in transplant recipients occur more frequently than BCC.

Transplant patients affected by SCC show a high tendency to develop subsequent SCC, with a proportion higher than 60% within 5 years after the occurrence of the first SCC [6]. A fraction of these patients experiences an unceasing onset of SCCs during the years, sometimes with only a few months interval between the occurrences of the lesions; this may lead to a severe impairment of professional career and personal life.

Risk Factors

Several risk factors for the occurrence of squamous cell carcinoma (SCC) in organ transplant recipients have been identified; these include both well-known environmental and intrinsic risk factors for SCC shared with the general population and some specific factors concerning transplant patients (Table 1).

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Table 1 Risk factors associated with the development of squamous cell carcinoma (SCC) in organ transplant recipients

Shared with the general population		
Enviromental	Genetic	Specific for organ transplant patients
Age	Ethnicity	Length and level of immunosuppression
Latitude	Skin type	Pre-transplantation history of SCC
Chronic sunlight exposure	Eye color	Polycystic kidney disease
(Human papillomavirus infection)	Hair color	Primary sclerosing cholangitis
(Smoking)	(Male sex)	
	(HLA-A11)	
	(p53 gene polymorphism)	
	(IL-10 gene promoter polymorphism)	
	(GST gene polymorphism)	

Older age at transplantation is the most important clinical predictor of SCC in organ recipients. Older patients not only have an increased risk, but also show a shorter mean interval between transplantation and development of the first SCC [7, 8]. The mean interval between transplantation and detection of the first skin cancer is less than 7 years for patients aged more than 50 years at transplantation and more than 11 years for patients younger than 50 years. The earlier onset of SCC in older patients following the induction of immunosuppression might represent the release of previously altered clones of cells accumulated over time that had previously been held under immune control. This difference may also be related to the greater cumulative exposure to environmental carcinogenic hazards, notably ultraviolet (UV) radiation.

Indeed, UV exposure is the primary risk factor for SCC both in the general population [9, 10] and in transplant recipients [11–14]. Patients with a higher cumulative sunlight exposure before to organ transplantation show a higher risk of SCC [3, 8, 15]. The cumulative risk of SCC after 10 years post transplantation rises from 11% to 33% for patients with low (less than 13,000 h) or high (more than 30,000 h) occupational sunlight exposure, respectively.

In keeping with these data, the cumulative risk for SCC in organ recipients was reported to be greater in countries with a high level of UV radiation, such as Australia (34% at 10 years) [2] or Spain (33% at 10 years) [3], compared with countries with limited sun exposure, such as Holland (7% at 10 years) [5] or Norway (7% at 10 years) [1]. Moreover, the preferential location of SCC on sun-exposed areas further supports the pathogenic role of sunlight. There are experimental data showing that UV light is a keratinocyte mutagen, often producing UV landmark mutations, such as cytosine to thymine transitions at cytosine-containing dipyrimidine sites, and acting as a tumour initiator and promoter [9, 16]. When these mutations affect the function of sufficient oncogenes, tumor-suppressive genes, and important housekeeping genes, neoplastic transformation of keratinocytes occurs.

In addition, UV light radiation can also induce immunological unresponsiveness. In particular, low doses of UV light radiation reduce the number and function of epidermal Langerhans' cells, impairing their role in the immune response against virus-infected cells and transformed cells. UV light radiation can also induce systemic immunosuppression by inducing the generation of soluble mediators, notably *cis*-urocanic acid and interleukin (IL)-10 [10]. Thus, sunlight exposure may exert an additive or potentiating immunosuppressive effect in transplant recipients who are already receiving chronic pharmacological immunosuppression.

The role of human papillomavirus (HPV) infection in the pathogenesis of post-transplant SCC remains unclear. A relationship between HPV and skin cancer has been proposed in renal transplant recipients since 30 years ago [17]. These patients experienced, in addition to an increased incidence of skin carcinomas and particularly SCC, a higher risk of viral warts compared to the general population [18]. Both SCC and warts favour sun-exposed areas. Several recent studies using degenerate polymerase chain reaction (PCR) reported a high prevalence (65%–83%) of HPV DNA in skin carcinomas from transplant patients [19–21]. These skin tumors were predominantly SCCs. Nevertheless, the detection of HPV DNA has been showed to be significant also in BCC (60%–75%) [19, 22]. Recently, it was shown that HPV 38, which was detected in nearly 50% of skin carcinomas but only in 10% of healthy skin, was able to actively support longevity or immortalization of cultured human skin keratinocytes [23].

However, even though the association between human papilloma virus (HPV) and anogenital SCC has been well established, to date the actual role of HPV in cutaneous SCC still remains controversial [19]. Several studies failed to find any relationship between the presence of warts and risk of SCC [8, 24, 25]. Most of SCC appears earlier in the post-transplant period than warts.

No specific individual type of HPV has emerged as strongly associated with skin cancer. Mixed infections are exceedingly common in SCC (63%) and BCC (55%) from transplant patients [19]. Moreover, HPV DNA was also detected in normal skin from transplant recipients (92%–94%) [26, 27] and, in a lesser extent, even from healthy people (35%–80%) [26–28]. The ubiquitousness of various HPV types in SCC, BCC, benign lesions, and normal skin of both transplant recipients and healthy people, with a high prevalence of mixed infections, points out the need to further investigate the exact mechanisms of HPV carcinogenesis. The role of HPV in the development of SCC will be distinctly elucidated when data from prospective studies become available.

Another potential environmental carcinogen is tobacco smoke. A history of having ever smoked tobacco (i.e., current and ex-smokers) has been found to be associated with the development and the number of SCCs, in comparison with lifelong non-smokers [29]. However, a number of other studies were not able to find such an association [30–32].

Patients with a history of SCC diagnosed and treated before transplantation show a higher risk of SCC development after transplantation [2, 29, 33]. The pre-transplantation history of SCC appears to be one of the strongest predictors of the occurrence of subsequent post-transplant SCC [29].

Additional risk factors for SCC occurrence in transplant recipients include genetic predisposal factors. SCC is more likely to develop in patients with Fitzpatrick skin types I, II, or III [15, 24]. Accordingly (although independently), blue or hazel eyes are associated with a higher risk of SCC [6, 18, 34]. Male sex has been related to a higher tendency to develop SCC in some [31, 35, 36] but not all investigations [4, 6, 29].

Even though there is little information on the risk of SCC in transplant patients from developing countries followed up over an extended period of time, it appears evident that ethnicity greatly influences the occurrence of skin cancer in those patients. The post-transplant incidence of SCC is rare in Arabs, Asians, and Africans [37, 38]. The role of the ethnic background is aptly demonstrated by the striking differences in the incidence of skin cancer among different ethnic groups living in the same geographical region. In South Africa, SCC has been reported to occur exclusively in white renal transplant recipients. Non-white patients residing in the same geographical region and exposed to the same immunosuppressive treatment do not develop SCC after transplantation. Protection may be conferred by either their skin type or unique genetic factors.

Some studies have investigated the association between specific HLA recipient antigens and the risk of SCC. Despite a conflicting report [39], only HLA-A11 has been consistently found to confer an increased risk for SCC development after transplantation [40, 41]. There are a variety of theoretical mechanisms by which HLA subtype might predispose to SCC development, from a higher immunogenicity leading to higher rates of rejection and therefore more intense immunosuppression to an association with increased susceptibility to oncogenic viruses, namely HPV. Nevertheless, the pathogenic role of HLA-A11 in SCC development remains speculative.

Specific gene polymorphisms have been described to be associated with SCC predisposition in transplant recipients, as well as the general population.

A common polymorphism that occurs in the p53 sequence, which results in the presence of an arginine instead of a proline at positione 72, has been linked to a higher SCC risk in renal transplant recipients [42, 43]. However, other studies have challenged this hypothesis [44, 45].

The association between SCC development and an IL-10 gene promoter polymorphism that leads to a higher production of IL-10 needs to be confirmed [46]. Because of its immunosuppressive and anti-inflammatory activities, it has been speculated that IL-10 may contribute to the escape of tumor cells from immunosurveillance and favour tumor growth.

A few studies reported an association between polymorphisms in the glutathione S-transferase (GST) supergene family and SCC risk in transplant patients. GST represents a group of enzymes involved in the detoxification of a variety of reactive and mutagenic compounds, including the products of UV-induced oxidative damage, that appears to be a key factor in UV-induced carcinogenesis. A genetically determined variation in this system may therefore have a role in determining a specific susceptibility to SCC occurrence in the transplant recipients, as well as the general population. A good deal of evidence has suggested a detrimental role of

GSTM1 null polymorphism [47, 48] and a protective role of GSTP1*A (or GSTP1 Val/Val) [47–49] in the SCC pathogenesis in transplant patients, but considerable controversy still remains [50].

The risk factors for SCC specifically related to organ transplantation encompass a chronic antigen stimulation from transplanted organs, the cumulative exposure to immunosuppression, resulting in an impairment of either the antitumor immunosurveillance or antiviral activity, and the direct oncogenic effect of the immunosuppressive agents by mechanisms independent of their immunosuppressive effects.

A presumptive deleterious effect exerted by a chronic antigen stimulation of the graft may contribute to the development of transplant-related lymphoma, but no data have supported its role in the pathogenesis of skin cancers.

Clearly, the chronic immunosuppressive state represents the critical feature differentiating organ transplant recipients from the general population. Immunosuppression should thus play a pivotal role in the increased risk for skin cancer in organ transplant patients. The risk of SCC increases with both the length and the level of immunosuppression. There is a steady rise of SCC cumulative incidence with time after transplantation, which is not only the result of a latency period for SCC development, because there are data concerning the decrease of SCC occurrence after cessation of immunosuppression in organ transplant recipients previously treated with immunosuppressive drugs for several years [51].

Many investigations have been conducted to identify the association between the development of skin cancer and a specific immunosuppressive drug, but the results are often conflicting [1, 12, 25, 52]. Several studies have showed no significant difference in the incidence of post-transplant skin cancer in diverse immunosuppressive regimens, suggesting that the risk of skin cancer in organ transplant recipients may be related to the cumulative immunosuppressive load, rather than to a specific immunosuppressive agent [1, 2]. A few studies reported a higher risk of SCC (but not of BCC) in organ transplant patients treated with an immunosuppressive therapy regimen thought to achieve a stronger immunosuppression compared to patients treated with other regimens [1, 53]. In particular, the adjusted (for known risk factors) relative risk for SCC development in patients on triple (cyclosporine, azathioprine, and prednisone) therapy compared to double therapy (azathioprine and prednisone) was 8.43 [95% confidence interval (CI), 1.3–54.8] in 262 renal recipients from UK [53] and 2.8 (95% CI, 1.4–5.3) in a series of 2,235 renal and heart recipients from Norway [1]. A relationship between higher doses of cyclosporine and the occurrence of skin cancer was demonstrated in a 5-year randomized, prospective study [54].

However, it is difficult to attribute a distinctive responsibility for SCC development to one particular drug rather than to a drug combination. Indeed, most studies are registry based and retrospective. An evaluation of the relative weight of one or more agents in inducing a skin cancer is exceedingly difficult to perform because of the variety of protocols with different drugs and different dosing regimens over different periods of clinical follow-up, according to graft function and rejection episodes.

To evaluate the global level of immunosuppression, various clinical measures were proposed as an indirect marker of the immunosuppressive load. Low CD4 cell

counts were found to be associated with the development of overall skin carcinomas in renal transplant recipients [55].

One study used the first year post heart transplantation rejection score, based on an adaptation of the International Society for Heart and Lung Transplantation (ISHLT) histological grading of rejection on endomyocardial biopsy, as an indirect marker of the level of immunosuppression in risk factor analysis for skin cancer in a series of 300 heart transplant patients. This score was calculated from the histological grading of rejection on endomyocardial biopsy obtained thoroughout the post-transplant period following established protocols (weekly during the 1st month, biweekly until the 3rd month, monthly until the 1st year; in the presence of grade 2 rejection, in the following 10 to 15 days). As a matter of fact, the level of immunosuppression is amended according to the presence of even subtle signs of cell infiltrate in the graft. Multivariate analysis showed that high rejection score at 1 year was independently associated with the development of SCC, but not with BCC [8].

Not surprisingly, many other studies evaluating only acute rejection episodes and not the chronic tendency to reject the graft organ assessed with serial graft biopsies, failed to detect any association between risk of SCC and the number of symptomatic rejection episodes [31, 35].

Conflicting results have been reported on the role of HLA mismatching in the SCC occurrence [31,35]. In one study, the rate of HLA mismatching has been found to be associated in organ transplant recipients with an increased risk of developing SCC, but not BCC, likely through an indirect effect on the level of immunosuppression [12].

Another attempt to evaluate the grade of overimmunosuppression was the weighted linear combination of azathioprine, cyclosporine, and corticosteroid cumulative dosages, a measure of the cumulative immunosuppressive drugs' dosages at different time points after transplantation [4]. This calculation, although not reproducible in the clinical practice, further supports the hypothesis that the risk of SCC, but not of BCC, in organ recipients is related to the level of global immunosuppression rather than to one specific drug.

Some immunosuppressive agents have been found to promote carcinogenesis by mechanisms independent of their immunosuppressive effects. Azathioprine is believed to act as a carcinogen by intercalation at the DNA level, inhibiting repair splicing and eliciting codon misreads [56]. Moreover, azathioprine is normally converted to 6-thioguanine, which is incorporated into DNA (0.02% substitution of DNA guanine in patients treated with 1–2 mg per kilogram of body weight) and can act as a endogenous UVA cromophore. Biologically relevant doses of UVA are thus able to generate reactive oxygen species that provoke chronic oxidative stress and eventually DNA mutations in the cells of patients treated with azathioprine [57].

Although cyclosporine has no genotoxic effect and no DNA-binding activity, an in vitro and in vivo experiment demonstrated that cyclosporine might directly promote tumor growth by a nonimmune mechanism via transforming growth factor-beta (TGF-β) receptors on the tumor itself [58]. Furthermore, cyclosporine is able to inhibit repair of UV-induced DNA damage and UV-induced apoptosis in human keratinocytes cell cultures [59].

Tacrolimus has been shown to increase the number of pulmonary metastases in both immunocompetent and immunodeficient mice, with an associated significant increased expression of TGF-β [60]. Conversely, tacrolimus was found to inhibit intercellular adhesion molecules and therefore prevent angioinvasion in cell culture, an effect that might limit tumor invasion and dissemination [61].

Rapamycin has a negative effect on malignant growth. In several animal models rapamycin is able to inhibit primary and metastatic tumor growth via cell-cycle arrest and reduced expression of vascular endothelial growth factor and TGF-β.

Even if some general trends are emerging, such as a lower risk of skin cancer for patients on the more recent calcineurin inhibitor tacrolimus compared to cyclosporine, large studies are warranted to evaluate the influence of the new immunosuppressive agents on the development and progression of SCC [38].

Despite remarkable differences in SCC incidence among heart, kidney, and liver transplant recipients, the type of organ transplanted is not likely to represent an independent risk factor for SCC occurrence. The increased incidence of SCC in heart compared to kidney recipients described in most studies is related to both older age at transplantation and higher level of immunosuppressive maintenance therapy [62, 63]. Liver transplant recipients, usually receiving a lower immunosuppressive load, show a less pronounced increase in risk of SCC compared with other organ transplant recipients. In our single-centre experience at Padua on 1,975 patients, the cumulative incidence of invasive SCC in liver ($n = 598$), kidney ($n = 812$) and heart ($n = 565$) transplant recipients is 1%, 2.5%, and 5% after 5 years and 4%, 5%, and 13% after 10 years post transplantation, respectively.

The role of the primary disease leading to organ failure before transplantation on the SCC occurrence has not been extensively investigated. There is some evidence that diabetic nephropathy might be independently associated with a lower risk of skin cancer compared with other types of pretransplant diseases leading to end-stage renal failure [35, 38]. In contrast, patients with polycystic kidney disease show a significantly higher risk of skin cancer development compared with patients with other pretransplant diseases [35, 38]. Patients with cholestatic liver disease and cirrhosis (and in particular primary sclerosing cholangitis [64]) have an increased risk of skin cancer [35].

Clinical and Histological Picture of SCC in OTRs

There are several histological subtypes of SCC, with the generic or conventional subtype being the most common.

Generic

Most SCCs belong to the generic or conventional subtype. Clinically, they are characterized by scaling, erythematous skin plaques that initially can be confused with a chronic inflammatory dermatitis (Figs. 1, 2). Lesions of SCCs are indurated and

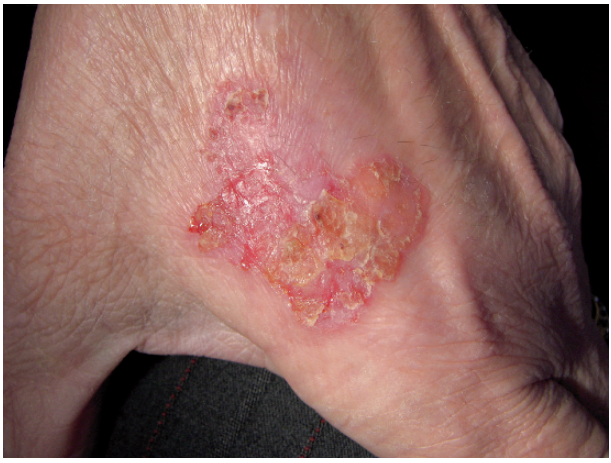

Fig. 1 Scaling, erythematous skin plaque growing on the dorsum of the left hand of a heart transplant recipient

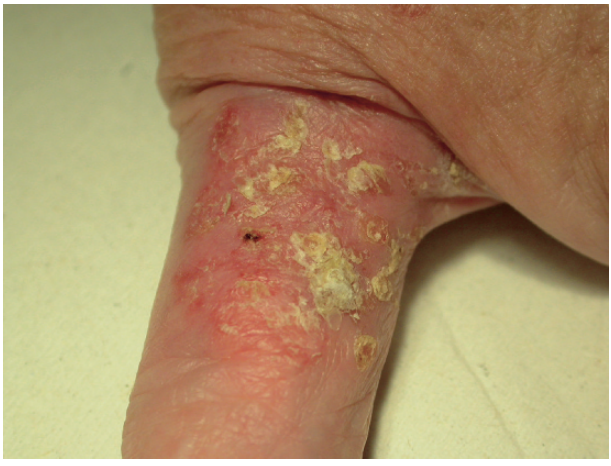

Fig. 2 Scaling plaque overlying a erythematous surface on the first finger of the left hand of a 56-year-old kidney transplant recipient

in immunocompetent patients have the characteristic of a slow-growing tumor that is painless and sometimes may be nodular [65]. The tumors can grow very rapidly in OTRs, forming exophytic lesions that tend to ulcerate (Fig. 3). Transplant SCCs are more commonly multiple and are often associated with multiple warts and premalignant keratoses. SCCs are often associated with signs of solar damage, notably elastosis, irregular pigmentation, teleangectasia, and leukokeratosis of the lower lip (Fig. 4). However, transplant patients tend to be younger at the time of diagnosis if

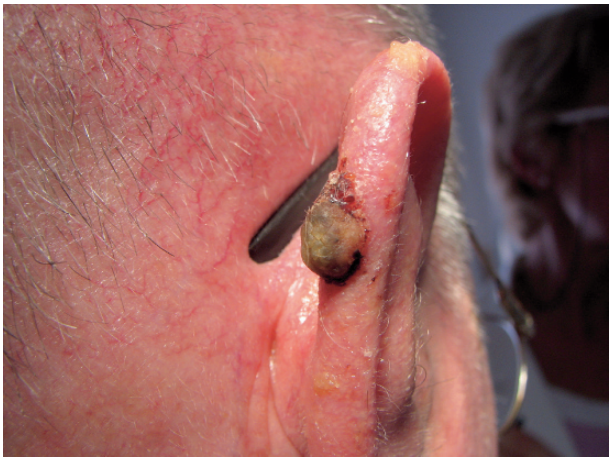

Fig. 3 A 1-cm nodule on the ear of a 67-year-old heart transplant recipient

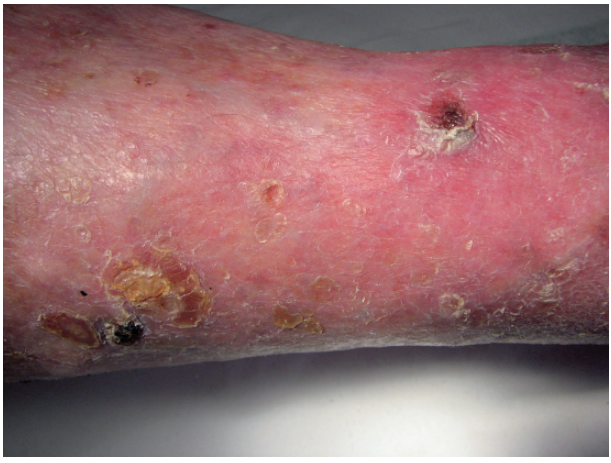

Fig. 4 A diffuse field cancerization with several squamous cell carcinomas in a lower limb of a 71-year-old heart transplant recipient

compared with immunocompetent individuals and therefore have generally less sun damage [66–71]. They can also be observed in association with other variants of SCC, notably Bowen's disease and keratoacanthoma.

The most common locations for developing SCC in transplant patients are sun-exposed sites, in particular head and neck, followed by upper extremity, trunk, and lower extremity; however, there is an increased incidence of lower trunk and upper extremity of SCCs as compared to immunocompetent patients (Fig. 5). The most common sites of SCC in the head and neck region are the scalp and the ear (Fig. 6).

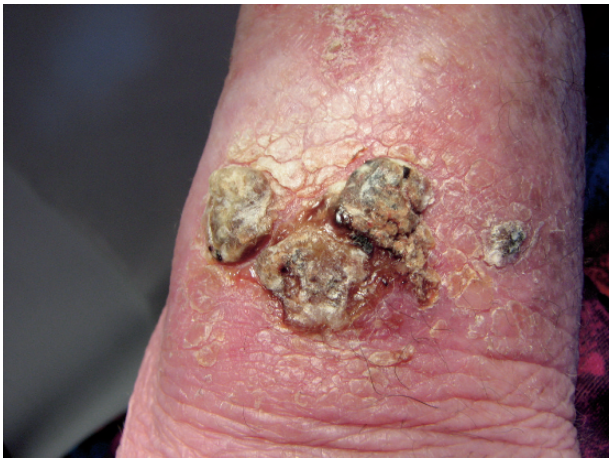

Fig. 5 A wide, hyperkeratotic squamous cell carcinoma on the upper limb of a renal transplant recipient

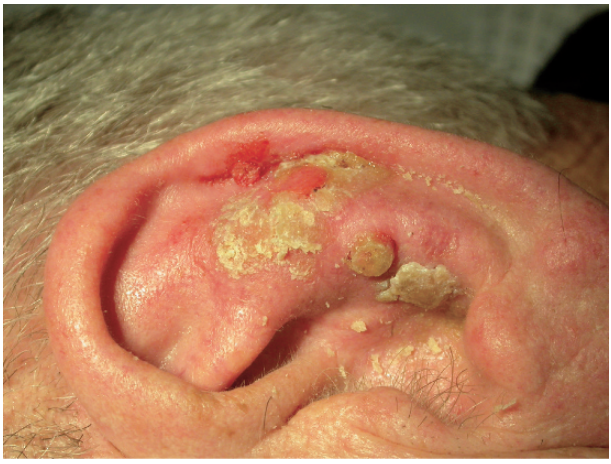

Fig. 6 Several nodules on the ear of a 62-year-old heart transplant recipient, developed in a 3-month period

In particular, on the scalp, SCCs in OTR are often associated with widespread severe actinic field damage. With regard to sex distribution, the most common sites are the head and neck for males and the trunk for females. SCCs of the ear and scalp are not generally observed in female patients, probably because of the protection of their hair, and this underlines the role of UV radiation in the development of this neoplasm [72, 73].

Clinical characteristics of SCCs at risk for invasive growth, recurrence, or metastasis in OTRs include multiple SCCs, tumor size more than 2 cm, indistinct clinical

borders, rapid growth, ulceration, and location on central face, eyelids, eyebrows, periorbital area, nose, lips, chin, mandible, preauricular and postauricular areas, temple and ear, genitalia, and digits [74, 75].

In the great majority of SCC developed in OTRs the histological characteristics are consistent with those observed in SCC of immunocompetent patients. Atypical keratinocytes develop within the epidermis and, subsequently, invade the dermal compartment. Tumor cells show enlarged, hyperchromatic, variably pleomorphic nuclei. Atypical mitoses are generally observed. The tumors tend to be associated with evidence of keratinization, which is responsible for keratin pearl formation. Intercellular bridges are commonly seen [76]. It has been suggested that the amount of atypia of SCC in OTRs is greater than in lesions from patients without a history of transplantation; however, a subsequent investigation has demonstrated that poorly differentiated SCCs are not more common in OTRs, compared with immunocompetent patients. Acantholytic changes, early dermal invasion, and an increased depth have been also demonstrated in SCCs of OTR if compared with immunocompetent patients. Verrucous features and the presence of invasive epidermal inclusion cyst-like nests are described more frequently in OTRs than in immunocompetent patients. Multinucleated giant cells are also more common in lesions from immunosuppressed patients than in immunocompetent patients [77–79].

Immunohistochemically, these tumors demonstrate positive antibody reactivity for cytokeratins. Vimentin antibody immunoreactivity may be observed in tumors that are poorly differentiated. A histological variant with a significant vascular component has been observed in OTRs [78]. Peritumoral inflammation is less intense in OTRs, and this may be responsible for the aggressiveness [79]. The presence of koilocytotosis in SCCs of OTRs was not observed more frequently than in lesions of immunocompetent patients. However, in another study koilocytosis associated with at least another epidermal feature suggestive of HPV infection (symmetry, central pointing, verrucous architecture, hyperkeratosis, parakeratosis, ectatic dermal capillaries, hypergranulosis) was more frequently observed in OTRs than in nontransplanted patients [79].

De Novo Squamous Cell Carcinoma

Squamous cell carcinoma arising without any definitive evidence of a precursor lesion or insult is denominated de novo SCC [80, 81]. In our experience, de novo SCCs occur in OTRs as well as in immunocompetent patients on either sun-damaged or sun-protected skin (Fig. 7). The lesions usually appear as a nodule or an elevated, infiltrated, and erythematous plaque with hyperkeratotic crusting and/ or ulceration. The tumor size varies from 1 to 5 cm. Histologically, the epidermis displays irregular acanthosis with variable hyperkeratosis and parakeratosis. Centrally, the lesion may be atrophic and /or ulcerated. In this tumor, strands of neoplastic squamous cells invade the dermal compartment in the absence of a clearly demonstrable precursor lesion [80, 81].

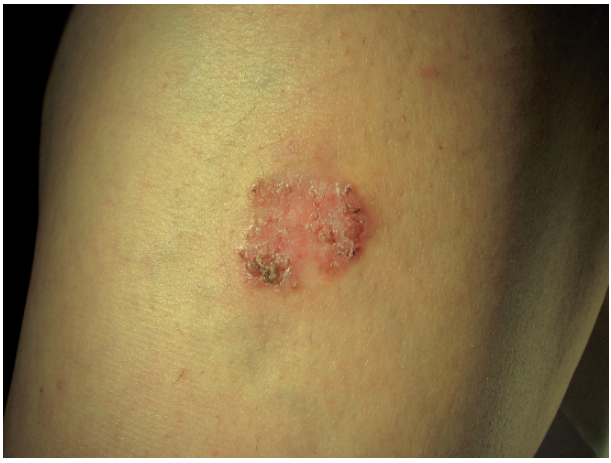

Fig. 7 Scaling and erythematous plaque on a lower limb of a 46-year-old kidney transplant recipient. The surrounding area is lacking any signs of actinic damage

Keratoacanthoma

Keratoacanthoma (KA) is considered a clinically distinct variant of well-differentiated SCC [82, 83]. KA occurring in immunosuppressed patients behaves more aggressively, and it should be considered a distinctive type of low-grade SCC [80, 81]. Clinically, KA is typically characterized by a very rapid enlargement over 1–2 months, followed by a stable period and then a slow regression over 3–6 months. KA usually presents as a solitary skin-coloured nodule, 1–2 cm in diameter, with a smooth crater and a central keratin plug on sun-exposed areas (Fig. 8).

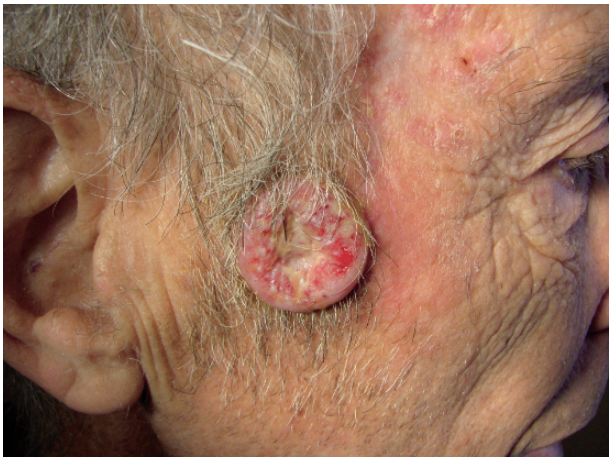

Fig. 8 A keratoacanthoma on the face of a 70-year-old heart transplant patient. The nodule developed in a 2-month period

Histologically, KAs are characterized by a proliferation of mature-appearing squamous cells showing both exophytic and endophytic growth with a typical cup shape. The lesion shows a central keratin crater enclosed by well-defined lips of epithelium growing down around the crater. Cytologically, the lesion is composed of large keratinocytes characterized by a pale and eosinophilic staining, and often a glassy cytoplasm. Squamous whorls formed by the cells with the glassy cytoplasm are commonly observed and show central foci of keratinization. Inflammatory infiltrates are also present and are composed of lymphocytes, histiocytes, plasma cells, and eosinophils associated with numerous neutrophils that focally form microabscesses. The cells of KA are superficially infiltrative and mitotically active, at least in early lesions, but generally lack the degree of cytological atypia observed in SCC. Furthermore, atypical mitoses are not found in KA [82, 83].

Bowen's Disease

Bowen's disease is considered by most dermatopathologists to be a squamous cell carcinoma in situ. Bowen's disease typically represents a slow-growing tumor that arises in sun-damaged skin. Clinically, it appears as a well-demarcated, slow-growing, erythematous scaly patch with frequent hyperkeratosis and crusting measuring a few millimeters to centimeters in diameter [84] (Figs. 9, 10). OTRs show a greater incidence of Bowen's disease with carcinoma than immunocompetent patients. Data from our institution and studies reported by other groups indicate that Bowen's disease represents from 20% to 25% of overall skin cancers in OTRs [7, 31]. The cumulative incidence is 1% and 4% after 5 and 10 years post transplantation, respectively. Bowen's disease in OTRs often occurs multifocally and carries the risk for progression into invasive and potentially fatal squamous cell carcinoma.

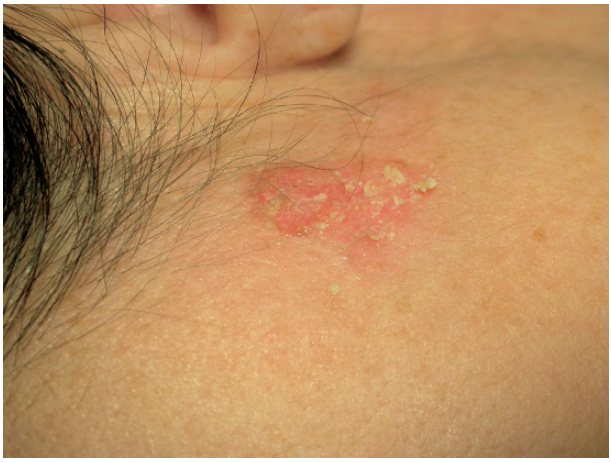

Fig. 9 Bowen's disease on the face of a 41-year-old kidney transplant patient

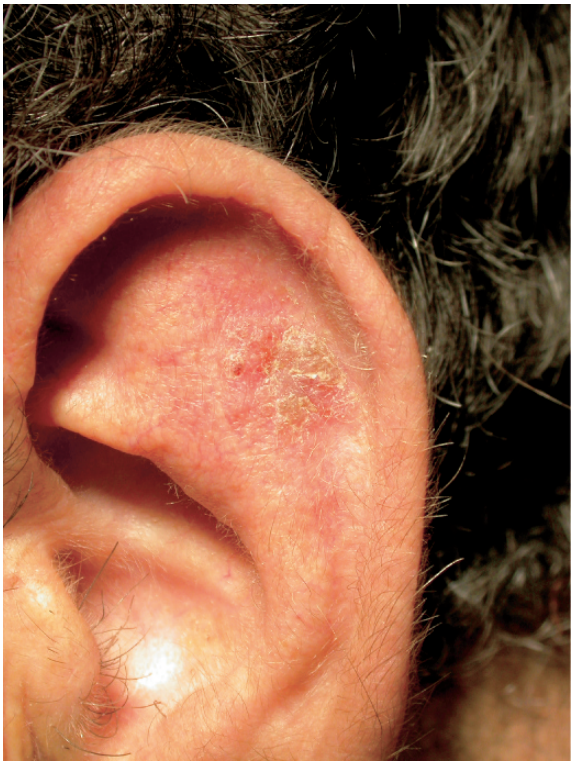

Fig. 10 Bowen's disease on the ear of a 68-year-old liver transplant patient

The most common site is the head and neck, followed by the lower limbs, upper limbs, and trunk. The locations of these lesions are supportive of the role of UV radiation in their etiology.

Histologically, the epidermis shows hyperkeratosis, parakeratosis, and acanthosis. The keratinocyte atypia is full thickness, and the neoplastic cells demonstrate loss of polarity and enlarged and hyperchromatic nuclei. Atypical mitoses are commonly observed. The follicular epithelium is frequently involved. When the neoplastic keratinocytes invade the dermis, the term bowenoid squamous cell carcinoma is used for this subtype of SCC [80, 81].

Spindle Cell Squamous Cell Carcinoma

The spindle cell subtype of SCC (SSCC) is a rare variant characterized by spindle cells infiltrating the underlying connective [76]. SSCC is considered an aggressive neoplasm. Compared to conventional SCCs, SSCCs exhibit less differentiation and higher grades of atypia. They tend to arise on the face and other sun-exposed areas and are more frequently observed in OTRs than in immunocompetent

patients [79]. These tumors may present as an exophytic mass and frequently show ulceration [85].

Histologically, SSCC is characterized by whorls of atypical squamous cells infiltrating the surrounding connective tissue. The squamous cells assume oval-to-spindled shapes and sometimes may exhibit signs of keratinization. The neoplastic squamous cells are characterized by hyperchromatic eosinophilic cytoplasm and elongated, pleomorphic, and vesicular nuclei with multiple nucleoli. Numerous mitotic figures are present. Immunohistochemistry may be useful in characterizing these neoplasms as SSCC stains positively with antibodies to cytokeratins, epithelial membrane antigen, and vimentin [86].

Aggressiveness

It is well documented that transplant-associated SCCs tend to be more invasive and more aggressive than tumors arising in nonimmunosuppressed persons, with higher frequencies of multiple and metastatic malignancies. Such tumors often grow rapidly and are often much larger and more aggressive than typical lesions, making metastatic disease a major cause of death in OTRs. They tend to recur locally in about 13% of adult transplant patients, generally during the first 6 months after excision, and the risk of metastasis from SCC in transplant recipients is approximately 7%, usually during the second year after excision (the metastatic rate in the general population is ∼2%) [87].

An unfavorable prognosis is associated with the presence of multiple tumors, a cephalic location, the presence of extracutaneous tumors, older age, and high exposure to sun (e.g., in outdoor workers). Sites particularly associated with elevated risk for recurrence or metastases include ear, lip/perioral, nose, periorbital, genitalia, and, in particular, the scalp (Fig. 11). Possible causes for the higher risk of metastatic disease in the scalp include the high vascularization of this area, the widespread severe actinic field damage, and the complex lymphatic drainage irroration [71]. Histological features of aggressive tumors include poor differentiation, a tumor thickness of more than 5 mm, and invasion of underlying tissue (hypodermis, nerves, cartilage, muscle, and bone). Patients with metastatic disease have a poor prognosis, with mortality rates ranging from 13% to 46% over a 2- to 4-year time period [88–90]. Martinez et al. also showed that the mean time from transplantation to the first metastasis was 10.7 years, but that metastases developed quickly after the diagnosis of the primary neoplasm, in a mean of only 1.4 years [89].

The primary site of metastasis of skin cancer in OTRs is the lymph nodes, as it is in immunocompetent patients [88]. Therefore, regional nodes should routinely be palpated in all patients with a history of SCCs. The head and neck region is the predominant sites of metastatic disease in the immunosuppressed population, as they are in immunocompetent patients [91].

A careful inspection of the skin surrounding the primary tumor is recommended as in-transit metastases tend to occur after high-risk SCC more frequently in OTRs

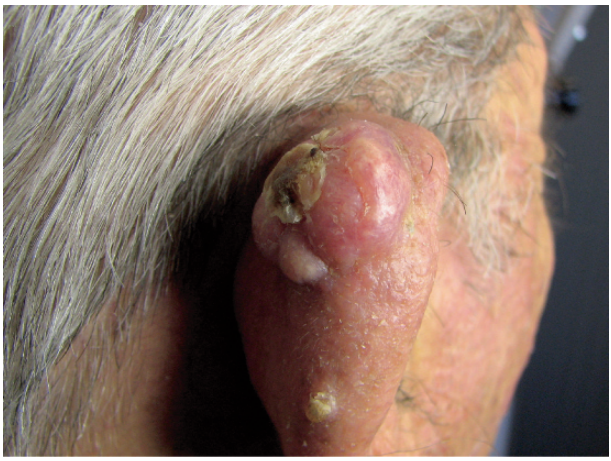

Fig. 11 A squamous cell carcinoma on the ear of a 72-year-old heart transplant patient. The nodule recurred within 1 month after a surgical excision

than in patients who have not received transplants (about 26% of OTRs) [92]. In-transit metastases have been defined by Carucci et al. as foci of cutaneous SCC originating within dermal or subcutaneous tissue clinically distinct from the primary tumor and occurring before the first echelon of regional lymph nodes [92]. They are generally gray to flesh-coloured subcutaneous papules measuring 2–6 mm in diameter with a mean distance from primary tumor or scar to metastatic lesion of 2.5 cm. In-transit metastases are generally associated with SCCs of the forehead and scalp and are probably the result of spread along lymphatic vessels. In-transit metastases are associated with a poor prognosis (33% mortality at 2 years), and it appears that immunosuppression predisposes to poor outcome with in-transit metastatic SCC. Systemic metastases are less common and are associated with a significantly worse prognosis. Lungs and bone are preferential metastatic sites for SCCs developed in OTRs [92, 93]. The overall 3- and 5-year disease survival for OTRs with metastatic SCC is 56% and 34%, respectively. This survival rate is higher than expected, given that 5-year survival for metastatic squamous cell carcinoma in immunocompetent patients is approximately 25% [94].

Decreasing immunosuppression after metastasis did not appear to have a clear beneficial effect in OTRs, although it is well documented that a less intense immunosuppression regimen may lower the incidence of cutaneous and internal malignancy. Therefore, minimizing the use of immunosuppressants should be considered in patients who tend to develop multiple SCCs as well as in those with metastatic disease [32, 51].

Patients with in-transit or regional nodal metastases have a better prognosis than those with distant nodal or systemic metastasis [92, 93]. Skin cancer metastatic to regional nodes, if limited, is a potentially curable disease. This fact supports the need for close follow-up of regional lymph nodes to detect metastasis at a manageable stage.

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Basal Cell Carcinoma

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Basal cell carcinoma (BCC) is a cutaneous, locally invasive, epidermal malignant tumor generally considered to originate from pluripotential immature cells of the epidermis and thus able to differentiate towards any of the epithelial skin structures. It is composed of cells similar to those in the basal layer of the epidermis and its appendages. Even though this tumor metastasizes only exceptionally [1], it causes considerable morbidity and costs to health services [2].

BCC in Organ Transplant Recipients

There is a paucity of reliable evidence in relationship to the incidence and risk factors of post-transplant BCCs for several reasons. First of all, most cancer registries either do not record BCCs or they are classified, along with squamous cell carcinomas (SCC), under the collective heading of nonmelanoma skin cancer (NMSC) [3,4] Moreover, many registries only enter the first NMSC for an individual when it is well known that post-transplant individuals often develop multiple skin cancers [5, 6]. Finally, some of these tumors, mainly those belonging to the superficial subtypes, are treated by family physicians with nonsurgical methods (cryotherapy, topical cytostatics, immune modifiers) without previous histopathological confirmation. As a consequence, data concerning epidemiology and specific risk factors of BCCs in organ transplant recipients (OTRs) are, in some ways, scarce and confusing. On the other hand, regarding the clinicopathological features of BCCs, only two studies specifically comparing BCCs in OTRs and the general population have been reported [7, 8].

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Epidemiological Aspects

BCC is by far the most common cancer worldwide in white-skinned populations and accounts for approximately 75% of all NMSC [9, 10]. However, its absolute incidence is difficult to determine because of both the marked geographic variability and the fact that tumor registries rarely record information on BCCs accurately.

In north Queensland, Australia, considered to be the geographic area with the highest rate of BCC in the world, the incidence rates were 2,058 per 100,000 inhabitants for men and 1,194 for women [11], while in the United States a lower incidence has been estimated, with a rate of up to 407 cases of BCC per 100,000 white men and 212 cases per 100,000 white women [12]. In Germany, the age-standardized incidences were 80.8 and 63.3 in 100,000 men and women, respectively [13].

OTRs are at increased risk for developing various cancers, including BCC. The latter, along with squamous cell carcinomas, account for more than 90% of all skin cancers in OTRs; therefore, skin cancer represents the most common malignancy among transplant-associated neoplastic diseases [5, 14], the risk being higher for heart transplant recipients, followed by renal and liver transplant recipients [15–17]. The risk of developing NMSC in this population of immunosuppressed individuals, when compared with the general population, has been reported to be increased up to 10- to 16 fold for BCCs [18, 19] and to 65- to 250 fold for SCCs [4, 17, 18]. As happens with SCCs, the incidence of BCCs rises with time after transplantation, but the risk seems to increase linearly for BCCs and exponentially for SCCs [20]. In this sense, in Spain the cumulative incidence of BCCs in kidney transplant recipients within 5 years post transplant was 14%, increasing to 40.6% after 10 years, while for the same period the cumulative incidence of SCC was 10% and 33%, respectively [21]. Other series have also shown an increased cumulative incidence of BCC, with a more or less linear tendency, with the passing of time after transplantation, irrespective of the organ transplanted [22–24].

Accordingly, the BCC/SCC ratio, usually considered to vary between 3:1 to 7:1 in the general population from different latitudes [25–27], has been reported in most studies to be reversed in OTRs [15, 20, 22, 23, 28–31]. However, in some Mediterranean countries the reversed ratio is less evident, both in renal and heart transplant recipients [21,30,32], and BCCs reportedly outnumber SCCs during the first 3 years after transplantation [30, 33].

The reason for this geographic variation remains under investigation but likely results from different genetic backgrounds, skin types, and sun-exposure habits at different latitudes. For as yet unknown reasons the BCC/SCC ratio appears to be higher in heart and liver than kidney transplant recipients [15, 24, 30, 32, 34, 35].

The mean time interval from transplant to BCC diagnosis varies broadly, depending on the mean follow-up considered, but is in general shorter than for SCC [15, 21, 23, 32, 36] as well as for heart compared to kidney transplant recipients [7,36], probably because of the older age and greater immunosuppression of patients receiving heart transplants.

Little is known about the occurrence of post-transplant skin tumors during childhood, but these seem to be extremely rare [37]; in three studies focused on skin diseases in children with organ transplants, no BCCs were observed [38–40].

Clinical and Pathological Aspects

Clinical Features

As in the general population, the clinical presentation of BCCs in OTRs varies considerably. Early tumors usually begin as small, translucent or pearly papules or nodules covered by thin epidermis with telangiectases, but other forms of presentation can be seen, such as a superficial ulcer resembling an excoriation by a fingernail or a small erythematous, lichenoid, and keratotic papule or plaque. In more advanced tumors, mainly three different clinical subtypes of BCCs exist.

Nodular basal cell carcinoma is the classic form, which most often presents as a pearly papule or nodule with overlaying telangiectases and a rolled border, at times exhibiting central crusting or ulceration (Fig. 1A). It may occasionally contain melanin, imparting a brown, blue, or black color to the lesion, which makes it sometimes difficult to differentiate from malignant melanoma (Fig. 1B). Occasionally, nodular BCCs may resemble enlarged pores or pits on the sebaceous skin of the central portion of the face.

Superficial basal cell carcinoma presents as a psoriasiform scaly erythematous patch or plaque with an atrophic epidermis covering the central zone and usually with small areas of superficial ulceration and crusts (Fig. 1C). As with the nodular subtype, it may contain small pigmented areas. *Morphea-like (morpheiform)* or *sclerodermiform basal cell carcinoma* typically appears as a flat or slightly depressed, pale, sclerotic, and indurated plaque with a slightly shiny surface and with clinically indistinct margins (Fig. 1D). In this subtype, ulceration and crusting are very uncommon.

The majority of BCCs are found on sun-exposed body areas, mainly the head and neck (80% of cases), with a particular predilection for the upper central part of the face, followed by the trunk [41]. However, the superficial type is found mainly on the trunk and the sclerodermiform type almost exclusively on the face.

There are very few studies comparing the clinicopathological features of BCCs occurring in OTRs with those appearing in immunocompetent individuals [7, 8]. From the clinical point of view, despite the fact that BCCs in OTRs follow the same trends as in the general population, some remarkable differences can be observed regarding age at diagnosis, anatomic distribution, and number of tumors. In two comparative case-series reports [7, 8] it was shown that BCCs develop in OTRs at a significantly younger age (15 years earlier) than in the general population, this trend being more pronounced for kidney than heart transplant recipients [7]. Differences were also found between BCCs in controls and OTRs regarding anatomic distribution, the proportion of BCCs developing in extracephalic locations being significantly higher in OTRs [37.5% vs. 24.5% [7]; 35% vs. 19% [8]], even though

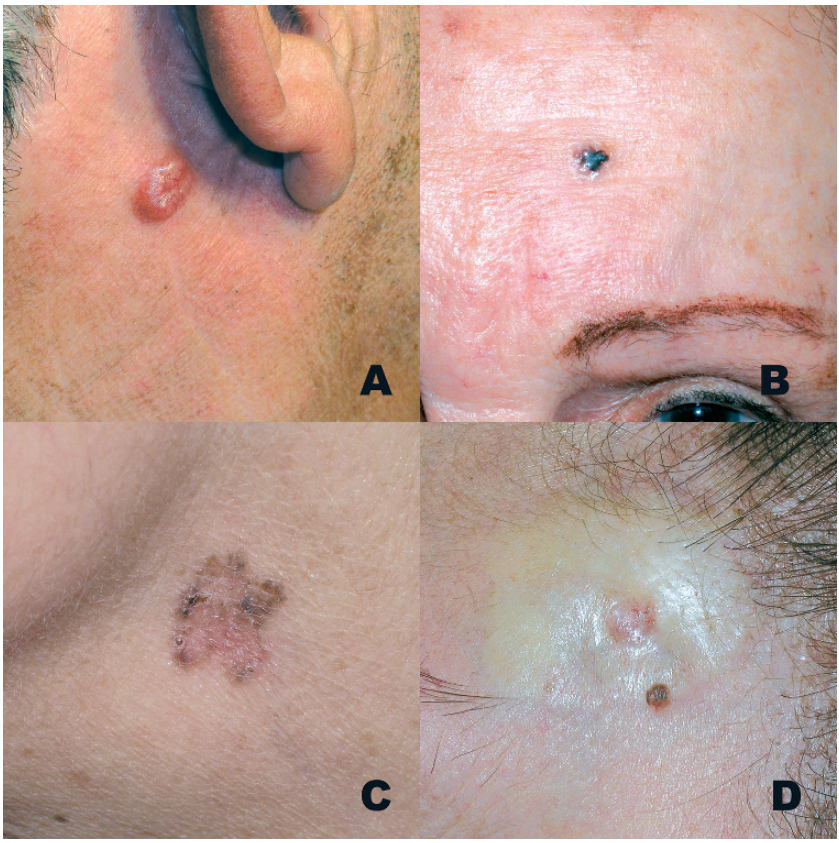

Fig. 1 Different clinical types of basal cell carcinoma. (**A**) Nodular basal cell carcinoma. (**B**) Pigmented nodular basal cell carcinoma. (**C**) Superficial basal cell carcinoma. (**D**) Morpheiform basal cell carcinoma

in OTRs the head and neck were still the most frequent locations of the tumors. BCCs on sun-protected sites such as genitalia, hand, or axilla were seen only in OTRs [7], although BCCs located in unusual sites, including the axilla, breasts, perianal area, genitalia, palms, and soles have also been reported in the general population [41].

Post-transplant BCCs are more commonly multiple than in immunocompetent patients. In a study over a 36-month period, 43% of OTRs had multiple BCCs whereas these occurred only in 21% [8] of immunocompetent patients.

Histopathological Features

The histopathological features of BCC show considerable variability. Based on its growth pattern, different subtypes have been recognized. The four major patterns

include nodular, multifocal superficial, micronodular, and morpheiform, although mixed patterns and other uncommon subtypes such as basosquamous, keratotic, granular cell, adamantinoid, clear cell, and basal cell carcinoma with matrical differentiation have also been described [41]. In a review of 1,039 consecutive cases in a nontransplant population, it was found that the most common subtypes were mixed (38.6%), followed by nodular (21%), superficial (17.4%), and micronodular (14.5%) [42].

All these forms share some characteristics: They are composed of irregular dermal masses of basaloid cells of variable size and shape surrounded by a peripheral layer of tumor cells with palisading nuclei. These tumor islands are surrounded by a stroma containing variable amounts of mucopolysaccharides. The individual tumor cells are usually uniform in appearance, with a hyperchromatic nucleus and a relatively small, poorly defined cytoplasm. Mitotic figures can be numerous and sometimes atypical. Most cases show connection with the epidermis, which can be ulcerated. In aggressive tumors, deep extension to the lower dermis occurs. More rare is the involvement of the subcutis, as well as the underlying cartilage in those located on the nose and ear. Perineural invasion is sometimes present and is considered a marker of aggressiveness. Calcification may be present in the center of keratin cysts that are seen in several histological subtypes. Most BCCs elicit a round cell inflammatory reaction of some degree, mainly composed of T cells, the majority of which are CD4+ [43].

Some differences exist among the different subtypes. *Nodular basal cell carcinoma* is characterized by rounded masses of tumor cells with well-defined peripheral contours and palisading of the cells at the periphery and a haphazard arrangement of the more central cells (Fig. 2A). Retraction spaces around the tumor islands separating them from the stroma are often seen. *Micronodular basal cell carcinoma* is quite similar to the nodular type, but the tumor islands are smaller and the peripheral palisading is not always obvious (Fig. 2B). It is considered a more aggressive variant than the nodular type, with a greater trend for local recurrences [44]. In *morpheiform or sclerosing basal cell carcinoma* the tumor is composed of narrow, elongated strands and small islands of tumor cells with an irregular outline that are embedded in a abundant, dense, fibrous stroma (Fig. 2C). Peripheral palisading is absent or poorly developed. In *superficial multifocal basal cell carcinoma* the tumor is composed of one or multiple small buds of basaloid cells, with peripheral palisading hanging from the undersurface of the epidermis and usually limited to the papillary dermis (Fig. 2D).

When compared with controls, the general histopathological features of BCCs in OTRs appear similar, except for the density of the peritumoral inflammatory cell infiltrate, which is significantly lower in OTRs than in controls, probably as a result of immunosuppressive treatment [7, 8]. In addition, squamous differentiation, basal layer dysplasia of contiguous epidermis, and associated viral changes were found more frequently in OTR BCCs in one study [8].

Regarding growth patterns, a nodular pattern was the most common in both groups, but a predominance of a micronodular and morpheiform patterns were seen less commonly in the OTR BCC group, whereas the superficial pattern was more

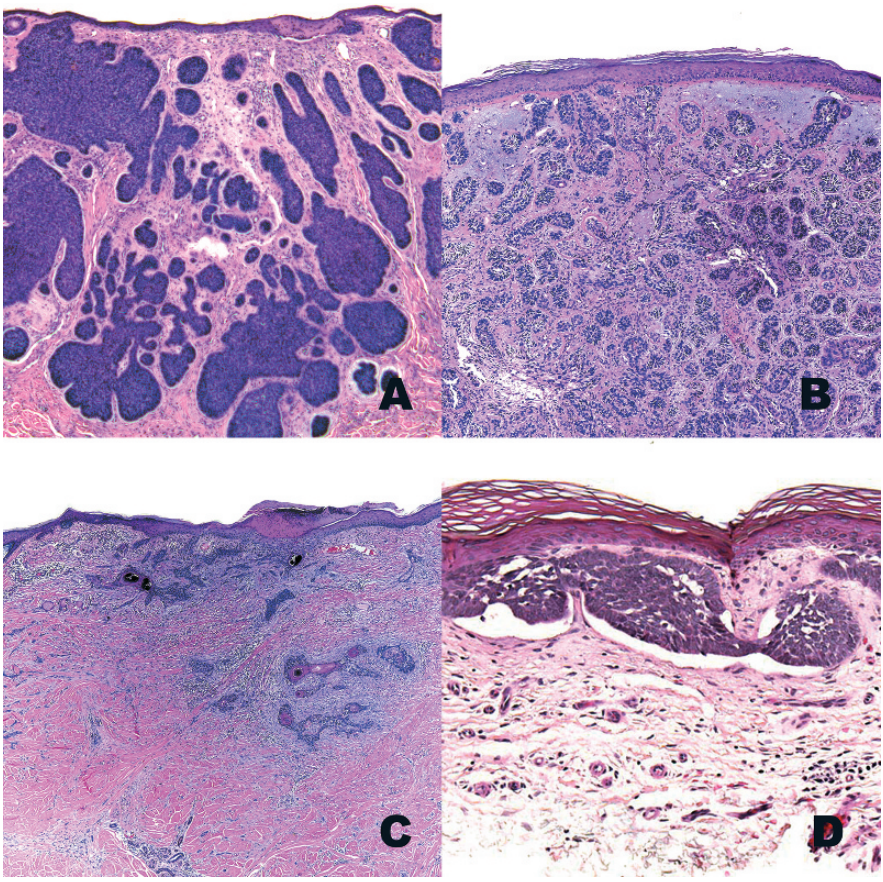

Fig. 2 Histopathological types of basal cell carcinoma. (**A**) Nodular type showing well defined round masses of tumoral cells with palisading of the cells at the periphery. (**B**) Micronodular type where the tumor islands are smaller and the peripheral palisading is not always well defined. (**C**) Morpheiform type; note the narrow, elongated strands and small islands of tumor cells embedded in a abundant, dense, fibrous stroma. (**D**) Superficial type characterized by multiple small buds of basaloid cells, with peripheral palisading hanging from the undersurface of the epidermis

frequent in transplant patients [7, 8], a fact also reported in other studies evaluating BCCs from immunosuppressed individuals including human immunodeficiency virus (HIV), chronic renal failure, and lymphoproliferative diseases [45–48].

Unexpectedly, it seems that BCCs in OTRs show overall fewer histological features of aggressiveness than controls as evaluated by the type of growth pattern (lower proportion of micronodular and morpheiform histological types), tumor thickness, presence of ulceration, and density of peritumoral infiltrate [7, 8], which is in agreement with the clinical behavior of these tumors in OTRs.

Different factors could explain the predominance of superficial BCCs in OTRs as compared with controls. First of all, this relative predominance is probably related to

the earlier age at presentation of BCCs in OTRs, as a similar trend occurs in BCCs appearing in the general population [49]; second, the closer surveillance of these patients could lead to an early diagnosis and intervention before progression into the invasive stage occurs. However, it is also possible that the overrepresentation of superficial lesions may indicate pathogenic differences between BCC arising in the setting of immunosuppression, as similar trends have been reported in some studies evaluating BCCs from other immunosuppressed populations, including HIV, chronic renal failure, and lymphoproliferative disease [45–48].

In summary, apart from an increased incidence, BCCs in OTRs show some clinicopathological differences from their ordinary counterparts, namely, a younger age at development, higher incidence of multiple tumors, male preponderance, less prevalence on the head and neck, more frequent distribution in extracephalic sites, and higher frequency of superficial subtypes [7, 8].

Clinical Course

In contrast to SCCs, the clinical behavior of BCCs in OTRs is similar to the BCC in the population at large, with a similar rate of recurrences after prolonged follow-up [8]. This finding is in agreement with the lower proportion of micronodular and morpheiform histological BCC types found in OTRs than in the general population as it is considered that these BCC subtypes have a worse prognosis than other BCC subtypes [42, 50].

Risk Factors

The relative contribution of constitutional and environmental risk factors in the development of BCCs in OTRs remains controversial.

In addition to the established risk factors known to play a role in the development of BCCs in the general population [41], other specific factors in OTRs such as age at transplantation, immunosuppression, and possibly human papilloma virus infection might play a role in this population.

Ultraviolet Radiation

As in the general population, UV radiation acting both as a mutagen and an immunosuppressant is probably one of the most important risk factors for NMSC in OTRs. In fact, different factors support an important role for actinic radiation. First, the highest cumulative incidence of NMSC (BCC and SCC) in OTRs is observed in countries with high sun exposure habits such as Australia [6, 22] and Spain [21, 30] and, among the OTR population of these countries, the cumulative incidence is especially high among those with a high occupational and recreational sun exposure

[21, 30]. In a population of kidney recipients from a Mediterranean area of Spain at 10 years post transplantation, in those subjects considered to have had a high occupational sun exposure before receiving the transplant, the cumulative incidence of NMSC was 85% versus 22% in the OTR population with no outdoor occupational sun exposure [21]. In another area of Spain, direct correlation between the total sun burden and the development of NMSC has also been reported [30].

Second, in spite of the higher frequency of extracephalic location for BCCs in OTRs than in the general population, most of them are located on sun-exposed skin areas [7, 8, 21, 30]. Furthermore, when comparing BCCs in OTRs and the population at large, the severity of solar elastosis in OTRs was high, comparable with that observed in immunocompetent patients [8], thus supporting a role for actinic radiation in cutaneous carcinogenesis in OTRs.

However, the precise relationship between the risk of developing BCC and the amount, timing, and pattern of exposure to UV radiation remains unclear. In the general population, a significant association between BCC development and recreational sun exposure during childhood and adolescence has been reported [51], and the risk has been shown to be more strongly associated with intermittent, intense ultraviolet exposure than with chronic cumulative exposure, considered to be associated with SCC development [52]. A similar trend has also been reported in Australian and Italian kidney and heart transplant recipients, respectively. In these studies, the OTRs born in cooler climates who had sunburn episodes during childhood were at particularly increased risk of BCC, whereas cumulative sun exposure was associated with SCC but not BCC [23, 53]. By contrast, in a cohort of 124 Spanish heart transplant recipients the predicted rate of developing BCCs among those with the highest total sun burden was highly significant [30].

Despite this, other factors are probably important, given that the proportion of BCCs developing in extracephalic locations in OTRs is significantly higher than that in controls [7]. Therefore sun exposure, although important, may play a lesser role in the development of BCC in OTRs in comparison with the general population.

Genetics

Apart from the genetic diseases known to be associated with the risk of BCC (Bazex's syndrome, Gorlin's syndrome, xeroderma pigmentosum) and the strong correlation with family history of skin cancer [51], the role played by some genetic factors such as HLA types and donor–recipient HLA mismatches in the risk of development of skin cancers in OTRs is controversial and needs to be clarified in large comparative studies [53–57]. A protective role of HLA-DR4+ against the development of BCCs has been suggested [58].

Host Factors

As in the general population [59–61], certain phenotype characteristics of OTRs, such as fair skin, blond hair, and light-color eyes, apart from influencing

responsiveness to UV radiation, have been shown to be independent risk factors, as has positive personal and/or family history of skin cancer [6, 23, 53]. Furthermore, the type of NMSC before transplantation is the strongest predictor of the type of NMSC after transplantation [53].

Age at Transplantation

No specific studies dealing with the influence of age at transplantation in the development of BCCs have been performed, but in several series from different countries the number of skin cancers was significantly correlated with both age at transplantation and duration of follow-up in heart, renal, and liver recipients [20–24, 29, 33, 36, 53]. In a study of kidney recipients it was reported that the relative risk for developing NMSC (BCC and SCC) increased by 1.06 when adding a year to the age at transplantation, implying that an increase of 10 years would double ($1.06^{10} = 1.8$) the risk [21]. In heart recipients, the risk of developing BCC was estimated to be 8.5 times higher in those receiving the transplant at the age of 59 or older than those who received the transplant before 43 years of age [23].

In addition, the age at transplantation influences the mean interval between transplantation and a diagnosis of NMSC (BCC and/or SCC), being shorter the older the patient is at transplantation [20, 36].

Immunosuppressive Treatment

Without doubt, immunosuppression is the critical feature differentiating OTRs from the general population. Two mechanisms have been proposed for the accelerated carcinogenesis associated with immunosuppression: decreased immunosurveillance, resulting in uncontrolled proliferation of abnormal cells, and a direct carcinogenic effect of some drugs, including azathioprine and cyclosporine.

The reduced immunosurveillance is likely to contribute to tumor progression in OTRs as supported by the significantly less inflammatory infiltrate reported in transplant BCCs [7, 8]. It has been hypothesized that immunosuppression could accelerate UV-ray damage given that, despite a lower mean age, solar elastosis was important and equivalent in perilesional skin of OTR BCCs compared with BCC in the general population [8].

In any event, the increased risk of nonmelanoma skin cancer with increasing duration of immunosuppression has been broadly reported [3, 17, 21, 22, 62]. Moreover, the level of immunosuppression and treatment of rejection episodes seems to be important because heart transplant recipients, who usually receive higher doses of immunosuppressants than liver and kidney recipients, are at a higher risk of developing NMSC [15–17, 22] , a finding further supported by a study showing a significantly higher incidence of skin cancer in kidney recipients receiving normal doses of cyclosporine than those receiving lower doses [63], and another study showing that

CD4 counts were significantly lower in transplant recipients with cutaneous carcinomas than in those without skin cancer [64]. However, there are few studies focused on the risk of specifically developing BCCs in OTRs related to immunosuppression. In this sense, the risk of developing BCCs was calculated to be 10 and 21 times higher in kidney and heart transplant recipients, respectively, than in the general population [65]. Nevertheless, in another study of heart recipients no relationship was found between the risk of BCC and the level of global immunosuppression [23].

The impact of different immunosuppressants regarding progression and possible induction of NMSC is still a matter of debate and is poorly studied in relationship with the development of BCCs. Some studies have shown that the use of triple regimen therapy (cyclosporine, azathiopirne, and predinosone) is linked to a greater risk for developing skin cancer than using a combination of prednisone and azathioprine only [17, 66]. On the other hand, tacrolimus-based regimens have a lower risk compared with cyclosporine-based regimens [67, 68]. In an Australian study, renal transplant recipients on cyclosporine, irrespective of other agents, had an increased risk of BCCs [53]. On the other hand, it has been recently demonstrated that sirolimus-based immunosuppressive therapy in renal transplant may protect against skin cancer [69, 70].

Human Papillomavirus Infection

The role of human papilloma virus (HPV) in the development of skin carcinomas in OTRs seems to be more important in SCCs than BCCs, since the former are frequently associated with viral warts and histopathological features of HPV infection [71]. Accordingly, in a Swedish case-control study of 249 renal transplant recipients an increased risk of NMSC in patients with warts appearing after transplantation was shown [72]. However, in other series the difference in the incidence of skin cancer in the group of patients with renal and heart transplants with viral warts was not statistically significant when compared with OTRs without viral warts [23, 33].

From a histopathological point of view, although the finding of signs of HPV infection in OTR SCC is frequent [71], the results in OTR BCCs are controversial. In this sense, two studies have yielded different results since no obvious histological signs of HPV infection were found in a series of 176 BCCs occurring in OTRs [7], while another author observed that these were present in 14 of 100 BCCs from kidney transplant recipients versus 2.4% of BCCs ($P < 0.001$) from immunocompetent patients [8].

Using sensitive molecular techniques for HPV DNA detection in BCC specimens, the results are contradictory. Although HPV DNA was found in 65% to 90% of OTR SCCs [73] in general, the percentage of HPV-positive BCCs from OTRs was low [74], quite similar to that reported in nonimmunosuppressed patients [75, 76]. Only in a small study was the prevalence of HPV-positive BCCs higher in OTRs than in a nonimmunosuppressed population [77]. In a recent study in the general

population, when stratified by HPV seropositivity, particularly beta-HPVs, the risk of developing an NMSC was significant only for SCC [78]. Therefore, based on these findings, the role of HPV in the development of BCCs in OTRs still remains unclear.

In summary, there is obviously a need for an epidemiological approach to determine what is the relative contribution of constitutional factors such as skin type, family history, past history, and environmental risk factors such as UV exposure, HPV infections, and immunosuppression that might be related to the development of BCCs in this population as well as the innate and classical immunological response.

Treatment and Prevention

Treatment

As in the general population, several different modalities, surgical and nonsurgical, are used in the treatment of BCCs in OTRs. The method chosen should mainly take into account the type of BCC and its extent.

Surgical approaches include the following. *Surgical excision* is the treatment of choice for most patients. It allows accurate diagnosis, histological examination of tumor margins to establish clearance, and assessment of the aggressiveness of the tumor. An excision margin of 4 mm around the tumor is recommended where possible [79]. *Mohs' micrographic surgery* is a specialised technique that uses horizontal frozen sectioning to examine serial sections of tissue until all margins are free of tumor. It offers the lowest 5-year recurrence rate of any treatment [80] and is strongly recommended for high-risk tumors and for locally recurrent BCCs.

Nonsurgical approaches include *curettage and cautery*, a destructive technique consisting of scraping away the lesion and cauterising bleeding points. It does not allow histological examination of tumor margins and can result in wounds that take longer to heal than excision wounds. It can be used to treat low-risk lesions (superficial, small, well-defined primary lesions on the neck, trunk, arms, and legs), but it is not appropriate for the management of recurrent, large, and morpheiform BCCs. Operator skill and appropriate choice of lesion are needed to ensure the best clearance rates (around 95%) [81]. *Cryosurgery* (liquid nitrogen at –196°C) is, as with curettage, only indicated for low-risk lesions, achieving similar cure rates [82]. However, no tissue is available for histological examination after cryosurgery. *Photodynamic therapy* (PDT) involves the topical application of a tumor-localizing photosensitizing agent (5-aminolevulinate) and further irradiation with visible light (usually in the wavelength range 620–670 nm) causing selective photodestruction of tumor cells. PDT is effective for superficial BCCs giving good healing and cosmesis, but must not be used for nodular or other types of BCCs because tumor thickness affects both uptake of photosensitiser and penetration of light source [83]. *Imiquimod 5%* is a topical immune response modifier recently licensed for the treatment of small superficial BCCs on the trunk, neck, arms, and

legs of adults with a normal immune system, achieving clearance rates ranging from 70% to 100% depending on frequency of application [84]. It appears to be relatively well tolerated, the incidence of local side effects being related to the frequency of application. More data are necessary to prove its efficacy and safety in transplant recipients [85].

Prevention

The most important steps for prevention of BCCs in OTRs are decreasing the risk factors and regular examination of the skin for the early diagnosis and treatment of skin cancers, avoiding further complications. So, any program of solid organ transplantation should provide educational information about these topics.

Because sun exposure is the main cause of skin cancer in OTRs, primary prevention should include avoidance of the sun and protection against sun exposure through the use of protective clothing and effective sunscreen for sun-exposed areas.

Secondary prevention for recipients at risk should include regular self-examination to detect early lesions and close follow-up by a dermatologist, at least once a year, to facilitate early diagnosis and treatment.

The use of systemic retinoids to prevent or delay the development of new BCCs in OTRs has not been assessed in a controlled trial, but these have been shown to be effective in the prevention of actinic keratoses and SCCs in recipients with multiple tumors [86].

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External Anogenital Premalignant and Malignant Disease

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Introduction

The external anogenital area comprises the anus, perianal skin, and the adjacent external genitalia including the vulva and vaginal introitus in the female, and the penis and scrotum in the male. Immunosuppressed organ transplant recipients (OTR) are prone to viral infections, and have an increased incidence of human papillomavirus (HPV) associated premalignant and malignant neoplasms, which specifically target the anogenital tract. The cumulative risk of the development of a solid-organ neoplasm is 5–6% [1–3]. Nearly all of these neoplasms occur on a background of premalignant disease (i.e., carcinoma in situ). The most common presentation of anogenital disease in OTR is condyloma accuminata or genital viral warts and these are regarded as a marker of immunosuppression in this group.

AGIN and Anogenital Malignancy: Association with Human Papillomavirus (HPV) Infection

Anogenital intraepithelial neoplasia (AGIN) is the collective term for pre-invasive squamous cell disease of the external anogenital tract, which has been classified using the WHO grading system where the degree of histological atypia is described as I – mild, II – moderate or III – severe (analogous to cervical intra-epithelial neoplasia [CIN]) [4]. Thus, the similar spectrum of changes seen at different sites may be referred to as vulvar intra-epithelial neoplasia (VIN), vaginal intra-epithelial neoplasia (VAIN), penile intra-epithelial neoplasia (PIN) or anal intra-epithelial neoplasia (AIN).

Most AGIN is associated with HPV infection and the natural history of AGIN in all types of OTR is to persist, recur and extend. Any anogenital skin and mucosa may be affected by disease but the cervix is a sentinel site of AGIN and less than 20% of VIN, VAIN or AIN occurs without previous cervical disease [5]. AGIN

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is usually multifocal and more extensive in OTR (Fig. 1) with lesions occurring synchronously, metachronously and sequentially. It is more likely to develop into invasive cancer in OTR even with correction for mortality from their immunosuppressive diseases. Squamous cell carcinoma (SCC) and its variants is the commonest type of neoplasm seen, and in OTR these are more often extensive, multifocal, undifferentiated, resistant to treatment, and present at a younger age compared to an immunocompetent population [6]. Since Euvrard and colleagues first reported the prevalence of external anogenital lesions in a large series of 1002 OTR in 1997, [7] there have been a number of other observational studies. In the UK a cohort study of 816 renal transplant recipients (RTR) referred for skin surveillance over a 10-year period found that 49 recipients (5.7%) had anogenital disease, of which 32 (3.7%) had non-dysplastic viral warts (Proby & Harwood, unpublished data). In the USA a 14-fold increased incidence of CIN in RTR was found [3]. In Australia a high incidence of CIN 3 was reported in 166 female lung transplant recipients giving an incidence rate of 30 per 1000 women compared with 6.2 per 1000 women screened in a large reference population [8]. In Scotland 49% of RTR ($n = 49$) were found to have CIN 3 compared with 10% controls ($n = 69$) [9]. Both AGIN and anogenital SCC are commonly associated with high risk oncogenic mucosal HPV types

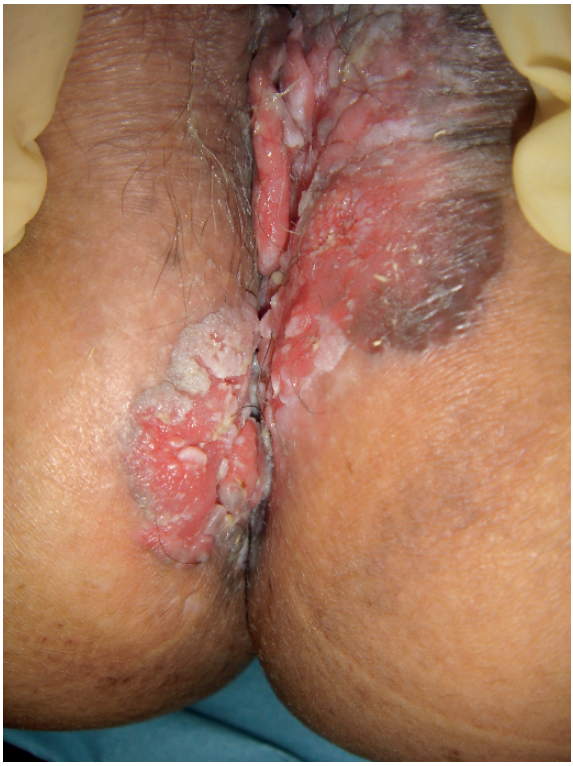

Fig. 1 Extensive vulvar and anal intra-epithelial neoplasia

(HR-HPV). For cervical disease, clinical, molecular and epidemiological investigations have combined to identify HPV as the necessary aetiological agent [10, 11]. The development of sensitive PCR methodology has demonstrated HR-HPV to be present in virtually all (99%) cases of SCC, most commonly HPV types 16, 18, 31, 33, 45 and 56 [12, 13]. A limited group of HR-HPV DNA is also found in 80–100% of biopsies of high-grade VIN and VAIN with >90% of cases of VIN involving HPV types 16, 18 or 33 [14]. Integration of HPV DNA into the host cell genome is the critical step in cervical carcinogenesis and integration of HPV-16 and HPV-18 DNA has also been demonstrated in nearly 40% of high-grade VIN with monoclonality of HPV genotype in over 80% of patients with multifocal disease [15]. Thus malignant transformation clearly needs additional genetic events, related to the genomic instability resulting in a diversity of transformed malignant clones.

Relative Risk of Anogenital Malignancy in OTR

Anogenital carcinomas are rare in the general population and until relatively recently have been neglected epidemiologically. Recent studies have suggested that VIN and vulval SCC are increasing in incidence with HPV-associated SCC occurring in younger women than previously reported. Over a 28-year period from 1973–2000 the incidence of VIN in a US cancer registry database increased by 411% and vulval cancer by 20% [16]. The current incidence of VIN is estimated to be 2.1 per 100,000 women per year and that of vulval cancer approximately 1.5–2 per 100,000 women per year [17]. The incidence of anal cancer in the general population is between 0.5 and 0.7/100,000 per year [18]. However, the estimated increased risk and incidence of anogenital malignancy in OTR compared to the general population varies in different studies. This results from the relatively small numbers of patients in reported studies along with the inclusion of other immunosuppressed groups such as HIV-positive patients and those on steroids for lupus erythematosus or sarcoid. Most epidemiological studies on anal cancer have tended to concentrate on high risk groups, e.g., HIV-positive men who have sex with men (MSM), and highlight a very high incidence of anal cancer of around 70/100,000 per year. This figure is similar to the estimated rate of 86.2/100,000 per year in liver transplant recipients [1]. However, whilst the incidence of anal cancer in renal OTR in the UK is not fully established it is thought to be significantly lower, around 14/100,000 per year [19].

Penn reported that anogenital cancer represented 2.8% of all cancers reported in 2150 RTR [20]. A study in Sweden of a cohort of 5931 OTR found a 20-fold excess risk of cancer of the vulva and vagina and a 10-fold excess risk of anal cancer [21]. Other workers have demonstrated a 30–100 fold increased incidence of anogenital cancer compared with the general population [22–26].

Characteristics of AGIN in OTR

Age. In OTR, anogenital malignancies present at a younger age with the average age for female OTR being 37 years, and for male OTR 45 years [20]. Female OTR

are more commonly affected by anogenital malignancy than males with a ratio of 2:1 and the mean interval between transplant and diagnosis is around 7 years [27]. The female:male ratio is even higher in patients who received allografts as children, in whom lesions often develop during early adulthood. Anogenital cancers are the fourth most frequent malignancy in patients who underwent transplantation in childhood [28, 29].

Time from transplantation and risk of progression. Anogenital cancer occurs at a longer time interval after renal transplantation compared to other cancers with an average of 88 months, compared with 56 months for all other post-transplant malignancies [26]. The duration of pretransplantation dialysis and uraemia in RTR is known to impair cellular immune responses and to increase the risk of skin malignancy [30, 31].

Untreated CIN 3 has a >12% risk of progression to invasive cancer in all women and the risk increases in OTR who have been immunosuppressed for more than 5 years [9]. VIN has around a 9% risk for progression in all untreated women [32]. VAIN has around a 5% risk of progression [5]. AIN of all grades can progress quickly into cancer in immunosuppressed patients (Table 1). PIN is the rarest type of AGIN but still carries a 36-fold increased risk of malignant transformation in HIV-positive men and transplant recipients [34–36].

HPV infection. The prevalence of HPV infection is high in transplant recipients prior to the transplantation and it further increases after iatrogenic immunosuppression is initiated. Whilst most AGIN in OTR is associated with HPV 16 or HPV 18, mixed infections that include low-grade subtypes 6 and 11 also occur [14]. Using highly sensitive, degenerate nested PCR technique mixed HPV infection with both alpha – (mucosal) and beta – (EV) papillomaviruses has been found in around 20% of patients, most of whom were immunosuppressed [37]. The detection of beta papillomavirus DNA in normal skin and plucked hair complicates the interpretation of this. Plucked anogenital hairs from 51 immunocompetent males contained β-HPV in over 25% of samples [38] and β-HPV DNA has been found in 92% of non-genital hair samples from one or more sites in a group of 26 RTR compared with 53% in 22 healthy volunteers [39].

The prevalence of anal HPV infection in the normal immunocompetent population has not been well studied. Koutsky et al. reported a prevalence of 15% for genital HPV infection in women [40]. In one series of OTR, the prevalence of previous anal HPV infection immediately prior to immunosuppression was shown to be 23% (14/60). Fifteen percent (9/60) were positive for high-risk HPV and 13% (8/60) for low-risk HPV [1]. An earlier case-controlled study showed 20% of RTR

Table 1 Factors increasing the rate of anogenital neoplasia in immunosuppressed patients

Prevalence and types of human papillomavirus (HPV) viruses present at anogenital sites
Degree and duration of immunosuppression
Sexual behaviour of the individual (and their partner(s))
Smoking
Type of contraception used

(27/133) had biopsy proven AIN (7/27 high-grade AIN) compared with 1% of the controls ($n = 145$). The prevalence of HPV 16 DNA as ascertained by PCR in established RTR was 47% (36/76) and 12% (18/145) in the control group [41].

Cigarette smoking. This is an important cofactor for the development of VIN in HPV16 seropositive women. Smoking is associated with an adjusted odds ratio of 6.4 for VIN and 3.0 for invasive vulval malignancy [42] and an increased risk of progression in all anogenital and head and neck cancer in a dose-dependent fashion [43].

Histopathology of Anogenital Intraepithelial Malignancy

The terminology of VIN (and AGIN) mirrors that of CIN and shares many histopathological features with CIN. By convention, other genital tumours of the vulva, e.g., basal cell carcinoma, Paget's disease and melanoma in situ, are excluded and VIN is generally accepted as referring only to squamous cell dysplasia in the vulva, i.e., SCC in situ. The characteristic pathological features of VIN include nuclear enlargement, pleomorphism, hyperchromasia and usually atypical mitoses. Although traditionally divided into VIN I, II, III for basal, partial and full thickness dysplasia, it has been further suggested that there are two main types.

1. *Warty-basaloid or undifferentiated VIN (HPV associated in younger patients)*: Warty-basaloid VIN has epithelial changes similar to CIN basaloid dysplastic cells with high nuclear-cytoplasmic ratios involving the basal, parabasal and intermediate squamous epithelial layers with or without koilocytosis in the surface layers (Fig. 2).

2. *Differentiated or Simplex VIN (HPV negative and associated with Lichen Sclerosus and keratinizing SCC in older women):* Conventionally warty-basaloid VIN

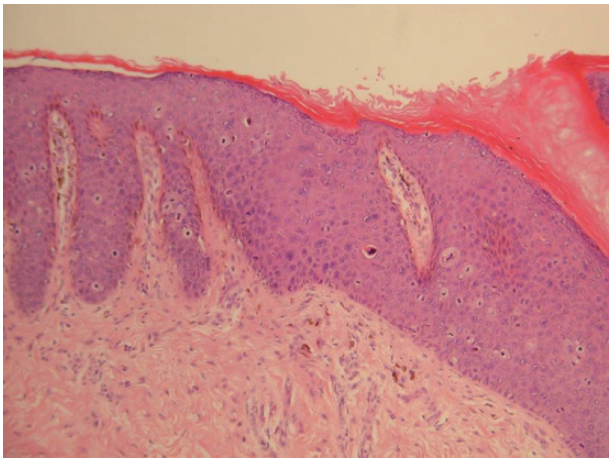

Fig. 2 Histological section of usual vulvar intraepithelial neoplasia (VIN) showing full-thickness epidermal dysplasia with numerous mitoses

has been graded using the WHO 3 grade system of mild, moderate and severe. In 2005 an altered grading system was proposed whereby low grade VIN corresponded to low-risk HPV infections, i.e., condyloma acuminate, and high grade VIN comprised VIN 2 and 3 and differentiated (simplex) VIN [44]. The International Society for the Study of Vulvar disease (ISSVD) has recently suggested further changes, combining VIN 2 & 3 into a single entity [45]. This was suggested due to the lack of biological potential for VIN 1 to progress and poor interobserver agreement in defining this entity. There is currently much potential for confusion with pathologists around the world using different classification systems and terminology. For example, whilst high grade (warty) VIN, so-called "bowenoid papulosis" (Fig. 3) can regress in a small subset of immunocompetent women there is no evidence that this occurs in immunosuppressed individuals, and the ISSVD advises pathologists not to use any "benign" terms such as bowenoid papulosis that imply regression or a low risk of progression.

Warty or basaloid SCC is the most common type of malignancy occurring in the anogenital skin of OTR. Whilst these cancers behave in a more invasive and aggressive manner in female OTR there are risks of either over-or under-reporting

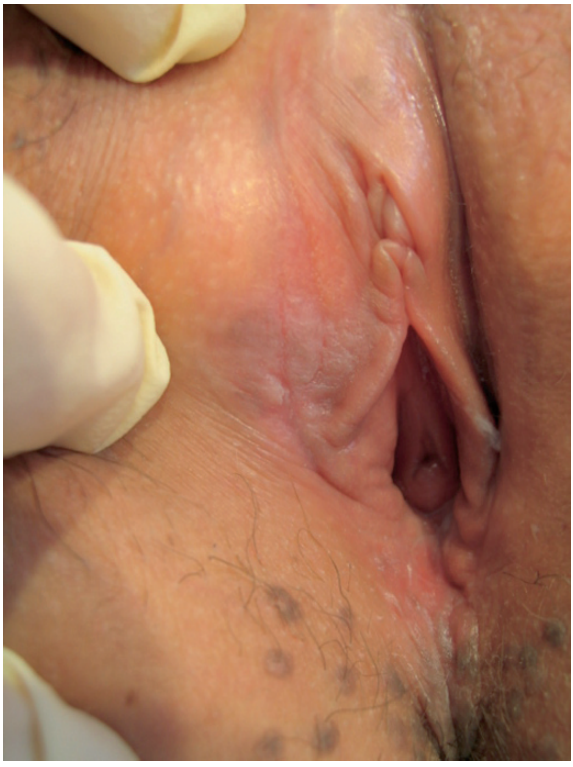

Fig. 3 Pigmented papules of VIN or "bowenoid papulosis' and adjacent hyperkeratotic papule of VIN, both containing high-risk human papillomavirus (HPV) type 16

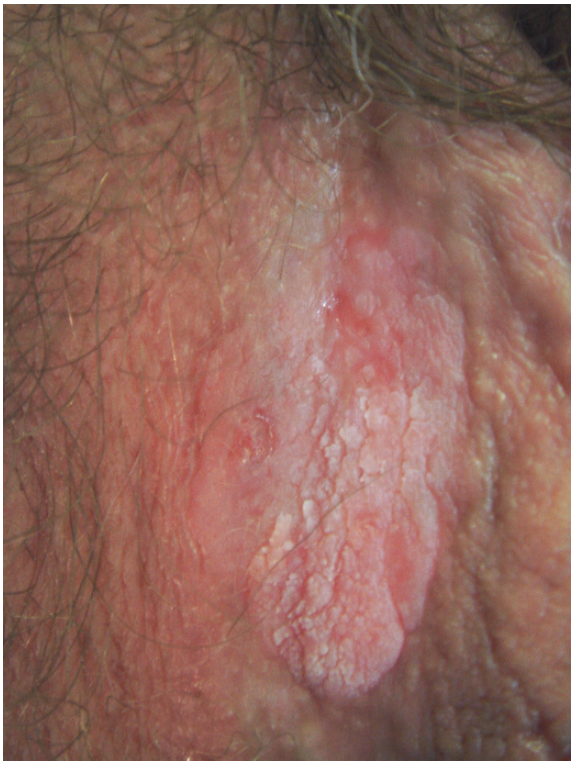

Fig. 4 Microinvasive squamous cell carcinoma in a plaque of VIN

of early or "micro invasion." If extension of VIN to underlying pilosebacous units, which occurs in about half of cases of high grade disease, is not recognised then tangential sections may be confused with invasion [46]. However, under reporting of stromal invasion can also be missed in 20% of patients without serial blocking [47]. Occult invasion was shown to occur in 22% of VIN III specimens at the time of initial treatment [48] (Fig. 4).

This emphasises the need for close communication between pathologists and clinicians, preferably in a multidisciplinary setting, to facilitate accurate reporting and interpreting of anogenital biopsies from OTR.

Clinical Features and Management of AGIN and Invasive Malignancy

There has been an increased awareness of the problem of anogenital malignancy in OTR within the last 10–20 years due to a better understanding of the molecular and genetic factors pertinent to oncogenic HPV types affecting anogenital skin, an

increase in the incidence of VIN and SCC occurring in young women, the development of prophylactic and therapeutic vaccines for cervical (and vulval) disease, and the availability of new topical treatments for AGIN such as the immune response modifier, 5% imiquimod cream.

Clinical Features

Lesions of AGIN are clinically diverse, varying from erythematous or pigmented papules or plaques, which are characteristically multifocal and pruritic to solitary, tender, eroded or ulcerated plaques (Fig. 5). Lesions are located widely over the anogenital skin, but frequently occur around the lower vestibule and periclitoral area in females, as well as perineum and perianal skin. Lesions of VAIN most commonly occur in the upper third of the vagina. Inflammatory dermatoses such as lichen sclerosus may coexist with AGIN and may cause additional diagnostic and management difficulties.

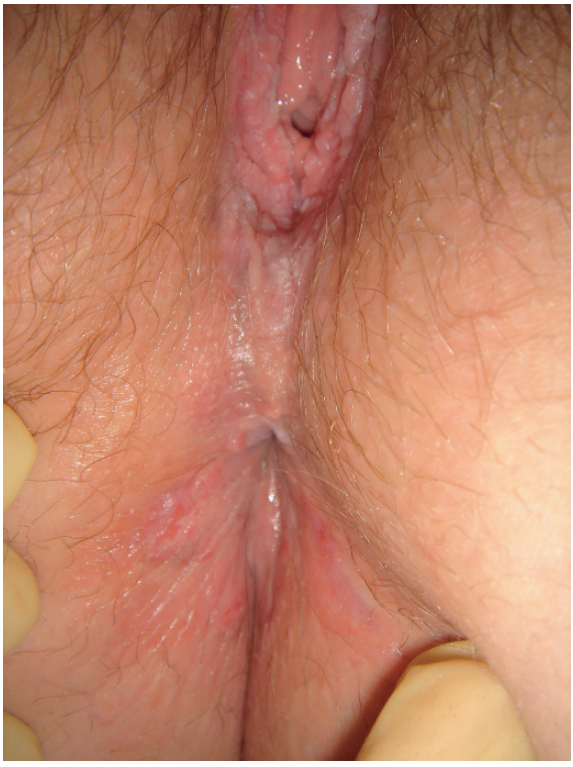

Fig. 5 Multiple white and erythematous papules and plaques of anal and vulvar intra-epithelial neoplasia

Management

Full clinical examination of a patient with AGIN should include colposcopy, cervical cytology, vaginal examination, and anoscopy. In the immunosuppressed anal, vulval, and cervical cytology can underestimate the severity of disease and should be carefully interpreted. Consequently, cytology is not currently recommended in place of punch biopsy and excision surgery [48, 49].

If several sites are involved then multiple field or mapping biopsies should be performed along with pretreatment clinical photographs. Repeat diagnostic biopsies, colposcopy, and anoscopy should be performed at regular intervals, particularly for any persistently pruritic areas, which could herald early or microinvasive change.

Psychosexual morbidity is high amongst patients with AGIN for a variety of reasons including previous multiple (often painful) surgical treatments (e.g., hysterectomy, partial vaginectomy, vulvectomy); a high risk of recurrence following treatment; fear of future malignancy and the need for frequent hospital follow-up attendances [50, 51]. A negative effect of vulvar surgery for patients is often irreversible. Over 50% of women suffer from psychosexual problems following radical or simple vulvectomy [52]. Management of psychosexual issues in OTR can be complex, time-consuming, and, unfortunately, often becomes a matter of secondary concern. Symptomatic relief with conservation of anatomy and functioning of the vulva can be achieved with careful repeated local resection combined with medical treatments. However this approach must be carefully weighed against the risks of a higher incidence of positive resection margins, recurrence, and missed occult invasion [53, 54]. Less aggressive surgery has also been proposed for AIN because of the high incidence of complications and morbidity following wide local excision, [55] although in one study 50% of the immunosuppressed patients ($n = 6$) developed invasive disease within 5 years [56]. The level of complex follow up for these patients is demanding and needs good collaboration of a multidisciplinary team of experts if OTR are to be properly monitored and treated. This is often not adequate, as highlighted by a report from one centre reporting that only 41% of their RTR patients had been adequately screened with respect to cervical cytology [9].

Following appropriate MDT discussion, treatment aims tailored to the specific affected sites are shown in Table 2.

Table 2 Treatment aims for anogenital intra-epithelial neoplasia (AGIN) in organ transplant recipients (OTR)

- Obtain representative histology from all affected areas and exclude invasive disease
- Provide symptom relief with topical therapy combined with surgical excision and/or laser ablation if appropriate
- Eradication of HPV infection with immune response modifiers (IRM's), antiviral agents, or therapeutic vaccination
- Conserve or restore normal epithelial architecture and function
- Stabilisation of involved and adjacent epithelium with oral retinoids
- Provide a sustained remission with regular surveillance and follow- up

Vulval Intraepithelial Neoplasia (VIN)

There is a dearth of prospective studies, clinical trials, or auditable standards that provide a rigorous evidence base for different treatment modalities currently used for AGIN [57] (Table 3).

Solitary well-demarcated lesions not encroaching on vital structures such as the clitoris are probably best treated with simple excision. All treatments have a significant risk (>50%) of recurrence especially in high-grade, multifocal disease with positive margins.

Medial to the pubic hair line, VIN lesions are usually thin, i.e., less than 0.4 mm thick and even if the minor vestibular glands are involved the depth is seldom >1 mm. Therefore, these lesions are amenable to treatment by CO_2 laser vaporisation, photodynamic therapy (PDT), 5-fluorouracil cream (5-FU), or surgery. In hair bearing areas, VIN extends down hair follicles to a mean depth of 1.4 mm and occasionally to >3 mm so focal skinning excision, laser treatment to 3 mm depth, or chemosurgery (5 FU followed by electrocautery) may be used [58]. Topical treatments with immune response modifiers (IRMs) can subsequently be used repeatedly as needed for residual areas of VIN for lesions on difficult sites such as the clitoris, or to treat recurrences following surgery. Five percent imiquimod cream has been shown in small uncontrolled studies in both immunocompetent and immunosuppressed patients to produce response rates of around 40% when used three times per week over a 16-week period [59–61]. However, inflammatory side effects restrict its use in many women. Although imiquimod can produce good clinical remission, post-treatment biopsies may show residual persistence of HPV DNA. In a small pilot study ($n = 10$) of topical Cidofovir for AGIN in immunocompetent women significant pain and ulceration was reported, and only 4/10 women showed complete regression of disease with histological and viral clearance [62]. Another study has, however, demonstrated lesion clearance and regression in 6 transplant recipients after intralesional or topical cidofovir [63]. There are a few case reports of oral isotretinoin used in combination with either interferon or cidofovir in VIN showing reduced lesion size [64, 65]. Photodynamic has been used with mixed results. It is

Table 3 Interventions for vulvar intra-epithelial neoplasia (VIN)

Surgical
Ablative
Chemodestruction
Photodestruction
Corticosteroids
Retinoids
Immunoe modulation
Antivirals
Vaccination

Source: From Todd RW, Luesley DM. Medical Management of Vulvar Intraepithelial Neoplasia. Journal of Lower Genital Tract Disease 2005; 9 (4): 206–212, by permission of Lippincott Williams & and Wilkins. AWAITED

very painful with all patients developing oedema and slough at treated sites. Poor cell-mediated immunity along with down-regulation of HLA class I may partially explain the lack of response of VIN to topical 5-ALA based PDT in OTR [66]. Those patients who respond best are able to mount a good post-treatment CD8 response.

Vaginal Intraepithelial Neoplasia (VAIN)

Lesions of VAIN most commonly occur in the upper third of the vagina and appear as white or red moist hyperkeratotic plaques, which may be secondarily eroded. In the immunosuppressed the rate of recurrence for VAIN is 55% compared with 25% in the immunocompetent population. Repeated surgery or aggressive laser procedures can easily produce stromal damage as the vaginal epithelium is very thin. Chemosurgery or CO_2 laser vaporisation are the preferred methods of treatment for easily accessible VAIN in the lower two thirds of the vagina (Fig. 6). Inaccessible sites, for example in the upper vagina, are best treated surgically with a

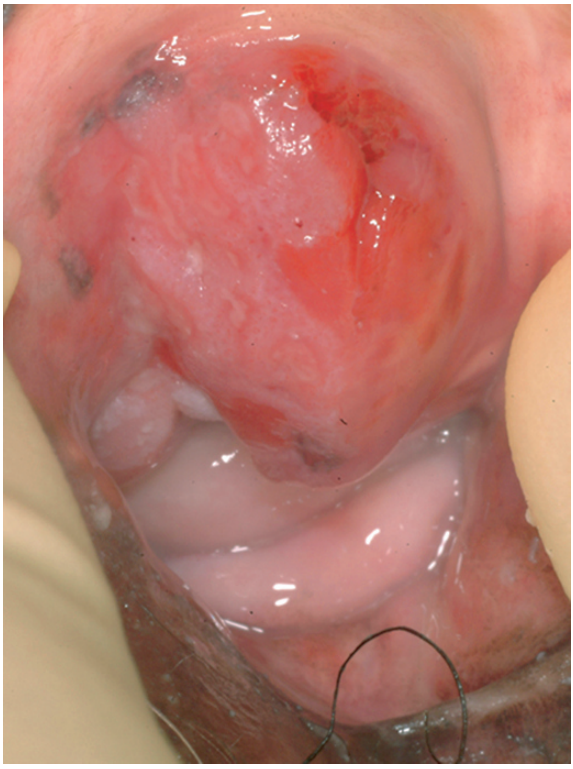

Fig. 6 A periurethral plaque of VAIN

partial vaginectomy [67]. In two small studies imiquimod and 5-FU cream have been shown to be potentially useful with clearance of VAIN rates of over 40% [67, 68] (similar to VIN).

Anal Intraepithelial Neoplasia (AIN)

The natural history of AIN and the much rarer PIN are not documented extensively in the immunosuppressed so standard treatment protocols or guidelines have yet to be determined. The risk of anal malignancy arising in AIN in OTR may have been overstated using data from other immunosuppressed groups, e.g., HIV-positive MSM. Nonetheless, AIN in OTR should be actively treated because of an increased risk of invasive disease. Using 3% acetic acid to identify lesions in the anal canal, surgical treatments include local excision, chemosurgery, mucosectomy, topical trichloroacetic acid, infra-red coagulation and laser destruction. Many of these treatments are painful with prolonged recovery times and high relapse rates. Topical therapy with imiquimod, has also been reported in a prospective, nonrandomized, open-label pilot study to be effective in treating AIN in 28 HIV-positive men with clinical and histological clearance in 77% without HPV clearance [69].

Adult OTR should be offered anoscopy and biopsy as part of their long-term follow up surveillance. Anal cytology using the Palefsky method has been shown to be of value for HIV-positive, bisexual or MSM, but does not yet have an established role in OTR. Once anal cancer is diagnosed standard treatment protocols include a combination of 5-fluorouracil and mitomycin C combined with radiotherapy. Abdominoperineal resection is usually required for local disease if lesions recur after chemoradiation.

Penile Intraepithelial Neoplasia (PIN)

Lesions of PIN are frequently located on the mucosal aspect of the glans or foreskin as sharply demarcated reddish plaques. Papular or pigmented lesions, previously described as Bowenoid papulosis, tend to be located on the penile shaft. PIN and penile cancer share most of the same risk factors as VIN, including smoking. Treatment modalities used in VIN (and side effects from such treatments) are all equally applicable to PIN. Whilst PIN (and penile cancer) is a rare condition it has been reported to occur more frequently in the uncircumcised [71]. The importance of screening female partners of men with PIN for occult CIN and AIN has been highlighted [72].

Invasive Vulvar Cancer

There are no specific recommendations for treating OTR with invasive vulvar cancer in comparison with immunocompetent individuals – where due to the rarity of vulvar cancers there are no large randomised controlled trials. Table 4 shows

Table 4 International Federation of Gynecology and Obstetrics (FIGO) staging of vulval carcinoma (1995)

Stage	Definition
1a	Tumour confined to vulva or vulva and perineum, 2 cm or less maximum dimension and with stromal invasion no greater than 1.0 mm
1b	As 1a but with stromal invasion greater than 1.0 mm
2	Tumour confined to the vulva or vulva and perineum; inguinal nodes not palpable
3	Tumour invades any of the following: lower urethra, vagina, anus, and/or inguinofemoral lymph nodes
4a	Tumour invades any of the following: bladder mucosa, rectal mucosa, upper urethral mucosa, is fixed to bone and/or bilateral lymph node metastases.
4b	Any distant metastasis including pelvic lymph nodes

Source: FIGO Committee on Gynecologic Oncology 2000 (122).

the current International Federation of Gynecology and Obstetrics (FIGO) staging of vulvar cancer incorporating representative histopathology [73]. The spread of vulvar cancer is predominantly lymphatic, with initial spread to the inguinal and femoral lymph nodes and then to the external iliac nodes. Drainage can occur to both groins from mid-line structures, particularly the perineum and clitoris. Following staging, treatment modalities for early stage T1 and T2 squamous cell vulvar cancer include wide local excision (surgical margin of 2 cm) with either unilateral or bilateral inguinofemoral lymphadenectomy via a triple incision technique [74]. A Cochrane systematic review showed groin recurrence to be higher following primary groin irradiation compared to surgery for early vulval cancer, and irradiation is currently not recommended as a single-modality treatment [75].

The involvement of the inguinofemoral lymph nodes in the staging system is important in prognosis. Noninvasive and minimally invasive methods of staging of lymph node metastases are being developed to reduce the high morbidity from surgery, particularly lymphoedema and wound breakdown. Even with modified surgical techniques and less invasive nodal staging, chronic leg oedema is still seen in 14–21% of patients [76].

Newer diagnostic techniques may improve the sensitivity of detection of metastases. The use of ultra-small-iron-oxide-particles (USIOP) in MRI lymphography improves the sensitivity and specificity for detecting micro-metastases in normal-sized lymph nodes [77]. Another promising technique is the ultrasound-guided fine-needle biopsy especially if carried out by an experienced investigator. Sentinel node biopsy followed by full lymphadenectomy is developing a high identification rate in tumour spread, especially using a combined technique with peritumoral-injected Technetium-99m-labelled nanocolloid and blue dye (isosulfan or methylene). However, the use of the sentinel lymph node procedure for staging is not yet standard care in the management of vulvar cancer [78].

Treatment

Radiotherapy and/or chemotherapy are generally reserved for locally advanced or metastatic disease. Pelvic exenteration can be avoided with a combination of preoperative radiotherapy and concurrent cisplatin/5-FU regimens aimed at reducing the size of the primary tumour and rendering the involved nodes more resectable. Postoperative cisplatin has also been shown to be a useful treatment following radical surgery with lymph node metastases in a prospective observational study on 14 patients [79].

Prevention of AGIN and HPV Vaccination

There is a concensus view that oral retinoids such as acitretin may prevent new skin cancers in OTR, but the drug is often poorly tolerated [80]. Limited clinical experience of low-dose oral retinoids (10–25 mg daily) to treat women with extensive AGIN and/or previous vulvar SCC suggests some clinical benefit.

The prevention of OTR-associated AGIN and subsequent malignancy is highly desirable. With the recent licensing of prophylactic HPV vaccines (see below) it would be logical to suggest that prospective OTR be screened for their HPV status prior to transplantation and vaccination discussed and offered (especially to children). However, it is not known at present whether vaccines would be ineffective together with iatrogenic immunosuppression.

As highlighted earlier, it is important that female OTR remain in cervical screening/colposcopy programmes despite vaccination status because 30% of cervical cancer is caused by non-16/18 types and mixed HPV infection has been demonstrated to occur particularly in immunosuppressed women.

HPV Vaccines

Phase II and III trials on prophylactic HPV vaccines for cervical cancer and genital warts have been shown to be both effective and safe [81, 82]. These vaccines consist of virus-like particles (VLPs) assembled from the major capsid protein L1 that appears morphologically identical to, and contains the major neutralising epitopes of the native virion. Two vaccines which have been recently licensed for use in the US, Europe and Asia-Pacific are Cervarix™, a bivalent HPV 16/18 vaccine from GlaxoSmithKline, and Gardasil™, a quadrivalent HPV 6/11/16/18 vaccine from Sanofi Pasteur MSD. In phase II trials, the bivalent vaccine has been shown to be highly effective in short term studies, preventing persistent infection with HPV 16 or 18 (detection of HPV 16 or 18 DNA in two consecutive samples six months apart) and preventing HPV 16/18- associated disease in 100% of vaccinated subjects [83]. In the phase III trials of the quadrivalent vaccine there was similarly 100% efficacy against the development of HPV 16/18 associated CIN 2/3 and HPV 6,11,16, or

18-related external genital warts (There have been no published reports of the use or effectiveness of these prophylactic vaccines in OTR). The Gardasil trial FDA data also showed 100% efficacy in preventing VIN and VAIN lesions in a large sub group of immunocompetent women ($n = 8641$) compared to controls [84].

Therapeutic vaccines are targeted at established HPV infections and anogenital disease, but results of these vaccines to date have been disappointing [85]. Therapeutic vaccines need to include some antigens from the expressed early HPV proteins (e.g., E2, E6, and E7) as well as capsid antigens. This has proved more challenging in terms of biodelivery and response. Lesions containing HR-HPV can evade immune recognition by down regulation of HLA class 1 and decreased numbers of Langerhans cells and CD8+T cells within lesions [86]. In AIN humoral and cellular immunity to HR-HPV can be induced with a heterologous prime-boost HPV oncogene vaccination, but there is no simple relationship between induction of systemic HPV-16 specific immunity and clinical outcome (lesion shrinkage or resolution). In addition lesions of high grade AGIN and invasive cancer are genetically unstable and can rapidly evolve additional immune escape mechanisms. A phase 2 study using vaccinia-expressed HPV 16 and 18 E6 and E7 (TA-HPV) in a small number of immunocompetent women with VIN and VAIN ($n = 12$) showed poor results with lesion shrinkage of around 40% and only 1 patient showing full regression of a single lesion [87]. Additional booster immunisations with TA-CIN (an HPV 16 L2E6E7 fusion protein) could provide additional clinical benefit, but again there was no direct correlation between clinical and immunological responses [88].

Other vaccine developments focus on synthetic peptides used alone or in combination. HspE7 (CoVal^{TM}) fusion protein has been developed by Nventa using recombinant DNA technology to covalently fuse a heat shock protein to an HPV E7 viral protein. This combination vaccine enhances the delivery of the E7 antigen to dendritic cells, which triggers an increase in functional CD8+ T cells independent of CD4+ T cells with subsequent tumour regression. In phase I\II trials in treating high grade AIN in HIV positive patients results demonstrated downgrading of dysplasia in 5/15 patients [89]. Other phase II trials of synthetic HLA-independent overlapping peptide vaccines containing HPV 16 E6 & E7 are currently underway for AGIN. Such new vaccines are relatively cheap to make as the E6 and E7 proteins are small (158 and 98 amino acids, respectively), and time-consuming expensive toxicity testing (inherent to the use of recombinant technologies) can be avoided [90].

Conclusion

Premalignant and malignant anogenital disease is increased in OTR when it is more difficult to treat and associated with considerable psychosexual morbidity. Treatment aims include stabilising the epithelium, excluding invasive disease, and trying to eradicate or minimise symptoms. Reducing the risk of malignant progression has to be balanced with tissue conservation and sexual function. Clinicians should manage these patients in a multidisciplinary setting with reduction or alteration of

immunosuppression whenever possible. Consideration should be given for introducing low dose systemic retinoids and topical (medical) treatment of co-existent multifocal AGIN undertaken. These strategies are currently considered "best practice" but need to be examined in a prospective randomized fashion to provide an expert evidence base. HPV-negative OTR, especially children, should be screened and offered preventative HPV vaccination. Future developments will bring new and hopefully more targeted therapeutic vaccines which will contribute to reducing the burden of anogenital HPV infection and associated malignancy in OTR.

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Kaposi's Sarcoma

Camille Francès and Céleste Lebbé

In 1872, a Viennese dermatologist, Moritz Kaposi, described multicentric, cutaneous, and extracutaneous neoplasms predominantly affecting older individuals with a protracted clinical course. This disease is now eponymously designated Kaposi's sarcoma (KS). Four recognized clinical subsets have been secondarily distinguished: the sporadic or classic subtype initially described by Kaposi, the endemic subtype observed in black Africans, the epidemic subtype in patients infected with human immunodeficiency virus, and the iatrogenic subtype in patients treated by immunosuppressive therapy, especially in organ transplant recipients. Whatever the clinical subset, KS ocurred in patients infected by human herpes virus type 8, also called Kaposi's sarcoma-associated virus, which was discovered in 1994 by Chang [1]. The level of immunosuppression is the main factor for development and progression of the disease.

Epidemiology

KS prevalence after organ transplantation varies greatly depending on the prevalence of HHV-8 infection in the general population. Table 1 summarizes the main data from the literature [2–12]. As expected, these KS prevalences in organ transplant recipients parallel the overall prevalences of HHV-8 infection in the different countries. An extremely low incidence of KS among transplant recipients (8.8 per 100,000 person-years) was found in a large national series from the United States [10]. This incidence was much lower than the prevalence previously published (4.3%) from the Cincinnati Transplant Tumor Registry, which collected data internationally [13]. In many countries, the geographic origin of transplant recipients with KS differs dramatically from that of the general population. In fact, post-transplantation KS mainly affects patients of black African, Mediterranean, or Carribean origin. Individuals from the same populations suffer from sporadic or endemic KS. In the United States, KS incidence significantly increased among

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Table 1 Prevalence of Kaposi's sarcoma (KS) in series of solid organ transplant recipients from different countries

Origin	Kidney % (patients, *n*)	Heart % (patients, *n*)	Liver % (patients, *n*)	Overall % (patients, *n*)
France [2]	0.45 (6,229)	0.41 (967)	1.24 (727)	0.52 (7,923)
Spain [3]	0.5 (788)	2.16 (231)		
Italy [4, 5]	1.2 (1,844)	1.6 (702)	3.1 (159)	1.4 (2,705)
Saoudi Arabia [6]	4.1 (630)			
South Africa [7]	0.5 (989)			
Israel [8, 9]	2.4 (330)	11 (18)		
USA [10]				0.02 (316,607)
USA (Pittsburgh) [11]			0.12 (1,657)	
Canada (Toronto) [12]	0.54 (1,300)	0.54 (189)	0.94 (426)	0.57 (2,099)

%, percentage; *n*, number of patients

Hispanics compared with that among non-Hispanics and in non-U.S. citizens compared with U.S. citizens [10].

Although low in absolute terms, the incidence of KS in organ transplant recipients nonetheless represented a 54-fold-higher risk compared with the general population [10]. In Italy, a risk 100 times greater has been estimated from a series of 1,721 renal, heart, and liver transplant recipients [4]. These recent results contrast with those from a Canadian study, which described a 400- to 500-fold increase in KS risk in transplant recipients compared with a control group of the same ethnicity. Nonetheless, that estimate was based on only 4 cases [14].

Comparative studies within an institution of KS prevalence according to the transplanted organ show variable results in different regions, but with generally similar prevalences after renal and cardiac transplantation and, in some series, a higher prevalence after liver transplantation [2].

KS risk increased with the recipient age at transplantation [10]. The number of mismatches at the HLA-B locus and a more aggressive immunosuppressive regimen were also associated with heightened risk [4, 10]. The male predominance, well known in sporadic, endemic, and epidemic KS, also exists in transplant KS: In the various relevant studies, the male/female ratio ranges from 2 to 40 [2, 10, 12, 13]. KS risk peaked in the 0- to 2-year period after transplantation and decreased after that period [10]. In Italy, a fivefold-higher KS risk was found in the first year after transplantation than in the subsequent periods [5]. The mean delay between organ transplantation and KS onset is 13 months, with a range of a few weeks to 18 years [15, 16].

Most cases of post-transplant KS apparently develop as a result of viral reactivation [17–24]. Indeed, more than 80% of transplant recipients with KS were seropositive for HHV-8 before transplantation (Table 2). In a prospective ongoing French national study, the percentage of patients with KS in the HHV-8-positive kidney recipients before transplantation was lower than 15%, 3 years after transplantation.

The high levels of drug-induced immunosuppression following transplantation probably lead to uncontrolled viral replication and/or expansion of tumor progenitor

Table 2 Prevalence of KS among transplant recipients who were seropositive for herpesvirus 8 (HHV-8) before transplantation

References	Type of T	Country	n° R + for HHV-8 before T/all R (%)	n° of KS patients/ + R for HHV-8 before T
Cattani et al. [18]	Kidney	Italy	26/175 (15)	6/26 (23%)
Francès et al. [19]	Kidney	France	32/400 (8)	9/32 (30%)
Andreoni et al. [20]	Kidney and liver	Italy	21/130 (16)	3/21 (14%)
Marcelin et al. [21]	liver	France	3/122 (2.5)	0/3 (0%)
Aseni et al. [22] Liver	Italy	4/459 (0.9)	4/4 (100%)	
Edmond et al. [23]	Heart	France	4/150 (2.7)	1/4 (25%)
Sachsenberg et al. [24]	Lung	Switzerland	1 recipient	1

T, transplantation; *n*, number; R, recipients; +, positive; %, percentage.

cells and progression to KS. The decline in KS incidence after 2 years post transplant may result from recovery of immunity as antirejection medications are tapered. Severe bacterial and/or *Pneumocystis carinii* infections were found to be associated with an increased KS risk among recipients who were seropositive for HHV-8 before transplantion [19]. This fact supports the main role of immunosuppression for KS development.

In some cases, KS developed in recipients probably infected by HHV-8 through the graft. Not only HHV-8 but also KS progenitor cells may be seeded after solid organ transplantation, survive in the recipients, and undergo neoplastic transformation and progression [25]. The percentage of KS among recipients who seroconverted for HHV-8 after transplantation is highly variable in the literature (0–50%) [26–28]. The discrepancy between these different studies probably arises from their retrospective nature, the small number of transplant recipients, and the different serologic tests used to detect seroconversion. A large ongoing French national cohort will prospectively examine the risk of post-transplant HHV-8 seroconversion and may be able to give a more accurate estimate of risk.

Clinical Features

Mucocutaneous lesions have been reported in more than 90% of all cases. As in other KS subsets, cutaneous lesions have a dark blue or purplish colour (Fig. 1). They may be more difficult to recognize on black skin (Fig. 2). They start as macules that progress and may coalesce to form large plaques or nodular and fungiform tumors. They are mainly localized on the lower limbs; they are also frequently seen on the trunk and the upper limbs. Face involvement is less frequent than in epidemic KS. Due to a Koebner's phenomenon, some lesions may be located on scars (Fig. 3), especially the transplantation scar [29]. Swelling of the lower limbs often antedate skin lesions by a few months [30]. At this initial edematous stage without skin lesions, serological tests for HHV8 may be useful for the diagnosis of KS. Oral lesions involve predominantly the palate with purple discolouration.

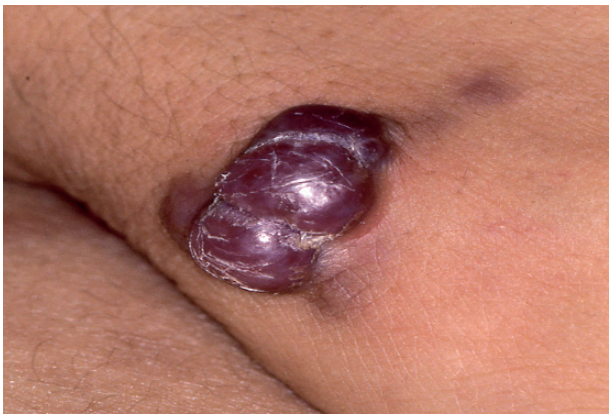

Fig. 1 Nodule of Kaposi's sarcoma on the leg

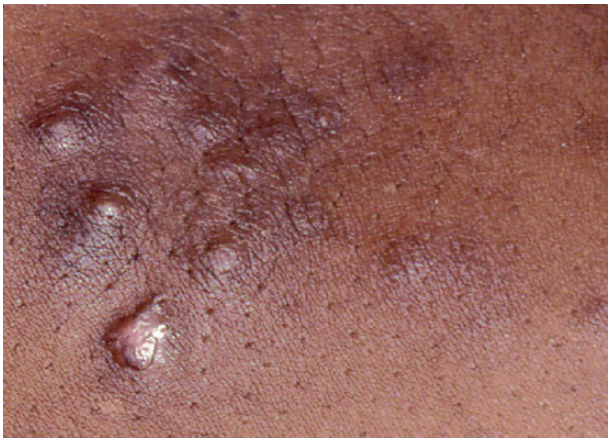

Fig. 2 Dark nodules of Kaposi's sarcoma on black skin

Gingival hyperplasia may occur and may be confused with hyperplasia induced by cyclosporin [31]. Genital mucosae or conjunctiva involvement is less frequent. Localized involvement of the uterine cervix has been reported [32].

Such mucocutaneous lesions induce a number of functional disorders: Walking can be hampered if edema or a large subcutaneous infiltrate is present; superinfection, particularly of ulcerated lesions, can be serious in immunosuppressed patients; distal paresthesias occur rarely, resulting from involvement of distal nerves by KS lesions [33].

Visceral KS predominantly affects the lymph nodes, gastrointestinal tract, and lungs. It is necessary to analyse histologically enlarged lymph nodes as there is a possibility of an associated lymphoma. Although KS can be present throughout the entire gastrointestinal tract, it most commonly localizes to the stomach and duodenum [33]. The lesions rarely cause clinical or biological symptoms, such as nausea,

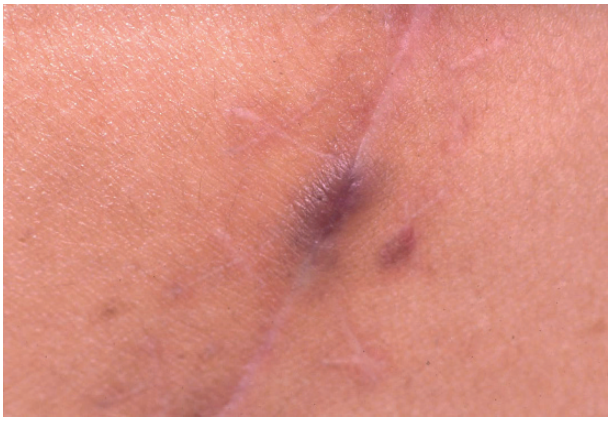

Fig. 3 Localization of Kaposi's sarcoma lesions on the scar of a liver transplantation

haemorrhage, perforation, or obstruction syndrome resulting from tumoral compression, or anemia; in most cases, KS lesions are detected at endoscopic examination showing more or less infiltrated red spots.

Pulmonary involvement appears at a more advanced stage of the disease; it may induce dyspnea, hypoxemia, and hypocapnia with diffuse interstitial infiltrates, pulmonary nodules with a bronchovascular distribution, and/or pleural effusions [34]. Many other localizations have been reported, especially in the hepatosplenic or cardiac areas. Bone involvement is rare; brain involvement has not yet been reported.

Diagnosis

Regardless of localization, KS diagnosis is confirmed histologically, with characteristic histopathological changes. KS is composed of a variable mixture of ectatic, irregularly shaped, round capillary and slit-like endothelium-lined vascular spaces and spindle-shaped cells accompanied by a variable inflammatory mononuclear cell infiltrate. Red blood cells and hemosiderin pigment are frequently present, often extravasated between the spindle cells. Sometimes the earliest patch and plaque stage lesions are difficult to distinguish from granulation tissue. Later, the spindle cells eventually become the predominant cell population, forming fascicles that compress the vascular slits, and the lesions become progressively nodular (Fig. 4).

KS cells show positive immunostaining for some endothelial cell markers such as $CD34^+$ and factor VIII. Most cells are of lymphatic endothelial cell origin [35]. Studies have shown varying monoclonality, oligoclonality, and polyclonality from lesions of various patients [36]. It is likely that KS starts as a hyperplastic polyclonal lesion that later gives rise to a clonal cell population only under specific circumstances, such as immunosuppression and selective pressures. The HHV-8 transcripts that are detected in most KS cells are primarily associated with latency; a few cells are undergoing lytic replication. In early stages, immunostaining of KS cells

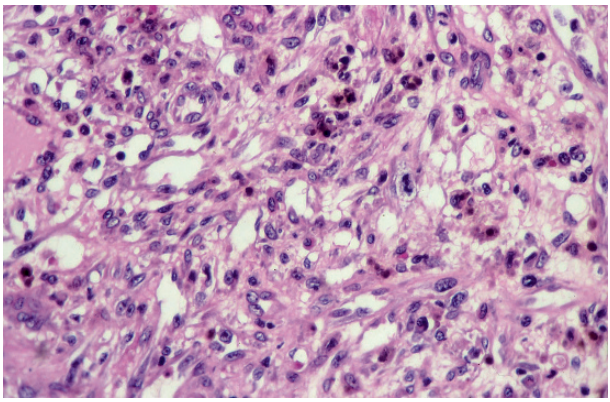

Fig. 4 Histopathology of Kaposi's sarcoma (KS) showing a proliferation of spindle cells with ectatic, vascular spaces

with antibodies directed against HHV-8 latent antigens may be useful for diagnosis of KS [37].

Initial Staging

The exhaustive clinical examination includes otorhinolaryngeal, ophthalmological, and genital examinations. A dated scheme with photographs of all mucocutaneous lesions makes it possible to accurately follow the evolution of skin lesions. Chest involvement is detected by radiography and computed tomography (CT). If thoracic disease is suspected, a bronchoscopy with bronchoalveolar lavage should be performed to confirm the diagnosis of KS and to exclude other diseases, especially opportunistic infections, which may be associated with lung KS. Gastrointestinal involvement is detected by esogastroduodenoscopy and, less frequently, by colonoscopy. Involvement of deep lymph nodes is detected by chest and abdominal CT.

Quantification of HHV-8 load in peripheral blood mononuclear cells has been found to be statistically associated with KS progression [38]. The detection of other viral, bacterial, fungal, or parasite infections arising from iatrogenic immunosuppression is not of purely theoretical interest; these infections are likely to be an aggravating factor for KS and should therefore be treated.

Based on thorough clinical examination, the disease may be classified into four stages, as reported by Al-Khader et al. in 1988 [39]. At stage 1, localized skin lesions involve only one limb; at stage 2, cutaneous involvement is still isolated but widespread skin lesions involve more than one limb; stage 3 has involvement of single or multiple viscera or lymph nodes; and stage 4 is characterized by any of the foregoing categories in the presence of either associated life-threatening infection or other neoplastic tumor. A lymphoma was reported in 2% of the 356 patients in

Penn's series [13]. This classification is simple, widely used in the literature, and allows for progression of KS. However, it does not take into account data essential for therapeutic decision such as functional disability, rate of development of KS lesions, and KS-linked vital risk, all of which have to be evaluated on a case-by-case basis.

Treatment and Prognosis

Until now, the cornerstone in treatment of post-transplant KS has been to taper down immunosuppressive regimens to the lowest possible level, whilst attempting to keep the allograft functional, which is of vital importance in case of liver or heart transplantation. The extent to which immunosuppressive drugs can be reduced depends on functional disability and the vital risk linked to KS or to failure of the transplanted organ. Associated infections must be treated whenever feasible. In one case, we observed that an extensive skin KS disappeared following treatment of tuberculosis without any reduction of immunosuppression, which was already considered as being at the lowest possible level [30]. Cutaneous and visceral lesions may not develop in parallel, but their evolution tends to run a roughly parallel course. Complete regression of KS lesions is not necessarily the ultimate aim; for instance, patients may prefer to keep a good renal function and accept that a few stable, largely asymptomatic, KS skin lesions are still present.

In the Cincinnati registry, the rate of remission of KS after just reducing the immunosuppressive therapy was 17% of 213 patients with mucocutaneous involvement and 16% of 143 patients with visceral involvement [13]. These percentages were probably underestimated in view of the long mean delays that we have observed between immunosuppressive therapy decrease and KS stabilization (3.6 months) or remission (11 months) [30].

In recent years, sirolimus has been demonstrated as possessing antineoplastic and immunosuppressive properties. These effects of sirolimus are due to a common mechanism. Sirolimus inhibits mTOR, which links mitogen-induced stimulation of protein synthesis and cell-cycle progression by activating p7056 kinase, a key enzyme in regulating gene translation [40]. Sirolimus has also an antiangiogenic activity [41]. Since 2004, more than 40 recipients with post-transplantation KS were treated successfully with sirolimus together with withdrawal of calcineurin inhibitors (CnI) [40, 42–47]. The switch from CnI to sirolimus was performed either immediately after KS diagnosis concomitant to withdrawal of other immunosuppressive drugs [40, 43, 44, 47], or, less often, after several months of tapering immunosuppressive therapy to the lowest possible level [41,42]. Only a few patients had visceral KS involvement [44–47]. It is likely that this switch must be performed as soon as possible after KS diagnosis, although further studies are needed to confirm this advice. The risk that KS may recur if the dose of sirolimus is increased suggests that for some patients regression of KS may be only the result of diminished immunosuppression and not the direct antineoplastic effect of sirolimus [48]. The beneficial effect of sirolimus was sometimes transient, with a KS progression

reported 2 to 24 months after KS remission, despite the lack of concomitant infectious or neoplastic event and stable trough blood levels of sirolimus [46, 49]. A few recipients with KS were refractory to sirolimus [46, 50]. Despite these last negative results, the switch from CnI to sirolimus is now considered as the first-line treatment of KS for transplant recipients. Monitoring P70(S6K) phosphorylation in peripheral blood mononuclear cells can help predict and monitor the biological effectiveness of rapamycin in recipients with KS, possibly allowing adjustment of the biologically active dose of the mTOR inhibitor [51].

Classical treatments are prescribed if sirolimus is ineffective or contraindicated. A small number of cutaneous or mucous lesions advocates for local treatment with cryotherapy, cryosurgery, laser, or surgical removal, with usually good cosmetic results. Intralesional chemotherapy is also recommended, but it is painful. Radiotherapy induces rapid regression of lesions, but increases the long-term risk of developing cutaneous carcinomas. Various single-agent or combination chemotherapies have been proposed, with none noticeably superior to another. The most commonly used single chemotherapies are vinblastine and bleomycin. In case of a rapidly progressing multivisceral involvement, liposomal anthracycline (doxorubicin, daunorubicin) or taxanes (paclitaxel, docetaxel) are usually prescribed. Alpha-interferon, which is widely used in endemic KS, is not recommended after an organ transplantation because of the rejection risk [52]. It seems to be better tolerated, however, after hepatic transplantation; it has been prescribed to treat recurrent viral hepatitis in the allograft [53] and in some isolated cases of KS [54].

Although in vitro studies showed that several antiherpetic molecules (foscavir, cidofovir, ganciclovir) may have an inhibitory action on HHV-8 replication, an action of these antiviral agents on KS in transplant recipients has not been demonstrated. Indeed, most of the tumor cells in KS are latently infected, and response to these agents is dependent on viral proteins that are exclusively expressed during lytic replication. Induction of HHV-8 into a lytic cascade may be a stategy for sensitizing tumor cells to antiherpesvirus drugs [37].

These potential therapies, associated with a more accurate modulation of immunosuppression according to KS severity, is likely to result in an improved prognosis for KS. The KS-associated mortality is probably lower than is reported in the literature. In the Cincinnati register, the percentage of deaths was 11% in patients with only cutaneous KS and 57% in patients with visceral involvement [13]. Usually, the cause of death was not accurately reported; rejection, opportunistic infections, or complications of a visceral KS involvement were counted together and labeled as KS linked. After renal transplantation, the percentage of returns to dialysis ranged from 21% to 58%, depending on the institution [2, 13].

Prevention

The goal in the future will be the prevention of KS in organ transplant recipients. Prevention should focus on HHV8-positive transplant recipients whatever the date of seroconversion. Today, HHV8 seropositivity as evidence of previous infection

should not exclude the possibility of organ transplantation. It is conceivable that sirolimus, prescribed early after transplantation, may be able to prevent development of KS. For recipients with a past history of KS, KS recurrence is frequent following transplantation although not inevitable [55,56].

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Malignant Melanoma

Beata Imko-Walczuk, Richard Turner, and Fenella Wojnarowska

Cutaneous malignant melanoma is a highly malignant tumour of the skin and is responsible for more deaths than any other skin cancer. Malignant melanoma arises from the malignant transformation of melanocytes at the dermal–epidermal junction or from the nevomelanocytes of melanocytic nevi that become invasive and may metastasise.

The immune system is thought to play a role in preventing or limiting malignant melanoma, predicting that immunosuppressed patients should be at increased risk for malignant melanoma. The increased relative risk for developing malignant melanoma in organ transplant recipients is still debated and varies as reported in different studies, although most show an increase. Malignant melanoma in organ transplant recipients has been less extensively reviewed in the literature than non-melanoma skin cancers, for which increased risk in organ transplant recipients is well documented.

Malignant melanoma is of concern in organ transplantation for three reasons: patients may develop a de novo malignant melanoma after transplantation, patients with a previous malignant melanoma may be candidates for organ transplantation, and malignant melanoma may be a result of transmission through a transplanted organ [1].

Clinical Aspects

Malignant melanoma may begin de novo or develop from a preexisting lesion, such as a congenital or atypical mole. The clinician must recognise malignant melanomas at the earliest possible stage, when the prognosis is good. Cutaneous malignant melanoma usually presents as a changed congenital or acquired pigmented lesion that shows asymmetry (A), border irregularity (B), colour variation (C), diameter enlargement (D), and evolution (or change) (E). Changes in shape and colour are important early signs and should be always taken as suspicious. The patient's

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description of changes in their mole is valuable and may be the earliest sign of malignant melanoma. Asymmetry of borders, changing colour, development of new red or pigmented halos around moles, and increase in diameter, height, or change in texture of the surface may all suggest development of melanoma. Ulceration, bleeding, and itch, pain, or tenderness may accompany the visual changes.

The ABCDE rule, based on the aforementioned features, and the seven-point checklist, with change in size, irregular shape, and irregular colour as major features, and largest diameter ≥ 7 mm, inflammation, oozing, and change in sensation as minor features, are important tools to aid recognition of malignant melanoma. Dermatoscopy can be useful in early diagnosis of malignant melanoma but requires training and experience. It allows visualisation of the distribution of melanin, which facilitates distinction of malignant melanoma from melanocytic naevi and other pigmented lesions.

The clinicopathological classification of malignant melanoma has evolved into six groups, based on proposals by Clark and McGovern [2]. The relative incidence of each type of malignant melanoma varies in different areas. In solid organ recipients, all the types of malignant melanoma existing in the general population may occur.

The following types of malignant melanoma are recognised: superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, and desmoplastic melanoma (Table 1).

Superficial Spreading Melanoma

Superficial spreading melanoma is the most common type of melanoma in patients with white skin. They are slightly more common in women than men, and present in patients from the teens upwards, but are most frequent in the fourth and fifth decade. Although any site can be affected, the commonest sites are the leg in women and the back in men. The lesions are usually a flat, variably pigmented lesion (Fig. 1). The colour may vary from brown to black, and can include grey, red, or white. Patients

Table 1 Clinical features of malignant melanoma

			Predominant sites	
Type of melanoma	Relative frequency	Male:female	Male	Female
Superficial spreading melanoma	50–70%	F > M (slightly higher)	Back	Leg
Nodular melanoma	15–35%	M > F	Trunk	Trunk
Lentigo maligna melanoma	5–15%	M = F	Face	Face
Acral lentiginous melanoma	10% whites	M > F	Feet > hands	Feet > hands
	50% African and Asian origin		Subungual	Subungual
Desmoplastic melanoma		F > M	Head and neck	Head and neck

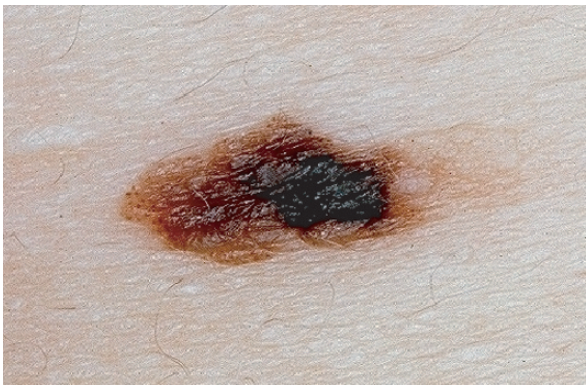

Fig. 1 Superficial spreading malignant melanoma

may describe areas of pigmentation or occasionally lesions that have disappeared (clinical regression). The size varies from a diameter of less than 5 mm to several centimetres.

Later the lesion may become palpable, indicating invasion, and in advanced lesions there may be nodules and bleeding.. Advanced lesions may ulcerate and bleed. An amelanotic variant has also been reported and may clinically be mistaken for a banal lesion or vitiligo.

Nodular Melanoma

Nodular melanoma is more frequent in men than in women and usually presents in the fifth and sixth decades. It most commonly arises on the trunk. This type of malignant melanoma grows rapidly and has often invaded deep into the dermis by the time of diagnosis. The clinical presentation is an elevated and dome-shaped nodule that often bleeds and ulcerates (Fig. 2). Nodular melanoma may initially be black or deep deeply pigmented, but often tumours lose pigmentation, causing difficulty with diagnosis.

Lentigo Maligna Melanoma

Lentigo maligna melanoma (Hutchinson's melanotic freckle) occurs in elderly patients and on sun-damaged skin. Lentigo maligna is a flat lesion with variable colour and areas of brown and black, and even red or pink. It has an irregular border that expands over many years. A small proportion progress to invasive disease, lentigo maligna melanoma. Clinically, this is apparent as the development of single or multiple raised nodules or plaques within the lesion (Fig. 3).

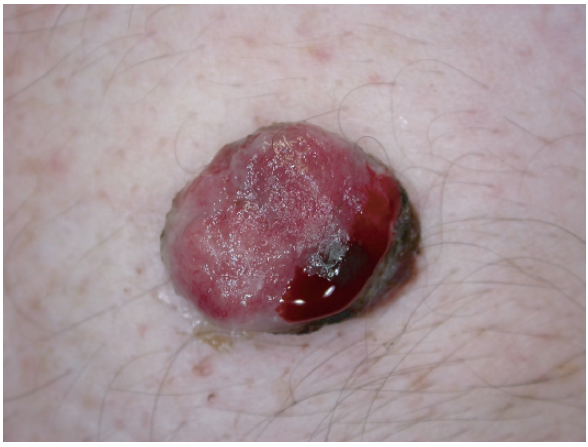

Fig. 2 Amelanotic, dome-shaped nodular melanoma

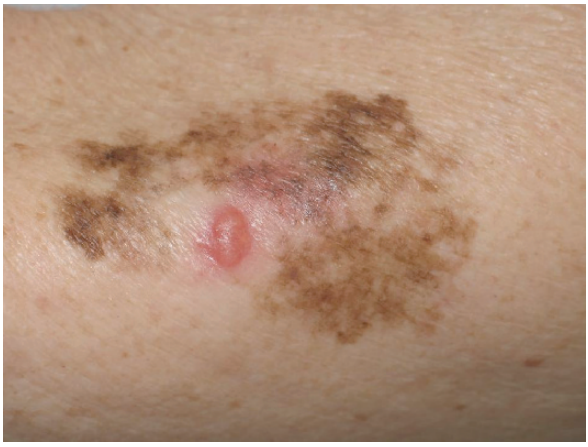

Fig. 3 Lentigo maligna melanoma

Acral Lentiginous Melanoma

Acral lentiginous melanoma (palmoplantar malignant melanoma) is more common in patients of African and Asian origin. It is more commonly found in elderly males. Acral lentiginous melanoma presents mainly on the sole of the foot, the palm of the hand, and under the nail. This type of malignant melanoma is characterized by an irregular large, flat, variably pigmented area, from which a raised pigmented and/or ulcerated area may arise (Fig. 4). Subungual melanomas may present as longitudinal streaks in the nail; pigmentation may spread to the proximal nail fold, and in late stages there may be destruction of the nail.

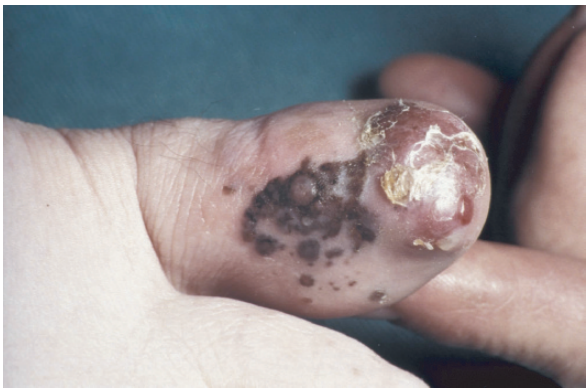

Fig. 4 Acral lentiginous melanoma

Desmoplastic Melanoma

Desmoplastic melanoma is rare and is usually found on the head and neck. The lesions are generally not pigmented; these present as a plaque or nodule and may be mistaken for a benign tumour or scar. Desmoplastic melanomas are often recurrent but rarely metastasise to lymph nodes. The lesions are not usually pigmented.

It is important to establish histologically if an invasive desmoplastic lesion has an exclusive desmoplastic vertical growth phase component, often associated with atypical lentiginous proliferation, or is a desmoplastic component of conventional melanoma. Sometimes the desmoplastic pattern is found only in a recurrence or in metastases from a more common type of melanoma.

Other Types of Malignant Melanoma

There are other pathological types in skin, such as naevoid, spitzoid, myxoid, and animal type. In addition, malignant melanoma may arise from mucosal surfaces or as an ocular melanoma.

Metastatic Melanoma

Recurrence and metastases occur in 25% of cases, usually in the first few years, but sometimes decades later. The risk of recurrence is related to the depth of invasion. Local recurrence and metastases do occur, but the majority of metastases occur through lymphatic spread initially. Metastatic disease occurs in areas of high blood flow, such as the lung, liver, and brain, and less commonly in bone and gastrointestinal tract.

Histopathology

Malignant melanocytes most likely develop from melanocytes in the basal area of the epidermis that may invade the underlying dermis. Cytologically malignant melanocytes are seen in the epidermis and invade the dermis. These cells may be seen not only in the overlying epidermis, but also spreading laterally, beyond the invasive component.

The main histological pointers to melanoma are contiguous moderate to severe cytological atypia, Pagetoid spread within the epidermis, architectural asymmetry, lack of deep maturation, and dermal mitotic activity.

Large atypical melanocytes first proliferate in the epidermis above the basement membrane zone. The term radial growth phase melanoma is used when the malignant cells are confined to the epidermis (in situ) or the papillary dermis (microinvasive), and are defined as having no dermal mitoses or dermal nests larger than any junctional nest; these lesions are almost always less than 0.76 mm thick, and have a good prognosis.

Tumours that have invaded the reticular dermis or deeper tissues have entered the vertical growth phase and have metastatic potential. There are mitoses and often nuclear pleomorphism, and the cells fail to maturate as the tumour extends downward into the dermis. Clark level 3 and 4 tumours (see following) are usually in the vertical growth phase.

The Breslow thickness is the vertical distance measured in millimetres from the granular cell layer to the deepest part of the tumour. This measurement is the most important histological determinant of prognosis [2, 3], with 95% 10-year survival in tumours less than 1 mm thick reducing to a 10-year survival of 30% to 50% in tumours greater than 3.5 mm thick. Clark levels (level I–V) assess tumour invasion by the deepest anatomic site involved within the dermis or subcutaneous tissues and also contribute to prediction of prognosis in thin lesions.

Incidence and Prognosis

The incidence of malignant melanoma in immunocompetent patients has increased significantly over past decades in white populations, although there is some evidence to suggest that incidence rates have now begun to stabilize or even decline. There is a huge geographic variation in relationship to skin type and latitude, so that northern parts of Australia have the highest rate.

The prognosis of malignant melanoma has continued to improve, primarily because patients are presenting at an earlier stage with smaller and therefore potentially curable lesions [4].

Risk Factors

In the immunocompetent population, risk factors for malignant melanoma are well documented. Risk factors include fair hair (blonde or red) and blue or green eyes.

Individuals with such features are usually of Fitzpatrick phototype 1 or 2, burning with very little sun exposure. Red-haired individuals have variants of melanocortin 1 receptor (MC1R) that are associated with a predominance of pheomelanin in hair and skin and reduced ability to produce eumelanin. This variant results in failure to tan and puts such individuals at risks from ultraviolet radiation. Such individuals may have increased numbers of freckles. All transplant recipients with these characteristics are at risk of malignant melanoma.

The presence of many naevi or dysplastic naevi [formerly known as the atypical mole syndrome: many common naevi (>100), on sun-exposed sites and also non-sun-exposed sites, such as the dorsum of the feet, buttocks, and anterior scalp and 4 or more atypical naevi], may also identify transplant recipients at risk of developing malignant melanoma. A history of previous malignant melanoma increases the risk of malignant melanoma.

Environmental factors are also important, including sun exposure, severe sunburns, particularly those in childhood, and use of sun lamps and/or sun beds. Immunosuppression must also be considered a risk factor and may compound other risk factors.

Several genetic factors have been identified in families with high prevalence of malignant melanoma, such as cyclin-dependent kinase inhibitor (CDKN)2A, also known as p16(INK4), and cyclin-dependent kinase (CDK4), which are cell-cycle inhibitors. Melanocortin 1 receptor (MC1R) is the gene for red hair and is common in Celtic and Nordic populations. These variants cause a predominant expression of phaeomelanin in hair and skin and reduced ability to produce a tan. These gene variants are associated with increased risk of malignant melanoma in several different populations and can be a cofactor with other susceptibility genes.

Malignant Melanoma in Organ Transplant Recipients

There are many reports of cutaneous melanoma in situ and malignant melanoma arising in organ transplant recipients. All clinicopathological types of melanoma have been reported, as well as mucosal malignant melanoma. The clinical appearance is the same as in immunocompetent patients, although in our experience some may look innocent clinically and under the dermatoscope.

The incidence of de novo malignant melanoma post transplantation is uncertain, as different groups have reported rates ranging from no increase to a 5-fold increase. In a study by Lindelof et al., following 5,356 patients in Sweden for more than 24 years, only 6 patients developed malignant melanoma with no increase in risk compared to the general population, taking age and gender in consideration [4], in line with earlier reports. Jensen et al. studied 2,561 kidney and heart transplant recipients in Norway over a 30-year period, during which time 12 patients developed malignant melanoma, a 3.4-fold-increased incidence in malignant melanoma for transplant recipients [5]. Jain et al. followed 1,000 liver transplant recipients for up to 8 years; 2 patients developed malignant melanoma, showing no increased risk in this group [6]. Using incidence data without age correction, Le Mire et al. found

an 8-fold-increased incidence of malignant melanoma, although the study also included in situ melanoma [7]. Similar results have been reported from other UK centers [8]. We reexamined the incidence in our Oxford cohort (Table 2), excluding in situ melanoma, and found a 5-fold increase in incidence (standardised incidence ratio, 5.0) [1]. Reports on an Irish cohort study, using standardized incidence ratios (SIRs), which correct for age differences in populations, similar to the Swedish and Norwegian studies, indicated a 6-fold increase in the incidence of malignant melanoma [9,10]. The incidence of malignant melanoma in African-American renal transplant recipients has been reported as 17.2 times higher than that for African Americans in the general population [11].

Table 2 Demographics and clinical features of Oxford transplant recipients with malignant melanoma

- Fourteen malignant melanoma patients
  - Ten men
  - Four women
- Mean age at first transplant: 40 years (range, 20–64 years)
- Mean age at diagnosis: 50 years (range, 36–73 years)
- Mean interval between transplant and development of malignant melanoma: 121 months (range, 15–248 months)
- Most patients skin type I–III (exception: one Asian patient with skin type V)
- Five patients had had immunosuppression before transplantation
- Risk factors
  - Most patients had a history of sunburn in childhood and/or excess sun exposure as a child or adult
  - Nine had multiple atypical naevi
  - One patient had a mother with two melanomas at the same age
  - Half the patients had other skin malignancies
- Most common site
  - Men: trunk and upper limb
  - Women: upper limb
- Type
  - Superficial spreading melanoma, 13 of 14 (Breslow thickness: 12/13, < 1 mm, 1/13, 1.5 mm)
  - Nodular melanoma, 1 of 14 (Breslow thickness, 4.5 mm)
- Course
  - Thirteen patients: no recurrences
  - One patient (with nodular melanoma, died 9 months after diagnosis)

Naevi and malignant melanoma are more common in paediatric transplant recipients than in adult recipients [12–14]. In an analysis of data from the Cincinnati Transplant Tumour Registry between 1968 and 1995, almost 4% of malignant melanoma occurred in children, whereas childhood malignant melanoma typically accounts for only 0.3%–0.4% of cases in the general population. Of skin cancers in the patients who had received transplants during childhood, 12% were malignant melanoma, compared to 5% in those who received transplants as adults [14].

History of Malignant Melanoma Before Transplant

The decision to transplant an organ to recipients with a history of malignant melanoma is difficult, as these patients are at a theoretically increased risk of developing new malignant melanomas, especially as they are immunosuppressed. In addition, immunosuppression may allow proliferation of micrometastases held in immunological check.

Four studies on patients with malignant melanoma before transplantation have been reported. An American study identified 31 patients who had malignant melanoma before transplantation, of whom 6 had a recurrence after transplantation; all died [15]. These patients had been free of recurrent disease before transplantation for up to 10 years. In a French report, 2 patients had had malignant melanoma 5 and 22 years before transplantation, respectively, and both had further malignant melanomas after transplantation. In an Australian report, 3 patients had had malignant melanoma 1, 9, and 27 years before transplantation, respectively, and all had further malignant melanomas after transplantation [16, 17]. In the recent European study, 9 patients had had melanoma before transplantation. Their mean age at diagnosis of melanoma was 44.9 years (range, 25.2–63.6 years). The mean interval between diagnosis and organ transplantation was 7.8 years (range, 0.4–32.5 years), and mean post-transplant follow-up was 5 years (range, 0.5–10.2 years). There were no melanoma-related deaths in this study, suggesting that a history of melanoma should not necessarily preclude subsequent organ transplantation [18].

The conventional recommendation has been to postpone an organ transplantation at least 5 years after removal of the malignant melanoma, but these reports do not support this view. No prospective study has addressed the question.

Malignant Melanoma as a Result of Transmission from Organ Donor

Donor-transmitted cancer may occur, and malignant melanoma is one of the most common donor-transmitted cancers in organ transplant recipients and the most common transmitted tumour causing distant metastasis [19, 20]. Often, the origin is unrecognised cerebral melanoma metastases misdiagnosed as primary brain tumours or cerebral vascular accidents.

The largest report of transmitted malignant melanoma is the one published by Penn. Of 20 organ transplant recipients with organs from 11 patients with malignant melanoma, 16 developed metastases, 11 died, and 1 had a deposit in the removed kidney [15]. With multiple transplants from each donor, and transmission to recipients not universal, transmission seems to occur in 50% to 100% of the recipients and is associated with a very high mortality [21–23]. In one study, no malignant melanoma developed from 4 organ donors with a history of malignant melanoma at a mean of 5 years before organ donation [24].

When a donor is identified as having transmitted malignant melanoma to a recipient, the other recipients of that donor's organs should be identified. If possible, their immunosuppression should be stopped and the donor organ removed. A subsequent retransplantation may be successful. This approach has been followed for a number of renal transplant recipients but has been reported only in one heart transplant recipient [25].

Treatment

Treatment of malignant melanoma in organ transplant recipients should follow established guidelines for malignant melanoma in immunocompetent patients. Metastatic disease should be managed by medical oncology centres, although the prognosis is very poor. Solitary metastases should be treated with surgery or radiotherapy.

There is no consensus as to whether reduction of immunosuppression in organ transplant recipients improves prognosis and whether dose reduction should be considered in all cases. Some authors consider that dose reduction is not indicated except in patients with metastatic disease. We always advise that immunosuppression is reduced if possible without detriment to graft function. There is no evidence as yet as to whether conversion to sirolimus is helpful.

Prognosis

Many pathological features have been postulated to influence prognosis, with tumour thickness, as established by Breslow, being the most significant single predictor of survival in clinical stage I melanomas. The prognosis for survival after 5 years in immunocompetent patients is 90% with tumours less than 1.5 mm thick, and 30% to 50% in those with tumours thicker than 4 mm. Tumour infiltration by lymphocytes is a good sign, and this may be relevant to the immunosuppressed patients whose immune response is reduced [26]. Certain body sites carry a poorer prognosis than others.

Organ transplant recipients with malignant melanoma might be anticipated to do worse than immunocompetent melanoma patients, and many reports support this view, although no formal meta-analysis has been performed. Other studies have reported mortality rates as high as 30% to 50% among organ transplant recipients with malignant melanoma, but in some previous studies a greater proportion of the melanomas were thicker [15, 16, 27]. In a study of 17 organ transplant recipients with malignant melanoma with a follow-up of 31 months, 4 died of metastases [16]. In a report from London, 2 deaths occurred in renal transplant recipients with malignant melanomas exceeding 2 mm Breslow thickness, and 1 patient unexpectedly developed metastases from lentigo maligna melanoma of Breslow thickness 0.4 mm [8]. Our own data demonstrate a better outcome (1 melanoma death), which

may be the result of regular surveillance and early detection of malignant melanoma with most of the malignant melanomas being thin ($<$ 1 mm) and of good prognosis [7]. Combined data from a European retrospective study where melanoma outcome in 100 transplantation-associated melanomas was compared with age-, sex-, tumour thickness-, and ulceration status-matched, immunocompetent controls from the American Joint Committee on Cancer (AJCC) melanoma database, suggest that outcome was similar to that of the general population for T1 and T2 tumours ($\leq$ 2 mm thickness) but was significantly worse for T3 and T4 tumours ($>$ 2 mm thickness) [18].

Prevention

All organ transplant patients and those awaiting transplantation should routinely be advised to protect their skin from the sun, and be advised on self-surveillance for early detection of skin cancers.

Dermatologists should play a significant role in the diagnosis and management of malignant melanoma in organ transplant recipients. All transplant recipients should be surveyed annually, and those at highest risk of malignant melanoma perhaps more frequently. High-risk individuals are fair-skinned individuals, recipients with major sun exposure, those with dysplastic naevi, and those with a previous malignant melanoma. Any changing or unusual pigmented lesion and lesions fulfilling the ABCDE rule or the seven-point checklist should be subjected to dermatoscopy, if available, and to an excision biopsy, so that malignant melanomas can be removed while still thin and with a good prognosis. In our experience, malignant melanomas discovered by a dermatologist at routine examination are often thin tumours. Regular complete skin surveillance can contribute to the early diagnosis of malignant melanoma in organ transplant recipients.

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Rare Skin Cancers

Jean Kanitakis

Several types of rare skin cancers have occasionally been reported in organ transplant recipients. The low incidence of these tumors in the general population makes it difficult to assess the true incidence in patients following organ transplantation; the fortuitous association of these malignancies with transplant-associated immunosuppression cannot be totally excluded, though it is conceivable (and probable) that immunosuppression favors their development, as it does for other cancers, such as nonmelanoma skin cancer, melanoma, lymphomas, and Kaposi's sarcoma, all of which have been shown to be truly increased by the observed versus the expected incidence in comprehensive skin cancer registries. The principal data concerning these rare tumors in the setting of organ transplantation are reviewed below.

Angiosarcomas

Angiosarcomas (AS) are rare malignancies developing from the blood or lymphatic vessels (mostly endothelial cells). They account for less than 1% of all sarcomas and present usually as red-violaceous plaques or tumors developing over the scalp, the trunk, or the limbs, mostly in elderly patients. Histologically, they consist of a dermal proliferation of more or less differentiated vascular channels or solid masses (Fig. 1). Diagnosis is made by pathological examination and is facilitated by the expression of endothelial markers such as CD31, CD34, and von Willebrand factor. The cause of AS is unclear; in contrast to Kaposi's sarcoma, they are not linked to human herpesvirus 8 (HHV-8) infection. Treatment includes surgical excision and radiotherapy, but the outcome is usually unfavorable.

Currently, 14 cases of AS (other than Kaposi's sarcoma) have been reported in organ transplant (mainly kidney) recipients, the majority of which affected the skin. The mean age of patients was 48 years (range, 31–71 years) [1–11]; 9 patients were men. AS appeared on average 6 years post graft (range, 0.7–12 years) and manifested clinically as tender violaceous hemorrhagic masses. Six tumors arose

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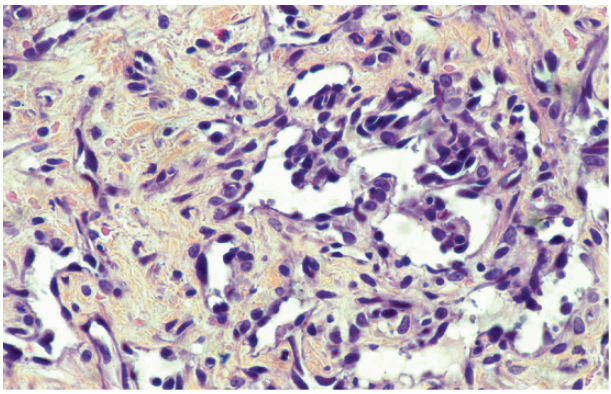

Fig. 1 Histopathological aspect of angiosarcoma: An infiltrative, vasoformative tumor is seen in the dermis, consisting of variously differentiated vascular structures lined by plump endothelial cells with hyperchromatic nuclei. Hematoxylin and eosin stain

within or adjacent to the arteriovenous fistula (AVF) performed for hemodialysis before renal transplantation [3–5, 7–9]. Other sites included the legs [10, 11], the scalp [6], and the transplant wound on the abdomen [1]. Histologically, they showed a wide morphological spectrum ranging from vasoformative to poorly differentiated tumors, with often an epithelioid appearance. Tumor cells expressed variably the endothelial markers, including endothelin [7]. Search for HHV-8 was performed in 2 cases and proved negative [11]. Despite aggressive multimodal treatment, the course was ominous with dissemination of the tumor and a fatal issue in half of cases on average 7 months after diagnosis. An association of Kaposi's sarcoma and a tumor diagnosed as (bullous) lymphangiosarcoma was observed in a renal transplant recipient [12].

Malignant Fibrous Histiocytoma/Atypical Fibroxanthoma

Malignant fibrous histiocytoma (MFH) is regarded as the most frequent type of soft tissue sarcoma, manifesting as a deep, subcutaneous or visceral mass. Atypical fibroxanthoma (AF) is considered as a superficial variant of MFH. It presents as a nodular, occasionally ulcerated tumor clinically mimicking basal or squamous cell carcinoma, lesions with which it may be associated. Pathologically it consists of a dermal proliferation of spindle-shaped or polygonal, often multinucleated cells (Fig. 2) expressing vimentin, CD99, and more variably the CD68 antigen. Sun exposure seems to be involved in the development of AF, as suggested by its usual appearance on sun-exposed areas (head and neck), its possible association with non-melanoma skin cancers, and by characteristic UV-induced mutations in p53 [13]. Therefore, sun protection could be a preventive measure. Treatment is surgical, as for aggressive SCC. Mohs' micrographic surgery provides lower recurrence rates.

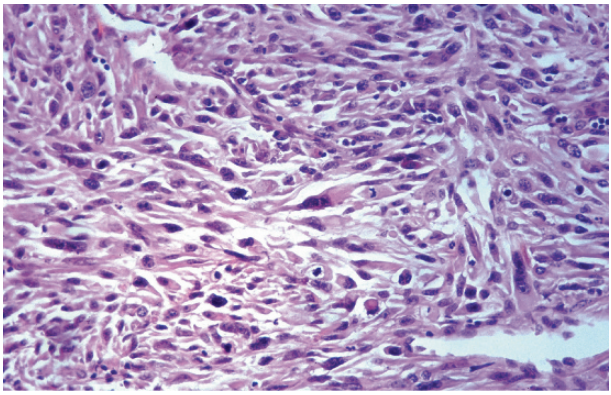

Fig. 2 Histopathological aspect of atypical fibroxanthoma: dermal proliferation of variously shaped, bizarre cells with ample cytoplasm and hyperchromatic, occasionally mitotic nuclei. Haematoxylin and eosin stain

Complementary methotrexate-based neoadjuvant chemotherapy has occasionally been used.

Ten transplant recipients (8 kidney and 2 heart) with cutaneous AF or MFH have been reported [14–22], and it has been estimated that the incidence of these tumors is increased over that of the general population [17]. All patients were men with a mean age of 58 years (range, 44–75). One patient developed multiple tumors [21]. In several patients the tumors were preceded by (or associated with) warts, premalignant keratoses, and skin carcinomas (in situ, basal, or squamous cell). The tumors appeared after a mean delay of 8.2 years post graft (range, 1.5–14), mostly over the head and neck and, more rarely, the limbs. Recurrences after surgical excision were often noted [14]– [17]. Widespread metastases with fatal outcome were reported in 2 patients with MFH [17, 20].

Dermatofibrosarcoma Protuberans

Although rare, dermatofibrosarcoma protuberans (DFSP) is the most common sarcoma of the skin. It usually affects young adults and presents as an indurated brown plaque that evolves slowly over several years, progressing into a multinodular tumor. Pathologically, it consists of a monomorphic proliferation of mesenchymal CD34+ spindle cells arranged in whorls, often with a cartwheel/storiform pattern (Fig. 3). The cause of DFSP is unclear. Tumor cells as a rule contain a t(17;22) translocation producing fusion of the platelet-derived growth factor B (PDGFB) gene with the gene encoding for collagen 1a1, with resulting increased synthesis and deposition of collagen. DFSP shows mainly local malignancy but may rarely metastasize (mainly to the lungs) and lead to death. Treatment includes wide excision with 2- to 3-cm lateral margins and must include the underlying aponeurosis.

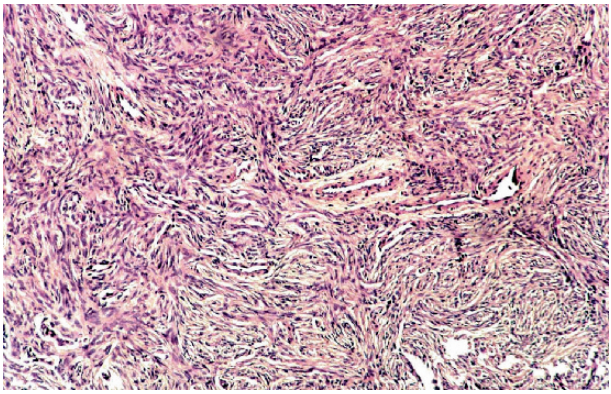

Fig. 3 Histopathological aspect of dermatofibrosarcoma protuberans: dermal proliferation of spindle-shaped cells with an inconspicuous cytoplasm, arranged in a storiform pattern. Haematoxylin and eosin stain

To date, three cases of DFSP have been reported in male renal transplant patients aged 34, 45, and 61 years, respectively; the lesions developed 3 to 11 years post graft over the chin [22], the arteriovenous fistula of the arm [23], and the shoulder [24]. The lesions were excised with wide safety margins and did not recur. Despite the small number of reported cases, DFSP could be overrepresented among renal transplant recipients [24].

Leiomyosarcoma

Leiomyosarcomas (LMS) are malignant tumors deriving from smooth muscle cells. Several cases of visceral post-transplant LMS have been reported, developing in the grafted organ and/or in host tissues, mostly in children [25]. LMS regularly express the Epstein–Barr virus (EBV) receptor (CD21) and harbor EBV (of host or donor origin); they are believed to develop as a consequence of EBV (re)activation [26]. The same EBV strain type may contribute to the development of smooth muscle tumors and lymphomas [27]. Cutaneous LMS present as indurated solitary nodules or plaques, affecting mainly the lower limbs. Histologically, they present as a poorly defined proliferation of spindle cells with eosinophilic cytoplasm and cigar-shaped vesicular nuclei with variable pleomorphism and mitotic activity (Fig. 4). Tumor cells express smooth muscle markers (such as desmin, smooth muscle actin, and caldesmon), allowing differentiation from other spindle cell sarcomas. The cause of LMS is unclear, even though some cases are associated with preceding trauma, radiation, or preexisting leiomyomas. Cutaneous LMS usually have a better prognosis than deep LMS, although local recurrences and systemic metastasis are possible [28]. Treatment is surgical with margins 2–5 cm wide; Mohs' micrographic surgery probably achieves better results.

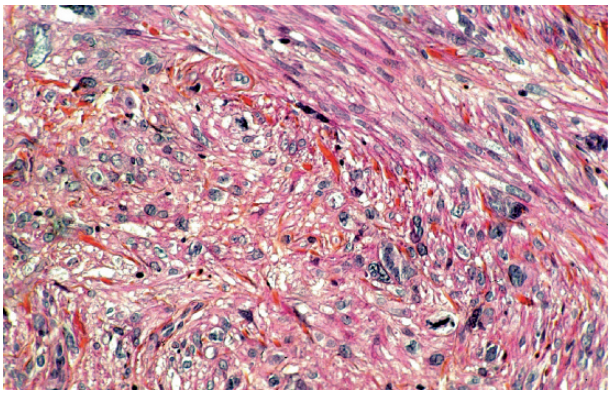

Fig. 4 Histopathological aspect of leiomyosarcoma: proliferation of eosinophilic spindle-shaped cells with blunt-ended, occasionally mitotic nuclei. Haematoxylin and eosin stain

Three cases of cutaneous LMS have been briefly reported so far in renal-grafted women. Two tumors developed on the legs 9 and 7 years post graft, respectively [28,29]; search for EBV, performed by in situ hybridization in one case [29], proved negative. One case was complicated by local recurrences and metastases necessitating leg amputation [28]. The third patient developed multiple EBV-positive LMS of the lung, liver, spleen, retroperitoneal lymph nodes, and the thigh 2 years post graft and died within 1 year [30].

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Merkel Cell Carcinoma

Jean Kanitakis

Merkel cell carcinoma (MCC) is a rare but aggressive skin cancer, first described by Toker in 1972 as "trabecular carcinoma of the skin" [1]. It is also known as primary neuroendocrine skin carcinoma, Toker tumor, cutaneous apudoma, or Merkel cell tumor. The origin of this malignancy from epidermal Merkel cells is likely but not unequivocally proven, and therefore the term primary neuroendocrine skin carcinoma seems more appropriate; however, the term Merkel cell carcinoma has prevailed in the literature and is adopted here. To date, several hundreds of MCC cases have been reported. An important percentage of these develop in immunocompromised patients, including solid organ transplant recipients (OTR). The salient features of MCC, with emphasis on those cases appearing in the setting of organ transplantation, are reviewed here.

Epidemiology

MCC is a rare tumor, even though its yearly incidence has reportedly increased threefold between 1986 and 2001 in the North American general population, where it reached 0.44 cases per 100,000 persons in 2001 [2]. Possible reasons for this trend include the increasing age of the general population, higher concurrent risk of immunosuppression, increasing rates of sun exposure, and possibly also improved detection and reporting. The mean patient age is 69 years [3]; more than 76% of them are 65 years or older, with almost 50% of patients being older than 75 years [4]; 97% of patients are Caucasians, and most of them are fair skinned. MCC shows a slight predilection for men (sex ratio, 1.4:1) [3].

The incidence of MCC after organ transplantation is not precisely known. Up till now 72 cases of MCC developing in OTR (OTR MCC) have been reported in a more or less detailed way [5–30] (Table 1), and some sporadic additional cases are mentioned in series of post-transplant skin cancers, although no sufficient clinical data were given on these patients [31,32]. The risk of developing MCC in renal graft

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Table 1 Clinical data of organ transplant recipient Merkel cell carcinoma (OTR MCC) cases published in the literature

Author	Reference	Patients (*n*)	Age (years)	Sex	OG	IST	DPG	Site	ASL	TRT	Course
Stempfle et al.	[5]	1	51	F	H	S, OKT3	nm	Forearm	nm	S	LM: A, 6 months
Formica et al.	[6]	1	54	M	K	Cs, S	3	Chest	nm	S, R	CM, LM: A, 18 months
Douds et al.	[7]	1	67	M	K	Cs, S	2	Thigh	0	S, R	VM: D, 2 years
Vazquez-Mazariego et al.	[8]	1	50	M	H	A, Cs, S	1	Arm	nm	nm	Nm
Gooptu et al.	[9]	1	56	F	K	A, Cs	4 **15**	Shin	nm	C	D, 1 year VM: D,
		1	55	M	K + L	A, Cs		Neck	SCC, W	S, R	6 months
Jonas et al.	[10]	1	50	F	L	Cs, S	1.9	nm	nm	S, R, C	D, 19 months
Williams et al.	[11]	1	65	M	K	A, Cs, S	7	Lower back	nm	S, R, C	A, 13 months
Plunkett et al.	[12]	1	55	M	K	A, S	5	Chest	SCC, BCC	S	LM: A, 8 months
Urbatsch et al.	[13]	1	40	M	K	A, Cs, S	3	Parotid	SCC	S, R	A, 5 years
		1	48	M	K	A, Cs, S	4	Forearm	NMSC	S, R, C	A, 2 years
		1	60	M	K	Cs, S	5	Forearm	NMSC	S	LM
Traest et al.	[14]	1	51	F	K	nm	19	Forearm	BD, W, P	S	nm
Silvestris et al.	[15]	1	55	M	K	A, Cs, S	8	Gluteus	BD	S, R	LM: A
Picciotto et al.	[16]	1	54	M	K	Cs, S	3	Thorax	nm	S, R	LM
Walsh	[17]	1	48	M	H	nm	nm	Ear	BD	S, R, C	LM, VM: D
		1	49	M	K	nm	nm	Hand	SCC	S	LM, VM: A, 9 months
Baron et al.	[18]	1	40	M	K	A, Cs, S	9	Gluteus	nm	R	A, 20 months
Friedlaender et al.	[19]	1	46	M	K	A, Cs, S	1	Cervical	nm	S, R, C	nm
Maksimyuk et al.	[20]	1	48	M	H	A,Cs,S	2	Shoulder	Appendage germ tumor	S	D[a]

Table 1 (continued)

Author	Reference	Patients (*n*)	Age (years)	Sex	OG	IST	DPG	Site	ASL	TRT	Course
Buell et al.	[21]	48/45[b]	Mean 47 (19–67)	69%M, 31% F	40 K 3 H 2 L 1 L/K 1 H/K	A, Cs, S ALG OKT3	Mean 6, 9	49% head/neck 45% limbs 15% torso	49% NMSC	S, R, C	LM, VM: 60% D
Bartsch et al.	[22]	1	59	M	K	nm	25	Ear	NMSC, KA	S	D, 6 months
Clark et al.	[23]	1	70–80	M	K	A, Cs, S	10	Forearm	SCC	S, R	LM, VM: D, 22 months
Morris et al.	[24]	1	72	M	K	Cs, M, S	4	Shoulder	nm	S	LM: D
Esen et al.	[25]	1	25	F	L	A, Cs, S	4	Finger	nm	S	A, 12 months
Ferreira et al.	[26]	1	72	F	K	A, Cs, S	11	Eye canthus (bilateral)	BCC	S	LM: D, 10 months
Kanitakis et al.	[27]	1	52	M	K	Cs, S	5	Shoulder	AMN	S	nm
		1	65	M	K	Cs, S	12	Shoulder	SCC, KA	S	LUM: A
Muirhead and Ritchie	[28]	1	63	M	L	A, Cs	2	Scalp	nm	S	LM, VM: D
Bensaleh et al.	[29]	1	69	M	L	S, T	6.5	Forehead	BCC, SCC, AK	R	D, 30 months
Bajetta et al.	[30]	1	59	M	L	T	3	Upper leg	nm	S, R	LM: D, 2 years

Sex (M, male; F, female); OG, organ grafted (K, kidney; H, heart; L, liver); IST, immunosuppressive treatment (A, azathioprine; ALG, antilymphocyte globulin; Cs, cyclosporine; M, mycophenolate mofetil; S, steroids; T, tacrolimus); DPG, delay post graft of tumor diagnosis (years); ASL, associated skin lesions (AK, actinic keratosis; BCC, basal cell carcinoma; BD, Bowen's disease; SCC, squamous cell carcinoma; NMSC, nonmelanoma skin cancer; W, warts; AMN, atypical melanocytic nevus; KA, keratoacanthoma); TRT, treatment (S, surgery; R, radiotherapy; C, chemotherapy); course (CM, cutaneous metastases; LM, lymph node metastases; LUM, lung metastases; VM, visceral metastases; A, alive; D, dead from MCC; nm, not mentioned/unavailable.

[a]From unrelated cause.

[b]48 MCC in 45 patients.

recipients has been estimated to 0.13 per 1,000 person-years [32]. More than half of all published OTR MCC cases were reported from the Israel Penn International Transplant Tumor Registry (IPITTR) (1968–2000), which contains data on 48 MCC developing in 45 OTR [21,33]. In this registry, OTR with MCC accounted for 0.9% of OTR with skin tumors in general [33]. Therefore, even though OTR MCC is more than 500 times less frequent than post-transplant nonmelanoma skin cancers (NMSC) [27,33] and 4 to 6 times less frequent than melanoma [33,34], its incidence seems to be increased as compared with the general population, where MCC is much rarer (65 fold) than melanoma [34]. In support of this contention is also the fact that among patients with MCC, the percentage of OTR (8%) is unexpectedly large [21].

Post-transplant MCC shows a significant predilection for men (sex ratio, 2.75:1), although this is (at least partly) the result of the predominance of men among OTR in general. For similar reasons, the large majority of OTR MCC (93%) have been observed in renal transplant recipients, followed by heart and liver transplant recipients, each of the latter two groups accounting for 4% of all OTR MCC cases. More than 80% of patients are Caucasians [21, 33]. The mean patient age at the time of diagnosis is 50 years (range, 19–72 years), much lower than patients with MCC in the general population. The mean delay for OTR MCC development post transplantation is 6.9 years (range, 0.4–25 years). Most patients were on double- or triple-agent immunosuppressive regimens associating azathioprine, steroids, and cyclosporine, more rarely tacrolimus and/or mycophenolate mofetil; some patients had received therapy with OKT3 or antilymphocyte globulin for induction or rejection [21].

Clinical Features and Staging

MCC has no distinctive clinical features: It usually presents as an asymptomatic papular or nodular hemispherical, well-circumscribed, firm tumor with a red, brown, or violaceous color (Fig. 1). It measures 2–3 cm in diameter and generally grows rapidly. The overlying skin may be telangiectatic or rarely ulcerated. The tumor appears mostly on the head and neck (41%–48%), the upper (19%) and lower limbs (16%), and the trunk (11%–23%). Because of the noncharacteristic aspect, the clinical diagnosis may be easily missed so that diagnosis is usually established upon histological examination. Staging workup at diagnosis of patients with MCC should include total body clinical examination, palpation of the draining region and determination of (regional) nodal status, sentinel lymph node biopsy (SLNB), blood cell count, erythrocyte sedimentation rate, liver enzymes, imaging tests [chest X-ray, abdominal and regional lymph node ultrasonography, brain computed tomography (CT) scan, and skeletal scintigraphy]. At presentation, 55% of patients have stage I disease (localized to the skin), 31% stage II (regional lymph node involvement), and 6% stage III (distant metastases) [35]; 3% of MCC cases present as metastasis of an unknown primary [3].

In OTR, the clinical presentation and distribution of MCC by anatomic site is similar to that found in the general population (head and neck, 49%; extremities,

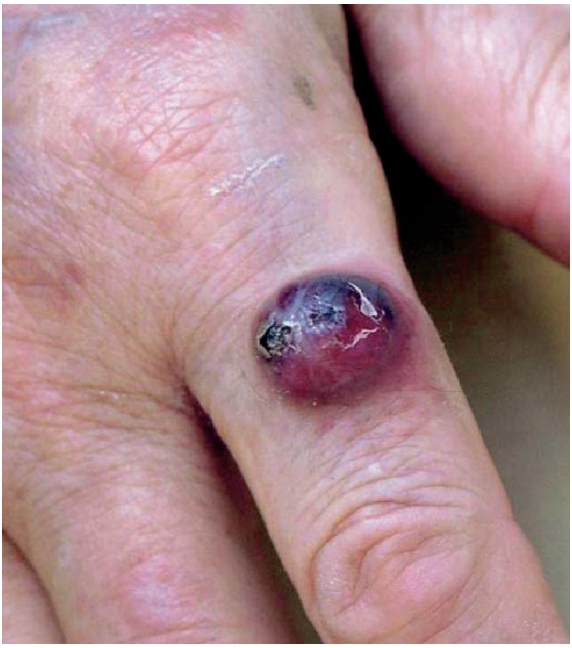

Fig. 1 Merkel cell carcinoma of the finger in an elderly woman

45%; trunk, 15%). Some OTR have multiple MCC [33]. At presentation, more OTR have stage II/III disease as compared with patients with MCC in the general population [21]. Most OTR with MCC also have other NMSC [9, 12–15, 21, 22, 33], including actinic keratoses, keratoacanthomas, basal and squamous cell carcinomas, and Bowen's disease, which are occasionally closely intermingled with MCC [13, 17, 27]. The vast majority of OTR MCC appear de novo after transplantation; exceptionally the tumor develops as a recurrence of a tumor diagnosed before transplantation [33].

Pathogenesis and Risk Factors

The precise cell of origin and pathogenesis of MCC remain unknown. MCC probably derive from epidermal Merkel cells, as suggested by several immunohistochemical and ultrastructural features shared between MCC and Merkel cells, even though this hypothesis does not explain the predominantly dermal location of MCC, where few, if any, Merkel cells are found in normal conditions. Dermal neuroendocrine cells or pluripotent epidermal stem cells could also be the cell of origin of the tumor. MCC appears to be favored by ultraviolet radiation, as suggested by the following facts: (a) propensity for sun-exposed skin, as happens with NMSC [4,21]; (b) association of MCC with other NMSC and with PUVA treatment for psoriasis

[9, 12–15, 17, 21, 22, 27, 33]; (c) higher age-adjusted incidence in zones with high sun exposure within the same ethnic population [4]; and (d) common genetic events between squamous cell carcinomas and MCC, including chromosomal imbalances and UVB-type mutations of the *p53* and H-*ras* genes [36].

Immunosuppression almost certainly is a predisposing factor, as suggested by the frequent association of MCC with immunodeficiency conditions, including human immunodeficiency virus (HIV) infection [37], leukemias/lymphomas [38], and iatrogenic immunosuppression for autoimmune diseases or organ transplantation. In a literature review on 420 MCC cases, 14.5% of patients were found to receive some type of immunosuppressive treatment [3]. The favoring role of immunosuppressants on MCC development is also upheld by the (temporary) regression of the tumor upon reduction or withdrawal of the immunosuppressive treatment [19, 28]. Immunosuppression could facilitate MCC development either by a direct (mutagenic) effect of immunosuppressive drugs on the parental cells of origin of MCC or through decreased immunosurveillance, allowing the development of oncogenic viruses, as happens with other tumors occurring in immunocompromised patients [e.g., Kaposi's sarcoma and human herpesvirus 8 (HHV-8), lymphoproliferative disorders, and Epstein–Barr virus (EBV), NMSC, and human papillomavirus (HPV)] [39]. This possibility prompted search for HPV and EBV in MCC, but neither of these viruses was detected in several MCC cases (including two OTR MCC) [40, 41]. Very recently, a new polyomavirus (called MCV) of a 5,387-base-pair genome was detected by digital transcriptome subtraction in cases of MCC. MCV seems to be integrated in the genome of tumor cells before their clonal expansion, suggesting that it may be a contributing factor in the pathogenesis of MCC [42]. Chronic arsenic ingestion has also been incriminated as an etiological factor of MCC [43].

Pathological Features

MCC is composed histologically of uniform, basophilic round cells with inconspicuous cytoplasm and large, often mitotic, nuclei (Fig. 2), arranged in solid sheets or more rarely in a trabecular or rosette-like pattern. Foci of necrosis and apoptotic cells are often present. Tumor cells are argyrophilic, showing positivity for the Grimelius stain [44]. MCC develops primarily in the dermis wherefrom it may extend to the hypodermis or the epidermis (10–30% of cases), occasionally with a pagetoid spread or formation of Pautrier-like microabscesses [27, 45]. Very rarely is the tumor entirely confined to the epidermis [46]. Three histological subtypes are recognized: The trabecular pattern consists of delicate ribbons of tumor cells; the small cell variant is composed of a hyperchromatic oat cell-like infiltrate with frequent crush artifact; and the intermediate type, which is the most common, consists of nodules and diffuse sheets of cells. The overlying epidermis may be normal but occasionally shows features of actinic keratosis or basal or squamous cell carcinoma [44]. These close associations with NMSC have also been observed in OTR MCC [17, 27]. Pathological features that have been found to be associated

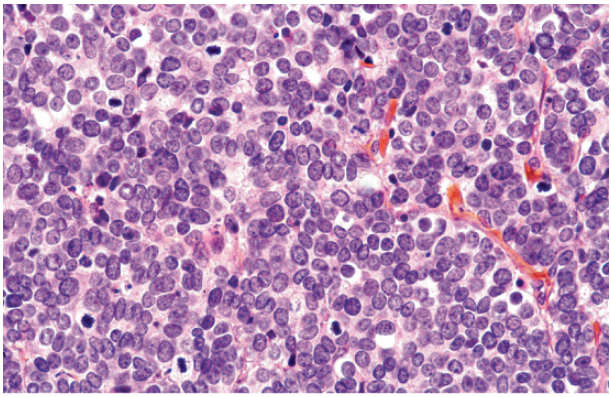

Fig. 2 Merkel cell carcinoma in a renal transplant patient. The tumor is composed of monomorphous basophilic cells with large, frequently mitotic, nuclei. Hematoxylin and eosin

with an unfavorable course include absence or paucity of peritumoral inflammatory infiltrate [47], high mitotic rate, and small cell size [48]. Tumor depth (Breslow thickness) does not seem to have an independent prognostic value [49].

MCC may histologically mimic other undifferentiated malignant round cell neoplasms [such as lymphomas, melanomas, or anaplastic (primary or metastatic) carcinomas]. The pathological diagnosis can readily be confirmed by immunohistochemistry or electron microscopy. MCC coexpress markers of epithelial and neuroendocrine differentiation, namely keratin 20 (in a characteristic dot-like paranuclear pattern) (Fig. 3), neuron-specific enolase, chromogranin (Fig. 4), synaptophysin, neurofilaments [50], CD117, and occasionally epithelial membrane antigen [27].

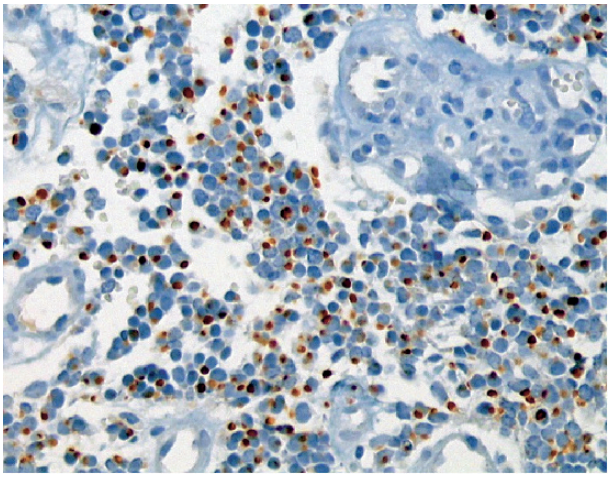

Fig. 3 Merkel cell carcinoma: Immunolabelling for keratin 20 shows a characteristic dot-like cytoplasmic reactivity within tumor cells. Immunoperoxidase revealed with aminoethylcarbazole

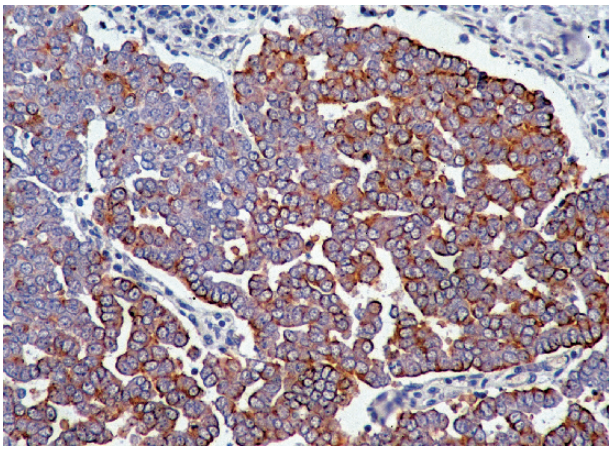

Fig. 4 Merkel cell carcinoma: immunolabelling for chromogranin shows a diffuse cytoplasmic reactivity within tumor cells. Immunoperoxidase revealed with aminoethylcarbazole

MCC must also be distinguished from cutaneous metastases of neuroendocrine carcinomas originating in other organs (lung, gastrointestinal tract). Keratin 20 positivity along with negativity for the thyroid transcription factor 1 and keratin 7 favor the diagnosis of MCC (rather than metastasis from a visceral neuroendocrine carcinoma). Electron microscopic examination shows characteristic dense-core (80–120 nm) neurosecretory granules within tumor cells and paranuclear whorls of intermediate filaments, accounting for the characteristic dot-like reactivity pattern obtained immunohistochemically with antibodies to keratin and neurofilaments.

Course and Prognosis

MCC is an aggressive tumor, even though exceptional cases with complete spontaneous regression have been described in nonimmunosuppressed, mostly (11/12) female patients [51]. The tumor shows frequent local recurrences (30%), spreads to regional lymph nodes, and produces distant metastases to the lungs, liver, central nervous system, bones, bone marrow, pancreas, and adrenal gland. The overall 5-year survival of MCC is 75%, 59%, and 25% at stages I, II, and III, respectively; better survival is associated with early-stage disease, limb localization, younger age, and female sex [4]. SLNB seems to be a valuable prognostic indicator, since node-positive patients have a threefold-higher risk for recurrence at 3 years compared with node-negative patients [52].

In OTR, MCC may temporarily regress following reduction of the immunosuppressive treatment (cyclosporine, azathioprine) [19, 28]. However, 31% of patients develop tumor recurrence with a mean interval of 58 months after excision of the primary [21]. Two-thirds of OTR MCC develop rapid lymphatic metastases to the regional lymph nodes and systemic metastases to the liver, bones, and lung.

Other tumors (including prostatic and ovarian carcinomas and overall NMSC and melanomas) are present in 49% of patients [33]. Prognosis is poor, apparently worse than in nonimmunosuppressed patients with MCC. In the IPITTR, 60% of OTR with MCC died of their tumor. The overall 1-, 3-, and 5-year mortality rate reached 20%, 51%, and 54%, respectively. MCC accounted for 4% of all fatalities from skin cancers in OTR [33].

Management and Prevention

Because of its relative rarity, no consensus guidelines based on controlled trials exist for the management of MCC. However, because of the high rate of recurrences and metastases, aggressive multimodality treatment seems necessary [53]. Wide surgical excision with generous margins (2–3 cm) including the skin, subcutaneous tissue, and the underlying fascia (when the tumor comes close to it) is the mainstay treatment for primary tumors. Mohs' micrographic surgery is a satisfactory alternative in cases where (because of anatomic localization, such as the face) wide excisions are not feasible. Large excision decreases local tumor recurrence rates, even though it may not improve overall survival. SLNB should be considered as it is helpful in staging and prognosis [52]. For SLNB-positive patients, complete lymphadenectomy is advisable, even though the benefit in survival remains to be seen. Adjuvant radiotherapy to the excision site, in-transit tissue, and regional lymph nodes (45–50 Gy at 2 Gy per fraction) should be considered [54, 55]; indeed, most studies concluded that combination treatment yields better results in terms of local and regional recurrence than surgery alone [3, 35, 55–57], especially for patients at stage II [58]. A recent study found a benefit not only on the time to first recurrence, but also on overall survival on multivariate Cox regression analysis [59]. In patients with advanced local regional disease and/or metastases, chemotherapy can be tried, pending adequate patient status [60]. The most commonly used regimens include cyclophosphamide/doxorubicin (or epirubicin)/vincristine with or without prednisone, and etoposide/cisplatin (or carboplatin), with similar overall response rates (60%–76%), responses that are nevertheless usually short lived. Toxic deaths are not exceptional, especially in older patients [61]. Even though MCC express CD117, they usually bear no activating c-*kit* mutations, rendering unlikely a benefit from imatinib mesylate treatment [62].

OTR with MCC have been treated with similar modalities (wide local excision, radical node dissection, radiation therapy, and chemotherapy with various combinations of etoposide, vincristine, cyclophosphamide, adriamycin, and carboplatin). SLNB has had limited use in OTR. An additional specific therapeutic measure to consider in OTR with MCC (as with any other aggressive cutaneous tumor) is revision of the immunosuppressive treatment. Reduction of individual immunosuppressants (cyclosporine and azathioprine) may result in temporary regression of metastatic MCC [19, 28] and should be tried whenever possible in OTR with MCC in view of the aggressiveness of the tumor. Switching from calcineurin- to

mTOR inhibitors (such as sirolimus) has been shown to decrease the rate of cutaneous carcinogenesis [63], probably via an antiangiogenic and a direct antitumor effect [64, 65]. Whether this results in a decreased risk for (further) MCC development is currently unknown, and will certainly be difficult to document in view of the relative rarity of MCC, but it can reasonably be hoped that this switch may have some benefit in OTR MCC.

In view of the likely involvement of UV radiation in the development of MCC, the measures of sun protection recommended for prevention of NMSC in general, along with the introduction of safer, less cancer-prone immunosuppressants (such as sirolimus and everolimus), should lead to a decrease of the incidence of MCC in OTR.

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Cutaneous Lymphomas

Deniz Seçkin and Günther F.L. Hofbauer

Introduction

Post-transplant lymphoproliferative disorders (PTLDs) are a clinically and morphologically heterogeneous group of lymphoid proliferations, affecting up to 5% of immunosuppressed patients following solid organ or bone marrow transplantation [1]. In transplant recipients, PTLDs are the second most common form of neoplasias after skin cancers [2], having a variable incidence that is 25- to 50 fold greater than that of the general population [3].

The clinical presentation of PTLDs can have a broad spectrum ranging from Epstein–Barr virus (EBV)-associated infectious mononucleosis-like disease to EBV-positive or EBV-negative lymphomas [4, 5]. According to the classification of the World Health Organization (WHO), PTLDs are divided into four major categories [6]: [1] early lesions, which encompass reactive lymphoplasmacytic hyperplasia and infectious mononucleosis-like lesions (mostly polyclonal); [2] polymorphous PTLD (usually monoclonal); [3] monomorphic PTLD (monoclonal), which should be classified according to the WHO classification of lymphoma; and [4] Hodgkin lymphoma and Hodgkin lymphoma-like PTLD. The Cincinnati Transplant Tumor Registry data showed that 86% of lymphomas were of B-cell origin, approximately 14% were of T-cell lineage, and less than 1% were of null-cell origin [7]. In contrast to B-cell PTLD, T-cell PTLD is usually a full-blown malignant process and usually not associated with EBV [8].

Post-transplant lymphoproliferative disorders usually present in extranodal sites, including the gastrointestinal tract, lungs, liver, lymph nodes, central nervous system, or the transplanted organ [7]. Isolated involvement of the skin is rare, as most patients with PTLD also have underlying internal organ involvement [5, 9].

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Clinical Picture

Cutaneous B-Cell Lymphoma

The clinical presentation of cutaneous B-cell lymphomas (CBCLs) in transplant recipients is diverse. The patients usually present with purple-red single or multiple nodules or tumors, which frequently ulcerate [5, 10–13]. Slowly growing erythematous, indurated plaques [4], erythematous maculopapular eruption [14], or plain single [15] or extensive ulceration can also be observed. The lymphoma lesions are usually firm, mobile, and asymptomatic [4, 12, 13]; however, they may sometimes be painful [10, 13, 16, 17], tender [4], or hypoesthesic [17]. They can be localized to a single anatomic region, or generalized [13]. Cutaneous lesions of the previously reported cases were localized on lower lip [11], chest wall [4], flank [14], upper [4] and lower extremities [10, 12, 18], forehead [11], and neck and back [13]. B-cell lymphoma has also been observed in the oral cavity as erythematous to cyanotic and hyperplastic gingival [19], a tongue ulcer [16], or chronic necrotic ulcer of the buccal mucosa and gingiva with foul-smelling discharge [17].

According to the WHO classification scheme [6], CBCLs in transplant recipients fall into the category of monomorphic PTLD. Previously reported cases showed morphological heterogeneity including diffuse large B-cell lymphoma [4, 13, 16, 17], plasmablastic lymphoma [13, 20], marginal zone lymphoma [13], extramedullary cutaneous plasmocytoma [12, 21], and isolated cutaneous lymphomatoid granulomatosis associated with an evolution to high-grade B-cell lymphoma [22]. Gingival Burkitt lymphoma has also been reported in this group of patients [23].

Although the number of reported cases is small, the prognosis in post-transplant CBCLs limited to the skin seems to be better than the one in identical extracutaneous forms [1, 4, 15]. However, exclusive cutaneous involvement may rarely lead to fulminant outcome [22].

Cutaneous T-Cell Lymphoma

The skin is an unusual site for primary or secondary extranodal involvement of post-transplant T-cell lymphomas. Cutaneous T-cell lymphomas (CTCLs) comprise 30% of all post-transplant cutaneous lymphomas [1].

The clinical features of the cutaneous lesions are the same as those observed in nonimmunosuppressed individuals, such as pruritic erythematous infiltrated plaques, erythroderma, solitary or several and sometimes ulcerated papules, and red-violaceous nodules or tumors [24, 25]. In a recent review, Ravat et al. [26] documented the previously reported 23 cases of post-transplant primary CTCLs. Five cases had erythrodermic CTCL, of which 2 cases had Sezary syndrome; 8 cases had primary cutaneous anaplastic large cell lymphoma; 2 cases had nonerythrodermic mycosis fungoides, of which 1 had the syringotropic variant; and 5 cases had peripheral T-cell lymphoma under the WHO classification. Of 5 cases with peripheral T-cell

lymphoma, 4 were pleomorphic and 1 was immunoblastic. One case was difficult to classify as the immunophenotype of the subcutaneous T-cell infiltrate was unusual for subcutaneous panniculitis-like T-cell lymphoma. The remaining 2 cases were subcutaneous panniculitis-like T-cell lymphoma of alpha/beta derivation with a $CD56^+$ phenotype and lymphomatoid papulosis.

In contrary to primary CTCLs in the general population, the majority of post-transplant primary T-cell lymphomas behave aggressively [1, 24, 26], leading to death in the first year after the diagnosis [24, 26]. Fourteen patients who had disease with an aggressive course died within 2 years of diagnosis (mean duration of survival from diagnosis was 10.2 months), while the remaining 9 had a relatively indolent course. The mean interval between transplantation and diagnosis was not different in the patients with poor prognosis and the long-term survivors. The prognoses associated with particular subsets of CTCLs in the general population may not apply to post-transplant counterparts as $CD30^+$ peripheral T-cell lymphomas, primary cutaneous anaplastic large cell lymphomas, and erythrodermic CTCLs without circulating Sezary cells may follow an aggressive course [24, 26].

Because both primary CBCLs and CTCLs in transplant recipients are very rare, these tumors might arise by coincidence rather as a consequence of immunosuppression. Nevertheless, concomitant involvement of other organs with secondary spread to the skin must be excluded. Treatment strategies in these patients should be planned after appropriate staging investigations are performed.

Histological Picture

Cutaneous B-Cell Lymphoma

Several types can be distinguished clinically and histologically, as summarized in the recent WHO-EORTC classification [27, 28].

Follicle center cell lymphoma shows centrocytes, few centroblasts, and many reactive T cells in a nodular of diffuse pattern. The epidermis is spared (grenz zone). Reactive follicle center structures may be apparent. With more advanced lesions, centroblast numbers increase while large numbers of follicle center cells lead to a monomorphic infiltrate. Immunohistochemical staining yields $CD19^+$, $CD20^+$, $CD22^+$, and $CD79A^+$ tumor cells. Additionally, clonal Ig rearrangements are present. In contrast to nodal lymphoma, the t(14;18) translocation is absent while bcl-2 protein is expressed only in a minority of cases.

Immunocytoma/marginal zone lymphoma shows small lymphocytes, lymphoplasmacytoid cells, and plasma cells in a diffuse or nodular pattern within the dermis while sparing the epidermis with a grenz zone. Tumor cells express monotypic Ig and CD79, but no CD5. Ig genes typically are clonally rearranged without specific translocations known yet.

Large B-cell lymphoma of the leg sports large B cells within the dermis sparing the epidermis in a diffuse pattern. Only few small and other reactive cells are seen.

Tumor cells stain $CD19^+$, $CD20^+$, $CD22^+$, and $CD79A^+$ with strong BCL2 protein expression. Ig rearrangement is mostly clonal.

Beyond these clearly defined primary CBCLs, provisional entities within the EORTC classification exist. Intravascular CBCL affects dermal and subcutaneous blood vessels in clusters of large tumor cell with occasional extravasation of tumor cells. Cutaneous plasmocytoma is defined by a clonal plasma cell population in the dermis. Ig is monotypic, CD38 is expressed, but CD20 or LCA is negative.

Cutaneous T-Cell Lymphoma

Histopathology in CTCL patients serves to confirm the diagnosis, a progression from patch to plaque or tumor stage, and to recognize relapsing lesions. Steroids both topical and systemic should be avoided several weeks before biopsy on account of their pronounced induction of lymphocyte apoptosis. Typical findings in CTCL with its most frequent type of mycosis fungoides include lymphocytes with convoluted and hyperchromatic nuclei, which may form intraepidermal microabscesses (Pautrier's microabscess). The underlying dermis presents with predominantly mononuclear cells displaying indented nuclei accompanied by a mixed inflammatory infiltrate of lymphocytes, plasma cells, and eosinophilic granulocytes. Lymphocytes in the epidermis seem larger than those in the dermis. In addition, moderate spongiosis of the epidermis with infiltrating lymphocytes (epidermotropism) sometimes with a lacuna or a seeming halo (halo cells). This contrast is sometimes referred to as "too much [inflammation] for too little [spongiosis]."

Lining-up of lymphocytes along the basal membrane in so-called Indian files is noted when five or more lymphocytes can be observed before entry into the epidermis. A predominantly mononuclear infiltrate may be seen perivascularly and especially with increasing thickness of clinical plaques also in a lichenoid pattern. Fibroplasia in the papillary dermis may be seen. The epidermis may be atrophic as in the poikilodermic variant of mycosis fungoides or regular or slightly spongiotic. The histological diagnosis of CTCL remains tricky and should thus be augmented by repeat biopsies from different locations and at different times as well as by immunohistochemistry and detection of clonality on Southern blot or by T-cell receptor polymerase chain reaction. Typical immunohistochemical staining or detection of clonality is not diagnostic in themselves but support interpretation of a biopsy as cutaneous lymphoma rather than the typical differential diagnoses of dermatitis or psoriasis.

The clinical variants of CTCL rely on histology for their classification. Epidermotropism is pronounced in pagetoid reticulosis, a clinical variant that by some authors is summarized under the umbrella of chronic actinic dermatosis rather than lymphoma nowadays. Mucin and cystic spaces surrounding and disrupting the pilosebaceous unit appear in follicular mucinosis.

In the erythrodermic patient, histology varies greatly. Lymphocytes accumulate perivascularly, while density of infiltrate and epidermotropism may need repeat biopsies for detection.

A pragmatic approach suggests to first distinguish between classical mycosis fungoides, its variants, and Sézary's syndrome on one side and different conditions on the other side; this will yield diagnosis in these common forms of CTCL, which make up about 60% of CTCL. Second, CD30 expression on immunohistochemistry separates large cell cutaneous $CD30^+$ lymphoma from the remaining $CD30^-$, rare conditions such as subcutaneous panniculitis-like T-cell lymphoma, $CD4^+$ large-cell CTCL, and $CD8^+$ CTCL.

Risk Factors

The development of PTLD is related to the immunosuppressive treatment that results in suppression of T-cell function, which is needed for graft preservation. Immunosuppressive drugs may exert direct mutagenic effects, but also decrease EBV-specific T cells that may result in polyclonal and monoclonal PTLDs [4, 25, 26]. B-cell PTLDs have a strong association with EBV infection [4, 13]; however, only rarely are T-cell PTLDs, including primary CTCLs, EBV related [24, 29]. In Japan, however, 30% of T-cell PTLDs are EBV positive, and coinfections with EBV and human T-cell lymphotrophic virus can be observed [30]. Epstein–Barr virus-negative patients who receive grafts from EBV-positive individuals may acquire a primary EBV infection from the donor and are at highest risk for developing PTLD [24, 31]. An increase in the peripheral blood EBV load often precedes the development of PTLD. Similarly, cytomegalovirus disease, especially in seronegative patients receiving a seropositive graft, is also a risk factor [32]. Human herpesvirus 8 (HHV-8)-associated primary cutaneous plasmablastic lymphoma has also been described in a renal transplant recipient [33], and there is strong molecular evidence of graft-related transmission of HHV-8 [34].

The type of immunosuppressive agent and type of transplant are known to be major factors in the development of PTLD. Use of calcineurin inhibitors, such as cyclosporine and tacrolimus, and treatment of graft rejection with high-dose steroids, antilymphocyte antibodies (OKT3), or antithymocyte globulin are associated with high risk of PTLD [5,31,35]. In both children and adults, PTLDs are more common after heart and lung transplantation than after kidney or liver transplantation [36]. It is possible that it is the duration and the intensity of immunosuppression rather than a specific drug or the allograft type that is important in the development of PTLD. To date, however, both primary CBCLs and CTCLs have been reported, mostly in renal transplant recipients [4, 13, 15, 25, 26].

Although PTLDs may occur at any time after transplantation, the risk is greatest within the first year when the immunosuppression is at the highest level and declines over time thereafter [36]. Interestingly, most cases of primary cutaneous lymphomas occur long after the first year post transplantation [5, 12, 13, 15, 20, 26].

In contrast to extracutaneous lymphomas, primary cutaneous lymphomas usually occur in men [1, 26].

A younger age at transplantation is another risk factor for PTLD. The higher incidence of PTLD in pediatric transplant recipients probably results from the increased risk of primary EBV infection after transplantation [36].

Treatment and Prevention Options

Cutaneous B-Cell Lymphoma

As a first step in organ transplant recipients, reduction of immunosuppression should be considered. Some post-transplant cutaneous lymphomas regress spontaneously following such a reduction of the immunosuppressive regimen. Follicle center cell lymphoma and marginal zone lymphoma carry a favourable prognosis. Cases with detection of *Borrelia* DNA have been reported in the general population [37]. Some authors thus advocate initial antibiotic therapy in all such cases, whether immunocompetent or immunosuppressed (e.g., doxycycline 2×100 mg daily for 3 weeks). Lesions can be excised, treated antibiotically, or irradiated as first line, while rituximab (anti-CD20), interferon-alpha, and steroids are second-line options for intralesional application. For multiple lesions, rituximab can also be considered for systemic application. CD20 expression should be verified in these cases.

Large cell CBCL carries a worse prognosis than those with a follicular architecture in the general population. No large series have been reported, leading to purely empirical recommendations. Radiotherapy, excision, and monochemotherapy such as liposomal doxorubicin, and polychemotherapy (CHOP, COP, MACOP) are employed.

Cutaneous T-Cell Lymphoma

Mycosis fungoides and variants should be treated according to clinical stage. Aggressive therapy shows no survival advantage [38]. Consequently, therapy should judiciously be escalated. Topical therapy is the start and employs topical steroids, PUVA photochemotherapy, and topical cytostatic drugs such as mechlorethamine [39] and BCNU [40] or radiotherapy using X-rays or electron-beam therapy. In immunosuppressed patients, PUVA should be considered carefully because of its known induction of cutaneous carcinogenesis. Narrow-band UVB has lately been successfully used as first-line phototherapy in CTCL. To date, narrow-band UVB has not been reported to induce cutaneous carcinogenesis and should thus be favored over PUVA therapy in organ transplant recipients, if any phototherapy is applied at all. However, phototherapy in CTCL has mainly been accomplished using PUVA, so there are extensive clinical data about PUVA efficacy and side effects. Thus, phototherapy in general and PUVA photochemotherapy in particular should carefully

be weighed in any immunosuppressed patient for its potential benefit and harm in every individual case. Topical cytostatics drugs are popular among U.S. and Scandinavian physicians, while PUVA is preferred in central Europe. Extracorporeal photopheresis is not adequate for early mycosis fungoides [41]. In more advanced stages, topical therapy is combined, such as with PUVA with or without systemic retinoids or recombinant interferon-alpha [42].

Lymphomatoid papulosis and large cell $CD30^+$ CTCLs carry a good prognosis in the general population, and there is a chance of spontaneous remission. However, post-transplant large cell $CD30^+$ CTCLs generally follow an aggressive course. Excision or radiotherapy is recommended for solitary or localized $CD30^+$ large cell T-cell lymphomas, and radiotherapy or methotrexate for multifocal $CD30^+$ large cell T-cell lymphoma with no waxing and waning. For multifocal $CD30^+$ large cell T-cell with waxing and waning or lymphomatoid papulosis, no therapy or methotrexate or phototherapy (narrow band UVB or PUVA) is chosen in the immunocompetent patient, but this is not recommended in organ transplant recipients as first-line therapy because of carcinogenicity.

Treatment of Sézary's syndrome in the general population is mostly reported in retrospective studies without clear definitions of diagnosis and stage, which prevents comparison of therapy reports [43]. First-line therapy typically comprises PUVA with interferon-alpha, extracorporeal photopheresis, and HN_2. Second-line therapies comprise bexaroten, chlorambucil with steroid, low-dose methotrexate, polychemotherapy, denileukin-diftitox, electron-beam therapy, and alemtuzumab (anti-CD52).

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Appendageal Malignancies

Catherine A. Harwood, Charlotte M. Proby, and Rino Cerio

Introduction

Appendageal tumours (ATs) are neoplasms in which differentiation occurs toward one or more adnexal structures of the skin. The increased incidence, clinicopathologic spectrum and prognosis of keratinocyte skin cancers in organ transplant recipients (OTR) have all been well documented in recent years. In contrast, there are few publications relating to post-transplant ATs. Available reports provide evidence for a probable increased incidence of at least some of these neoplasms in OTRs. However, most published studies are essentially anecdotal observations or small case series, few data are available from systematic transplant cohort studies, and there is a dearth of reliable information on the prevalence of ATs in the general population. Such factors place major limitations on attempts to accurately assess relative risk of these tumours in OTRs. Nonetheless, an appreciation of the clinicopathological features of ATs arising in OTRs is important as, although some may simulate more common cutaneous malignancies, their prognosis may be significantly different, and they may occasionally represent a source of considerable morbidity and mortality. In reviewing information on post-transplant ATs, this section will draw on evidence from the published literature together with additional unpublished data from a cohort of over 1000 renal transplant recipients under surveillance at Barts and the London NHS Trust (BLT), London, U.K.

Classification of Appendageal Tumours

Various mesenchymal neoplasms may theoretically be included in the category of appendageal tumours, but the term is usually reserved for those with origin from, or differentiation towards, epithelial adnexal structures [1]. Embryologically, skin

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appendages are derived from the ectoderm (reviewed in [2]). Hair follicles originate from epidermal basal layer cells that protrude into the dermis and are surrounded by a condensation of mesenchymal cells; sebaceous glands, aside from those in the labia minora and glans penis, are hair follicle-dependent and originate as a budding of the sebaceous gland primordium; eccrine glands originate from epidermal epithelial germs but contain less mesenchymal aggregation than pilosebaceous units; apocrine glands develop from an upper bulge in hair follicles.

The current World Health Organization (WHO) classification categorises ATs into three groups, usually with benign and malignant counterparts ([1]; Table 1): 1) those with apocrine or eccrine differentiation; 2) those with follicular differentiation, and 3) those with sebaceous differentiation. Many of these tumours have a rather confusing array of synonyms as listed in Table 1, and may display more than one line of differentiation (hybrid or composite tumours). Whilst BCC should probably be included as an AT, specifically as trichoblastic carcinoma, its traditional categorisation as a keratinocyte malignancy will be maintained in this chapter.

Clinicopatholgical Features: An Overview

Clinical features: For both benign and malignant ATs, diagnosis is more often made on the basis of histology rather than clinical features. In general, the anatomic distribution of ATs parallels that of the normal structures, for example, apocrine tumours in the axillae and anogenital areas and follicular tumours on hair-bearing skin. Benign tumours tend to occur in younger individuals than carcinomas and are often smooth-surfaced, skin-coloured or reddish-brown papules or nodules. Most malignant ATs present as irregular, ulcerated plaques [1], but given the rather subtle clinical appearances of many malignant ATs, it is not unusual that they are misdiagnosed as more common skin cancers such as BCC and SCC or cutaneous metastases from internal neoplasms. Diagnosis of certain ATs may have important implications, as some are markers for syndromes associated with internal malignancies, even in OTRs. For example, the appearance of sebaceous tumours has been described as a presenting feature of Muir Torre syndrome in OTRs [3]. Most benign ATs arise *de novo*, but some may arise in organoid naevi (naevus sebaceous), hamartomas involving epidermis, dermis and adnexae (e.g., [4]). Appendageal malignancies also most commonly arise *de novo*, although may occasionally result from malignant transformation in a benign counterpart (e.g., [5, 6]). Most low-grade carcinomas do not metastasise. Haematogenous spread is the major route for metastasis for some tumours such as adenoid cystic carcinoma, but most spread by either lymphatic or haematogenous dissemination. Malignancies with eccrine differentiation commonly metastasise to the skin [1].

Histopathology and immune profile: Diagnosing ATs may be challenging even for experienced dermatopathologists, and the detailed practical considerations of histological evaluation are beyond the scope of this chapter, but have recently been extensively reviewed [2, 7]. An overview only is presented here.

Table 1 WHO classification of appendageal tumours and occurrence in organ transplant recipients

Differentiation	WHO histological classification[a]	ICD-O Code	Synonyms	No of cases	References
Apocrine and eccrine					
Malignant ($n = 19$)	Tubular carcinoma	8211/3	–	0	
	Microcystic adnexal carcinoma[b]	8407/3	Sclerosing sweat duct carcinoma; eccrine epithelioma; syringomatous carcinoma	4	1 (×2); 5 (×1); 6 (×1)
	Porocarcinoma[b]	8409/3	Malignant eccrine poroma; malignant hidroacanthoma simplex; poroepithelioma; epidermotropic eccrine carcinoma	6 (+ in situ × 2)	BLT* (×5 + in situ ×2), 12 (×1)
	Spiradenocarcinoma	8403/3	Malignant spiroadenoma	0	
	Malignant mixed tumour	8940/3	Malignant apocrine mixed tumour; malignant chondroid syringoma	0	
	Hidradenocarcinoma[b]	8400/3	Clear cell papillary carcinoma; nodular hidradenocarcinoma; malignant eccrine acrospiroma; primary mucoepidermoid carcinoma of skin; clear cell eccrine carcinoma; malignant nodular clear cell hidroadenoma	6	1 (×2), BLT (×2); 3 (×1); 9 (×1)
	Mucinous carcinoma	8480/3	Primary cutaneous mucinous carcinoma; colloid, gelatinous and adenocystic carcinoma	1	1 (×1)
	Digital papillary carcinoma	8408/3	Digital papillary adenocarcinoma; aggressive digital papillary adenoma	0	
	Adenoid cystic carcinoma	8200/3	–	1	13 (×1)
	Apocrine carcinoma	8402/3	Apocrine adenocarcinoma; apocrine gland carcinoma	1	1 (×1)
	Paget disease of breast	8540/3	–	0	
	Extramammary Paget disease	8542/3	–	0	

Table 1 (continued)

Differentiation	WHO histological classification[a]	ICD-O Code	Synonyms	No of cases	References
Benign ($n = 11$)	Hidrocystoma	8404/0	Apocrine gland cyst; apocrine cystadenoma	1	1 (×1)
	Syringoma	8407/0	Eccrine syringoma; lymphangioma tuberosum multiplex	0	
	Poroma	8409/0	Eccrine poroma; hidroacanthoma simplex; dermal duct tumour; syringoacanthoma	3	1 (×1); BLT (×1); 10 (×1)
	Syringofibradenoma	8392/0	Eccrine syringofibradenoma; eccrine syringofibroadenomatous hyperplasia; acrosyringeal adenomatosis	1	BLT (×1)
	Hidradenoma	8402/0	Clear cell hidreadenoma; nodular hidradenoma; poroid hidradenoma; acrospiroma; solid-cystic hidradenoma	0	
	Spiradenoma	8403/2	-	1	BLT (×1)
	Cylindroma	8200/0	Cylindrospiradenoma; spiradenomcyclindroma	0	
	Tubular adenoma	8211/0	Apocrine adenoma; tubular adenoma	0	
	Tubular papillary adenoma	8263/0	Tubulopapillary hidradenoma; papillary tubular adenoma	0	
	Syringocystadenoma papilliferum[b]	8406/0	Syringoadenoma	5	1 (×3); BLT (×1); 3 (×1)
	Hidradenoma papilliferum	8405/0	–	0	
	Mixed tumour (chondroid syringoma)	8940/0	Chondroid syringoma; mixed tumour of skin	0	

Table 1 (continued)

Differentiation	WHO histological classification[a]	ICD-O Code	Synonyms	No of cases	References
Follicular					
Malignant ($n = 2$)	Pilomatrical carcinoma	8110/3	Pilomatrix carcinoma; metrical carcinoma; invasive pilomatrixoma; malignant pilomatrixoma; matrix carcinoma	0	
	Proliferating tricholemmal tumour	8103/1	Epidermoid carcinoma in sebaceous cyst; subepidermal acanthoma; proliferating epidermoid cyst; invasive hair matrix tumour of the scalp; trichochlamydocarcinoma; giant hair matrix tumour; proliferating tricholemmal cyst; proliferating pilar cyst; proliferating follicular cystic neoplasm; proliferating tricholemmal cystic squamous cell carcinoma; proliferating isthmic cystic carcinoma	2	7 (×1); 11 (×1)
Benign ($n = 9$)	Trichoblastoma	8100/0	Trichoepithelioma; trichoblastic fibroma; adamantinoid trichoblastoma; trichogerminoma; sclerosing epithelial hamartoma	3	1 (×2); BLT (×1)
	Pilomatricoma	8110/0	Pilomatrixoma; calcifying epithelioma of Malherbe; benign calcifying epithelioma	3	1 (×3)
	Tricholemmoma	8102/0	Trichilemmoma	0	
	Trichofolliculoma	8101/0	–	2	BLT (×2)
	Pilar sheath acanthoma		Infundibuloisthmicoma	0	
	Tumour of the follicular infundibulum		Infundibular tumour	1	1 (×1)
	Fibrofolliculoma/ trichodiscoma	8391/0	Mantleoma	0	

Table 1 (continued)

Differentiation	WHO histological classification[a]	ICD-O Code	Synonyms	No of cases	References
Sebaceous					
Benign ($n = 5$)	Sebaceous adenoma[b]	8410/0	-	5	1 (×3); BLT (×1); 14 (multiple)
Malignant ($n = 11$)	Sebaceoma	8410/0	Sebaceous epithelioma; basal cell epithelioma with sebaceous differentiation; sebomatricoma	0	
	Cystic sebaceous tumour	8410/0		1	4 (×1)
	Sebaceous carcinoma[b]	8410/3	–	10	1+2 (×5), BLT (×3), 8 (×1); 14 (×1)

BLT, unpublished data relating to tumours observed and histologically confirmed at least once in a cohort of >1000 organ transplant recipients attending Barts and the London NHS Trust.

[a]As defined in the World Health Organisation Classification of Tumours: Skin Tumours (Edited by LeBoit PE, Burg G, Weedon D, Sarasin A; IARC Press, Lyon, 2006) and Tumours of Soft Tissue and Bone (Edited by Fletcher CDM, Unni KK, Mertens F; IARC Press, Lyon, 2002).

[b]Specifically discussed in the text.

1. Harwood et al. [27]; 2. Harwood et al. [29]; 3. Ramsay et al. [20]; 4. Mudhar et al. [26]; 5. Snow et al. [22]; 6. Happenreijs et al. [18]; 7. Garrett and Scott [24]; 8. Bordea et al. [25]; 9. Blohme and Larko [16]; 10. Euvrard et al. [21]; 11. Oh et al. [17]; 12. Plunkett et al. [23]; 13. Lindelof et al. [71]; 14. Levi et al. [3].

Morphological features are critical in the evaluation of ATs. The histogenesis of many ATs is unknown, but pathology may give clues as to likely differentiation. The most clear-cut evidence of differentiation is in follicular tumours, where signs such as trichohyaline granules and follicular papillae may occur. Sebaceous differentiation is suggested by the presence of holocrine secretion and "foamy" cells with coarse cytoplasm and starry nuclei (Mulberry cells). Apocrine differentiation may manifest with decapitation secretion of columnar luminal cells with eosinophilic cytoplasmic granules and eccentric, basally located nuclei. However, this may not be straightforward in tumours of ductal origin since eccrine and apocine ducts are very similar, and columnar cells of eccrine secretory coils can resemble poorly differentiated apocrine secretory cells [1].

The utility of immunohistochemical, other special stains, and ultrastructural studies in terms of diagnostic value and yield is variable (reviewed in [2]). Sebaceous glands are immunoreactive to low molecular weight cytokeratin (LMWK), epithelial membrane antigen (EMA), and the lymphatic marker D2-40. Sweat gland tumours non-specifically express oestrogen and progesterone receptors. Eccrine secretory cells express LMWK, EMA, carcinoembryonic antigen, and S100 protein, and associated myopeitheial cells express smooth muscle actin, p63, and calponin. Eccrine excretory cells stain with high molecular weight keratin and cytokeratin 14. Apocrine cells contain periodic acid Schiff (PAS)-positive and diastase resistant granules and iron pigment that stains with Prussian blue. They express androgen receptors, and luminal cells are positive for gross cystic disease fluid protein 15 (GCDFP-15), EMA and CEA. Apocrine secretory cells are positive for LMWK, GCDFP-15 and androgen receptors, but are oestrogen receptor/progesterone/BCL2 negative.

Epidemiology

Given the greatly increased incidence of the keratinocyte skin cancer, it might be predicted that the incidence of ATs would also be high in OTRs, but reliable evidence for this is limited and, until recently, largely based on case reports and case series. For example, there are reports of ATs developing in the context of immunosuppression including HIV infection, other immunosuppressed states [5, 8–15], as well as organ transplantation [3, 16–26].

In 2003 we reported the first systematic analysis of an association between organ transplantation and ATs in a retrospective comparison of consecutive tumours from OTRs and immunocompetent individuals presenting to our institution (BLT) over a 6-year period, 1993–1998. ATs in 178 immunocompetent people were identified from a population base of over 600,000 (0.03%) served by our institute, compared with 21 / 650 (3%) OTRs under surveillance [27]. The OTRs were all renal transplant recipients with a mean duration of transplantation of 9.8 years (range 1–28 years). Anatomical distribution was similar in both groups, with 67% and 74% respectively presenting on the head and neck. Just 20 / 231 (8.7%) lesions were malignant, but malignancy was significantly over-represented in the transplant

cohort: 10 / 23 (43%) OTR ATs were malignant compared with 8/195 (4%) immunocompetent ATs ($p < 0.0001$). Sebaceous tumours were more common in OTRs compared with immunocompetent patients (30% versus 6%, $p < 0.0001$). Indeed, sebaceous tumours were the most frequent subtype among OTRs and the least frequent in immunocompetent individuals. There were few differences between the two groups with respect to the degree of cytological atypia in malignant lesions, host inflammatory response or histological evidence of human papillomavirus (HPV) infection.

In the 15-year period up to mid-2007, our cohort of OTRs has increased to over 1000 individuals. We have now recorded 23 invasive malignant ATs in 21 OTRs (8 sebaceous carcinomas, 5 porocarcinomas, 4 hidradenocarcinomas, 2 microcystic adnexal carcinomas, one mucinous carcinoma and one apocrine adenocarcinoma) and 2 porocarcinomas-in-situ. These data, together with those pooled from the published literature, are presented in Table 1. Of the 32 invasive malignant ATs reported (excluding our two *in situ* porocarcinomas), 17 (59%) were of apocrine/eccrine origin, the most common being eccrine porocarcinoma; 11 (34%) were sebaceous in origin, of which 10 were sebaceous carcinomas; and 2 (6%) were follicular malignancies.

Pathogenesis

The aetiology of most OTR ATs is unclear, but immunosuppressive drugs, ultraviolet radiation (UVR), genetic factors, and HPV infection are plausible contributory factors.

Immunosuppressive drugs may play a role through mechanisms independent of their immunosuppressive effects. For example, ciclosporin is implicated in causing hyperplasia and dysplasia of the pilar matrix [19, 28] and azathioprine may have more specific effects on sebaceous tumour development [29], possibly enhanced by its interaction with UVA [30]. However, the occurrence of ATs in other immunosuppressed states such as HIV infection, suggests that the reduction in immune surveillance *per se*, rather than direct carcinogenic effects, is likely to be the most important factor in the contribution of drugs to the pathogenesis of ATs.

Evidence that ***ultraviolet radiation*** plays a role in AT formation includes the predilection of ATs for the head and neck, and the occurrence of many ATs in OTRs with other UVR-associated skin cancers [27]. These observations may be in part confounded by the increased density of pilosebaceous apparatus in the head and neck area. However, eccrine sweat glands are found on all body sites, yet eccrine tumours reported in OTRs to date have usually arisen on UVR exposed sites [27]. It may also be pertinent that malignant ATs are more common in patients who are older at transplantation and therefore have a potentially greater lifetime cumulative UVR exposure, a feature also associated with increased risk of post-transplant SCC. As in the case of keratinocyte skin cancers, mechanisms are likely to involve both direct mutagenesis by UVR and indirect effects on immune surveillance [31–34].

Genetic predisposition to ATs is well recognised and includes, for example, the occurrence of sebaceous tumours in Muir-Torre syndrome [35] due to mutation of

DNA mismatch repair genes (see below), and the appearance of multiple cylindromas and other ATs associated with loss of the tumour suppressor cylindromatosis gene on chromosome 16q [36,37]. Multiple trichoepitheliomas have been mapped to chromosome 9p21 [38], and sporadic cases may also be associated with deletions in 9q22.3, a region containing the *PTCH* gene [39]. Trichilemmomas have mutations in PTEN, the gene involved in Cowden syndrome [40]. To what extent these genes contribute to post-transplant ATs is unknown.

Human papillomaviruses have been proposed as co-carcinogens in post-transplant SCC (see chapter by Herbert Pfister), and there are limited data for a role in some ATs. In animal models, harvest mouse papillomavirus has been found in spontaneous trichoepitheliomas and sebaceous carcinomas arising in European harvest mice [41] and FVB/N K14-HPV16E7 transgenic mice develop sebaceous epitheliomas [42]. In a series of eyelid sebaceous carcinomas from Japan, 13/21 (62%) tumours were HPV DNA positive by in situ hybridisation with probes for HPV-6, 11,16,18, 31 and/or-33 [43], but positive signal in the nucleus was also observed in the cells of surrounding normal sebaceous glands and epidermis. Beta-HPVs and HPV-6 were detected in all of 11 trichoepitheliomas examined in one series [44], but not in 25 paraffin-embedded trichilemmomas in another [45]. HPV-20, HPV-23, and HPV-DL332 were detected in a solitary eyelid syringoma [46] and sebaceous carcinoma of the vulva may be HPV-associated [47, 48]. Data from plantar skin suggests that HPV 63 targets keratinocytes resident in or around the eccrine ducts [49], and clinicopathological evidence suggested the involvement of HPV in a case of mixed tubulopapillary hidradenoma combined with syringocystadenoma papilliferum, although HPV was not detected in studies limited to HPV types 2, 6/11, 16 and 18 [50]. In few of these examples has the involvement of HPV been systematically examined at an epidemiological and/or functional level, and the significance of the associations described remain unclear.

Specific Appendageal Tumours

Based on the information presented in Table 1, only the most common appendageal neoplasms, defined as those reported on at least four occasions in OTRs (microcystic adnexal carcinoma, porocarcinoma, hidradenocarcinoma, syringocystadenoma papiliiferum, sebaceous adenoma, and sebaceous carcinoma), are discussed in further detail:

Eccrine and Apocrine Tumours

Eccrine sweat glands are widely distributed in the skin, whereas apocrine glands are concentrated in the axillae, inguinal, and anogenital regions as well as the umbilicus, eyelid (Moll's glands), areola and external auditory meatus. Review of the literature (Table 1) confirms that tumours of eccrine/apocrine origin are collectively the most common post-transplant appendageal neoplasms. The histological spectrum of

these sweat gland tumours is wide and complicated by the existence of lesions with composite or mixed differentiation. In addition, tumours previously assumed to be of eccrine origin are now recognised to have apocrine counterparts [7]. So called "apoeccrine glands" are also recognised and are found in the axillary regions and within lesions of organoid naevi. Their existence may explain the origin of some appendageal tumours that have both eccrine and apocrine components, including syringocystadenoma papilliferum (SCAP).

Microcystic adnexal carcinomas (Fig. 1) are low grade adenomcarcinomas differentiated towards ducts [51]. Although generally believed to be of eccrine origin, there is evidence to support a mixed appendageal lineage. They usually occur as solitary facial lesions with a predilection for the upper and lower lip in women. They are locally infiltrative and destructive and invade nerves with high local recurrence rates, but rarely, metastasise. Histologically they consist of poorly circumscribed proliferations of rather bland epithelial cells infiltrating the dermis and subcutaneous tissue. These malignant cells are small and basaloid or clear, and have minimal cytological atypia. Keratin-filled microcysts are often present in the superficial dermis, and tubular and ductal structures dominate the deeper parts of the lesion. The tumour stroma is sclerotic [1]. Differential diagnoses include desmoplastic trichoepithelioma and morphoeic BCC. In superficial skin biopsies, microcystic adnexal carcinoma may be misdiagnosed as syringoma and the base of the lesion must be assessed in all cases.

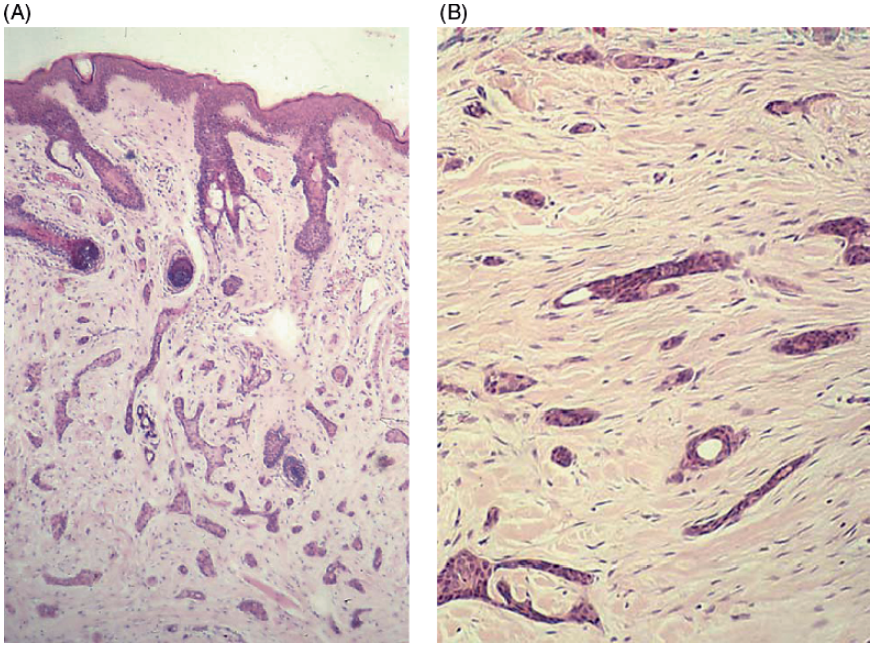

Fig. 1 Microcystic adnexal carcinoma. Photomicrographs at (**A**) low power and (**B**) high power show nests and cords of pleomorphic tumour cells with extensive luminal formation. Reproduced with permission from: Harwood et al. [27]

Porocarcinomas (Fig. 2A–F) are the commonest malignant tumours related to sweat gland ducts and show both intraepidermal and dermal components [51]. They usually present as nodulo-ulcerative plaques and are aggressive tumours with potential for both local recurrence and distant metastases. Although up to 50% have been described as arising in pre-existing benign poromas [51], this is not our experience in OTRs, and the five invasive and two in situ lesions that have presented to our institution have all apparently arisen *de novo* (Table 1). Furthermore, previous reports indicate that up to 50% of porocarcinomas occur on the lower limbs with 20% or fewer on the head and neck [52], whereas the majority of the OTR-associated lesions in our series were located on the head and neck. Histologically they are characterised by asymmetrical, solid growth pattern with infiltrative or pushing borders and neoplastic cells with basaloid features and varying degrees of cytological atypia, hyperchromatic nuclei, and prominent nucleoli. Foci of squamous and spindle cell differentiation are common. Evidence of eccrine differentiation in the form of CEA positive ductal formation is present in most cases. The main differential diagnosis is SCC, which lacks ductal formation and intracytoplasmic lumina.

Hidradenocarcinoma (Fig. 3A,B): usually arise *de novo* and, while historically considered eccrine, evidence now suggests that they can be of eccrine or apocrine origin. They may appear anywhere on the skin and usually present as slow-growing, solitary dermal or subcutaneous nodules. Histologically they are multinodular, solid tumours with ductal structures and intracytoplasmic tubular vacuoles with areas of necrosis. Usually there is no connection between the epidermis and the tumour, but the surface may be ulcerated. They may have an aggressive course with local recurrence and/or metastasis. These tumours may occasionally simulate metastatic clear cell carcinomas including renal, lung or thyroid carcinomas. Clinical history, morphological appearances and immunoshistochemical profiles all contribute to differentiation of these entities.

Syringocystadenoma papilliferum (Fig. 4): These are benign hamartomatous tumours and usually occur as solitary, verrucous lesions, most commonly on the face and scalp. Origin is variably described as eccrine, apocrine, or apoeccrine and they may arise within pre-existing organoid naevi in up to one-third of cases [53]. Multiple epithelial invaginations extend from the epidermis and consist of a dermal fibrovascular core in which a marked plasmacytic inflammatory cell infiltrate is common. These papillary structures are lined by two layers of epithelial cells; luminal columnar cells with decapitation secretion and outer cuboidal cells. It is unclear whether the apparent over-representation of these tumours in OTRs simply reflects the fact that they are among the more common forms of AT usually diagnosed.

Sebaceous Tumours

Sebaceous gland hyperplasia (SGH) (Fig. 5A,B): Although not a true neoplasm, SGH deserves specific mention given its high prevalence as a post-transplant appendageal abnormality. SGH was first reported in association with organ transplantation in 1996 and cyclosporin has most frequently been implicated in its pathogenesis [54]. In the BLT cohort, we observed SGH in 187/815 (22%) of individuals,

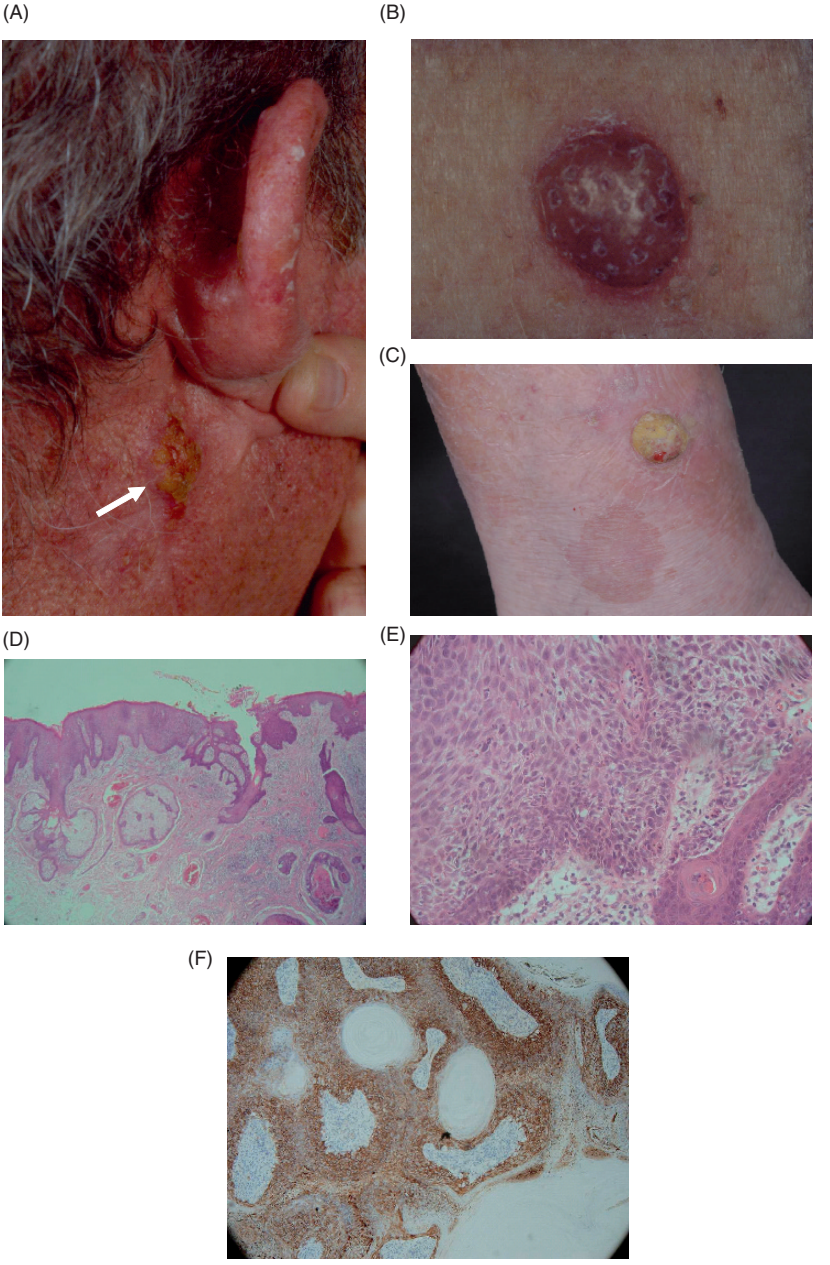

Fig. 2 Eccrine porocarcinoma. Clinical appearances may be variable, and include (**A**) a slowly growing, ulcerated plaque (arrowed) behind the ear of a 69 year old male renal transplant; (**B**) a tender, reddish-purple, non-ulcerated nodule on the scapula of a 55 year old female renal transplant recipient; (**C**) a rapidly growing, tender, ulcerated nodule on the leg of a 60 year old female renal transplant recipient. All three patients had been transplanted for over 15 years and had a history of at least 3 cutaneous SCC, and the lesions were thought to represent SCC. In each case diagnosis was made after histological examination. (**D**) Photomicrograph at low power showing both intraepithelial and dermal invasive component of an eccrine porocarcinoma from an immunosuppressed individual and (**E**) at higher power showing ductal structures surrounded by monomorphic clearer cells. (**F**) EMA positive immunolabelling supports the diagnosis

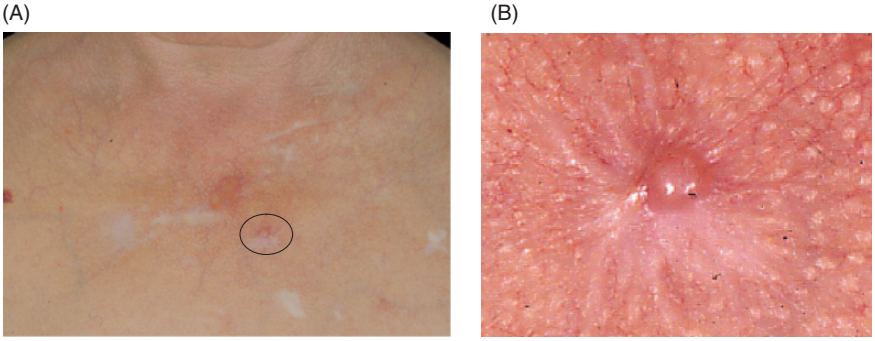

Fig. 3 Hidradenocarcinoma. This renal transplant recipient had a past history of multiple SCC. A skin coloured, non-tender, rapidly growing nodule on his upper chest was excised and confirmed to be an eccrine carcinoma. This recurred locally within 3 months as shown in (**A**) and (**B**), and was widely excised with no further recurrence. Scar from previous surgery for SCC are clearly visible. Reproduced with permission from: Harwood et al. [27]

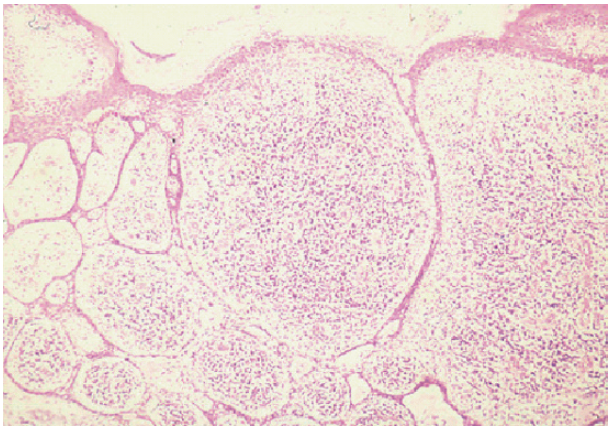

Fig. 4 Syringocystadenoma papilliferum. Photomicograph at low power showing the cystic space opens to the surface. Tumour is lined by squamous epithelium in the upper portion and luminal cells which are columnar except for the periphery where they tend to be cuboidal. Reproduced with permission from: Harwood et al. [27]

including 13% of non-caucasian OTRs [55], a prevalence similar to that of 17% reported in Ireland [56] and 17.4% in a French liver transplant cohort [57]. Clinically, SGH are usually present on the head and neck (particularly forehead, cheeks and nose), tend to be multiple, and individual lesions are generally <5 mm in size and have the appearance of a distinctive "rosette" of whitish-yellow papules arranged around a dilated hair follicle with a central dell. Larger lesions, particularly if solitary, are most commonly mistaken for BCC or even molluscum contagiosum. Histologically the appearances are of enlarged, but otherwise normal, sebaceous glands around a central follicle.

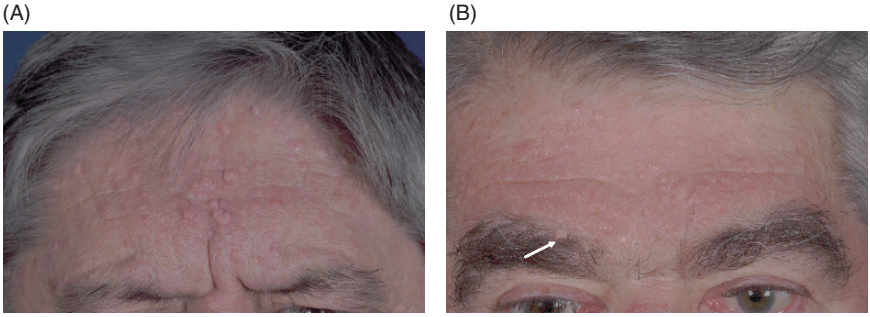

Fig. 5 Sebaceous gland hyperplasia. This is a common finding post-transplant. Lesions may be solitary or multiple as in (**A**) and (**B**). Larger lesions may be clinically confused with BCC, as with the lesion arrowed in (**B**) which was excised and confirmed histologically as sebaceous hyperplasia

A possible association between SGH and keratinocyte skin cancer (KSC) risk has recently been reported [58]. In our cohort, 56% (99/176) of patients with KSC had SGH compared with 14% (88/617) without KSC. Treatment of SGH in OTRs has not been systematically evaluated, despite the potential negative impact of SGH on quality of life [56]. In our experience, topical and systemic retinoids were unsuccessful in improving cosmesis in severe SGH (>20 individual lesions). Although cryotherapy, trichloroacetic acid electrodessication helped a minority, these approaches were associated with significant scarring; photodynamic therapy with methylaminolaevulinic acid may be a more promising strategy [59], and carbon dioxide laser has also been reported as effective [60, 61].

Sebaceous adenomas have been reported in five OTRs (Table 1) and usually present as solitary yellowish or skin coloured papules, often with a scale or crust, on the head and neck region. Multiple lesions in OTRs have also been reported, usually in the context of an underlying Muir Torre syndrome [8, 12]. Histology is characterised by well-circumscribed lobules of sebocytes with a peripheral zone of undifferentiated basaloid cells (Fig. 1). Unlike SGH, these lobules do not centre on a follicular infundibulum. There is usually a connection to overlying epidermis, and the lesion may be covered with a plug of keratin or disintegrated sebocytes.

Sebaceous carcinomas (Fig. 6A–D): Sebaceomas have not been reported in OTRs, but at least 10 post-transplant sebaceous carcinomas have been observed (Table 1; 7 published cases together with 3 unreported tumours from our own institution). It is likely that their incidence may be underestimated in OTRs, perhaps foremost because such lesions are misclassified as SCC with sebaceous differentiation. However, in our experience, sebaceous differentiation is rare in SCCs, even in OTRs [62]. Sebaceous carcinomas are often categorised into ocular and extra-ocular, although there are few biological differences between the two groups. The majority of post-transplant cases reported are extra-ocular. Most present as painless, red nodules on the head and neck and enlarge slowly, although some may grow rapidly and ulcerate. Histologically, sebocytic differentiation in sebaceous carcinomas is characterised by multivesicular, vacuolated clear cytoplasm. The dermal tumour lobules

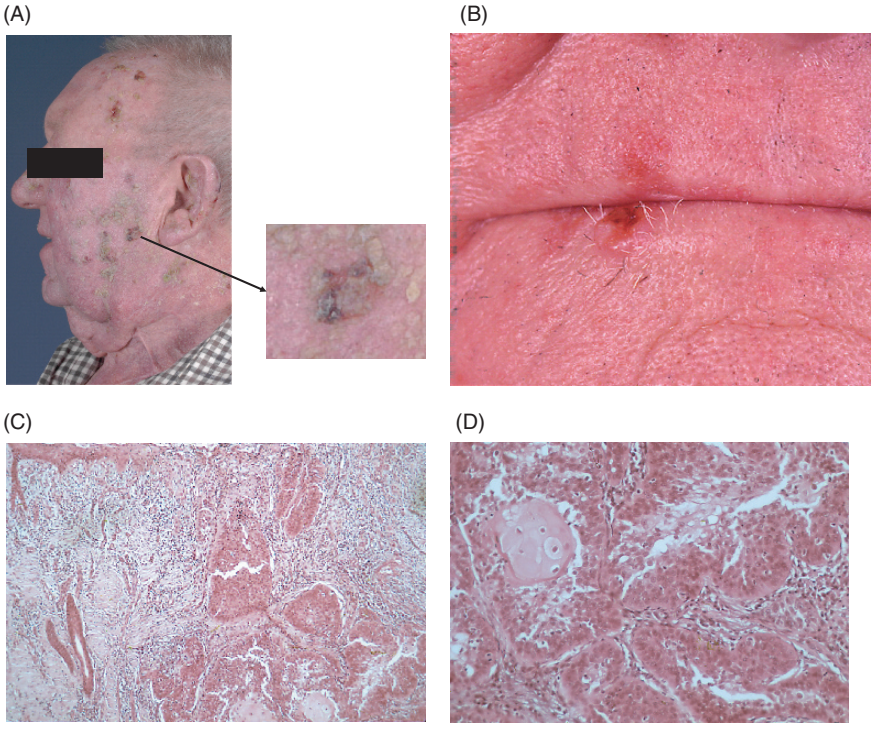

Fig. 6 Sebaceous carcinoma. Sebaceous carcainomas in transplant recipients may also simulate SCC. (**A**) This renal transplant recipient had an enormous skin cancer burden (47 SCC, 4 BCC, 2 porocarcinoma, and Merkel cell carcinoma – the latter his eventual cause of death). He also had 2 sebacceous carcinomas, one of which is highlighted here, and was thought clinically to be an SCC. (**B**) This renal transplant recipient also had 2 sebaceous carcinomas, including this ulcerated nodule on his lower lip (Reproduced with permission from: Harwood et al. [27]). (**C**) Photomicrograph of sebaceous carcinoma at low power (Reproduced with permission from: Harwood et al. [27]), and (**D**) at high power showing nuclear and cytoplasmic pleomorphism (Reproduced with permission from: Harwood et al. [27]). Sebaceous differentiation can be seen and the presence of lipid was confirmed on fresh tissue with Oil Red O and Sudan Black. Cytokeratin and epithelial membrane antigen were positive on immunohistochemistry

consist of variably atypical polygonal cells with a fibrovascular stroma. Centrally the tumour cell nests may be necrotic. Well-differentiated sebaceous carcinomas have abundant cytoplasm and oval vesicular nuclei with distinct nucleoli and variable mitiotic figures. In poorly differentiated sebaceous carcinomas, intracellular vacuoles may not be so easily seen, and immunohistochemical stains such as oil-red-O or Sudan IV may be required for identification.

Recent research interest in post-transplant sebaceous carcinoma has focused on the possible role of azathioprine in their aetiology, and the important advances in our understanding of this area are worthy of more detailed discussion. Sebaceous carcinomas are rare in the general population but have an established association with Muir-Torre syndrome (MTS), an autosomal dominant genodermatosis charac-

terised by the occurrence of sebaceous tumours and internal malignancy [63]. Inherited mutations in genes encoding post-replicative DNA mismatch repair proteins have been detected in these tumours and result in a mutator phenotype, manifesting as microsatellite instability [35, 64, 65]. Sebaceous gland tumours in patients with MTS have been reported following organ transplantation [8], and in our BLT cohort, one of four OTRs had a history of gastric carcinoid, and a second had a history of colonic adenomas, possibly in keeping with MTS [29]. We found evidence of microsatellite instability suggestive of DNA mismatch repair defects in a proportion of transplant-associated sebaceous carcinomas consistent with the notion either that immunosuppression unmasks a latent MTS phenotype, or that there is a biologically relevant interaction between DNA mismatch repair proteins and immunosuppressive drugs, most plausibly azathioprine [29]

The possible role of azathioprine is of particular interest as experimental models suggest that chronic exposure to azathioprine could select for cells with a mismatch repair deficit as a mechanism for evading its cytotoxic effects [66]. Azathioprine is partly metabolised to 6-thioguanine (6-TG) and, after incorporation into DNA, thioguanine is methylated to form S6-methylthioguanine, which directs incorporation of thymine or cytosine during DNA replication. The resultant S6-methylthioguanine-thymine mispairs are recognised by the post-replicative MMR system, and DNA that includes the mismatched T is removed, but reinsertion of T opposite S6-methylthioguanine by repair DNA polymerase (as it always assumes the template is correct) provokes a futile T insertion / removal cycle postulated to lead to cell death perhaps by allowing introduction of double stranded DNA breaks or simply by stalling DNA replication. Cells may avoid the cytotoxicity of alkylating agents by losing DNA MMR [67], and chronic exposure to azathioprine might thereby select for cells with a mutator phenotype with consequent increased mutation rate and tumorigenesis [68].

Whilst UVB is the major UV carcinogen in SCCs and BCCs, there is evidence that in sebaceous glands at least, UVA may be more relevant since it penetrates more deeply into the dermis [69]. In this respect, it is notable that azathioprine causes 6-TG to be incorporated into DNA where it can become methylated to produce a potentially promutagenic DNA lesion or interact with UVA [30]. In the latter photochemical reaction, biologically relevant doses of UVA combine with DNA 6-TG and molecular oxygen to generate mutagenic reactive oxygen species and at least one novel and potentially promutagenic DNA lesion, guanine-6-sulfonate (GSO_3). Photochemical DNA 6-TG damage also occurs in the clinical situation and the skin of patients taking azathioprine is selectively hypersensitive to UVA [30, 70].

Management and Outcome

There are very few published data relating to prognosis of transplant ATs. Outcome data for ATs arising since 1993 in the BLT cohort (mean of more than 10 years) suggests a generally good prognosis following complete surgical excision of benign

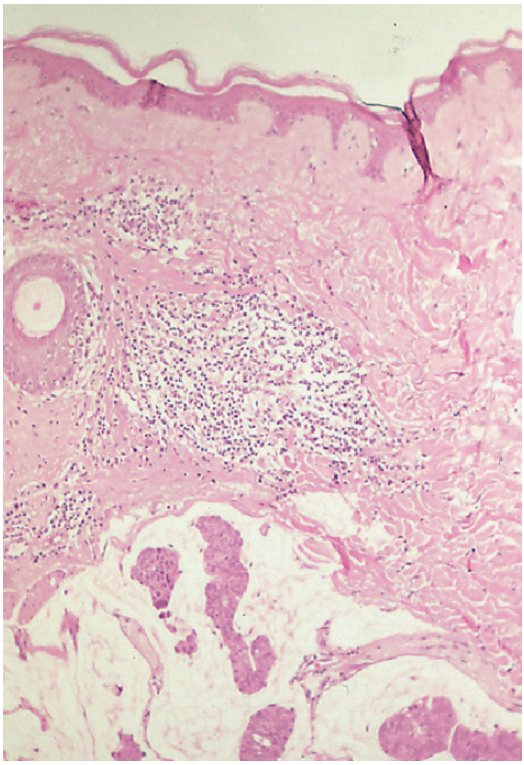

Fig. 7 Primary mucinous carcinoma. Photomicrograph demonstrates that this is composed of atypical cords and lobules of epithelial cells compartmentalised by delicate fibrous septae into islands of epithelial cells floating in a pool of pale-staining mucin. Glandular differentiation can be seen often with a cribriform pattern. Cutaneous secondaries from an adenocarcinoma were excluded after thorough clinical investigation. Reproduced with permission from: Harwood et al. [27]

ATs, with no evidence of recurrence. The relatively small number of malignant tumours in our cohort (21 invasive malignancies) precludes accurate prediction of outcome for individual tumour types. However, only one patient – with mucinous carcinoma (Fig. 7) – died from metastatic disease and one other developed skin metastases from eccrine porocarcinoma but died from unrelated medical causes. Review of the literature (Table 1) reveals a total of approximately 2 further AT - related deaths in OTRs, but in the absence of robust data relating to outcome in the immunocompetent population, it remains uncertain whether this represents a worse prognosis in OTRs.

For most benign ATs, local complete surgical excision is usually curative. Reported treatment for low-risk malignant ATs is usually wide local excision or, in certain circumstances where cosmetic considerations are paramount or the lesion is recurrent, Mohs surgery. General principles relating to management of high-risk transplant skin tumours in such as subgroups of SCC, Merkel cell cancer, and melanoma,

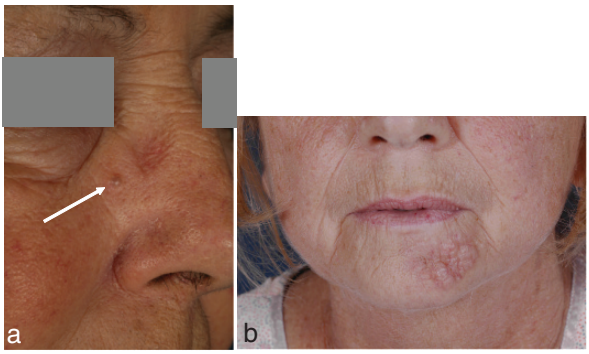

Fig. 8 Two examples of lesions in organ transplant recipients thought to represent BCCs clinically and only diagnosed histologically: (a) A small pearly nodule present for 4 months on the left cheek of this famale renal transplant recipient proved to be a hidrocystoma; (b) this slowly growing, asymptomatic plaque on the chin of a 47-years-old female renal transplant recipient was thought to represent a large BCC, but repeated diagnostic biopsies and final excision confirmed as a benign desmoplastic trichoepithelioma

also pertain to the management of more aggressive ATs, such as sebaceous carcinoma. The importance of a multidisciplinary management approach cannot be overemphasised, ideally with a dermatopathologist alongside dermatologists, transplant clinicians, plastic surgeons, oncologists, and clinical nurse specialists. For high-risk tumours, consideration should be given to radical surgery of the primary lesion, staging with sentinel lymph node biopsy, reduction of immunouppression, and possibly conversion to agents with anti-carcinogenic properties such as rapamycin. Due to the small number of metastatic cases, there exist few chemo- or radiotherapeutic protocols for treatment of metastatic disease, as discussed in Chapter by Steve Nicholson.

Unfortunately, given the rarity of malignant ATs in OTRs, few of these management strategies are evidence-based, and the likelihood of future prospective, randomised, controlled studies to guide decision-making is low. Such studies are scarce, even in the general population, and cannot be guaranteed to be informative if extrapolated to OTRs. A more pragmatic approach to providing information on management and outcome of transplant ATs might be the establishment of an international "rare transplant skin tumour" database with the aim of collating detailed retrospective and prospective data on such tumours. Such a database might prove particularly useful in gauging incidence and prevalence of ATs if based on well-defined and monitored patient cohorts, and may provide a resource of at least anecdotal information relating to outcome and treatment.

In summary, skin appendageal tumours following organ transplantation are uncommon compared with keratinocyte skin tumours, but their incidence appears to be increased over that of the general population. Their significance lies in the diagnostic confusion they may cause (Fig. 8) and their associated morbidity and occasional mortality. It is therefore of particular importance that clinicians involved in managing OTRs should be alert to their existence and possible clinicopathological presentations.

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Part IV
Prophylaxis and Therapy

The Role of the Transplant Physician in the Management of Skin Cancers After Organ Transplantation

Emmanuel Morelon, Emmanuel Mahe, and Jean-Louis Touraine

The Role of the Transplant Clinician

Although prognosis in organ transplantation has significantly improved with the development of new immunosuppressive therapies that have dramatically reduced the incidence of acute rejection, post-transplant malignancies are still one of the main complications that may occur after organ transplantation. Most of these malignancies are skin cancers, which are not only frequent, but also multiple, recurrent, and aggressive [1]. In kidney transplant recipients, the overall incidence is 20 times higher for squamous cell carcinoma and 10 times higher for basal cell carcinoma than in the general population [2]. Thus, about 50% of kidney transplant patients develop some form of skin cancer after transplantation and 5% of these skin cancer patients die of their malignancies [3]. The prophylaxis of skin cancers should therefore remain a top priority in the management of long-term post-transplant complications. It is based on a multidisciplinary approach that takes into account the main skin cancer risk factors, including overimmunosuppression, the type of immunosuppressive drugs administered, sun exposure, the possible presence of human papillomavirus, and the patient's skin phototype, with a higher risk for fair-skinned individuals [4]. The role of the transplant clinician therefore comprises three main objectives.

1. To ensure optimal immunosuppression in the transplant patient by the following means:
   - Monitoring of graft function by routine biopsy, serum creatinine measurement, or liver function tests, depending on the organ transplanted.
   - Monitoring of immunosuppressive drug concentrations by trough level measurement or pharmacokinetic tests.
   - Monitoring for side effects of immunosuppressive therapy and for the consequences of overimmunosuppression.

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At best, the prophylaxis of skin cancers requires the reduction of immunosuppressive therapy or the inclusion of mTOR inhibitors in the immunosuppressive regimen. The choice of the appropriate combination of immunosuppressive drugs and the stage at which immunosuppression should be reduced is discussed in detail below. The minimization of immunosuppression must be carefully monitored to avoid the occurrence of ongoing silent acute or chronic rejection that might impair transplanted organ function and shorten the half-life of the graft. Protocol biopsies seem a useful tool for detecting subclinical rejection, which may occur during the reduction of immunosuppression.

2. To establish close collaboration with dermatologists for post-transplant skin cancer surveillance.

   The principal screening test for skin cancers is examination of the skin by dermatologists, who possess the greatest expertise in the early diagnosis of premalignant and malignant skin lesions [5]. These examinations should be performed at least once a year for the preemptive diagnosis, and at least every 6 months for the secondary prevention of skin cancers [3,6]. The annual visit to the transplant centre for a complete evaluation of graft function and post-transplant complications is the appropriate time for the examination by the dermatologist. The surveillance and management of skin cancers by a highly qualified dermatologist with great experience of transplant skin care [5], should be done in conjunction with the transplant clinician to ensure adequate immunosuppression. It constitutes the transplant patient's best chance to reduce the risk of developing new skin cancer and to avoid the degradation of organ function resulting from inadequate immunosuppression.

3. To inform the patient fully about the risks of skin cancer and how to avoid it.
   Ultraviolet radiation remains the main avoidable risk factor for skin carcinomas. Patients must be informed before and after transplantation about the risks of skin cancers and the methods of sun protection, including the avoidance of sun exposure, the use of appropriate clothing, sunscreen application, and self-examination [3, 5, 7]. Clear oral and written advice about sun protection must be provided. Because patient management during the year after transplantation focuses on graft rejection and the prevention of infection, it seems important to educate transplant recipients more thoroughly about sun protection immediately after the initial period and at all post-transplant follow-up visits.

The compliance of renal transplant patients with sun avoidance varies greatly [7–10]. The inclusion of skin specialists in transplant teams probably increases the impact of the sun protection message, as suggested in a recent sun protection survey in which most of the 445 responders [11] remembered that they had been informed, on several occasions, of the need for sun protection, and 78% remembered being similarly informed about how to reduce sun exposure. This information had been given by dermatologists to 80% of patients and by transplant physicians to 52% [9]. However, although most patients are aware of the need for protection against the sun, and of ways of reducing sun exposure, only a few of them adopt adequate sun pro-

tection measures. Surprisingly, most patients did not know why these measures were necessary, but stated that they wish to obtain more information about avoiding sun exposure [9].

The use of sunscreens is limited by cost and cosmetic acceptability. In addition, even though there is substantial evidence that UV protection does reduce the risk of carcinomas and probably also of melanoma, doubts are repeatedly raised concerning the true efficacy of sunscreens in preventing cutaneous malignancy [12]. In particular, the level of sun protection filtration [13] stated on sunscreen products is usually not achieved, mainly because the products are not applied often enough; an application every 2 h is recommended [14, 15]. Also, the use of sunscreens during sunbathing tends to increase the duration of exposure to doses of ultraviolet radiation above the sunburn threshold [13]. Last, sunscreens may encourage prolonged sun exposure because they delay sunburn. Consequently, it is not only necessary to increase the use of high-SPF sunscreens in transplant recipients but also to stress the need for patients to reduce exposure to sunlight and wear protective clothing, whether or not they use a sunscreen [16]. All this advice is more efficiently transmitted to the patient by dermatologists.

In conclusion, well-organized cooperation between transplant physicians and dermatologists is the best way of ensuring early detection of pretumoral skin lesions that may lead to the alteration of immunosuppression, and of educating transplant recipients sufficiently well to increase their compliance with sun protection measures [17].

Immunosuppression and Post-Transplant Cancer

The most important contribution a transplant physician can make to the prevention and treatment of skin cancer is immunosuppression management. Indeed, the appearance of skin cancer following a transplant is directly linked to immunosuppression. Immunosuppression generally favours oncogenic virus-related infections: type 8 human herpesvirus (HHV-8) associated with Kaposi's sarcoma [18], and papillomavirus possibly associated with squamous cell carcinoma (SCC) [1]. The key role played by immunosuppression in the development of skin cancer is well illustrated by the Dantal study, which shows that lowering dosages of cyclosporine 1 year after transplantation causes a decrease in the incidence of cancer [19]. The role of immunosuppression is also well illustrated by the lower SSC recurrence rate when immunosuppression is reduced [20]. Immunosuppression also reduces the patient's immunosurveillance, which could explain the more aggressive growth of these tumors.

In addition to the global role that immunosuppression plays in the development of cancer, certain immunosuppressants have direct oncogenic effects that enhance this process [21]. Cyclosporine modifies the structure of cultured tumor cells, affects metastasis, and may also decrease DNA-repair ability [11, 22, 23]. Azathioprine encourages the damage of DNA and reduces DNA-repair ability [21].

In contrast, mTOR inhibitors have antineoplastic properties that make them the most appropriate immunosuppressive drugs in the prophylactic treatment of nonmelanoma skin cancers [24]. These properties are the following: inhibition of the growth of tumor cells [25], antiangiogenic properties via vascular endothelial growth factor (VEGF), which decreases the rate of metastasis [26], and a proapoptotic effect on tumor cells [21]. These experimental data have recently been confirmed in humans. Patients treated with an mTOR inhibitor-based immunosuppression have a lower incidence of post-transplant cancer, whether they receive the treatment at the beginning of transplantation [27] or after switching from a calcineurin inhibitor to an mTOR inhibitor [28]. Finally, mTOR inhibitors are particularly effective in treating KS [29].

The aforementioned data show that mTOR inhibitors are well suited for primary and secondary prevention of nonmelanoma skin cancers after transplantation.

Drawbacks in the Use of mTOR Inhibitors

The use of mTOR inhibitors remains limited because they increase the renal toxicity of calcineurin inhibitors (CNI) and have numerous side effects.

Renal Toxicity Caused by CNI–mTOR Inhibitor Combination

Although mTOR inhibitors do not decrease renal function when used alone, they do increase intratubular concentration of CNI, thus increasing their nephrotoxicity [30, 31]. It is therefore necessary to considerably reduce the dosage of CNI, by at least 50% to 75%, to avoid this combined nephrotoxicity. Another option is the use of mTOR inhibitors alone as base immunosuppressive therapy. In this case, mTOR inhibitors are usually combined with corticosteroids and with mycophenolate mofetil. The advantage of treating patients with mTOR inhibitors as base therapy is the improvement of renal function in comparison with CNI-based protocols [32–35].

mTOR Inhibitor Side Effects

The second drawback of mTOR inhibitors is their side effects: arthralgia, aphtosis, edema in the lower limbs, inflammatory acne, cutaneous rashes, angioedema [36], impaired wound healing, lymphocele, drug-related pneumopathy [37, 38], diarrhea, asthenia, hyperlipidemia, thrombopenia, anemia [39], leukopenia, and proteinuria [34, 40, 41]. For the most part, these side effects are dose dependent, and some of them occur more frequently when the initiation of the mTOR inhibitor treatment is delayed following transplantation. This is the case for aphtosis [36], anemia (in most

cases inflammatory) [39], and pneumonitis [37,38]. Nephrotic-range proteinuria can occur when CNI is replaced by mTOR inhibitors [40, 42].

These numerous side effects lead to withdrawal of mTOR inhibitors in 15% to 30% of patients [43]. The dose reduction of mTOR inhibitors should decrease the incidence and severity of side effects.

The randomized CONVERT study that included 830 renal transplant patients has shown that patients with creatinine clearance lower than 40 ml/min and proteinuria greater than 0.5 g/day before administering sirolimus are at high risk for mTOR inhibitor side effects. Interestingly, most side effects appeared in the 6 months following the introduction of the sirolimus. The same study showed that the switch from CNI to sirolimus is not associated with an increased risk of rejection [44]. Other studies have suggested that poor renal function and proteinuria greater than 0.8 g/day are linked to sirolimus toxicity [45, 46]. It is of utmost importance to better understand the physiopathology and risk factors of mTOR inhibitor side effects to improve the management of their use in organ transplant recipients. The mechanisms of the aforementioned side effects could be related to cell proliferation inhibition (diarrhea, leukopenia, impaired wound healing), to a decreased antiinflammatory response caused by inhibition of interleukin 10 (IL-10) synthesis leading to anemia [39], arthritis, drug-related pneumopathy, and inflammatory acne. In addition, certain side effects such as edema, angioedema, and proteinuria could be caused by an increase in capillary permeability related to VEGF. Side effects could be limited by reducing the dose and excluding patients with proteinuria or altered renal function.

Finally, it is particularly important to warn patients of any possible side effects and to monitor sirolimus trough levels frequently to adapt dosage, particularly during the first 3 months after the first administration. The sirolimus trough level in base therapy ranges from 6 to 12 ng/ml.

Switching from CNI to Sirolimus

The best method for switching from CNI to mTOR inhibitors has not been determined. The switch from CNI to sirolimus can be accomplished either in steps or abruptly. Switching from cyclosporine to sirolimus can be done over a 4-week period, as published [47]. In that case, sirolimus at 2 mg/day is used as a starting dose, and cyclosporine is tapered down by 25% every week. Sirolimus blood trough levels are monitored every week, and daily dosage is modified until satisfactory trough levels are reached. Tacrolimus can be switched to sirolimus in the same way. The advantage of this stepwise switching process is that by gradually raising sirolimus dosage, it is easier to prevent dose-related side effects. However, this stepwise transition increases the risk of a short period of excessive immunosuppression. The alternative is to replace CNI for sirolimus in a 1-week period, with a loading dose of sirolimus (4 mg per day for 2 days), followed by a maintenance dose adapted to reach 6–12 ng/ml. In this case, CNI is tapered down by 50% at sirolimus introduction and withdrawn after 1 week.

Choice and Dosage of mTOR Inhibitors

The mTOR inhibitors sirolimus and everolimus have the same antitumor and immunosuppressive properties. The main difference between the two is their pharmacokinetics. The elimination half-life of sirolimus is 62 h and the elimination half-life of everolimus is 28 h. For base immunosuppressive therapy, the mTOR inhibitors used have mostly been sirolimus, which explains the larger number of publications and clinical studies on the subject in comparison with everolimus. The targeted trough level of sirolimus as a base immunosuppressive therapy is from 6 to 12 ng/ml depending on the time of transplantation and on the side effects. Everolimus has mainly been used in association with low doses of CNI, at a targeted trough level ranging from 3 to 10 ng/ml. There is no particular reason to choose one or the other molecule for the treatment of skin cancer in organ transplant patients, and there are no comparative studies of the two drugs. It is thus the transplant physician's choice, based on experience, published studies, availability of blood monitoring, and the existence (or not) of a combination with a CNI. For renal transplant patients, the antineoplastic effects of sirolimus in patients with nonmelanoma skin cancer or KS have been shown in several prospective studies [27, 28], and several prospective studies are underway. Everolimus has been used in heart and in renal transplant patients in association with CNI [31]. Pending the results of the ongoing studies that evaluate the role of mTOR inhibitors in post-transplant skin cancers, it seems logical to recommend sirolimus in base therapy for renal transplant patients with nonmelanoma skin cancer or KS and to recommend everolimus for heart transplant patients in combination with a low-dose CNI.

Assessment of the Transplant Recipient Before Immunosuppression Modification in Patients with Skin Cancer

The therapeutic modifications to immunosuppressive treatment for patients with nonmelanoma skin cancer or KS are the following: decrease of immunosuppression, switch from CNI to mTOR inhibitors, and no change in immunosuppression. The immunosuppressive regimen will depend mostly on the function of the graft, any contraindication related to mTOR inhibitors, the risk of skin cancer recurrence, and risk factors for other cancers.

Assessment of Graft Function and Risk of Rejection

This evaluation is essential because the main risk of lowering immunosuppression is acute rejection or the aggravation of a preexisting chronic active rejection. In the prospective and randomized Dantal study, reducing cyclosporine dosage led to a significant decrease in the incidence of skin cancer, but also to an increase in the incidence of acute rejection [19]. The risk of rejection should be carefully assessed in heart transplant patients as there is a life-threatening risk of losing the graft. For any grafted patient, the risk of acute rejection should be balanced with the

anticipated advantages of lowering immunosuppression and the risk of skin cancer recurrence. In contrast, in case of CNI-related renal toxicity and no signs of rejection, decreasing cyclosporine or tacrolimus dosage, or replacing them completely with an mTOR inhibitor, could be beneficial for graft function and increased graft survival [43].

The criteria enabling an assessment of the patient's immune status and the risk of acute rejection associated with immunosuppression alteration are anti-HLA immunisation before transplantation (PRA level, number of transplantations), the number of acute rejections after the graft, the period after the graft, the time since the last acute rejection, the presence of anti-HLA antibodies, and the patient's age (as the risk of acute rejection diminishes with age). Infections or post-transplant malignancies related to immunosuppression should also be considered. Last, graft function should be carefully evaluated before deciding on the best treatment option. For renal transplant patients, the assessment of the graft includes at minimum a measurement of glomerular filtration rate, its evolution over time, and the measurement of proteinuria. Finally, a biopsy of the graft is recommended if there is deterioration of renal function or in the presence of proteinuria. Transplant biopsy should be performed systematically before administering mTOR inhibitors to monitor for any subclinical rejection and as a reference point before immunosuppression modification.

Assessment of Intolerance to mTOR Inhibitors

The assessment of the risks of intolerance to mTOR inhibitors is essential because mTOR inhibitor side effects are the main drawback in their use. Clinical studies related to the switch from CNI to mTOR inhibitors have for the most part been conducted to eliminate the nephrotoxicity of CNI. These studies established the risk factors for intolerance to mTOR inhibitors. The two main criteria seem to be renal function and proteinuria before the switch. Consequently, a glomerular filtration rate below 40 ml/min seems to be a risk factor leading to serious mTOR inhibitor side effects [44]. Additionally, proteinuria greater than 0.8 g/day before the switch exposes the patient to an unacceptable increase of proteinuria following the administration of the mTOR inhibitors [42, 46]. Finally, blood lipids, blood cell count, glycemia, and liver enzymes must be determined before the switch. The contraindications related to mTOR inhibitors are a glomerular filtration rate (GFR) inferior to 35–40 ml/min, proteinuria more than 0.5 g/day, hyperlipidemia (fasting total cholesterol >7.8 mmol/l, fasting triglycerides >4.5 mmol/l, allergy to macrolides, a history of sirolimus-associated pneumonitis, thrombopenia (platelet count $<100{,}000/\text{mm}^3$), and leukopenia (white blood cell count $<2{,}000/\text{mm}^3$).

Choosing Immunosuppressants for Patients Developing KS

The dramatic efficacy of mTOR inhibitors in treating post-transplant KS [29, 48] and the early occurrence of these tumours after the graft make them the best immunosuppressant for the treatment of post-transplant KS. The switch from CNI to mTOR inhibitors should take place as soon as possible to avoid the spread of

cutaneous lesions or the development of visceral lesions. The only partial efficacy of the switch to sirolimus in renal transplant patients with KS and the recurrence of lesions under sirolimus could be related in part to its late introduction in patients with advanced lesions [49]. The early introduction of mTOR inhibitors after the transplant, a GFR over 40 ml/min, and the absence of proteinuria in transplant recipients at this period of transplantation are all factors associated with a low incidence of side effects. In the majority of published case studies, all patients remained on the treatment [29]. CNI should be replaced by mTOR inhibitors within 1–4 weeks. The sirolimus initial dose should be from 2 to 4 mg per day to reach trough levels of 8–12 ng/ml at this stage of transplantation. CNI dosage should be reduced by at least 50% when the sirolimus is introduced. Sirolimus and cyclosporine or tacrolimus blood trough levels should be monitored weekly to avoid the risk of insufficient or excessive immunosuppression. This immunosuppressive regimen should also include corticoids (5–10 mg daily) and mycophenolate mofetil (MMF) (1–2 g daily). MMF dosages should be adapted to the AUC (30–60 mg h/l) and to gastrointestinal and haematological side effects. The combination of sirolimus with MMF induces diarrhea, leukopenia, and anemia more frequently than the combination of cyclosporine with MMF [34]. In case of gastrointestinal or haematological side effects, a reduction of MMF dosage first is suggested to preserve the antineoplastic effects of the sirolimus.

Choosing Immunosuppressants for Patients with Nonmelanoma Skin Cancer

Should Immunosuppression Be Modified from the First Episode of Nonmelanoma Skin Cancer?

The optimal timing for immunosuppression modification for nonmelanoma skin cancers has not yet been established. Immunosuppression has to be reduced for recurrent cancer cases involving repeated surgical excisions that can leave scars on the face and hands [20]. At the first episode of nonmelanoma skin cancer, however, the medical attitude is not evidence based. SCC often has a good prognosis in transplant patients if the surgery is performed early on. In addition, decreasing immunosuppression always increases the risk of rejection, and the risk–benefit ratio of a modification in immunosuppression for the first cancer episode varies from one individual to the next. This ratio depends mainly on the immunological risk for the transplant patient.

However, it is also important to take into account the fact that the recurrence of nonmelanoma skin cancer in patients having received renal or heart transplants is relatively constant for a 5-year period following the diagnosis of the first episode [50]. The risk of recurrence is related to the period of transplantation, to the skin phototype, to eye and hair color, and to multiple lesions at the initial diagnosis. Reducing immunosuppression and sun exposure are factors that lower the

risk of recurrence [50]. Pending the results of the current prospective studies (the TUMORAPA study, for instance) that assesses the efficacy of mTOR inhibitors in the treatment of the first episodes of SCC, the aforementioned factors should be taken into account for each patient. The decision should be reached through a close collaboration between the dermatologist and the transplant physician after discussing the risks of the different options with the patient. A modification of immunosuppression is easier for those patients with a recurrent nonmelanoma skin cancer or for those who have already developed other post-transplant tumors or are at risk of developing them [20].

Choosing immunosuppressants, and their dosage and association, will depend on immunological risk, on the status of the graft, and on any contraindications related to mTOR inhibitors. The following steps for immunosuppression modification are suggested:

Patient with Good Graft Function and Neither Acute Rejection nor Chronic Active Rejection

Two possibilities depend on the existence of contraindications related to mTOR inhibitors.

Lack of contraindications related to mTOR inhibitors. The switch from CNI to mTOR inhibitors should be suggested as an initial option to patients. The switch will take place over a 4- to 6-week period, as described previously. Targeted sirolimus blood trough levels are from 6 to 12 ng/ml depending on the post-transplant period. The other associated immunosuppressants should be maintained regardless of whether the patient is on bi- or tri therapy to avoid the risk of rejection. If the patient is initially administered MMF and cyclosporine, MMF dosage will have to be adjusted after the switch based on MMF pharmacokinetic studies. Indeed, the AUC of MMF decreases when it is associated with cyclosporine and increases after cyclosporine withdrawal [51]. If mTOR inhibitor-related side effects do occur after the switch, their dosage should be lowered to reach the minimum targeted trough level. Lowering dosage, statin, or erythropoietin treatment is often enough to control the side effects at this early stage. Mouth ulcers are usually temporary and can be treated efficiently with mouthwashes containing clometazol [52]. In cases of sirolimus-associated pneumonitis, it is recommended to stop mTOR inhibitors definitively [38]. If recurrence of nonmelanoma skin cancer occurs after the switch to mTOR inhibitors, a reduction of MMF dosage, or its withdrawal, should be considered. In case of side effect-induced mTOR inhibitor withdrawal, immunosuppression can be lowered as indicated in the following paragraph.

Contraindications or intolerance related to mTOR inhibitors. For patients unable to be treated with mTOR inhibitors and not presenting a rejection, the best option is to lower immunosuppression. Immunosuppression must be lowered very progressively to avoid the risk of rejection. There are multiple combinations possible for lowering immunosuppression [20]. The patient can remain on tri-therapy

with dose reduction of the three immunosuppressive drugs. The patient can also be switched from tri therapy to bi therapy. Azathioprine (AZA) [53] dosage should be reduced or withdrawn first because of its oncogenic properties. Lowering the dosage of cyclosporine has the advantage of lowering its nephrotoxicity but also increases the risk of rejection if lowered too quickly. One alternative would be to replace AZA by MMF, which is a nononcogenic immunosuppressant [18], and concurrently decrease the dosage of cyclosporine. In the event of a nonmelanoma skin cancer recurrence following this initial alteration of immunosuppression, MMF dosage could be decreased or withdrawn completely. This strategy for lowering immunosuppression by reducing CNI on MMF therapy has a lower risk of rejection [54] and should be evaluated in a prospective manner for skin cancer. It is particularly important to monitor graft function during the process of lowering immunosuppression and during the following months to diagnose early any graft rejection.

Patient with Renal Graft Dysfunction and with No Signs of Rejection

These patients have a renal dysfunction and the graft biopsy has revealed tubular atrophy and fibrosis with no active chronic rejection, that is, no allograft glomerulopathy, no infiltration of the graft by mononuclear cells, and no peritubular capillary C4d deposits. If renal function is not significantly altered, with glomerular filtration rate above 35–40 ml/min and proteinuria below 0.5 g/24 h, a switch from CNI to mTOR inhibitors may be recommended as renal lesions are probably related to the toxicity of cyclosporine or tacrolimus. The conversion from CNI to sirolimus or to everolimus can be associated with a significant improvement in renal function [43, 44]. The conversion for mTOR inhibitors can be accomplished in the same manner as that already described. Sirolimus blood trough levels, and doses of corticoids, AZA, and MMF, are the same as described for patients with a good functional graft. Similarly, if the patient does not tolerate mTOR inhibitors, or if they are contraindicated, CNI dosage should be decreased first to reduce both nephrotoxicity and immunosuppression. Recurrence of cancer after these changes should lead to the subsequent decrease of MMF dosage or to complete CNI withdrawal.

Patient with Graft Chronic Rejection

Chronic rejection of renal allograft can be diagnosed by biopsy revealing fibrosis and tubular atrophy associated with lymphocyte infiltration and/or a positive C4d staining in peritubular capillaries and/or glomerular capillary double contour aspect. These lesions are classified as chronic active rejection in the Banff 2005 classification [55]. The decrease of immunosuppression in chronic active rejection is contraindicated by the high risk for graft function and survival, and in particular for the first case of SCC. Chronic active rejection can be treated by increasing the

doses of steroids, switching the patient from AZA to MMF, or increasing the doses of CNI. However, the risk of such alteration of immunosuppression in patients suffering from recurrent SCC is to increase their incidence or severity. A therapeutic approach that could to be initiated on a case-by-case basis is the introduction of an mTOR inhibitor (trough level, 8–12 ng/ml) to take advantage of its antineoplastic properties accompanied by a 50% to 75% reduction in CNI dosage to limit the combined nephrotoxicity of these two drugs. The dosage of MMF should be maintained initially and then adapted in accordance with the AUC. These changes to immunosuppressive treatment, which do not reduce and may even increase immunosuppression, could both control the process of rejection and the recurrence of nonmelanoma skin cancer by the use of mTOR inhibitors. Such an immunosuppressive regimen should be at best evaluated in randomized studies.

Conclusion

The appearance of skin cancer after transplantation is a sign of excessive immunosuppression that can expose the patient to multiple recurrent skin cancers or the appearance of other tumours. If possible, immunosuppression modification should include a switch from CNI to mTOR inhibitors. If this process fails or if there are contraindications related to mTOR inhibitors, immunosuppression should be lowered. Adjusting immunosuppression to the lowest level consistent with safely maintaining graft function may also decrease the development of premalignant skin lesions. Any change should be monitored through more frequent follow-up visits to screen and prevent side effects and detect all signs of rejection. These changes in treatment will be well accepted by the patient if he or she is informed of the risks and advantages by the dermatologist in collaboration with the transplant physician.

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Pretransplantation Dermatologic Screening and Prophylaxis

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Background

In the United States, more than 29,000 patients receive solid organ transplants annually, and the number worldwide is many times higher. Organ transplantation offers increased quality of life and prolongs survival for hundreds of thousands of patients with debilitating end-stage organ disease. This "gift of life" is possible with potent systemic immunosuppression, which prevents post-transplant immunological rejection of the donor allograft. However, the immunosuppression is not specific for prevention of allograft rejection, and many adverse effects accrue with prolonged survival. Primary among these is skin cancer, the most common malignancy of solid organ transplant recipients.

Skin cancer is considered a preventable malignancy. Effective sun protection with ultraviolet radiation-blocking sunscreen and protective clothing is the primary preventive strategy for lowering the risk of melanoma and nonmelanoma skin cancer (NMSC). Although the evidence showing the efficacy of sun protection practice for preventing skin cancer is suboptimal, several studies have documented a decrease in actinic keratosis and squamous cell carcinoma (SCC) for photoprotected patients [1–3]. Because solid organ transplant recipients experience accelerated formation of skin cancer, they provide a unique opportunity to study primary skin cancer prevention.

In this chapter, I describe the phenomenon of accelerated skin cancer formation in organ transplant recipients and present strategies for ameliorating this phenomenon. The indications for, rationale supporting, and efficacy of early pretransplantation skin cancer screening and prophylactic treatment regimens are outlined. The practicalities of implementing these preventive health care strategies are explored. Last, skin cancer as a potential contraindication to organ transplantation is discussed.

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Pretransplantation Dermatologic Screening and Prophylaxis

The European best practice guidelines for renal transplantation, written by the Expert Group on Renal Transplantation, recommended education of all transplant patients regarding the risk of skin cancer and institution of primary prevention (avoidance of sun exposure, use of sun-protective clothing, and effective application of sunscreen) to prevent SCC [4]. The European best practice guidelines also recommended referral to a dermatologist and active treatment and follow-up for patients with premalignant skin lesions such as actinic keratosis and warts. Close follow-up and early treatment of precancerous and cancerous lesions on a regular basis were recommended.

Little in the medical literature describes the cost-effectiveness and usefulness of dermatologic screening and prophylactic practices in the pretransplantation period. However, two recent studies from the United Kingdom showed that closely monitored cohorts of patients heavily educated in photoprotection and skin cancer awareness had better outcomes from melanoma and superior skin cancer awareness when compared with patients who did not receive care in a dedicated transplant dermatology program [5, 6]. Given the importance of innate immunity for control of cutaneous dysplasia and skin cancer, the greatest opportunity for effective dermatologic intervention is the pretransplantation period, before institution of potent systemic immunosuppression that may render therapeutic interventions less efficacious.

Identification and Treatment of Preexisting Dermatologic Conditions

Dermatologic screening of patients before transplantation offers advantageous and early identification of treatable infections, cutaneous diseases, and premalignant and malignant skin lesions. These conditions may be managed more easily in the pretransplantation period, before introduction of potent systemic immunosuppression. Infections that may complicate the intense postoperative immunosuppressive period (e.g., fungal, viral, and bacterial infections) can be managed in the earliest stage of manifestation, when the patient's immune system is not suppressed. Additionally, unsuspected cutaneous malignancies commonly are found during a full skin examination of older patients, the predominant candidates for organ transplantation. Treatment of overt malignancies and actinic keratosis and removal of severely atypical nevi before transplantation would be advantageous. Furthermore, examinations would identify patients with a high risk of skin problems after transplantation.

Assessment of Risk of New Skin Cancer after Transplantation

Patients with high risk of skin cancer after transplantation readily are identifiable by several basic phenotypic and historical features [7]. As in the general population, transplant candidates with fair skin and a history of extensive sun exposure have

significantly increased risk of skin cancer. In a multivariate analysis of liver transplant patients and skin cancer, Herrero et al. [8] showed that lower Fitzpatrick skin type (I, II, or III) and higher total sun burden correlated with increased risk of skin cancer development after transplantation. The total sun burden score was calculated by quantifying the intensity of sun exposure (i.e., determined by location of residence, occupational sun exposure, and holiday sun exposure) over four different 20-year periods during the patient's life. Increased sun exposure with each factor correlated with an increased total sun burden, and increased exposure was independently correlated with the risk of NMSC development. These findings support those of Euvrard et al. [9], who determined that factors such as transplantation before 1984, multiple tumors at first consultation, blue or hazel eye color and blond hair, and fair skin predicted the development of multiple tumors in kidney and heart transplant patients after the first SCC developed. The minimal risk of skin cancer for patients with darker skin and high Fitzpatrick skin type also has been documented [10].

Moreover, specific causes of pretransplantation end-organ failure correlate with the risk of skin cancer. My colleagues and I found that polycystic kidney disease and cholestatic liver disease as a cause of transplantation correlated with an increased risk of skin cancer, whereas patients with diabetes mellitus had a lower risk of skin cancer after kidney transplantation [11]. Another study showed that patients undergoing transplantation for alcoholic liver disease had an elevated risk of post-transplantation malignancy [12].

Ideally, any system for pretransplantation dermatologic screening and prophylaxis would provide a risk stratification assessment for skin cancer in the posttransplantation period. I outlined an example system in an earlier publication about the establishment of specialty clinics for organ transplant recipients [13]. Evaluation of every candidate before transplantation is a wonderful idea, but there are multiple logistic barriers to this approach. Therefore, systems that quantify a transplant patient's risk of post-transplantation malignancy may help target those with the highest risk to receive preventive screening prophylactic regimens.

Institution of Prophylactic Treatment Before Transplantation

Although no published trials have tested this strategy, organ transplant candidates have a prime opportunity for cancer prevention with prophylactic therapy and skin cancer education. Many patients with end-stage organ failure are in a partially or completely debilitated state; thus, activities involving extensive sun exposure are pursued infrequently. However, a patient on dialysis must travel to a dialysis center several times weekly, and cumulative damage from incidental sun exposure during travel may be substantial. Patients with chronic liver disease may be ambulatory and thus may accumulate photodamage. Patients with end-stage cardiac failure are likely to be the least active and to be confined to indoor activities. Nonetheless, an opportunity to educate patients about sun protection exists, and ideally patients will establish a pattern of behavior that incorporates sun protection practices before

transplantation. The cost-effectiveness and implementation of this approach may be suboptimal, as discussed next.

The most basic form of pretransplantation prophylactic therapy involves daily application of sunscreen (with a high sun protection factor) to all photoexposed areas. This is an essential preventive strategy for all transplant patients, particularly those with high risk of skin cancer development. In addition to sunscreen application, sun-protective clothing, hats, sunglasses, and behavioral strategies that promote sun avoidance during peak sun hours (10:00 A.M.–4:00 P.M.) are components of a comprehensive program of sun protection that should be taught to all potential transplant candidates.

In addition to sun protection practices, a reasonable strategy for high-risk transplant candidates may include topical and systemic pharmacologic agents that reverse photodamage and treat precancerous actinic keratosis. Topical application of 5-fluorouracil cream is among the most effective prophylactic strategies for transplant patients. However, this treatment modality has not been studied in the pretransplantation setting. Likewise, administration of photodynamic therapy or imiquimod cream to eradicate subclinical or early actinic damage may be a reasonable prophylactic and therapeutic strategy during the pretransplantation period.

Photodynamic therapy has been used prophylactically in the post-transplantation period with mixed results. de Graaf et al. [14] showed in a randomized trial that photodynamic therapy does not prevent development of cutaneous SCC. However, photodynamic therapy effectively reduces the presence of actinic keratosis for patients after transplantation [15]. Prophylactic photodynamic therapy also effectively increases the length of time (in the post-transplantation period) before new actinic keratoses appear on treated surfaces [16].

Topical imiquimod cream reduces actinic keratosis in transplant patients, but it has not been studied in the pretransplantation setting [17]. Systemic chemotherapy with *PTGS2* (*COX2*) inhibitors may be considered on theoretical grounds, but long-term administration is associated with renal toxicity. Indirect evidence (based on surrogate biological markers) from a phase I trial of difluoromethylornithine in organ transplant recipients showed that administration of difluoromethylornithine was associated with decreased levels of ornithine decarboxylase [18].

Another attractive strategy is vaccination against skin cancer during the pretransplantation period, when the patient is immunocompetent. This could be accomplished by using a human papillomavirus vaccine, a version of which has been approved recently for prevention of genital warts and cervical cancer. However, the subtypes of human papillomavirus present in organ transplant recipients are much broader than those found in cervical carcinoma, which increases the difficulty of creating a broad-based antigenic vaccine. Oseroff [19] postulated that photodynamic therapy may act as an in situ vaccination strategy. Phototoxic destruction of cells releases tumor antigens into an inflammatory milieu, thereby creating the potential for memory immune activation; this may lessen risk of recurrent and new primary skin cancer after treatment. This theoretical memory immune activation could apply to treatment with imiquimod cream or 5-fluorouracil cream, although it is unproven at this time.

Skin Education During the Pretransplantation Period

In addition to the sun protection strategies outlined above, all transplant candidates should receive education about post-transplantation skin cancer risk and instructions for performing a self-skin examination. Self-skin examination may facilitate early detection of cutaneous malignancies, which can be treated more effectively in terms of cost and cure rate. The possible benefit of intensive skin screening has been shown recently in a closely followed cohort of renal transplant recipients in the United Kingdom [5]. Examination of the entire cutaneous surface on a monthly basis is optimal for early detection and may be combined with a breast examination for women and testicle examination for men. Any suspicious lesions identified during self-skin examination should be reported promptly to health care providers who can evaluate and manage the conditions appropriately.

There are multiple studies that show the poor understanding among transplant recipients about skin cancer risk and the need for photoprotection. In a study by Cowen and Billingsley [20], only 41% of transplant recipients were able to recall skin cancer education at any point in the past. A more recent study showed that only 11% of transplant patients knew what the sun protection factor numbers associated with sunscreens meant [21]. Of that group, 62% of patients did not apply any sun protection, and only 5% of patients routinely applied creams with high sun protection factors. Another study showed that 88% of organ transplant patients (queried in a survey) were unaware of their increased risk of developing skin cancer, and 35% reported sunburn [22]. In addition, patients felt that, despite the risk of skin cancer, people with a tan looked better and healthier. The patients who most need sun protection are the least compliant with sun protection practices [23]. However, improved compliance with sun protection practices and skin cancer awareness has been shown by transplant recipients as a result of attending an organized specialty dermatology clinic [6].

Two recent studies using educational intervention strategies with organ transplant recipients showed that beneficial results may occur as a result of specific educational efforts. In a study at Mayo Clinic, patients who received intensive educational reinforcement were significantly more compliant with recommendations for sun protection behavior than patients who did not receive intensive educational reinforcement ($P = 0.007$) [24]. In a study of a Scottish cardiac transplant cohort, considerable increases in the mean knowledge score and behavioral score were measured after dermatologic assessment and education [25]. Thus, intensive educational efforts aimed particularly at high-risk transplant patients may improve knowledge and sun protection behavioral compliance.

The optimal timing of delivery of sun protection education is unknown. It is possible that patients suffering end-organ failure in the pretransplantation period may not retain health care information as well as they do in the post-transplantation period, when they may be feeling better. A major skin cancer education campaign, coordinated through the International Transplant Skin Cancer Collaborative, has been targeting transplant patients for intensive education through their transplant coordinators and physicians. The After Transplantation – Reduce Incidence of

Skin Cancer (AT-RISC) initiative has created patient and professional informational pamphlets that are available cost free on their web site [26]. All transplant programs should include information about skin cancer, sun protection, and self-skin examination in the program's pretransplantation educational material. This step will allow patients in the pretransplantation period to be aware of this risk and to pursue opportunities for skin cancer prevention that are based on their individualized risk factors.

Selection of Immunosuppressant Regimen on the Basis of Skin Cancer Risk Factors

The medical literature essentially does not discuss customization of immunosuppressant regimens on the basis of pretransplantation history of skin cancer or perceived high risk of skin cancer after transplantation. However, modification of immunosuppressant regimens (reduction of immunosuppression or switching to medications that are less associated with cancer) is a reasonable strategy in the post-transplantation period [27]. With this in mind, transplant candidates particularly prone to skin cancer reasonably could receive immunosuppressant regimens tailored to minimize cancer risk. Treatment regimens could include more frequent medication reevaluation and reduction of immunosuppression to the lowest possible level without permitting allograft rejection. Use of sirolimus as an immunosuppressant medication could be considered because of its lower association with skin cancer [28–30]. It is not clear which immunosuppressant medication is the most cancer promoting because evidence in the literature is contradictory. Therefore, the primary goal is to reduce immunosuppression to the lowest level necessary to maintain healthy allograft function, not to select a specific immunosuppressant regimen that addresses skin cancer risks.

Practical Implementation of Dermatologic Screening and Prophylaxis

Ideally, all transplant candidates would be evaluated and their skin conditions would be optimized before transplantation. Furthermore, the level of health care intervention would be individualized using stratification schemes based on known pretransplantation risk factors for posttransplantation malignancy. Although this would likely lead to improved cutaneous health for transplant patients after surgery, the logistics of implementing such a vision are challenging. Implementation of a preventive health care approach to skin cancer for organ transplant recipients will vary on the basis of center-related logistics, insurance considerations, and the unique realities of each country. A typical transplant program in the United States has 350 new patients undergoing transplantation each year, and thousands of candidates are awaiting transplantation throughout the country. Moreover, 5,000

to 10,000 patients in the United States have previously undergone transplantation. On a practical basis, the ability of dermatologists to deliver customized care to each of these patient groups is limited. In addition, the thousands of patients on waiting lists at each medical center generally are quite ill with end-stage organ disease that requires hemodialysis, hospitalization, or substantial medical therapy.

At Mayo Clinic, pretransplantation patients are educated by transplant coordinators and through written material. Consultations are reserved for patients with special risk factors that require direct evaluation and examination by a dermatologist. Sun protection and self-skin examination messages are provided to all transplant candidates at the earliest possible time. However, dermatologic examinations of transplant patients are not performed until the 4-month post-transplantation visit, when patients are feeling better; at that stage, the number of patients is smaller, and they can be managed successfully. Multiple ways to organize the logistics of dermatologic intervention for solid organ transplantation have been outlined recently [31]. Transplant programs in the United Kingdom have used a nurse-led skin cancer surveillance program to expand the availability of dermatologic education and screening for transplant patients [13, 32].

Skin Cancer as a Contraindication to Organ Transplantation

Many patients who present for consideration of solid organ transplantation have a history of skin cancer, the most common malignancy in humans. Avoiding skin cancer recurrence or metastasis is paramount because solid organ allografts are allocated to patients with the greatest likelihood of prolonged survival. For most patients, risk of recurrence or metastasis is minimal, and transplantation would be appropriate. Conversely, patients with metastatic skin cancer are inappropriate candidates for solid organ transplantation. Between these extremes are patients with a history of high-risk skin cancer with variable metastatic potential who may harbor clinically and radiologically occult residual microscopic disease. In the worst case scenario, occult metastatic skin cancer cells could grow with systemic immunosuppression and result in an increased risk of recurrence or metastasis [33]. Patients with a history of skin cancer have a high risk of developing de novo primary skin cancers at sites other than those of previous carcinomas. Patients with a high number of prior skin cancers perhaps should be evaluated carefully before transplantation; however, most will be appropriate candidates for transplantation.

My colleagues and I recently published a review article outlining the issues surrounding the evaluation of transplant candidates who have a history of skin cancer [34]. We described appropriate evaluation of these patients, prognostic factors associated with skin cancer that may assist in determining the appropriateness of transplantation, and the limitations of the data available to guide these decisions. The content of this section is modified from that review.

Evaluation of Transplant Candidates with a History of Skin Cancer

Given the disparity between the supply of organs and the escalating number of potential transplant recipients, available organs must be transplanted into patients with the greatest likelihood of benefit. Estimating the risk of recurrence, metastasis, or occurrence of new primary skin cancers for candidates of solid organ transplantation who have a history of skin cancer involves a review of their medical history, a physical examination, and critical assessment of the clinical and histological details of any previous skin cancers. For patients with a history of skin cancer of indeterminate risk, the clinical, surgical, and histological details of the prior skin cancer may

Table 1 Pretransplantation skin cancer assessment

Cancer type	May receive transplant	Consult with transplant dermatologist	Should not receive transplant	Reevaluation interval after primary tumor diagnosis (if transplant was denied), years
Basal cell carcinoma				
Primary	X			
Metastatic, in remission			X	5
Metastatic, not in remission			X	NA
Squamous cell carcinoma				
Primary, low risk	X			
Primary, high risk		X		3
Metastatic, in remission			X	3–5
Metastatic, not in remission			X	NA
Melanoma				
In situ	X			
Stage I		X		3–10
Stage II			X	5–10
Stage III			X	10
Stage IV			X	10
Merkel cell carcinoma				
Primary		X		2–3
Metastatic, in remission			X	3–5
Metastatic, not in remission			X	NA
Kaposi sarcoma		X		NA
Atypical fibroxanthoma		X		3
Dermatofibrosarcoma protuberans	X			
Sebaceous carcinoma		X		3
Eccrine carcinoma		X		3
Microcystic adnexal carcinoma		X		3
Extramammary Paget disease		X		3

NA, not applicable.
From Otley et al. [34]. Used with permission.

be assessed by transplant dermatologists, Mohs' surgeons, and general dermatologists. They may provide an estimate of prognosis, taking into account the time that has elapsed since the occurrence of the primary skin cancer. Radiologic examination may be used to exclude occult residual disease, although there are limitations in the sensitivity and specificity of radiologic techniques for cutaneous malignancies. Positron emission tomography is the most sensitive imaging technique available to ascertain the presence of metastatic melanoma, high-risk SCC, or Merkel cell carcinoma, whereas computed tomography provides more specific information about hypermetabolic foci identified on positron emission tomograms.

Prognostic Factors for Pretransplantation Skin Cancer

Table 1 shows general recommendations when considering transplantation for patients with skin cancer and specifies the appropriate time frames for reevaluation after the occurrence of the primary tumor. Because metastasis of aggressive cutaneous malignancies usually occurs soon after primary occurrence, the risk of recurrence and metastasis lessens dramatically with time. After most of the risk has passed, reevaluation would be appropriate to update the prognostic data for such patients. However, caution is advised when examining all cancers with metastatic potential because the available data are derived from nonimmunosuppressed patients; thus, the risk of metastasis could be higher in immunosuppressed transplant recipients.

Risk of Recurrence and Death from Pretransplantation Basal Cell Carcinoma

The risk of metastasis from basal cell carcinoma (BCC) is exceedingly low, and almost all patients with a history of BCC are appropriate candidates for solid organ transplantation. In rare instances of metastatic BCC, treatment is difficult, and it therefore is an absolute contraindication to transplantation unless [1] a disease-free interval greater than 5 years has passed since the last manifestation of disease, and [2] complete restaging has shown no residual focus of tumor.

Risk of Multiple De Novo NMSCs after Transplantation

Although a lethal outcome from NMSC is uncommon, pretransplantation BCC or SCC is a marker of increased risk of multiple de novo NMSCs after transplantation. Among nonimmunosuppressed patients, 44% of patients with a history of BCC developed another BCC within 3 years, whereas SCC developed in 6% of patients with prior BCC within 3 years [35]. Among nonimmunosuppressed patients with a history of SCC, new primary BCC developed in 45% and new primary SCC

developed in 18% [35]. In addition, among patients with a history of 3 or more NMSCs, the 3-year risk of another NMSC was 93%. Although rarely lethal, multiple NMSCs in transplant patients may decrease quality of life, and high-risk skin cancers may occasionally be life threatening. Pretransplantation NMSC, however, is not a contraindication to transplantation unless the patient has an extraordinarily high number of NMSCs or individual high-risk NMSCs. Rather, an organ transplant candidate with a history of multiple NMSCs should have aggressive preventive treatment implemented, including sun protection under the close supervision of an experienced dermatologist, before the patient can be listed for an organ transplant.

Risk of Recurrence and Death from Pretransplantation SCC

For nonimmunosuppressed patients, the risk of metastatic spread of cutaneous SCC is 3.6% at 3 years [36]. In contrast, the rate of metastasis for transplant recipients is nearly 7% [7]. When assessing the likelihood of recurrence, metastasis, or death from a pretransplantation SCC, clinicians must weigh a complex group of factors, including the histological findings, clinical presentation, treatment history, and anatomic site. In general, patients with truly high-risk SCC and a high risk of metastasis usually have multiple simultaneous risk factors, and delay of transplantation may be a reasonable approach. However, the risk of metastatic disease with cutaneous SCC passes quickly, with 90% of the risk occurring in the first 3 years. After 3 years, the likelihood of recurrence could be reevaluated, and the presence of occult metastatic disease could be reassessed [37].

Patients with metastatic skin cancer have a poor prognosis. The 3-year survival of immunosuppressed patients with metastatic SCC is 56%, and the 5-year survival rate is 34% [38]. Because of the high risk of recurrence from metastatic SCC, a waiting period of at least 3 to 5 years is appropriate before conducting a comprehensive reevaluation for transplantation.

Risk of Recurrence and Death from Pretransplantation Melanoma

Most patients with cutaneous melanoma have prolonged survival, with an average 5-year disease-free survival rate of 80% to 85%. Thus, most patients with low-risk melanoma or a remote history of invasive melanoma would be reasonable candidates for solid organ transplantation. The most accurate system for quantifying the probability of survival for a patient with melanoma is the 2001 staging system of the American Joint Committee on Cancer [39, 40]. Melanoma in situ, which includes lentigo maligna, is categorized as stage Tis and has a theoretical disease-specific survival rate of 100%. These tumors have no metastatic risk if the histological evaluation is accurate. Therefore, excluding patients with in situ melanoma from consideration for transplantation is unwarranted.

Clinical stage I or II melanoma has an increasing risk of recurrence and death that correlates with increasing tumor thickness and histological evidence of ulceration. Patients with melanoma that is less than 1 mm thick and without ulceration (stage I) have an excellent prognosis and a 5-year survival rate of 95% or greater. Although metastatic potential always exists, patients with stage I melanoma could be considered for transplantation after a few years. Patients with thick primary melanomas (>4 mm) have a high risk of metastasis, a poor prognosis, and 5-year survival rate of 45% to 67%. For patients with a history of stage II or III melanoma, transplantation should be delayed for 5 to 10 years. Any consideration of transplantation for patients with stage II or III melanoma should be performed with the knowledge that late recurrence could occur after 10 to 20 years. A history of metastatic melanoma is an absolute contraindication to transplantation without an extended disease-free interval.

The potentially adverse influence of systemic transplant-related immunosuppression on outcome has not been reliably quantified. The Israel Penn International Transplant Tumor Registry provided data for 30 patients with melanoma and showed a 19% incidence of recurrence after transplantation [41]. This rate is within the expected range of recurrence. Further data from the tumor registry show a 30% mortality rate among patients with melanoma that developed after transplantation, approximately 50% higher than the mortality rate noted in the general nonimmunosuppressed population [41]. However, 69% of the patients had melanoma with a Breslow thickness more than 0.75 mm; thus, a 30% mortality rate may be in accordance with expectations. Study authors recommended waiting 5 years after a melanoma diagnosis before considering transplantation. A patient's prognosis may be assessed individually with case-specific details and with a risk–benefit analysis to weigh potential outcomes (positive and negative outcomes, with and without transplantation).

The Future of Pretransplantation Dermatologic Care

Ideally, with advancements in technology and care, the evaluation and management of organ transplant candidates will be vastly different from that of today. One can imagine using a comprehensive genome-based risk assessment system for skin cancer and other conditions. For patients with increased risk, intensive pretransplantation vaccination with human papillomavirus vaccines or skin cancer antigen vaccines would create immunological memory that is capable of reducing the risk of skin cancer after immunosuppression. Comprehensive evaluation and chemopreventive strategies would be implemented before transplantation, and effective, customized sun protection and self-examination programs could be established. Last, the immunosuppressant regimen would be specific for each patient to allow tolerance of the donor organ but not widespread systemic immunosuppression. Customized care based on validated phenotypic and genotypic risk factors would allow clinicians to concentrate the most intensive and effective prophylactic interventions on the patients who are most in need.

Summary

Currently, the pretransplantation dermatologic evaluation and prophylaxis programs of most organ transplant centers are based on established principles of screening and prophylaxis for the general dermatology population. With integration of skin cancer information into comprehensive transplant educational materials and education of all providers involved in the care of transplant patients, an effective multidisciplinary preventive approach to dermatologic disease is possible. For patients with phenotypic and historical factors that indicate a high risk of post-transplantation malignancy, aggressive screening and administration of prophylactic regimens may prove effective at reducing the risk of numerous NMSCs in the post-transplantation setting. Considerable progress needs to be made before preventive strategies in this high-risk patient population are optimized.

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Aftercare – A Multi-disciplinary Approach

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General Considerations

During the past decades, organ transplantation has become a frequently performed routine procedure offered to patients with end-stage organ failure. Transplant recipients live longer, have a good quality of life, and represent therefore a rapidly growing population. Although the follow-up, and especially the immunosuppressive therapy, are maintained by specialized transplant centers, such patients increasingly appear in private offices or hospitals that lack particular expertise in transplant medicine. Thus, there is need for an educational network for physicians and health care workers and for guidelines how to care best for this cohort of patients.

Qualitative and Quantitative Requirements

A sufficient number of regional transplant centers is necessary as part of a country's medical infrastructure to allow all organ graft recipients quick access to adequate health care institutions, where transplant professionals must be available at any time. The center may also be consulted by doctors from general hospitals when organ graft recipients are admitted. The requirements for a transplant team include transplant surgeons and physicians with experience in various fields dependent on the type of the transplanted organ. Also, operating rooms and intensive care units have to be constantly available in case of emergency, and permanent access to sophisticated radiologic imaging techniques is required. In addition, experts of various medical specialties such as dermatology, neurology, and oncology/hematology are necessary to meet the complex needs of graft recipients with their broad spectrum of comorbidities. Furthermore, the contributions of dieticians, health care professionals, social workers, and the nursing staff are essential for the success of aftercare.

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Hence, the maintenance of this patient cohort is a challenge that can only be accomplished by multi-disciplinary cooperation.

Objectives of Aftercare

Post-transplant aftercare must handle all types of problems occurring in organ graft recipients, but the primary goal is the prolongation of graft and patient survival (Fig. 1). In those types of organs, where replacement therapies are either not available or may only be supportive for a limited time period, such as cardiac or lung transplantation, the loss of graft function leads to a patient's death unless the person can be regrafted immediately. Therefore, graft survival and patient survival may be identical in such cases. However, this does not apply to renal or pancreatic transplantation, where dialysis or insulin therapy can be performed over years until a first or second graft becomes available. Although still experimental, the transplantation of hands, face, or larynx, that is, body parts that are not indispensable for life, underlines the perception that quality of life is equally important and has to be considered as having value of highest significance.

To achieve successful transplantation with long-standing graft function, effective immunosuppression is required to prevent acute and chronic rejection. The selection of drugs and their dosing is therefore one of the key elements of maintenance therapy, with a large impact on the frequency or severity of infections and the risk of tumor development. To find a well-balanced state between overimmunosuppression and lack of protection against rejection is the major challenge in aftercare. However, if complications occur in the post-transplant course, their early detection is critical

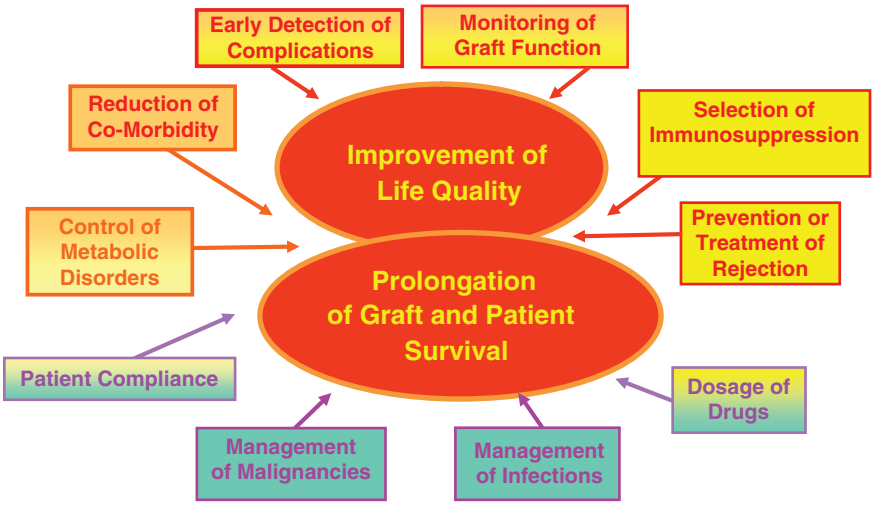

Fig. 1 Objectives of aftercare

for immediate therapeutic interventions, which can be important to protect graft function and to prevent life threatening conditions.

Monitoring of graft function is therefore urgently needed, and various established protocols adapted for the various organs are available [1–7]. Metabolic disorders and hypertension are quite common after transplantation and need to be managed because of their possible impact on morbidity and cardiovascular mortality. Last not least, patient compliance, an important contributor to long-term success, needs to be controlled and ameliorated, if necessary, during post-transplant aftercare.

Outpatient Management

The management of aftercare varies among countries and institutions and may be performed by surgeons or physicians. Many of the aspects and screening approaches that have to be covered by aftercare are also pre transplant issues, and it might be that the same expert who had carried out the requested pre transplant investigations is later monitoring the same patient after transplantation.

Each of the post-transplant periods has different issues that have to be considered (Table 1). The first weeks after transplantation, when patients are most susceptible to infections [8], acute rejections, and complications from the surgery, can be quite critical. Therefore, in this period most of the patients will stay under the care of a dedicated transplant centre where they can be seen frequently for outpatient follow-up. After this initial period, most patients return to their referring physicians for long-term care, with whom the specialists of the particular transplant centre should be in good cooperation and communication. Regular follow-up of transplant recipients is also required during the intermediate and late post-transplant period to manage complications typical for the respective timeframe (see Table 1). However, the customs in aftercare are dependent on the regional infrastructure and

Table 1 Timetable of prevalent complications after solid organ transplantation

Early post-transplant period
Surgical complications
Acute rejections
Bacterial infections
Intermediate post-transplant period
Viral or fungal infections
Drug-related side effects
Loss of patient compliance
Late post-transplant period
Hypertension, cardiovascular disease
Development of malignancies
Chronic allograft dysfunction
Recurrence of disease
Chronic rejection

may vary quite a bit between different centres, particularly in their view about how frequently an individual patient should present at the responsible transplant unit. Minimal requirements and recommendations for outpatient surveillance have been developed [2–6]. At the Vienna Transplant Center, for instance, allograft recipients are seen at a minimum every 3 to 4 months.

Maintenance Immunosuppression: From Standardized Protocols to Individually Tailored Therapy

The choice of substances used for the primary protocol in de novo patients depends on the type of organ and the patient's individual immunological risk, which might be estimated by the medical history (with possible former graft losses), the matching of blood groups and HLA types, or the amount of preformed panel reactive antibodies, but it is also influenced by centre politics, regional differences, personal experiences, and reimbursement practices.

If graft function remains stable throughout the early postoperative weeks and months, subsequent reduction of immunosuppression is desirable. This reduction can be achieved by both reduction of the number of immunosuppressive substances and their respective dosing by aiming for lower blood target levels. Although the use of drug combinations may vary considerably among the centres, there is a general consensus that their selection should be adjusted to a patient's individual requirements. Conversion from one drug to another is now regarded as a safe procedure, if done properly, and can be performed without putting a patient at risk [9]. Consequently, substances with more or less impact on certain factors such as blood pressure, metabolic disorders, tumors, repeated infections, or specific side effects may be used or avoided to achieve an individually tailored immunosuppression [10]. Minimizing the various side effects of drugs will also improve patient compliance and medical adherence.

Early and Late Complications After Transplantation

Although complications may appear at any time, their prevalent occurrence is generally observed at certain periods after transplantation (see Table 1). Thus, it is mainly the early post-transplant period when acute transplant-related problems such as surgical complication or acute rejections have to be managed. Also, infections display a typical time course with line infections, wound inflammation, and urinary tract infections typically becoming apparent during the first month after the operation [8]. Those early infections are predominantly caused by bacterial pathogens, whereas viral or fungal infections occur somewhat later in the course.

The intermediate post-transplant period is characterized by attempts to minimize immunosuppression. Naturally, this leads to an increased risk for late acute

rejections but at the same time drug-related problems may occur because of the long term application, leading to loss in patient compliance, as they can alter the patients' appearance, including hirsutism, gingival hyperplasia, weight gain, cushingoid facies, hand tremors, alopecia, and skin disorders [11].

Metabolic disturbances are common side effects of various drugs including immunosuppressive agents, but can also be related to the underlying disease or to comorbidities associated with chronic organ failure. Consequently they may be both the underlying cause for transplantation or a consequence of grafting that has to be accepted. Nevertheless, complications such as hyperlipidemia, post-transplant diabetes, or hyperuricemia may have a negative impact on the half-life of grafted organs and thus hamper the long-term success. To manage such metabolic disorders, dietary restrictions and concomitant drug therapies are generally recommended. Additional drugs may be necessary to control blood pressure, to reduce inflammation, or to decrease fluid retention that may appear with or without electrolyte abnormalities. Concomitant problems may also concern hematological disorders or hepatic involvement.

Late complications are arteriosclerosis and cardiovascular disease, the primary cause for death with a functioning graft. De novo or recurrent renal disease, musculoskeletal problems, and steroid-induced cataracts and osteoporosis can also be observed [1, 12]. Skin problems in organ recipients result not only from the induced immunosuppression but also from specific adverse effects of the immunosuppressive drugs themselves [13]. The management of all these conditions is necessary in the routine follow-up of all transplant patients and involves the respective specialists in a multi-disciplinary approach.

Post-Transplant Infections

One of the major problems in immunocompromised transplant patients is infections, and particularly in the early post-transplantation period it is one of the major determinants for the outcome of solid organ transplantation. The incidence of infections varies according to the type of transplanted organ, the current or recent degree of immunosuppression, the need of additional antirejection therapies, the occurrence of technical or surgical complications, and, in the long run, to graft function [14, 15]. Generally, there are similar patterns of infections in all types of transplant recipients and consistent timeframes for the occurrence of different pathogens [8].

Infections may be of fungal, bacterial, and viral origin. The immunosuppressive drugs are potent antiinflammatory agents; therefore, in immunocompromised patients, inflammatory processes may be suppressed and the clinical course of infection may be mitigated. To not underestimate the severity of an infective process, even nonspecific signs or early symptoms should freely prompt the physician to perform simple blood tests, radiographs, and blood and urine cultures. The nature of infectious diseases in transplant recipients may be altered, and as a consequence, the rationale in evaluating and treating disease in transplant recipients is one of

heightened suspicion [16]. The process of evaluation involves specialists for microbiology, infectious diseases, radiology and the whole spectrum of internal medicine, as well as the dermatologist, because the skin and the subcutaneous tissue occupy a central position in any consideration of infection in the immunocompromised host: conventional skin infections may be increased in incidence and severity, may be widespread or extensive, and followed by systemic disease. There may be systemic infections metastatic to the skin from a noncutaneous portal of entry, or the skin may be the site of infections with true opportunists. In any case, the diagnosis of an infectious process must be confirmed by identification of the presumed pathogen, either by direct examination, culture, or molecular methods. Multiple infections with bacteria, viruses, fungi, and/or parasites may develop in the same patients simultaneously, even within the same lesion.

Prophylactic Measures

Patients may be given antibacterial prophylaxis, in particular before invasive surgical or dental procedures are done [17, 18]. As a general measure patients should avoid contact with people with infections, especially during the early months after transplant [17], in particular those with chickenpox and shingles and other viral infections [19]. Household members should have influenza vaccination [19]. Prophylactic measures to avoid fungal and bacterial contamination must be performed by the patient; for example, gardening and handling pets may bear some risks for uncommon or opportunistic infections with pathogens that under normal conditions are not human pathogens [20]. In children, routine childhood vaccination should be performed before transplantation; live vaccines are forbidden [21].

Post-Transplant Cancer

According to the data from the Cincinnati Transplant Tumor Registry and the existing literature, the predominant tumors are lymphomas and lymphoproliferations, carcinomas of the skin, lips, and the cervix of the uterus, Kaposi's sarcoma, hepatocellular carcinomas, renal carcinomas, and various sarcomas [22, 23]. Interestingly, the incidence of tumors frequently observed in the general population such as lung and breast cancer seems to be not increased in transplant patients [24, 25]. The relative risk for the development of various cancers can either be compared to the age-adjusted rate in the general population [26], or calculated, as for kidney transplant recipients in comparison with patients on the waiting list [27]. It depends on the type of organ transplanted and the related immunosuppressive regimens; however, there are no definitive data on the individual immunosuppressants [28]. For nonmelanoma skin cancers (NMSC), for instance, the overall degree of immunosuppression has been shown to be relevant [29]. Cancer risk is also related to the patients' underlying conditions, such as the age of the patient at the time of transplantation and individual

or family history [28]. For NMSC, in particular, the cumulative lifetime sun exposure is essential, as well as age at time of transplantation [30, 31]. All these factors have to be assessed, and individual adaptation of existing screening schedules for transplant patients is required.

Nonmelanoma skin cancers comprise the most frequent cancers worldwide in the white population with increasing incidences [32], with the highest rates reported for Australia, where, according to a survey, the incidence of treated NMSC in 2002 was more than five times the incidence of all cancers combined [33]. In transplant patients, the incidence of NMSC is increased 65- to 250- fold compared to the general population, and NMSC account for approximately 50% of malignancies [25, 34]. The 10-year incidence of NMSC in heart and kidney transplant recipients varies from 7.8% (Sweden) to 43% (Australia) (heart transplant recipients) and 7% (Norway) to 33.3% (Australia) (kidney transplant recipients) respectively, depending on the geographic location and other pathogenesis factors such as ultraviolet radiation (UVR) and immunosuppressant therapy [35].

Dermatological Care and Skin Cancer Assessment in Organ Transplant Recipients

With regard to skin cancer, the American Society of Transplantation (AST) recommendations for outpatient surveillance of renal transplant recipients list the need for self-examination of the skin once a month and skin examination by a physician annually [2]. Particular guidelines have been developed for the treatment of skin cancer in organ transplant recipients [36]. Therefore, organized dermatological care for these patients is mandatory, first as a preventive measure to improve counseling leading to improved compliance with regard to photoprotection and increased skin cancer awareness in the patients [37], and second to have regular clinical investigations and early therapeutic care.

This concern is particularly true in patients with NMSC in their history because they are at increased risk for the development of subsequent skin tumors [38]. In most cases this occurs in three basic clinic settings: in multidisciplinary transplant clinics, where dermatological care is on site and automatically an integral part of the overall health care of the patients, in designated dermatology transplant subspecialty clinics, and as an integration of transplant recipient care within an existing dermatology clinic [39]. In most of these settings, pre transplant screening for NMSC is offered; ideally, as carried out at the Mayo Clinic transplant centre, patients on transplant waiting lists are determined whether they are at high risk for skin cancer on the basis of a skin cancer risk factor self-assessment, and if one of the criteria is applicable, should have baseline screening examination [40]. These criteria include fair, easily burned skin (Fitzpatrick type I and II), blue, green, or hazel eyes, red or blond hair, extensive freckling or nevi, outdoor occupation or extensive prior history of sun exposure, positive family history of skin cancer, prior personal history of skin cancer or precancer, and significant clinical photodamage [40]. At the Mayo

Clinic, follow-up visits are scheduled according to a triage system: Patients without history of NMSC or actinic keratoses (AK) have annual examinations by transplant physicians until lesions arise; those at risk are seen annually by a dermatologist. Transplant patients with a history of AK or one NMSC are seen at 6-month intervals, and those with a history of multiple NMSC, high-risk squamous cell carcinomas (SCC), or metastatic SCC or melanoma require 2- to 4-month intervals [40]. In other centers, follow-up is also based on risk assessment, with patients at high risk for skin cancer examined every 3 months, patients with moderate risks every 6 months, and those with low risk annually [39].

Early diagnosis and treatment of all types of skin cancers is the best method to alter the course and prevent the patients from metastatic disease; therefore, regular screening for skin cancers and total body examination by a dermatologist as well as education concerning regular self-examination supported by patient folders has to be part of the follow-up in transplant patients. Malignant and premalignant skin lesions have to be treated accurately; counseling includes avoidance of sun exposure and the use of protective clothing and sunscreens [2].

Living with a Transplanted Organ

Most of the patients immediately have substantial benefit from their transplanted organ because transplantation provides patients with a good quality of life, which may be comparable to the age-matched general population. Patients will be able to return to normal or near-normal activities 6 to 12 months after transplantation, including carrying out a regular job and sports exercise. In general, the recovery process after transplantation can be enhanced and a good health status maintained by an appropriate diet that should generally be low in fat, sugar, and salt, which will help to control weight and blood sugar, limit fluid retention, and control blood pressure.

Following transplantation, fertility is usually restored within an average of 6 months [41]. Therefore, women of childbearing age must be informed that they are able to conceive and they must be advised to carry out effective contraception, particularly for the first 2 years after transplantation. In particular, for renal transplant recipients with good renal function pregnancy is feasible after this period, although there is a certain risk for deteriorating graft function. For most of the more recently developed immunosuppressive agents the experience in pregnancy is quite limited, so that a more conventional drug combination should be preferred. Gravidity in a transplanted woman is always considered a high-risk pregnancy and requires the whole medical spectrum, especially a close monitoring by both the obstetrician and the transplant physician, and later the pediatrician.

Special attention and considerations should be paid to children with a transplant according to body size, level of understanding, familial and social conditions, and children's rights. In children, a special license is mandatory for any type of medication. In this particular patient group, a significant number of late graft losses result

from noncompliance, which emphasizes the need for continuous education as well as psychological support [21].

Older patients may have fewer rejections but are at higher risk for complications associated with overimmunosuppression. Compared to younger transplant recipients they will display more comorbidities and age-related problems, but there are substantial differences between patients with different organs transplanted.

Psychological Aspects, Patient Compliance, and Educational Needs

To live with an organ of a deceased donor can be a substantial psychological burden for a graft recipient. Patients may feel responsible for the death of someone else and guilty for having the subsequent benefit. They sometimes need professional help and supervision to overcome their objections and to accept their altered life situation.

Professional support may also be necessary to improve patient compliance. Most important is lifelong adherence for intake of the immunosuppressive therapy, resulting in the maintenance of graft function and long-term success. Therefore, support services, psychological counseling, and education provided by health care workers and the after-transplant physicians are essential for identifying predictors of noncompliance [42]. Graft recipients should understand the importance of follow-up visits, their medication, and preventive measures including vaccinations. Particularly in recipients in whom drug abuse or alcohol consumption has caused their organ disease, specific attention should be drawn on possible signs of addiction to allow early inclusion into adequate programs [43]. Patients should also receive counseling and guidance on how to optimize their health through physical activity, exercise and training, nutrition, and other usual measures of lifestyle modification.

Future Outlook and Upcoming Needs

At present the number of transplantations is limited by the availability of donors, but several approaches are being undertaken to expand the source of organs, including the development of donation programs and research in xenotransplantation or genetic engineering. Because of the increasing life expectancy of the general population, it can clearly be expected that the number of potential graft recipients will increase over time. In the future there will be a change in the patient profile; improved transplant medicine such as advanced diagnostic techniques and enhanced means for the control of life-threatening post-transplant infections and problems have led to expanded inclusion criteria for patients who need transplantation. Increasingly older patients are put on the transplantation waiting lists, and various comorbidities including human immunodeficiency virus (HIV) infection are no longer absolute reasons for an exclusion [44]. There are also an increasing number of patients who even undergo more than one retransplantation because of

transplant failure, or receive an additional allograft because of medication-related organ failure other than the transplant.

All the foregoing concerns imply a challenge for post-transplant aftercare programs within the scope of quantitative expansion and qualitative demands. To meet those expectations, the interdisciplinary cooperation between all fields of medicine and health care and its constant amelioration will be an important contribution.

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Evaluation of Patient Education

Luigi Naldi and Fabrizia Sassi

Health Education and Behavioural Changes

Patient education is the process of enabling individuals to make informed decisions about their personal health-related behaviour. It aims to improve health by encouraging compliance with medical treatment regimens and promoting healthy lifestyles [1]. Patients have a right to receive appropriate education, so they can utilize their knowledge to participate in decision-making processes [2]. Professionals in health care have a commitment to provide unbiased and evidence-based information to help educate patients. Virtually every patient encounter should include some element of patient education. The physician who uses a variety of strategies (e.g., verbal messages, printed materials, computerized information) and involves staff in patient education efforts is most likely to be a successful patient educator [3].

Building a partnership with patients over time and understanding the perceptions, values, and beliefs that influence their health behaviours are the underlying principles of clinical practice that provide the foundation for helping patients to change. It is in the context of "continuity of care" that theories of health behaviour are most beneficial [4].

Behavioural Models

Behavioural change for patients is a complex process and requires more than the simple acquisition of knowledge. In fact, the choices that patients make may be influenced by many factors beyond knowledge, including personal, familial, spiritual, and social issues. Several educational models based on behavioural theories have been developed to explain individuals' health-related behaviour, including the Stages of Change Model, the Health Belief Model, the Health Promotion Model, the Precede-Proceed Model, the Health Information Model, and many others. These models help explain what happens during the patient education process, making

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narrative or causal sense out of the observed changes [5]. To conduct fruitful educational research, theoretical assumptions need to be articulated and elaborated. The Stages of Change Model and the Health Belief Model are most commonly used in research.

Stages of Change Model

The stages of change model (Fig. 1) recognizes that behavioural change often involves a temporal sequence of different processes and that successful patient education and support must acknowledge these processes and be stage specific [6]. Five distinct stages related to behaviour are recognised: pre-contemplation, contemplation, preparation, action, and maintenance. Based on these stages, the model assumes that 1) change is a process that unfolds over time through a sequence of the five stages, 2) patients tend to remain in early stages without planned interventions, and 3) patients are most likely to progress through the stages if offered education and support specific to their current stage.

The Health Belief Model

The health belief model, based on the work of Rosenstock et al. [7], proposes that decisions about health-related behaviours involve a balance between a value (i.e., the desire to become well or avoid illness) and an expectation (i.e., whether or not an action will benefit a patient's health). The model (Fig. 2) states that individuals take action to ward off, screen for, or control unhealthy conditions if they believe 1) they are susceptible to the condition (perceived susceptibility), 2) the condition has potentially serious consequences (perceived severity), 3) an available course of action will help to reduce their susceptibility to or the severity of the condition (perceived benefits), and 4) the benefits of taking action outweigh the anticipated barriers (perceived barriers). Possible barriers include cost, danger, discomfort, inconvenience, and time. Perceived threat is the term often used to express the combined effects of the patient's level of perceived susceptibility and perceived severity. Factors that influence perceived benefits include non-health-related benefits (e.g., smoking cessation saves money). A patient could have a high level of perceived susceptibility and severity (i.e., perceived threat) but not take action unless the action is perceived as potentially effective.

Study Design to Evaluate Educational Interventions

When the research focus is on the effectiveness of alternative educational approaches, the best study design, not different from the evaluation of other interventions, would be a randomised controlled trial, sometimes with cluster rather than individual randomisation.

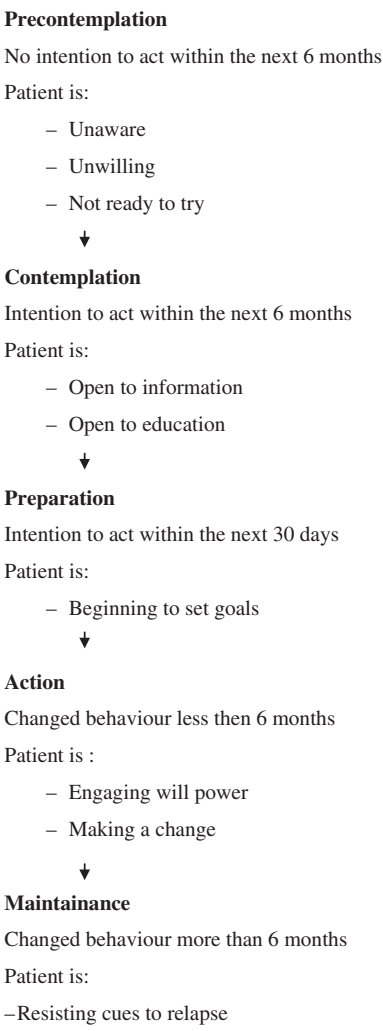

Fig. 1 The Stages of Change Model. Behavioural change often involves a temporal sequence of different processes. Patient education and support must acknowledge these processes and be stage specific

If the focus is on implementation and assessment, then the evaluation of the interventions would involve activities somewhat similar to those required in an audit cycle where data are systematically and successively collected to evaluate progress towards a series of planned changes [8]. The paradigm of "action research" first introduced in 1946 by Kurt Lewin, a social scientist concerned with intergroup relations and minority problems in the United States, may be particularly relevant in this respect [9]. Action research, a style of research rather than a specific method, focuses on change and improvement; involves a cyclical process of col-

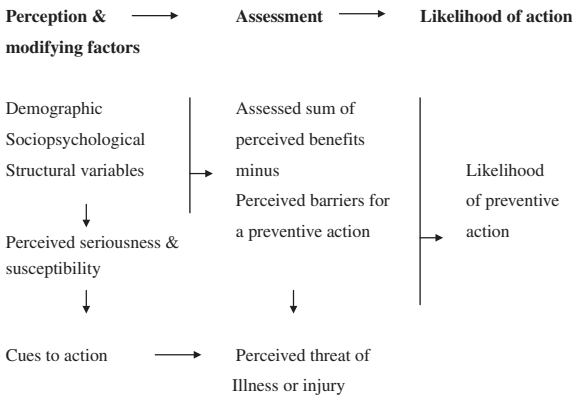

Fig. 2 The Health Belief Model postulates that decisions about health-related behaviours involve a balance between a value (i.e., the desire to become well or avoid illness) and an expectation (i.e., whether or not an action will benefit a patient's health)

lecting, feeding back, and reflecting on data; explicitly and proactively involves participants (such as clinicians, managers, and service users) in the research process; looks reflectively at questions that arise from practice; and is educational for both researchers and participants. According to the paradigm of action research, researchers work explicitly with and for people rather than undertake research on them.

Education of Organ Transplant Recipients

Organ transplant patients are a particularly important target for health education. To give an example, nonadherence to the immunosuppressive regimen is a major risk factor for poor outcome after transplantation. In spite of such an adverse consequence, considerable variability within and between subjects has been observed in the degree of compliance with immunosuppressive drugs. In a study of renal transplant recipients, noncompliance rates during the first year after transplantation were 23% for cyclosporine, 13% for azathioprine; 23% for prednisone, and 36% for atenolol. Except for a better compliance for prednisone in men as compared with women, no consistent relationship between compliance on the one hand and several demographic variables, graft function, or quality of life on the other hand was found [10]. Surprisingly, few studies have examined nonadherence intervention in this context. The available evidence suggests that educational-behavioural interventions may increase adherence in nonadherent renal transplant patients, at least in the short term [11, 12].

Skin Complications of Transplant Procedures and Patient Education

Organ transplant recipients have a remarkably increased risk of developing skin cancer; they have a higher rate of acute and chronic skin infections, including opportunistic ones such as deep fungal infections; and they may experience minor changes in skin aspects, such as hypertrichosis, which are nonetheless quite disabling. All these complications call for adequate screening and patient education (Fig. 3).

Skin cancer has been the focus of particular interest in recent years. Ultraviolet radiation is one of the major cofactors, and wide consensus exists that patients should be taught to adopt sun protection practices [13]. Recently, a randomised controlled trial documented that an intensive educational program based on repetitive written instructions about skin cancer produced measurable improvement in patient knowledge and sun-protective behaviour after 3 and 10 months compared to standard episode-of-care-based education [14]. Similar results after 6 months were obtained in a nonrandomised controlled study where 50 of 118 patients attending a cardiac transplant clinic were seen by a dermatologist for education about skin cancer risk, sun protection measures, and skin cancer screening. Specialist advice improved self-reported knowledge of skin cancer risk and sun protective behaviour [15].

Fig. 3 Example of written instructions for transplant patients

Education of patients should start as soon as transplantation is recognised as a potential treatment and should continue long term. It has been documented that when advice and literature on avoidance of ultraviolet light are given at the time of transplantation, only a minority of patients remain aware of the risks and adopt adequate sun protection measures long term. A survey conducted in Yorkshire County, United Kingdom, in 1998, showed that despite advice and literature given to newly transplanted patients at the time of discharge from hospital, only 54% of patients remembered receiving advice. Renal physicians and nurses gave advice to the majority, with dermatologists providing advice only in 17% of cases. The use of sun-protective measures such as sun avoidance and protective clothing was poor, and the use of sun barrier creams was inappropriate. Only 30% of patients knew why extra precautions against sunlight were necessary [16]. Similar lack of awareness and unsatisfactory behaviour has been documented in patients in organ transplant recipients from several other countries, including the United States, Poland, and Ireland [17–19]. In Ireland, compliance with sun protective measures and sunscreen use was poorest in those groups at higher risk for nonmelanoma skin cancer, that is, males older than 50 years engaged in outdoor occupations. This observation points to the need for assessing behaviour before designing an educational intervention and for specifically targeting high-risk or less-compliant groups by educational interventions [18].

There may be a secular trend toward a kind of improvement in sun protection behaviour in some countries. We have already mentioned the survey conducted in Yorkshire in 1998 showing limited knowledge and adoption of sun-protective measures by organ transplant recipients. The survey was conducted again in 2005 [20]. Compared with data obtained in 1998, a significant improvement in the compliance of renal transplant recipients with skin protection measures was documented, not apparently related to more intensive educational efforts.

Interpersonal motives related to appearance and the social image or prototype of a tanned person being healthy may mitigate risk perception of the health problem in organ transplant patients. Data from a survey conducted in the United States in 2003, comparing 200 organ transplant recipients with a sample of the general population, showed that organ transplant recipients had a stronger belief compared to the general population that the appearance of a tan was attractive and that people looked “healthier” with a tan [21].

Final Remarks

The key to managing skin complications in organ transplant recipients lies in a multidisciplinary approach encompassing patient education, skin screening in the immediate post-transplant period, regular follow-up, and rapid referral to a dermatologist once skin lesions suspicious for skin cancer are diagnosed.

Health professionals, and dermatologists in particular, need to take a more active role in raising the awareness of organ transplant recipients to their increased risk of skin cancer. More innovative ways to educate and involve patients should be considered, including audiovisual materials and interactive web-based education.

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Sunscreens and Sun Protection

Jason Fallon and Gillian M. Murphy

Susceptibility to Adverse Effects of the Sun

Some sun exposure is the norm for human beings. Exposure to ultraviolet radiation (UVR) varies widely depending on behaviour, culture, occupation, and geographic location, particularly latitude and altitude. UV exposure increases by 8% for every 1,000 m in altitude. Ozone depletion of 10% has occurred, mainly affecting sun exposure in countries in the Southern Hemisphere and leading to measurable increase in UVC at the Earth's surface [1]. One in two Australians, and one in five Americans in the United States, may now expect to develop skin cancer in their lifetime [2]. Skin cancer is the commonest malignancy in the Western world, even in northern latitudes, because such countries are inhabited by people with light-coloured skin.

Evolution has led to a wide variety of skin colours originally well adapted to the local environment. Not surprisingly, black-skinned races flourished in areas of low latitude and high ultraviolet radiation, as eumelanin in black skin is highly photoprotective. It is hypothesized that lighter skin colour developed because, as competition for food increased, those with paler skin were able to survive at higher latitudes, needing less UVR to synthesize vitamin D; thus, pale skin was an advantage as people moved away from high sun exposure [3, 4]. A second factor thought to be an important in selecting skin colour in different latitudes is photolysis of folic acid [5]. Black skin protects against this effect even at low latitude, but moving to areas of reduced UV exposure area leads to less photolysis of folic acid even with pale skin. Thus, there was selection pressure only for pale skin in moving to higher latitudes. Hundreds of millennia later, history has led to societies with racial types geographically dislocated from their origins, for example, Celtic skin types in the tropics and subtropics of Australia, and African Americans widely located in temperate and northern latitudes. It is important to differentiate risk from excessive amounts of UV radiation and risks from UV deprivation in some situations for different racial subtypes. Vitamin D deficiency may be a problem for dark-skinned

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Table 1 Skin type

With 30 min of midday sun in June on a nonacclimatised skin at 40° latitude:	
Skin type I	Always burns, never tans
Skin type II	Always burns, tans poorly
Skin type III	Tans well, rarely burns
Skin type IV	Always tans, never burns
Skin type V	Indian/Asian skin
Skin type VI	Black skin

races in countries at high latitudes, especially where cultural behaviour leads to sun avoidance and restricts intake of animal fats.

Skin colour was divided clinically by Thomas Fitzpatrick into six different types [6, 7] (Table 1). Skin types I and II are typical of the Celtic populations of Ireland and Scotland. Epidemiological studies of skin cancer prevalence where such people emigrated to the New World centuries ago show that those with skin types I and II account for the majority of skin cancers in Australia, New Zealand, and the United States. Now the genetic basis of skin and hair colour is being unravelled [5], and polymorphisms of the melanocortin 1 receptor are known to be direct risk factors for skin cancer [8]. In the immunosuppressed organ transplant population, skin cancer risks are greatly increased [9]. As in the normal population the major risk is for light-skinned individuals with skin types I and II and directly linked to UV dose. Those in tropical areas are at greater risk than in temperate zones, risk being directly related to level of exposure.

In Northern Europe, where populations are fair skinned, cancer prevalence is high even though solar irradiance is relatively low. Ireland, Scotland, and Denmark show very high prevalence of skin cancers of the nonmelanoma and also malignant melanoma types. By contrast, Mediterranean populations rarely develop squamous cell carcinomas, although basal cell carcinomas and malignant melanoma do occur. Individual susceptibility to skin cancer is determined by both genetic and acquired factors. Genetic factors include the genes that encode skin colour, genes that encode DNA-repair enzymes, tumour suppressor genes, genes for DNA methylation and oxidative stress, and genes encoding immunosuppression and immune responsiveness.

Systemic Immunosuppression and Carcinogenesis

In the context of this book, the major exogenous factor conferring susceptibility to skin cancers is the protocol of immunosuppression required to maintain a transplanted solid organ. Usually this comprises a calcineurin inhibitor such as tacrolimus and an antimetabolite such as mycophenolate mofetil (older regimens included cyclosporine and azathioprine) and a low dose of steroid. Azathioprine, the first major successful transplant drug, enabled 50% renal allograft survival for 12 months when used as a single agent. Undoubtedly azathioprine led to a significant

increase in skin cancers in transplant patients. Addition of cyclosporine increased graft survival dramatically and also led to more skin cancers. Transplant survival (renal) now stands at 85% to 90% at 5 years, the best results coming from live donors. Average renal graft survival is now 18.5 years. Triple therapy enables maintenance of a transplanted organ for up to 30 years [10]. Patients may undergo two, three, or even four transplants. Patients therefore spend decades exposed to significant systemic immunosuppression. Sirolimus, an alternative to tacrolimus, is a new agent, appropriate for many patients, that shows promise in reducing susceptibility to skin cancers; sirolimus is in the process of being evaluated to assess such benefit.

Consequent to long-term immunosuppression, sun-exposed skin develops myriads of viral warts which, after variable periods of time, dependent on age, duration of transplant, and skin colour, transform into dysplasia and invasive skin cancers [11], squamous cell carcinoma by far outnumbering all other types. Chronically immunosuppressed renal transplant patients develop nonmelanoma skin carcinomas at a rate 250 times that of the general population, largely on sun-exposed sites. Also less frequently, in all skin types, mucosal sites and anogenital skin develop squamous cell carcinomas related to human papillomavirus (HPV) types 16, 18, 31, and 33; these high-risk viral types thrive in conditions of long-term immunosuppression and lead to cervical cancers, penile and urethral carcinomas, and anogenital cancers [12]. Chronic sun exposure encourages HPV to thrive on the skin, and HPV of intermediate-risk and so-called epidermodysplasia verruciformis (EV) types of HPV are repeatedly found in association with squamous cell carcinomas on UV-exposed skin [13, 14], irrespective of *p53* mutation, Thus, not only does systemic immunosuppression encourage HPV, but local immunosuppression from sun exposure and UV-induced DNA damage combine to cause cancers together with other tumour suppressor genes [15]. HPV thriving in such an unfettered environment also assists in preventing apoptosis of UV-damaged cells as a mechanism of ensuring its own survival [16]. Total avoidance of UVR prevents skin cancer on exposed sites. Transplant patients with skin types I–IV, but especially I and II, therefore, need very special protection from UVR. It is exceptional to see skin cancers in brown- and black-skinned patients. Such patients by contrast may develop genital cancers and Kaposi's sarcoma [17], unrelated to UV exposure.

Photoprotection Strategies

Ultraviolet radiation intensity depends on time of day, time of year, geographic location, and human behaviour. Regardless of location, staying indoors at times of high solar intensity has a huge impact on lowering lifetime exposure. Strategies to minimise lifetime sun exposure could be as follows. Infants should be kept completely out of direct sunlight. Epidemiological evidence suggests that exposure of children to UVR predisposes to later basal cell carcinoma and malignant melanoma in adult life. Sunburn should be avoided in children by avoiding strong sunlight, playing in shade, avoiding 2 h either side of midday, and wearing protective clothing.

In northern latitudes, most UVR is acquired from March to October [18]; thus, if outdoors, exposed skin should be protected by sunscreens. This use should be as an adjunct to clothing and behaviour designed to cut down excess UVR. Adults who work indoors accumulate most UVR at weekends and on summer vacation. Targeting these times will therefore make an enormous impact on lifetime dose reduction.

Minor alterations in behaviour can greatly reduce UVR. Outdoor workers have more difficult problems, but the wearing of a hat with a 4-in. brim cuts the UVR dose to the face by 70% [19], and keeping a shirt on rather than taking it off costs nothing and may prevent developing severe sunburn [20]. A comparative study of gardeners in Denmark and Ireland showed the Irish gardeners took much less sun [21], as measured by wrist-worn electronic dosimeters. The skin type and latitude of both groups was similar; the difference was that Irish gardeners took lunch from noon until 1 P.M. whereas the Danes lunched earlier and were outdoors when the sun was at its zenith. Effectiveness of shade has also been evaluated [22]; sitting in the perimeter of a tree with light foliage may give very little protection, but sitting by the trunk of a large tree with dense cover gives very good protection. Similarly, sitting under an umbrella on a Mediterranean beach with a clear blue sky may give the individual a feeling of protection, but Rayleigh scattering of UV by particles in the atmosphere and reflection of UVR leads only to 50% reduction of UV dose to the skin, which could lead to severe sunburn in areas with intense UVR. Snow reflects 80% UV, sand 15% UV, sea foam 25%, and ground 10%. [1, 2]. Therefore, even in winter in snow, severe sunburn may occur, particularly at altitude.

Sunscreens

Sunscreens have been widely available for more than half a century [23]. Initially sunscreen chemicals were UVB-absorbing agents as the danger of excessive sun exposure was perceived to stem from UVB alone. Sunscreens are evaluated in laboratory conditions using 2 mg/cm^2 thickness and a solar simulator with defined filters to reproduce midday sun [24, 25]. The sun protection factor (SPF) is a measure of UVB protection. The SPF number is derived by the ratio of the UV dose that causes just perceptible erythema with sunscreen, divided by the UV dose causing similar erythema without sunscreen. A panel of 20 people is tested, and the SPF is the mean of the group.

In the 1980s increasing awareness of the detrimental effects of UVA spilled over from scientific research into industry, and UVA-absorbing chemicals began to be added; by the 1990s, broad-spectrum sunscreens started to be widely available, and now in the 21st century any sunscreen without broad-spectrum protection is regarded as a poor sunscreen. Methods of measuring UVA protection range from in vitro transmission tests [26] to in vivo measurements of the early phase of delayed tanning, so-called persistent pigment darkening (PPD) [27].

A sunscreen is defined as a product with an SPF of 2 and higher. Sunblock is a product with a physical block and an SPF of 12 or higher. Products often contain

a mixture of physical and chemical block ingredients, and most sunscreens with SPF greater than 20 contain both UVB and UVA sunscreens. In the United States sunscreens are licensed as drugs and are approved up to SPF 30 [28], although this is under review. In Europe, sunscreens are regulated as cosmetics and are now available as products with SPF up to 50+ [29]. Chemical sunscreens have the ability to be absorbed by the skin, and UV radiation is absorbed by the products, enabling it to be harmlessly dissipated as heat or fluorescence. A physical block sits on the skin's surface and does not have the ability to be absorbed into the skin. Light is either absorbed into the sunblock material or is reflected away. Sunscreen ingredients are strictly controlled in Europe and in the United States [30]. Permitted chemicals used in cosmetics including sunscreens are listed by the European Union (EU) [31] and U.S. Food and Drugs Administration (FDA) [32]. Other than allergic contact dermatitis and irritant effects on the skin, no serious side effects have been caused by sunscreen use. Claims that sunscreens caused skin cancer are unsubstantiated [33]. Clinical trials show a reduction of actinic keratoses and squamous cell carcinomas in immunocompetent people [34, 35]

Sunscreen Application Technique Is Critical to Its Efficacy

Using a sunscreen to prolong sun exposure could be a problem if the sunscreen only protects against sunburn and enables cumulative UVA exposure leading to skin damage, photoageing, skin cancer, and even pseudoporphyria. Inadequate application of a sunscreen has an enormous impact on efficacy. The relationship between UV transmission and SPF is logarithmic (Lambert–Beer law); thus, using less sunscreen reduces the SPF by much more than one might expect. Most people apply less than half the required amount of sunscreen, some even less than 20% of that recommended; the net effect is to dramatically reduce photoprotection.

A further area of concern is actually failing to apply sunscreen at all to exposed areas, the likelihood of sunburn rises with increased exposure. The most frequently nonprotected areas are close to the eyes, the hairline, the scalp, ears, back of neck, and upper midback. Sunscreens are also applied irregularly, with some areas better protected than others. It is therefore best to apply the sunscreen before leaving home, reapply on reaching the destination, and continuing to reapply every 2 h and after swimming. The ideal situation is to wear clothing that does not permit transmission of UV; the need for sunscreen is thereby dramatically reduced, and the margin for error reduced. Thick fabrics with tight weave are best. Some clothing companies give a UV protection factor (UPF), giving the wearer definite information about the protective properties of the garment. Compliance with sunscreen use is variable. A recent study indicated that the highest risk, most UV-exposed renal transplant patients, who already had developed skin cancer, were the worst at complying with photoprotective measures, especially sunscreen use [36]. Renal and other solid organ transplant patients are at very significant risk of squamous cell carcinomas [9], and also basal cell carcinomas [37], and have a six- to eightfold-increased risk of

malignant melanoma [38, 39]. Photoprotective measures of all types are required, and regular reinforcement of these strategies is required as not all comply well with this advice.

The amount of sunscreen applied to the skin is the most important factor that determines efficacy. Sunscreens combine ingredients in a variety of combinations to produce a product that confers stability and optimal UV protection. High-factor sunscreens over SPF 30 inevitably contain good UVA protection. The best sunscreens contain high UVB protection, indicated by the SPF number, and high UVA protection, indicated by a high PPD number, or increasingly the star system; one star is poor protection and four or five stars excellent UVA protection. When sunscreens are adequately applied before exposure to the sun and then reapplied every 2 h with ongoing sun exposure in combination with photoprotective behaviour and clothing, excellent photoprotection may be achieved. No oral agents offer adequate protection against sunburn, and beta-carotene proved disappointing as an anticancer drug [34]. New research gives hope for the future that strategies will emerge which may alter pigment production to photoprotective eumelanin, even in current skin type 1, such that natural photoprotective tanning may be augmented in humans with a consequent decrease in skin cancer risk [40, 41].

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Surgical Intervention for Skin Cancer in Organ Transplant Recipients

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An important complication of solid organ transplantation and chemical immunosuppression is the potential for the development of multiple skin cancers, especially squamous cell carcinomas [1]. The magnitude and severity of these malignancies are troublesome. They are often multiple, are associated with verrucal lesions, are more likely to occur at a younger age, and have a higher recurrence rate than in those who do not receive transplants, are capable of rapid growth, have an aggressive histological growth pattern [2, 3], and are prone to metastasis on the trunk and extremities as well as the head and neck.

More than half of patients receiving organ transplants develop tumors. In most transplant recipients, these tumors occur de novo and are associated with a history of sun exposure, the types of immunosuppressant medications, the degree of immunosuppression, and sometimes the human papillomavirus or human herpesvirus 8 [4]. Usually, in patients with skin cancer, basal cell carcinoma occurs more frequently than squamous cell carcinoma, but in transplant recipients, this frequency is reversed. Compared with generally observed occurrence rates, squamous cell carcinomas are increased between 65- and 250 fold, basal cell carcinomas 10 fold, malignant melanoma 3- to 5 fold, and Kaposi's sarcoma 84 fold. The severity of Merkel cell carcinoma is increased, as is that of atypical fibroxanthoma. Cancers typically begin 7 to 8 years after the patient receives the transplant, but this interval is shorter, 3 years or less, in heart transplant recipients. The number of malignancies and the percentage of patients involved increase with the duration of immunosuppression. For example, in Australia, 7% of patients had skin cancer after 1 year and 70% after 20 years [5]. The trend is the same in the Netherlands but to a lesser degree; 0.2% had skin cancer after 1 year and 40% after 20 years.

The surgical approach to skin cancers in transplant recipients is detailed below. In general, treatment is based on high-risk factors such as rapid growth, potential for or presence of metastasis, and the number of occurrences of the same type of cancer present at the time of the initial dermatological examination. Standard surgical treatments include electrodesiccation and curettage, excision with or without

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Table 1 Management of skin carcinomas in transplant recipients

Tumor type	Surgical treatment
Superficial basal cell carcinoma, Bowen disease	Electrodesiccation and curettage
Basal cell and squamous cell carcinoma, keratocanthoma	Electrodesiccation and curettage, excision, or Mohs surgery
Multiple squamous cell carcinomas	Electrodesiccation and curettage, excision, or Mohs surgery
High-risk squamous cell carcinomas	Excision or Mohs surgery
Local recurrence of squamous cell carcinoma	Excision or Mohs surgery
Metastatic squamous cell carcinoma	Excision

Source: Modified from Euvrard et al. [4]. Used with permission.

frozen-section margin control, wide excision (as defined for each tumor), and Mohs micrographic surgery (Table 1).

Unique situations such as the "transplant hand" may require a different approach. The indications for sentinel lymph node biopsy are influenced by each tumor's potential for metastasis. Adjunctive therapy may include the following treatments, singly or in combination: topical and systemic anticancer and antiviral agents, radiotherapy and chemotherapy, and reduction of immunotherapy. These therapeutic options are discussed elsewhere in this book.

Surgical Management of Skin Cancer in Organ Transplant Recipients

Shelley and Shelley [6] have suggested that for patients with chronic skin problems dermatologists should "Make friends with these patients as you will be seeing them for a long time. They need your help, not for months but for years." A comment by Brown [16] echoes this sentiment for organ transplant recipients: "You can never be faulted for following these high-risk patients too closely." These are good starting points for the surgical management of skin cancer in organ transplant recipients. They need to be evaluated frequently, sometimes as often as every 3 to 4 weeks (Table 2). The surgical management of skin cancer in organ transplant recipients depends on the type of malignancy, its extent, and its high-risk features.

The skin cancers that occur commonly in these patients and are amenable to surgical intervention are squamous cell carcinoma, basal cell carcinoma, malignant melanoma, Merkel cell carcinoma, atypical fibroxanthoma, malignant fibrous histiocytoma, angiosarcoma, and verrucal carcinoma (a special type of squamous cell carcinoma) [7]. Kaposi's sarcoma usually occurs at multiple sites, and these tumors are not treated surgically except for the occasional isolated lesion [8].

Stasko and colleagues [9] have produced a user-friendly algorithm that is helpful for managing squamous cell carcinomas in organ transplant recipients. Superficial low-risk tumors (small, occurring on the cheeks, scalp, neck, trunk, and extremities) that are slow growing can be managed by aggressive electrodesiccation and

Table 2 Guidelines for follow-up intervals for organ transplant recipients[a]

Patient risk factors	Frequency of skin examination
No risk factors except immunosuppression	Initial exam + exam every 12–24 months
Risk factors but no history of premalignant or malignant lesions	Initial exam + exam every 6–12 months
Actinic keratoses or warts	Initial exam + treatment + exam every 3–6 months
One basal cell carcinoma	Initial exam + treatment + exam every 3–6 months
One squamous cell carcinoma	Initial exam + treatment + exam every 3–6 months
Multiple nonmelanoma skin cancer	Initial exam + treatment + exam every 3 months
High-risk squamous cell carcinoma	Initial exam + treatment + exam every 3 months
Metastatic squamous cell carcinoma	Initial exam + treatment + exam every 1–3 months

[a]Because of the increasingly high risk of skin cancer development from the time of transplantation, periodic skin evaluation is recommended for the life of the patient.
Source: Stasko et al. [8]. Used with permission.

curettage or laser surgery. Larger tumors may require surgical excision, including resection of subcutaneous fat with frozen-section margin control. High-risk tumors (large, rapidly growing, recurrent, poorly differentiated, occurring on the central face, periorbital area, pre- and postauricular areas, and genitalia) are best managed by Mohs micrographic surgery. Sentinel lymph node biopsy or elective lymph node dissection with adjunctive radiotherapy is sometimes indicated.

Because certain tumors may seed during surgery (e.g., squamous cell carcinoma associated with human papillomavirus or malignant fibrous histiocytomas), ring block anesthesia may be more appropriate than anesthetic injection through the tumor.

Organ transplant recipients and their skin cancers have been subdivided into several groups by Berg and Otley [10]:

1. Early cutaneous carcinogenesis (isolated nonmelanoma skin cancers). These may be treated by traditional surgical methods, including electrodesiccation and curettage, excision, or Mohs surgery.
2. Moderate cutaneous carcinogenesis. These patients develop multiple cutaneous carcinomas each year. Aggressive standard surgical therapy, that is, using standard treatments with increased diligence, is appropriate for these patients.
3. Catastrophic cutaneous carcinogenesis. These patients develop more than 100 squamous cell carcinomas per year with the associated potential for metastasis and mortality. Aggressive frequent tumor removal is indicated with evaluations every 1 to 3 months. Mega sessions (excision of numerous skin cancers in a single session), as outlined by Martinez and Otley [11], may be indicated in these patients.

Table 3 Characteristics of high-risk squamous cell carcinoma

Multiple, rapid recurrences
High-risk location
Large size (>2 cm)
History of aggressive growth
High grade (Broders 3 or 4)
Deep invasion (>4–6 mm), especially into fat

Source: Data from Rowe et al. [12].

Berg and Otley [10] recommend identifying high-risk squamous cell carcinoma on the basis of the risk factors defined by Rowe et al. [12] (Table 3). The factors that portend potential aggressive behavior include perineural spread, depth greater than 4 mm, size larger than 2 cm, poor differentiation, previous treatment, and location in high-risk sites (ear, lip, periorbital area, and temple). In the study by Rowe et al. [12], the high-risk tumors most likely to metastasize were found on the head and neck, but in organ transplant recipients, the lesions on the trunk and extremities also had increased potential for metastasis. Because guidelines for surgical margins in organ transplant recipients have not been fully defined, squamous cell lesions with potential for aggressive behavior are best treated by frozen-section margin control or Mohs micrographic surgery if available. In selected cases, surgical staging may be appropriate using sentinel lymph node biopsy or elective lymph node dissection. Adjunctive radiotherapy may be considered for recurrent cancers.

Squamous cell carcinomas of the ear and lip are considered aggressive in organ transplant recipients, and their management includes wide surgical excision or Mohs micrographic surgery and the consideration of sentinel lymph node biopsy. Complete removal of the invasive component on the lip may be followed by carbon dioxide laser vermilionectomy of the adjacent actinic cheilitis [10], thus reducing the risk of subsequent squamous cell carcinoma.

In-transit metastatic squamous cell carcinoma may be predictive of metastasis and is treated by wide local excision plus adjunctive radiotherapy. The prognosis for these tumors is poor [13].

Squamous cell carcinomas on the backs of hands of transplant recipients in association with multiple verrucal lesions have been termed the "transplant hand." Surgical excision of the total surface of the back of the affected hand from the wrist to the nail folds is followed by skin grafts from skin that has not been exposed to the sun. This procedure has been reported by two groups [5, 6]. The reason given for the resurfacing was the development of multiple skin cancers in a short time on the back of transplant recipients' hands [14]. Other resurfacing techniques, such as carbon dioxide laser resurfacing, dermabrasion, or chemical peel, may not destroy the follicular extensions of the malignant epidermis and are probably of limited value in these patients.

Atypical fibroxanthomas and their counterpart, malignant fibrous histiocytomas, are at risk for metastasis and should be treated by wide local excision (1–3 cm),

including subcutaneous fat, or Mohs micrographic surgery. Their incidence may be greater in transplant recipients than in control patients [8].

Merkel cell carcinomas are more aggressive in organ transplant recipients. Fifty-six percent of patients with Merkel cell carcinomas died within 2 years of this diagnosis, compared with 25% to 35% of other surgical patients who do not receive immunosuppressive agents. Transplant recipients with Merkel cell carcinomas are treated in the typical manner for this type of tumor by wide local excision (2–3 cm) including subcutaneous fat or Mohs micrographic surgery plus sentinel lymph node biopsy and postoperative radiotherapy.

Sarcomas have been reported in organ transplant recipients, but whether sarcomas are more common or more aggressive in transplant recipients than in skin cancer patients who have not undergone transplantation is not clear. These sarcomas include angiosarcoma and dermatofibrosarcoma protuberans. The surgical management for these in transplant recipients does not differ from that in patients with skin cancer. Either wide local excision including subcutaneous fat or Mohs micrographic surgery (the treatment of choice for dermatofibrosarcoma protuberans) is appropriate, plus consideration of adjunctive therapy for angiosarcomas. The prognosis for this tumor is poor despite therapy.

The occurrence of malignant melanoma is slightly increased in organ transplant recipients and may be more aggressive (i.e., rapid growth), so prompt wide excisional surgery is appropriate, with margins determined by Breslow thickness and consideration of sentinel lymph node dissection. For metastatic malignant melanoma, a lymph node dissection is indicated and adjunctive therapy is considered. This is one of the skin cancers that may be transmitted inadvertently from the donor.

Basal cell carcinomas are 10 times more common in these patients, but there is no literature indicating that they carry a worse prognosis than nontransplant patients. Standard treatment is indicated, which includes electrodesiccation and curettage, excision for low-risk tumors (small primary tumors at low-risk sites and low-risk histology, i.e., nodular or superficial), excisional frozen-margin control with subcutaneous fat, or Mohs surgery for high-risk tumors (recurrent, large tumors at high-risk sites and/or high-risk histology such as morpheaform, micronodular, or metatypical).

The indications for antibiotic prophylaxis are the same for organ transplant recipients as for patients undergoing skin cancer surgery.

Conclusions

Organ transplant recipients are at a higher risk of several types of skin cancer, especially squamous cell carcinomas. Marks [15] compared these tumors in transplant recipients to alluvial gold, the type that is easily mined by the first people to discover it on the surfaces in large amounts: “Once that has been removed, it becomes increasingly difficult to find more gold, often requiring extensive mining.” He went

on to say that the area of skin cancer in organ transplant recipients was alluvial gold at the moment and that it should be mined while it was easy. Therefore, surgical management of the skin cancers in transplant recipients should be frequent and aggressive with consideration of appropriate adjunctive treatment in selected cases.

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Topical Treatment of Field Cancerization

Claas Ulrich

The concept of "field cancerization" was established by Slaughter [1] in 1953 to describe the presence of histologically abnormal tissue surrounding an invasive squamous cell carcinoma from the upper gastrointestinal tract. The term was proposed to explain the development of multiple primary tumours and locally recurrent cancer; this specifically accounts for organ systems such as the skin (head and neck) cancers, in the ENT areas (oral cavity, oropharynx, and larynx), but also the lung, vulva, esophagus, cervix, breast, skin, colon, and bladder.

The specific pathophysiology of "field cancerization" appears to be similar for all relevant organ systems described so far. After acquiring a (single- or multi-hit) genetic alteration, a stem cell forms a clonal unit of dysplastic cells (initial phase). This formation ("dysplastic patch") may take place in close connection with the initial genetic alteration or might follow years later. The primarily horizontal expansion of a "patch" to an expanding dysplastic field is the next step towards malignancy (expansion phase).

Actinic keratoses (AK) are the earliest, clinically detectable lesions equivalent to spots or regions of dysplastic keratinocytes [2]. The incidence of AK is significantly higher in transplant recipients compared with age-matched controls, implying impaired immune elimination of previously damaged keratinocytes [3, 4]. These areas of "field dysplasia" account for much of the skin-cancer-related morbidity and mortality in organ transplant recipients (OTR) and, in recent years, have become the key target of most dermatological initiatives to reduce the skin cancer burden in OTR.

The most important clinical implication in the management of AK and field cancerization is that unspecific, primarily destructive therapies of individual primary lesions with surgery, cryotherapy, curettage, or laser frequently lead towards "new cancers." These "new cancers" are often misinterpreted as "local recurrence" or new tumours by the clinician, which leads towards a repetitive and often enough frustrating cycle of destructive, lesion-adapted treatments followed by the clinical

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occurrence of new lesions. In contrast to immunocompetent patients, immunosuppressed patients have a highly accelerated rate of AK development and progression into invasive squamous cell carcinoma (SCC). Management strategies that counteract the effects of systemic immunosuppression via the induction of a locally restricted, tumour-specific immune response, the induction of apoptosis in dysplastic keratinocytes, or the use of phototoxic agents can provide the advantage of treating large clinical and subclinical lesions in UV-exposed dermatomes associated with "field carcinogenesis." Topically applied imiquimod, 5-fluorouracil, photodynamic therapy, and diclofenac 3% gel are promising noninvasive alternative treatment modalities that are applicable to larger treatment areas. Different studies performed so far in OTR showed interesting treatment results of these comparably specific drugs.

Imiquimod

Imiquimod is one of the first described members of a novel class of Toll-like receptor 7 agonists that serve as immune response modifiers (IRM) with the ability to stimulate the innate and acquired cellular immune system. It belongs to a family of imidazoquinolines that via stimulation of the innate and possibly acquired immunity have potent antiviral and antitumor properties in vivo [5, 6].

The local application of imiquimod leads towards an activation of epidermal antigen-presenting Langerhans' cells (APCs) which, after migration to regional lymph nodes, activate antigen-specific cytotoxic T lymphocytes (CTL) and natural killer cells (NK). Both cell types are crucial for the skin's local surveillance against intracellularly located viruses or aberrant keratinocytes. This ability to enhance adaptive immune responses (CTL) as well as innate immunological responses [interferon-alpha (IFN-α)] seems to play the key role for the substance's antitumour and antiviral activity. Imiquimod 5% cream is effective in the treatment of AK in immunocompetent patients, as evidenced in multiple randomized controlled clinical trials, and recurrence of lesions is found to be low in patients who achieve clearance [7, 8]. Reports of imiquimod treatment of individual cases and small, non-placebo-controlled case series suggest that efficacy is similar in transplant recipients with BCC or even invasive SCC. A small, open-label study found imiquimod 5% cream to be safe and effective in the treatment of superficial and nodular basal cell carcinomas in transplant patients [9].

Three placebo-controlled studies in renal transplant recipients and one large multicenter study in kidney, heart, and liver transplant recipients found imiquimod to be safe when treating areas of field cancerization not exceeding 100 cm^2 and two sachets per application [10, 11]. For all studies published so far on the use of imiquimod in OTR, the drug was applied on the treatment area on 3 nonconsecutive days per week for 16 weeks. A randomized, blinded, placebo-controlled study evaluated imiquimod or placebo applied three times a week for 16 weeks to comparable areas of clinically atypical skin on dorsal hand or forearm of 21

high-risk renal transplant recipients with skin cancer [10]. At week 16, biopsy samples were collected from preassigned sites in the treatment and control areas and were examined for dysplasia. Of the 14 patients receiving imiquimod, 7 patients (1 taking placebo) had reduced skin atypia, 7 patients (none taking placebo) had reduced viral warts, and 5 using imiquimod (1 taking placebo) showed less dysplasia histologically. In 1 year, fewer invasive squamous skin cancers arose in the imiquimod-treated skin areas than in control areas. The authors concluded that topical 5% imiquimod cream was effective in reducing cutaneous dysplasia and the frequency of invasive SCC developing in high-risk renal transplant recipients [10]. A recent multicentre, placebo-controlled safety and efficacy study performed by the Skin Cancer in Organ-transplant Patients (SCOPE) research network enrolled 43 patients in six European transplant centres. Patients applied two sachets of topical imiquimod or vehicle cream 3 times per week to a 100-cm^2 field. Dosing continued for 16 weeks regardless of lesion clearance. All patients were strictly assessed for safety variables that included adverse events, local skin reactions, laboratory results, vital signs, dosage of immunosuppressive medication, and indication of graft rejection. A blinded independent expert committee was responsible for safety monitoring and final safety assessment. Neither graft rejections nor laboratory or clinical trends for deterioration of graft function were detected. Among patients randomized to imiquimod, the histologically confirmed complete clearance rate was 62.1%, compared to a complete clearance rate of 0% in the vehicle group [12].

To exclude graft rejections induced through the Th1 immune response of the immune response modifier imiquimod, all patients were monitored for changes in hematology and serum chemistry. Specifically, levels of serum creatinine, C-reactive protein, and proteinuria were monitored for renal transplant recipients; levels of gamma-glutamyl-transpeptidase, glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), and bilirubin were monitored for liver transplant recipients; and heart transplant recipients were monitored specifically for GOT and GPT, white cell blood count, serum creatinine, hemoglobin, and signs of heart failure. Three transplant physicians comprised a blinded, independent safety committee to monitor for any signs of graft rejection in each transplant group. In all studies published with imiquimod in OTR, no side effects of the IRM on the function of the graft were observed.

Treatment with imiquimod is often associated with local skin reactions which may be considered important in achieving optimal efficacy [13]. Most commonly reported local side effects include erythema, edema, pruritus, and erosion in OTR [12]. Systemic side effects of topical imiquimod are rare but have been described in patients with autoimmune diseases [14].

Photodynamic Therapy

Topical photodynamic therapy (PDT) is a well established, (photo-)toxic treatment of sporadic AK and BCC, and has also been used for Bowen's disease and other skin malignancies [15]. MAL (methyl aminolaevulinate), as a succeeding

molecule to the widely used 5-ALA (5-aminolevulinic acid), appears to be more lipophilic and has therefore an improved skin penetration. Furthermore, MAL has been described to bear a higher selectivity for neoplastic cells as compared to 5-ALA.

Two studies have shown MAL PDT to be effective in the treatment of AK in transplant recipients [16, 17]. An open-label intrapatient randomized study examined the prevention potential of MAL PDT in 27 renal transplant patients with AK (two circular contralateral areas; 5 cm diameter) [16]. The treatment area surface but not the control area was debrided, and MAL cream (160 mg) was applied for 3 h before illumination by red light (570–670 nm; light dose, 75 J cm^2). The mean time to occurrence of the first new lesion was significantly longer in treated than control areas [9.6 vs. 6.8 months; treatment difference, 2.9 (95% confidence interval, 0.2–5.5) months; $P = 0.034$]. Over 12 months, 62% (16/26) of treated areas were free of new lesions compared with 35% (9/26) in control areas.

To evaluate the preventive effect of PDT on the development of new SCC, a further randomized-controlled trial with paired observations in 40 organ transplant recipients was performed [18]. The treatment area consisted of a randomly assigned forearm and the corresponding hand, whereas the other forearm and hand served as the control area. After 2 years of follow-up, no statistically significant difference was found in the occurrence of new SCC between the treated and untreated arms. The number of keratotic skin lesions increased in both treatment and control arms. The authors discuss that the transplant-associated impairment of the cutaneous immunosurveillance fails to create a state in which immunosurveillance and eradication of residual tumour cells after PDT are impaired, resulting in a reduced response to PDT in post-transplant compared with sporadic or nontransplant AK [18]. This finding was supported by a prospective, open, comparative trial with aminolevulinic acid (ALA) PDT for AK in 20 immunosuppressed transplant recipients and 20 immunocompetent controls. The overall complete response rate at 12 weeks was 8% and 68% in the respective groups ($P < 0.05$) [19].

In a recently published open-label, single-centre, randomized study, eight organ transplant recipients with epidermal dysplasia were treated with either two cycles of topical MAL PDT or fluorouracil (5-FU) cream, which was applied twice daily for 3 weeks to a clinically and histologically comparable area [20]. PDT was found to be significantly more effective than 5-FU in achieving complete resolution (CRR, 89% vs. 11%). Cosmetic outcome and patient preference were also superior in the PDT-treated group [20].

Pain is the most common side effect of PDT. It is usually related to tissue damage by reactive oxygen species and is therefore restricted to the illuminated area. This tissue damage is followed by the development of erosions, crust formation, and healing over 2–6 weeks and occasionally dyspigmentation, which usually resolves within 6 months. Nevertheless, PDT allows a safe treatment of larger dysplastic areas, which is especially useful in a transplant patient population characteristically showing widespread and numerous lesions.

5-Fluorouracil

5-FU is still the most widely used topical chemotherapeutic agent for extensive epidermal dysplasia. Its cytotoxic mode of action involves the inhibition of thymidylate synthetase and consequently DNA synthesis [21]. 5-FU is widely used in the treatment of AK in both transplant and nontransplant patients. However, only a few trials evaluating the efficacy of 5-FU-based AK treatments in the transplant population were published so far. In a small study, five long-time renal transplantation patients with multiple areas of biopsy-proven carcinoma in situ (CIS) of the lower extremities were treated with a combination of a imiquimod cream and 5-FU, resulting in clearing of the CIS areas. It was hypothesized that cytokines induced by imiquimod may improve the therapeutic efficacy of topical 5% 5-FU in the treatment of CIS [22].

Interestingly a recent open-label, single-centre, randomized study comparing topical MAL-PDT with 5-FU cream in the treatment of post-transplant epidermal dysplasia found PDT to be more effective and cosmetically acceptable and preferred treatment [20]. Local side effects of topical 5-FU are dependent on the concentration of the active drug and include transient erythema, inflammation, and pain. In some instances the inflammation can persist and result in ulceration and secondary wound healing.

Diclofenac Sodium Gel

Diclofenac is a nonsteroidal antiinflammatory drug (NSAID), reducing the production of prostaglandins by inhibiting the inducible cyclooxygenase 2 (COX-2) enzyme. There is increasing evidence that cyclooxygenase-2 (COX-2) plays an important role during the development and progression of nonmelanoma skin cancers [23]. COX-2 is normally undetectable in most epithelial tissues. However, both growth factors and pro-inflammatory cytokines may result in its overexpression as it has been documented in actinic keratoses and invasive squamous cell carcinoma [24,25]. Sun damage, AK, and invasive SCC have been linked with increasing levels of prostaglandins and COX-2 activity, paralleling increased levels of dysplasia [26]. Furthermore, COX-2 immunopositivity correlates with hypoxia and higher proliferating endothelial cell fractions, indicating an involvement of COX-2 in skin tumour angiogenesis. A correlation between COX-2 levels with vascular endothelial growth factor expression and tumour vascularization has been previously shown [27, 28]. Most interestingly, studies on human cell lines revealed a causal, Bcl-2 dependent linkage between COX-2 inhibition and anti-apoptosis [29], which, especially for preinvasive SCC, could be of specific importance. Furthermore, diclofenac may act via an overexpression of metalloproteinases, which would have keratolytic and collagenolytic effects [30,31]. Diclofenac has been shown to inhibit murine angiogene-

sis, regulate apoptosis, and induce cell-cycle arrest, and has a significant antitumour effect in murine colon-26 growth [32].

Topical diclofenac 3% gel (solaraze; in 2.5% hyaluronic acid) (HA) is licensed for the treatment of actinic keratoses by the FDA and European Medicines Agency (EMEA) and is currently widely used as an efficient treatment of AK in the non-transplant population. Inducing only mild signs of inflammation and other side effects (including stinging, burning, pain), diclofenac gel offers potential advantages over cytotoxic agents such as 5-FU in most patients. Despite the disadvantage of a longer treatment period (16 weeks) and similar clinical efficacy, a greater number of patients therefore expressed significant satisfaction with diclofenac gel when compared to the 5-fluorouracil cream in a recently published trial [33].

The use of diclofenac 3% gel in OTR was recently evaluated in a small, open-label study on six OTR (three kidney, one liver, and two heart transplant patients) with histories of multiple NMSCs and extensive AKs. Diclofenac 3% gel showed a 50% complete clearance rate and 83% partial response rate ($\geq$75% lesion reduction). Patients were treated with diclofenac 3% gel twice daily for 16 weeks. Complete and partial clearance rates of AKs were assessed after 16 weeks, and biopsies were performed 4 weeks post therapy. Local adverse events at the site of application were very mild to moderate and included mild erythema and marginal erosion [34]. Another single-centre, placebo-controlled study on 32 OTR [kidney ($\pm$ pancreas), liver, or heart transplantation] following the same 16-week, two times per day application scheme showed an overall complete clearance in the diclofenac arm of 50% compared to 0% in the vehicle group. Laboratory parameters were carefully checked throughout the whole study period and were found to be generally stable and unaffected by the study drug. No systemic side effects, especially changes of the serum creatinine, were reported. However, further studies are needed before recommending NSAIDs for use on skin areas exceeding 100 cm^2 [35].

Conclusion

The accelerated skin carcinogenesis seen in immunocompromised patients makes them the ideal population for studying short-term efficacy rates in the clearance of AK and long-term prevention of invasive squamous cell carcinoma.

The aforementioned studies add to the findings of other trials that show the benefits of so-called field or topical therapies compared with nontopical therapies (e.g., cryotherapy) that are unspecific, more destructive, and provide limited short-term efficacy in immunosuppressed patients with actinic field dysplasia. In addition, it becomes obvious that the transplant population would benefit from a self-applied, effective, convenient, and safe method to treat clinical and subclinical AKs. However, comparative data regarding efficacy, side-effect profile, and patient preference are needed to allow clear advice on the choice of treatments. Furthermore, there exists a potential for combination therapies with two or more agents, and this should be discussed if debridement of actinic hyperkeratosis before application of

a field-managing drug, as performed before PDT, would also increase the efficacy in imiquimod, 5-FU, and diclofenac regimens. Further long-term follow-up of these patients will provide evidence that the eradication of all actinic damage can prevent these patients from developing invasive SCC in the future.

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Destructive Management of Skin Cancers in Organ Transplant Recipients

Jonathan Ng, Alvin Chong, and Peter Foley

Introduction

Destructive therapies, namely cryotherapy and curettage and electrodessication (C&ED), play an important role in the day-to-day management of skin malignancies and premalignant lesions in the organ transplant recipient (OTR).

Advantages

When compared with other modalities such as surgical excision, topical immunomodulatory agents, or radiotherapy, destructive therapies are simple, inexpensive, and quick procedures that are readily carried out in the clinician's office. Destructive therapies are particularly useful when dealing with large numbers of lesions, which in the OTR can arise within a relative short period of time and for which other therapies may become impractical. Destructive therapies also provide an alternative when surgery is not suitable, for example, in patients with concurrent medical conditions such as pacemakers or coagulopathies, at body sites at which scar contractures may be a problem (e.g., digits), for patients who refuse to have surgery, or those with incurable tumours where treatment is aimed at palliation only.

Disadvantages

The main drawbacks of destructive therapies relate to the issue of efficacy. Although both cryotherapy and C&ED have been widely used for decades in the general population to treat nonmelanoma skin cancers and related premalignant conditions, with many reports exalting their efficacy present in the literature, by and large the evidence is based on noncontrolled prospective or retrospective series with

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varying selection criteria, treatment protocols, and follow-up periods. Randomized controlled trials are certainly scarce in the literature. Therefore, the excellent reported cure rates and recurrence rates of both cryotherapy and C&ED need to be interpreted with caution. In addition, specific studies examining the efficacy of these modalities in the transplant population are lacking, and most guidelines regarding cryotherapy and C&ED in the OTR draw primarily on expert opinion. The other main disadvantage of destructive therapies is the unpredictable cosmetic result, which may include hyper- and hypopigmentation and hypertrophic or atrophic scarring.

Cryotherapy

Cryotherapy (or cryosurgery) is the destruction of tissue by the direct application of a cryogenic agent. Liquid nitrogen, generally applied with an open spray technique, is the most commonly used cryogenic agent because it is easy to use, inexpensive, and readily available.

Cryotherapy causes tissue destruction through multiple proposed mechanisms, including physical damage of cellular components by ice crystals, osmotic damage during thawing caused by uneven intracellular ice formation during freezing, ischaemic damage from cold injury to small vessels, and immunological stimulation from release of antigenic components in freezing. The extent of injury is proportional to the rate of freezing, the coldest temperature achieved, and the length of freeze time. A repeated freeze–thaw cycle produces much greater tissue damage than a single freeze as a result of the increased conductivity and impaired circulation of previously frozen tissue, allowing for a faster and greater degree of cold penetration [1].

The cosmetic outcome following cryotherapy is generally considered good or excellent despite the not inconsiderable risk of dyschromia [2, 3]. In one study that reported a lesion response rate of less than 70% for cryotherapy, 29% of responding lesions demonstrated hypopigmentation following cryotherapy [3]. The choice of cryotherapy in darker-skinned individuals requires careful consideration, as hypopigmentation is a frequent result. Figures 1 and 2 demonstrate blister formation post cryotherapy and residual hypopigmentation in an OTR.

Curettage and Electrodessication

Curettage relies on the target material being more fragile or having a cleavage plane that separates it from the surrounding normal skin. Cicatrical lesions such as morphoeic BCCs therefore cannot be curetted. In addition, curettage is also inappropriate for anatomic sites where there is very thin and mobile skin, such as the eyelids, lips, or genitalia, because the curette may penetrate into subcutaneous tissue during the procedure.

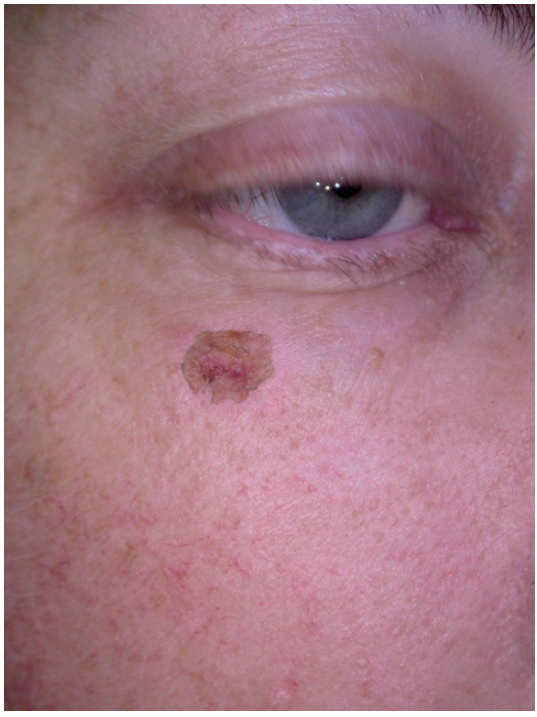

Fig. 1 Blister formation post cryotherapy for actinic keratosis

The technique of curettage is usually performed in conjunction with electrodessication, although sometimes curettage is carried out alone. The purposes of electrodessication are multiple, including causing thermal destruction of tumour cells unreached by the curette, aiding haemostasis, and provoking an inflammatory response that is believed to facilitate clearance of any residual tumour. Electrodessication, as opposed to electrocautery, refers to a monoterminal, high-voltage, low-amperage current with oscillations damped, which is superficially destructive. In contrast, electrocautery utilizes biterminal, low-voltage, high-amperage currents that cause deeper tissue destruction. The optimal number of treatment cycles is not clearly delineated, with one to three cycles being generally used, depending on the lesion and the clinician.

An additional advantage of C&ED over cryotherapy is the ability to obtain a histological diagnosis from tissue obtained during the procedure. C&ED is heavily operator dependent, and better cure rates are seen with increasing physician experience.

The cosmetic outcome following C&ED has qualitatively been considered "good" to "excellent" in many reports in the literature [4], but direct comparisons with surgical excision are lacking. On the trunk, C&ED most often leaves a pale flat macule (Fig. 3), but on occasions this may be followed by a pink, elevated, or depressed

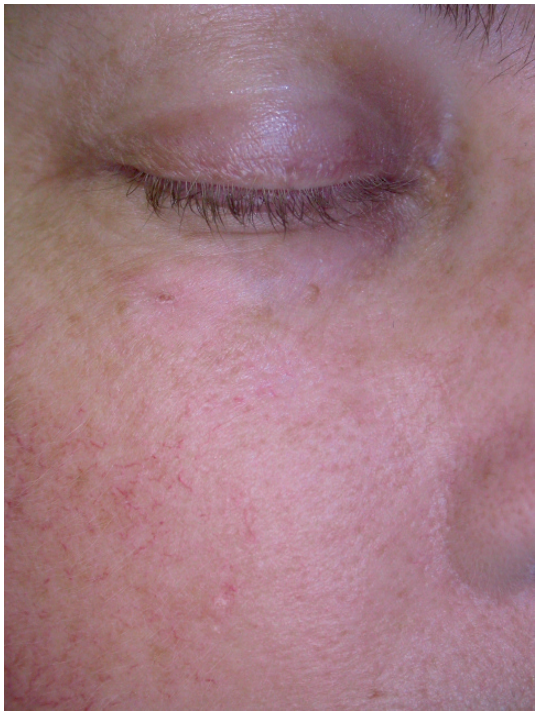

Fig. 2 Hypopigmentation post cryotherapy for actinic keratosis

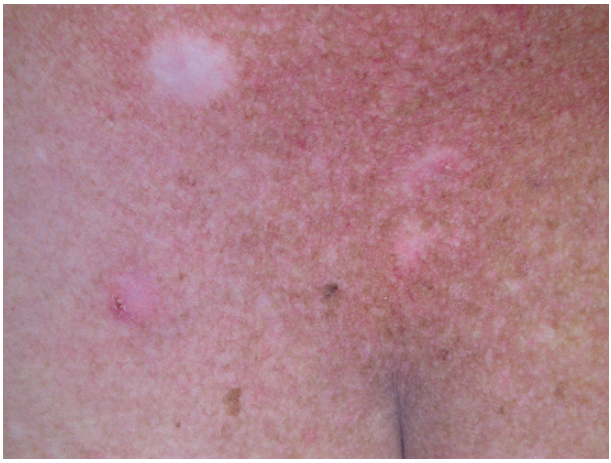

Fig. 3 Postinflammatory hypopigmentation and scarring (chest) after curettage and diathermy for Bowen's disease in renal transplant patient

scar that may persist for months or be permanent. Over the face, C&ED generally results in a fine white patch but may rarely produce a firm rope-like scar from wound contracture [5].

Use of Destructive Therapies in the Transplant Population

In a survey of U.S. dermatologists managing OTRs, both cryotherapy and C&ED are routinely used, primarily for actinic keratoses and early or superficial nonmelanotic skin cancers. A few clinicians specifically stated that C&ED is reserved for lesions on the trunk and limbs [6].

Basal Cell Carcinoma

Lesion Selection

Both cryotherapy and C&ED are appropriate first-line treatments in the OTR, provided careful selection of tumours is made [7]. The characteristics of BCCs suitable for destructive treatments in non-OTRs include the following:

- Size less than 0.5 cm diameter in high-risk areas (the lips, alar creases, inner canthi, and periauricular regions);
- Size less than 1 cm diameter in middle-risk areas (forehead, temples, scalp);
- Size less than 2 cm diameter in low-risk areas (trunk, limbs);
- Nonaggressive subtypes, i.e., not morphoeic, fibrosing, or otherwise poorly defined BCCs.

In general, the authors would prefer to only use C&ED on lesions that are not on the head and neck because the recurrence rate is high on the head and neck, and even more so in transplant recipients.

Efficacy

Cryotherapy has been shown, primarily in noncontrolled prospective studies, to be effective in treating BCCs in the general population with cure rates consistently above 90% [8, 9]. Several randomized studies suggested somewhat more variable outcomes, with recurrence rates reported from 6.25% (at 1 year) [10] and 13% (at 1 year) [11] to 39% (at 2 years) [12]. A 5-year follow-up study of superficial BCC treated with cryotherapy compared with photodynamic therapy reported a recurrence rate of 20% in the cryotherapy arm [13].

The experience of cryotherapy in the transplant population is largely anecdotal. In one series of 59 superficial BCCs (32.8% in head and neck region, and 67.2% in extracephalic regions), it was commented that the majority were “successfully managed” with cryotherapy, but no cure rates or recurrence rates were reported [14].

C&ED used for BCCs has consistently provided 5-year cure rates above 90% in the general population [4]. The main factors influencing efficacy are anatomic locations and lesion size. In reference to the risk groups outlined above, the cure rate of BCCs is greater than 96% for low-risk areas, 94.7% for lesions less than 1 cm and 77.3% for lesions larger than 1 cm in middle-risk areas, and 94.7% for lesions less than 0.5 cm and 77.3% for lesions larger than 1 cm in high-risk areas. BCCs larger than 2 cm in diameter are associated with a cure rate of 84% irrespective of anatomic site [9].

Procedure

A biopsy should be performed before cryotherapy in suspected BCCs to confirm diagnosis and favourable subtypes. Double freeze–thaw cycles have been shown to be superior in cure rate (95.3%) to single cycles (79.4%) on facial BCCs in non-OTR [15]. The recommended cryotherapy technique consists of repeated freeze–thaw cycles with 3- to 5-mm margins.

Cosmetic Outcome

In the general population, the cosmetic outcome post cryotherapy for BCCs has been qualitatively stated as "good" in most reports in the literature [8]. In comparison studies, the cosmetic outcome for cryotherapy has been found to be no different to radiotherapy [12], inferior to surgical excision [10], and significantly inferior to photodynamic therapy [11].

C&ED is probably best avoided in cosmetically important areas such as the face, given its potentially unpredictable outcome [5].

Other Considerations

C&ED is particularly useful in treating BCCs on the legs of older patients, where excision would require skin graft repair.

In cases of recurrent BCCs, the preferred treatment is Mohs' surgery as further cryotherapy or C&ED leads to low response rates.

Squamous Cell Carcinoma

Lesion Selection

Two international groups have published guidelines regarding the treatment of SCCs in the transplant population and have recommended C&ED as an acceptable modality in "less aggressive SCCs" [16, 17]. Cryotherapy to −50°C, either alone or

in combination with C&ED, is also considered appropriate therapy for this select group of SCCs.

The characteristics of "less aggressive SCCs" include the following:

- Size of less than 0.6 cm diameter for locations in the "mask" areas of the face (central face, eyelids, eyebrows, periorbital, nose, lips, chin, mandible, preauricular and postauricular areas, temple, ear), genitalia, hands, and feet;
- Size less than 1.0 cm over the cheeks, forehead, neck, and scalp;
- Size less than 2.0 cm over the trunk and extremities;
- Static or slowly growing tumours;
- Nonulcerated tumours;
- Distinct, well-defined clinical margins;
- Lack of satellite lesions;
- Histology showing in situ tumour, keratoacanthoma type, well-differentiated tumour, invasion limited to papillary dermis, absence of neurotropism, and absence of perivascular or intravascular invasion.

Efficacy

The cure rates of SCCs with cryotherapy in non-OTRs have been reported consistently to be greater than 95% [9]. The exceptions are SCCs in the head and neck region, especially lips, ears, periocular regions, and the scalp, where even with strict selection criteria some recurrences are observed. In fact, an Australian retrospective study on deaths from SCCs found that 76.5% originated from the head and neck [18]. As a result, although the SCC guidelines for OTRs have recommended cryotherapy or C&ED as an acceptable modality, the authors have some reservations in recommending non-margin-controlled treatment for any head and neck tumour, particularly SCCs.

In the general population, controversy remains over the efficacy of C&ED in SCCs. The cure rates have been reported to be more than 96% for lesions of all sizes, and up to 100% in smaller lesions less than 2 cm in diameter, at 5 years [4]. However, the available data are primarily based on uncontrolled studies.

Specific data regarding OTRs are scarce in the literature. The first published series examining the specific efficacy of C&ED in SCCs of OTRs included retrospective analysis of 211 SCCs in 48 OTRs treated with C&ED. Recurrence was observed in 13 of the 211 SCCs (6%) and 10 of the 48 patients. The majority of recurrences occurred within 12 weeks of the procedure, and all were within 40 weeks, which seems to suggest that these were residual SCCs. The recurrence rate was not associated with gender, age, type of transplantation, or follow-up time, but was associated with the number of SCCs treated ($P = 0.01$). Such results are encouraging, especially given an adequate follow-up period (mean, 50 months). Importantly, the SCCs selected were of the low-risk category, that is, less than 2 cm in diameter, present for less than 3 months, and did not appear to infiltrate

into deeper tissues [19]. An early case report also described successful treatment of multiple SCCs by C&ED in a transplant recipient [20].

Procedure

For cryotherapy, histological confirmation to analyse for high-risk features is essential before the procedure. The recommended technique is repeated freeze–thaw cycles with 5-mm margins.

Cosmetic Outcome

No specific data have been published for cosmetic outcome following cryotherapy or C&ED of SCC in the general or transplant populations. One would not expect different outcomes to those reported for BCC.

Other Considerations

Residual or recurrent SCCs are recommended for excision as repeat cryotherapy leads to low response rates.

There are suggestions that SCCs of the scalp in the transplant setting constitute a special high-risk group. In one case where two SCCs of scalp in a lung transplant recipient were treated with C&ED, eight cutaneous metastases (biopsy proven) subsequently developed 7–11 months later on the scalp, with the patient eventually dying 38 months after the initial procedure. In the same report, a renal transplant recipient whose SCC of the scalp had initially been treated with curettage became recurrent and subsequently developed cervical lymph node metastasis [21].

Bowen's Disease

Lesion Selection

Cryotherapy has been recommended in an expert opinion article as appropriate first-line therapy for Bowen's disease (BD) in the OTR [7]. Specific studies are not available in the transplant population. Body sites seem not to affect the cure rates of BD by cryotherapy. The size of lesions also does not seem to adversely affect the outcome, and treatment may indeed be undertaken with overlapping fields.

In the authors' experience, the restrictions on C&ED for BD are primarily due to anatomic locations. The thin skin of the eyelids, lips, and genital areas precludes its use as the curette is likely to penetrate the dermis to deeper tissues. As with SCCs, BD of the scalp may be associated with occult deep follicular involvement and

therefore is probably best not treated with superficial methods such as cryotherapy or CE&D. A recognized problem for cryotherapy in BD is slow healing, especially for lesion larger than 20 mm in diameter and those on the lower legs.

Procedure

Histological confirmation of BD is recommended before treatment. BD can be treated effectively with a single freeze–thaw cycle of cryotherapy. A freeze time of 30 s with a 3-mm margin is advised.

The optimal number of treatment cycles for BD with C&ED is not well defined in the literature.

Cure Rates

The reported cure rates of BD by cryotherapy in the general population vary widely in the literature, ranging from 66% to 97% depending on treatment protocols [9]. A recent clinical trial compared Metvix photodynamic therapy (PDT), cryotherapy, and 5-fluorouracil topically in treating BD. For the cryotherapy arm, the lesion clearance rate was 86%, with a recurrence rate at 12 months of 21% overall, and 35% over the face/scalp regions. Such figures suggested a relatively modest efficacy of cryotherapy in BD only, compared to previous reports [22].

Information on the efficacy of C&ED in BD in the literature consists essentially of retrospective series in non-OTRs. Cure rates of 80% up to 98% have been reported, although follow-up duration was generally inadequate or not stated [23, 24].

Cosmetic Outcome

In a trial in non-OTRs comparing PDT with cryotherapy and 5-fluorouracil for Bowen's disease, cosmetic outcome for the cryotherapy arm was "good" or "excellent" in 66% at 3 months, maintaining that level of response at 62% at 12 months [22].

Special Considerations

Care needs to be taken when selecting locally destructive methods for the treatment of Bowen's disease on certain sites. In particular, the legs can be very slow to heal using these modalities, especially in patients with peripheral vascular disease or diabetes. Recurrence rates following cryotherapy on the scalp is higher than usually accepted, presumably a result of follicular extension of the neoplasm.

Actinic Keratosis

In the transplant setting, an aggressive approach to managing actinic keratoses is advocated to prevent progression into invasive SCCs [25].

Lesion Selection

Both cryotherapy and C&ED have been advocated as first-line therapy for actinic keratoses in the OTR by a number of expert opinion papers [7, 26, 27].

In contrast with BCCs and SCCs, at least in the general population, there appeared to be no significant differences anatomically in the cure rates of actinic keratoses (AKs) using cryotherapy or C&ED, for example, comparing those of the facial regions with that of the upper limbs [28, 29].

Hyperkeratotic and hypertrophic AKs or those suspected of increased atypia may better be treated with C&ED, so that an histological specimen could be obtained to rule out SCC.

Procedure

The required freeze time for AKs is generally proportional to the palpable depth of the lesions and is usually recommended to be 5–15 s. Thicker lesions may require double freeze–thaw cycles.

Cure Rates

With careful clinical diagnosis, single freeze cycle cryotherapy has been reported to achieve a cure rate of as much as 98.8% in the general population [28]. Lower response rates for cryotherapy have been found in comparator studies [2, 3, 30]. Thicker lesions tend not to respond as well to cryotherapy. Little information is available on the efficacy of C&ED, although it is often used to treat the thicker AKs resistant to cryotherapy.

In the transplant population, the recurrence of AKs has been commented to be “common” (exact rate not stated) and generally occurring a few months after destructive therapies [31].

Cosmetic Outcome

Cosmetic outcome following cryotherapy is highly variable and is both operator- and patient dependent. In two studies comparing MAL PDT with cryotherapy for AKs in non-OTRs, cosmetic outcome was “excellent” in 51% of patients following

a single freeze–thaw cycle of cryotherapy in one study [2] and "good" or "excellent" in 81% following double freeze–thaw cryotherapy in the other [30].

Other Considerations

Any AK lesions that persist following destructive therapy should be biopsied to rule out SCC [32]. A recent case report highlighted such an instance: A biopsy-proven AK of the scalp in a renal transplant recipient was treated initially with curettage alone, but the area failed to heal. Subsequently, a further biopsy revealed moderately differentiated SCC, necessitating Mohs' micrographic surgical excision [21].

Widespread AKs may be treated with cryotherapy alone, but are probably more efficiently dealt with by field treatments such as PDT and topical 5-fluorouracil. In extensive AKs, 5-fluorouracil may be used as a pre treatment to cryotherapy, applying for 10 days before the procedure to make the lesions more easily visible. Furthermore, photographic mapping may be considered before cryotherapy for future surveillance of recurrence.

Keratoacanthoma

Few data are available for cryotherapy in KAs. A cure rate of 87.5% was reported in a small series of eight KAs in non-OTRs [33]. It is probably best to debulk a suspected KA with curettage or shave excision before cryotherapy, which would provide a specimen for histological confirmation. Cryotherapy with double freeze–thaw cycles of 30 s or more with a 3- to 5-mm margin is recommended.

C&ED of KAs seems to produce acceptable cure rates provided that strict clinical diagnostic criteria are adhered to and that histological examination of the largest, deepest curetted fragment confirms the diagnosis. A series of 111 KAs in non-OTRs found four recurrences 3 to 26 months post C&ED [34], but the reliability of the report is compromised by its short follow-up period. C&ED in KAs is probably best avoided in previously treated lesions, any lesions on the lips or ears, and lesions larger than 1 cm in diameter on other areas of the head.

Close follow-up should be carried out following destructive therapy to move to immediate excision should any recurrence occur.

Approach to Therapies

As evidence for the efficacy in destructive therapies is relatively scarce in the transplant population, the choice of therapies, whether alone or in combination, is rather dependent on the clinician's preference.

One author manages low-risk lesions (actinic keratoses and porokeratoses) in OTRs with cryotherapy, and moderate-risk lesions (well-differentiated SCCs on

trunks and limbs that do not invade past the papillary dermis) with C&ED. High-risk lesions (recurrent SCC, fast growth, larger diameter, location on scalp, ears, and lips, aggressive histology) should be managed with a margin-controlled technique such as surgical excision, Mohs' micrographic surgery, or radiotherapy [35].

In another expert opinion article, a combination approach is taken. Small skin cancers less than 0.5 cm were treated with C&ED three times followed by cryotherapy. For lesions larger than 0.5 cm, complete excision was recommended [27].

Other Destructive Therapies

Although not considered in any depth in this chapter, an expert opinion article has advocated the use of chemical peels in managing larger numbers of actinic keratoses. For lighter peels, alpha-hydroxy acid or beta-hydroxy acid may be used, and for medium peel, trichloroacetic acid is used. For a deeper effect, laser resurfacing with the carbon dioxide laser or dermabrasion may be considered [27].

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Systemic Chemoprevention

Stephen Shumack

Cutaneous neoplasms in organ transplant recipients (OTR) have traditionally been managed by early detection and treatment. Prevention is also a key factor in managing these patients, and to date this has largely been achieved through education, sun avoidance measures, and early detection with regular clinical reviews. Lately, systemic chemoprevention has emerged as an important modality for treatment whereby clinicians can hope to reduce and/or delay the development of cutaneous neoplasms in these patients.

However, the framework within which we make the decisions concerning systemic chemoprevention in OTR is still in its infancy, which is not surprising given that this particular area of transplant medicine is relatively new. Fortunately, this is now an area where more research is being undertaken, and therefore we should be able to develop clear guidelines in the years to come. Currently, oral retinoids are empirically the most commonly used systemic agent for the prevention of skin cancers in OTR, and they are increasingly perceived as being useful in this regard [1]. Sirolimus is another agent that is under investigation for such use.

Oral Retinoids

The mechanism of action of retinoids (acitretin, isotretinoin) in the context of systemic chemoprevention for OTR remains under investigation. Retinoids alter gene transcription through their action on the cellular retinoid receptors. The established effects of retinoids include immunomodulation, antikeratinization, induction of apoptosis, and antiproliferation [2]. As early as 1988, there was a reported beneficial effect of etretinate in the management of malignant cutaneous neoplasms in six renal transplant recipients in an open-label, uncontrolled study [3]. Acitretin, an oral retinoid that is an active metabolite of etretinate, has replaced the use of the latter. A number of small uncontrolled studies suggest that acitretin may be also effective in the management of cutaneous neoplasms in OTR. Data regarding the

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benefits and risks of using oral retinoids in OTR are few. Chen et al. reviewed the medical literature from randomised controlled trials on the use of oral retinoids as a preventive agent for skin cancers in OTR [4]. They found only three trials that were suitable for full review [5–7]; all three involved the use of acitretin.

The main outcome of interest for acitretin usage is the effect it has on the number of skin cancers: squamous cell carcinomas (SCC), basal cell carcinomas (BCC), and melanomas. Other outcomes of interest include a reduction of premalignant lesions such as actinic keratoses and a reduction in number of skin biopsies and excisions needed. Adverse outcomes include cheilitis, asteotosis, alopecia, paronychia, nocturnal visual impairment, myalgia, hypercholesterolaemia, liver impairment, and bony abnormalities. There is no published evidence regarding retinoids producing an adverse outcome so far as the transplanted organ is concerned in these patients.

The trial by George et al. was a cross-over study [5], whereas those by Bouwes Bavinck et al. and de Sevaux. et al were parallel in design [6, 7]. Two trials were open label [5, 7], while one involved blinding of investigators, participants, and outcome assessors. Blinding is not an easy process for studies involving oral retinoids because of the occurrence of cheilitis and dryness in the treatment group. All three trials assessed outcome measures and adverse events in a similar way. Skin cancer counts were assessed clinically, with suspicious lesions biopsied and histopathologically confirmed. Adverse events were determined by clinical history and laboratory tests (renal function, liver function). Radiologic tests (spinal, hip, and ankle radiographs) were also performed in two of the trials [5, 7].

Trial participants were adult Caucasian renal transplant patients and were, on average, 10 to 15 years post transplantation. All three trials had different inclusion criteria. One trial included those with 3 or more SCC/BCCs in the past 5 years or 10 or more actinic keratoses at the time of entry into the trial [5]. One included those with at least 10 keratotic lesions on hands and forearms [6], while the remaining trial included any renal transplant recipient in a stable condition [7]. Similar exclusion criteria were used in all three trials (pregnancy, hyperlipidaemia, impaired renal or liver function, increased alcohol intake).

Protocols for treatment and follow-up were different in all three trials. Patients were treated with 30 mg acitretin or placebo daily for 6 months by Bouwes Bavinck et al., followed by 6 months of follow-up [6]. De Sevaux et al. divided the subjects into two treatment groups: One group was treated with acitretin 0.4 mg/kg/day for 12 months, while the other was treated with 0.4 mg/kg/day for the first 3 months followed by a lower dose of 0.2 mg/kg/day for the remaining 9 months. Both groups had a subsequent follow-up period of 12 months [7]. In the study by George et al., subjects were treated with acitretin 25 mg daily or placebo at the start [5]. The dose was then adjusted according to the occurrence or severity of adverse events, being increased in some to 50 mg daily, while decreased to 25 mg on alternate days in others. Treatment period was for 12 months and then crossed over. The participants were followed up for 2 years.

There was a significant reduction in the number of new skin cancers developing in the two trials comparing acitretin with placebo [5, 6]. In the study reported by Bouwes Bavinck et al., 9 of 19 subjects developed 18 new skin cancers (15 SCCs,

1 Bowen's disease, 2 BCCs) as opposed to 2 of 19 subjects developing 2 SCCs in the treatment group [6]. The difference between both groups was significant both in terms of number of subjects developing skin cancers and in terms of number of new lesions developing. George et al. reported 46 SCCs developing in 6 subjects during the period of acitretin treatment, compared to 65 SCCs developing in 15 subjects during the drug-free period. This difference in the number of SCCs developing was significant. The development of BCC was also reduced significantly in this study. The dose comparison trial reported by de Sevaux et al. found no significant differences in the numbers of malignant lesions developing between the groups with differing dose regimens [7]. However, no more details were provided on the actual number of neoplasms developing in both groups. Statistical analyses were also not provided. The occurrence of premalignant lesions was reportedly reduced in the two in which this was considered [6, 7]. Bouwes Bavinck et al. reported a 13.4% decline of keratotic lesions in the acitretin group compared to an increase of 28.2% in the placebo group. The other study did not provide any numbers for analysis [7].

The findings suggest that acitretin may be beneficial in some OTR in the prevention of both malignant and premalignant skin lesions. However, caution has to be exercised before coming to any conclusions because the number of trials evaluated is small and they have their limitations. The sample size of each trial was also small, ranging from 23 to 44 subjects. Given that blinding is difficult and allocation concealment may not have been able to be fully implemented in these studies, the possibility of ascertainment bias during clinical assessment of primary outcome cannot be excluded.

In addition, the reduction in the number of skin cancers appears modest. However, the short treatment and follow-up periods in these trials may have led to an underestimation of treatment effect. It is possible that longer treatment periods may lead to a more clinically meaningful and sustained reduction in skin cancers. The suggestion that acitretin can be part of a long-term management plan of skin cancer in OTR can be inferred from a clinical impression in the study by Bouwes Bavinck et al. [6] that a "rebound" effect on the development of skin cancers was present when acitretin was ceased. This has been confirmed in a 16 year retrospective study of low dose retinoids in the prevention of SCCs in OTR where the SCCs were significantly reduced for 3 years after treatment commenced, and where this trend persisted for up to 8 years [8].

Risk–benefit profiles of different patient populations and doses used can be further clarified in future trials. At present, it seems that acitretin is more beneficial in OTR with a history of skin cancer, as has been reported by Bouwes Bavinck et al. in their subgroup analysis [6]. In addition, the study by de Sevaux et al. suggests no difference in the doses examined (0.4 mg/kg/day vs. 0.2 mg/kg/day) [7].

Adverse effects were the cause of withdrawal of a relatively large proportion of subjects treated with acitretin. Four of 19 subjects treated with acitretin withdrew from the study reported by Bouwes Bavinck et al. [6]; 2 developed a rash, 1 had hyperlipidaemia, and the fourth withdrew due to dysphagia from stomach cancer. George et al. reported 9 of 23 subjects withdrawing because of adverse events: 3 had headaches, 2 musculoskeletal complaints, and the remaining 4 had

hyperlipidaemia, gastritis, paronychia, and a twofold increase in serum liver enzymes, respectively [5]. In the study by de Sevaux et al., 1 subject from the high-dose group withdrew due to severe headache, and 1 subject in the low-dose group withdrew due to severe mucocutaneous side effects [7]. Cheilitis was the most common symptom reported by between 70% and 100% of subjects who remained in the trials. Other frequently reported but tolerable symptoms included alopecia (44%–47%), headache (40%), myalgia (20%–35%), rash (30%), photosensitivity (30%), dry eyes (30%), palmoplantar desquamation (20%), epistaxis (20%), nail changes (15%), and pruritus (10%). All these symptoms resolved after cessation of therapy. Hyperlipidaemia occurred in at least 4 subjects in all three trials, leading to the withdrawal of 2 subjects from the studies. There was no observable deterioration of renal or liver function in any of the trials. Radiologic changes were also absent in the two trials in which these were monitored. Unexpected adverse events were reported by de Sevaux et al., with 3 acitretin-treated female subjects developing recurrent urinary tract infections (UTI) during the study. All three subjects had no prior history of recurrent UTI, and it was presumed to be the result of thinning of the urethral mucosa by acitretin. Two subjects developed an unexplained anemia during the study, which resolved spontaneously at the end of the trial.

Recently, solid OTR with nonmelanoma skin cancers (NMSC) has been listed as one of the specific indications for oral retinoid treatment [9]. The initiation of treatment through a gradual dose escalation to an effective dose was recommended to allow time for patients to become used to the mucocutaneous adverse effects. In a separate survey of 28 dermatologists, it was found that maintenance doses for acitretin ranged between 10 and 50 mg daily, with 25 mg daily being the most frequently used dosage [10]. More than two-thirds of dermatologists surveyed would use oral retinoids for OTR with five or more low-risk SCC, two or more high-risk SCC, or those with in transit, nodal, or systemic SCC. At least a third of dermatologists surveyed would also use oral retinoids in OTR with extensive actinic keratosis (AK), two to five low-risk SCC, or one high-risk SCC.

Sirolimus

Sirolimus (SRL) is a relatively new immunosuppressant. It is increasingly used in OTR as part of the antirejection regimen. It mainly acts by blockade of the multifunctional serine-threonine kinase-mammalian target of rapamycin (mTOR) to cause immunosuppression. It was also found to exhibit antiproliferative effects on a majority of rapidly dividing cells [11]. Furthermore, it dampens transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) signal transduction, which is the likely reason for inhibiting metastatic progression. Hence, it appears that SRL may have an antineoplastic effect that is independent of its immunosuppressive properties. In a review undertaken by Matthew et al., it was found that there was a significantly lower incidence of skin cancer at the 2 years post renal transplantation point in patients treated with SRL in continuous

combination with cyclosporine (CsA) compared to those on placebo [12]. An evaluation of 1,008 renal transplant patients on sirolimus therapy over a 10-year timeframe found that the incidence of skin cancers was 1.9% [13], significantly less than the 7% reported among other renal transplant patient cohorts. The results so far are therefore encouraging, and they certainly validate further efforts to better understand the chemopreventative potential of SRL. Clinical trials investigating the long-term conversion to SRL in patients at high risk of skin cancer are underway.

Beta-Carotene

Beta-carotene is another systemic agent that has been studied for its effects in the prevention of NMSC, and it could potentially be used in OTR. However, studies shows that beta-carotene does not affect the development of, or the rates of, NMSC in community-based populations [14, 15].

Summary

In summary, OTR provide a clinical model for the use of a chemopreventive against skin cancer development. These patients develop skin cancers more rapidly and in far greater numbers than the general community, and therefore chemopreventative agents and sun protective measures are vitally important strategies to improve their morbidity and mortality.

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Management of Metastatic Skin Cancers in Organ Transplant Recipients

Steve Nicholson

The Scope of the Problem

Metastatic skin cancer in the organ transplant population cannot be regarded as a common entity.

The experience of the Royal London Hospital (C.A. Harwood and C.M. Proby, personal communication) is useful to quantify the problem.

Of 1000 organ transplant recipients (OTRs) under surveillance between 1989 and 2007, 22 patients developed confirmed metastatic disease from skin cancer (excluding individuals with Kaposi's sarcoma), namely:

Squamous cell carcinoma = 16 (including 3 of vulval origin)
Melanoma = 3
Merkel cell cancer = 1
Eccrine porocarcinoma = 2

There were 12 deaths resulting from metastatic disease (or approximately 1% of the cohort).

It is this rarity that makes the formulation of evidence-based guidance so difficult.

General Principles

Changes in Immunosuppression

It is often said, rightly or wrongly, that metastatic disease is itself immunosuppressive. Although this is used (rather glibly) to explain intercurrent infection and general debility, there is laboratory evidence of impaired cellular immune responses in patients with metastatic cancer [1–3], and this may bolster a decision to reduce immunosuppression.

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Use of Steroids

Corticosteroids are used in patients with metastatic disease for a variety of reasons, but most commonly:

a. As part of the anti-emetic regimen for those receiving cytotoxic chemotherapy;
b. To improve symptoms, including pain, anorexia, and nausea;
c. As immediate treatment for three oncological emergencies (spinal cord compression, superior vena cava obstruction, and symptomatic brain metastases).

The doses, although often intermittent, are generally rather higher than those used as part of an immunosuppressive regimen, and this may allow a corresponding reduction in the doses of the other components of such a regimen.

Surgery

The involvement of lymph nodes at the draining nodal basin (AJCC stage III in melanoma terms) is not metastatic disease in the same sense as distant dissemination, but it does illustrate the importance of surgery in locoregional control. Surgical management of distant metastatic disease still has a role to play in the following.

a. Management of spinal cord compression: Given the limited radiosensitivity of some skin tumours, decompressive surgery should always be considered as both the quickest and potentially the most effective strategy [4].
b. Metastatectomy for palliation: notably where distant cutaneous or subcutaneous metastases are causing pain or are at risk of bleeding.
c. Metastatectomy for disease control: The presence of a solitary brain metastasis should always prompt the discussion of the appropriateness of craniotomy. This decision will depend on the extent of extracranial metastatic disease (and its response to treatment) and the fitness of the patient. Metastatectomy elsewhere usually depends on the ability of the surgeon to obtain macroscopic clearance, liver, bone, and lung being typical sites (see Melanoma, below).

Skin-Directed Therapy

Disseminated disease may still be amenable to skin-directed therapy, either in combination with systemic therapy or as the sole modality where metastases are restricted to the skin. Approaches include the use of Psoralen plus Ultraviolet A (PUVA) therapy in cutaneous T-cell lymphoma, topical therapy for Kaposi's sarcoma (KS), and intralesional treatment for melanoma, basal cell carcinoma, or KS.

Radiotherapy

Radiotherapy has a particular role in the management of three oncological emergencies, namely spinal cord compression, superior vena cava obstruction, and symptomatic brain metastases. It also has value in the management of painful metastases (particularly to bone) and where skin or subcutaneous deposits are unresectable and in danger of bleeding.

Drug Therapy

The guiding principle of drug therapy for metastatic skin cancer in OTR is to balance the potential antitumour activity of the treatment with the potential for harm, both to the graft and to the patient. The pattern may be exacerbated in some circumstances either by increased toxicity as a result of subnormal function of the graft organ or by compounding the toxicity of the antineoplastic drug with that of the immunosuppressants.

The details relating to specific tumours (below) illustrate that the strategy that is emerging as first-line therapy in the majority of cases will be a reduction in calcineurin inhibitors coupled with the introduction of mTOR inhibitors at either transplant-preserving or antineoplastic levels [5].

Retinoids continue to have a role in a variety of tumours, and their use is to be recommended ahead of the use of cytotoxic chemotherapy [6].

Chemotherapy itself is inevitably limited by both graft function and immunosuppressive medication. Examples given below are for guidance only.

Palliative Care

The involvement of specialist palliative care clinicians and nurses is desirable once metastatic disease has been diagnosed, and it should not be left until the patient has deteriorated to a preterminal condition. Palliative care involves dealing with the psychological needs of patients and their families, as well as symptom control issues, and the former are best addressed when there is time in hand. Palliative care should be regarded as an integral part of the management of patients with metastatic skin cancer, not an alternative to it [7].

Clinical Trials

Patients with metastatic disease should be offered entry into clinical trials where possible. The absence of trials specific to some skin cancers should not deter clinicians from offering patients with rarer diseases referral to a phase I unit, as most phase I trials are not tumour specific. Clinical trials are preferable to *ad hoc*

treatment as they have been shown to benefit patients even where the trial treatment is not better than standard management, probably because of the more systematised care, including regimented follow-up and prearranged investigations [8].

Management Issues with Specific Cancers

Squamous Cell Carcinoma

The magnitude of the problem of cutaneous squamous cell carcinoma (SCC) in OTR is such that investigators have drawn on experience in other tumours to develop some guide to the management of metastatic disease [9].

Changes in Immunosuppression

There is mounting evidence of the importance of mTOR inhibitors as the first line of management when SCC develops in OTR. This includes use of rapamycin itself (sirolimus, as it is now formally known) and its derivatives temsirolimus (formerly CCI-779) and everolimus [10, 11]. It is likely that when future patients present with metastatic disease they will already have had their immunosuppressives changed to include an mTOR inhibitor, and a question that could usefully be addressed in prospective clinical trials is whether a change from an immunosuppressive dose to a "cytotoxic" dose should be the first manoeuvre in this situation.

Drug Therapy

Retinoids have a track record in both secondary prevention of new skin SCCs (in OTR) and as part of combination treatment for locally advanced and metastatic SCC in the immunocompetent population [6, 12–14]. There is good reason, therefore, to believe that retinoids may have value in the treatment of metastatic disease, probably dosed as high as patient tolerance will allow. Information on the combining of mTOR inhibitors with retinoids is lacking in the literature, but this, too, would be a logical strategy in patients with advanced disease.

Retinoids have been used in some of the better-documented trials of combination therapy for advanced squamous cell carcinoma. Oral retinoid plus daily interferon-α produced a response rate of 25% in 8 patients with metastatic disease [12], while 13-*cis* RA, thrice-weekly interferon-α, and weekly cisplatin chemotherapy had a response rate of 17% among 23 patients with metastatic disease [13]. These are well-conducted studies, but the inclusion of interferon in the regimen limits their utility in OTR.

Conventional chemotherapy regimens that may be called upon are listed below, together with their limitations in OTRs. There are no direct data on any of these regimens in OTRs.

Published regimen	References	Limitations and attributes
Cisplatin + fluorouracil (5-FU)	[15]	Nephrotoxicity and potential cardiotoxicity
Cisplatin + 5-FU + bleomycin	[16]	Nephrotoxic, potential cardiotoxicity, and pulmonary toxicity
Cisplatin + doxorubicin	[17]	Nephrotoxicity and cardiotoxicity; doxorubicin is excreted hepatically
Taxol, ifosfamide, carboplatin	[18]	Nephrotoxic and given with high fluid loads
Oral 5-FU (capecitabine or tegafur would be more contemporary options)	[19]	Potential cardiotoxicity

Basal Cell Carcinoma

The concept of metastatic basal cell carcinoma (BCC) does not sit well with our understanding of the indolent nature of this tumour, but the increased frequency of BCC in OTR coupled with the potential acceleration of the disease under immunosuppressive conditions makes this a real possibility.

Where metastatic disease has occurred in the immunocompetent population, there is a body of evidence that platinum-based chemotherapy (typically cisplatin with doxorubicin) is highly effective [17, 20–22]. Unfortunately, use of such a regimen in OTR is likely to be difficult. Interferons are also active against BCC [23] and equally undesirable in OTR. The option of conversion to mTOR inhibitors therefore needs to be considered, and there is some evidence in favour of this strategy [24].

Kaposi's Sarcoma

There is rather more of an evidence base for the treatment for immunosuppressed patients with metastatic Kaposi's sarcoma (KS) than exists for other metastatic skin cancers, although it may be argued that the problems experienced by patients with human immunodeficiency virus (HIV) infection do not directly relate to those seen in organ transplant recipients.

Changes in Immunosuppression

It is clear that a change in immunosuppression to sirolimus or one of its newer derivatives should be the first line of management for metastatic KS in a transplant patient [25, 26].

Skin-Directed Therapy

There is good evidence of the modest efficacy of topical retinoids in HIV-infected populations who have multiple lesions, and this is one approach for patients who have metastatic disease that is confined to the skin. The overall response rate is between 35% and 50% [27, 28].

Another approach to the management of cutaneous and oral cavity dissemination is intralesional therapy. Intralesional interferon-α should be given weekly for 6 weeks at a dose of 1 M IU/lesion (probably to no more than five lesions at a time) [29]. Intralesional vinblastine repeated every 4 weeks is associated with complete remissions in up to 75% of patients [30], and bleomycin, vincristine, and liposomal doxorubicin have all been used intralesionally [31, 32].

Both radiotherapy and photodynamic therapy may have a role in the palliation of metastatic skin KS, but the ideal schedule for either of these modalities has not been established.

Drug Therapy

Where systemic metastases develop, systemic therapy is likely, eventually, to be required.

A useful alternative to chemotherapy is thalidomide, which has a response rate of about 40% [33, 34]. Data on its combination with immunosuppression are not available.

Liposomal anthracyclines have supplanted bleomycin-vincristine combinations on the basis of randomised trials showing higher response rates and lower toxicity [35, 36]. Liposomal doxorubicin 20 mg/m^2 given fortnightly will produce objective responses in 45% to 60% of patients, with bone marrow suppression and palmar-plantar erythrodysaesthesia being side effects that commonly cause problems. Cardiotoxicity is not a significant feature of the liposomal preparations, so these should be safe in cardiac transplant patients, and renal function is not an issue. Anthracyclines are excreted by the liver, and their use in liver transplant recipients may be best avoided.

Etoposide is given orally, 50 mg bid for days 1–3 (increasing to days 1–5 according to response and tolerance), repeated every 21 days. Its response rate is in excess of 70% [37, 38]. Docetaxel is given intravenously at a dose of 25 mg/m^2 weekly for 8 weeks, followed by fortnightly administrations in responders (42% when used as second-line therapy) [39]. There are no reports of either of these drugs being used to treat KS in OTRs, but graft function is unlikely to be compromised.

Malignant Melanoma

The therapeutic choices available for patients with metastatic melanoma are limited, regardless of immune status. The goal for stage IV therapy is primarily palliative and focuses on regression of the tumour masses with an improvement in symptoms.

Changes in Immunosuppression

Sirolimus inhibits metastatic tumour growth in mouse models of malignant melanoma at doses used for immunosuppression [40]. Sirolimus and the newer mTOR inhibitors therefore have the unusual feature of being studied as both antirejection and antimelanoma drugs. Their use in metastatic melanoma has identified disease stabilisation as the best response [41, 42], but this is the same "signal" of potential activity that has led to the studies involving sorafenib (see below). It is possible, therefore, that combination of mTOR inhibitors with chemotherapy or with other new agents may yield more valuable responses [43].

Surgical Management

There are several scenarios where metastatic disease may be amenable to surgical control. The data on these clinical indications are derived from the immunocompetent population, but there is good reason to assume that metastatectomy may have a role in OTR, particularly where immunosuppression is being altered concurrently.

i. Resectable central nervous system (CNS) disease: There is good evidence that patients with solitary brain metastases should undergo resection (without postoperative radiotherapy) where possible [44, 45]. This selection will usually apply to patients who have only CNS disease, but may occasionally include patients with extracranial metastases who have good performance status.
ii. Resectable gastrointestinal (GI) metastases: There is an apparent survival advantage for resection of GI disease (including hepatic metastases) where surgery leads to complete remission. Median survival for this highly selected group of patients is of the order of 12 months, with up to 35% surviving to 5 years [46–48]. Referral to a surgeon with experience and an interest in metastatectomy is essential.
iii. Resectable pulmonary metastases: A favourable outcome from pulmonary metastatectomy is associated with complete surgical resection, a longer disease-free interval, and a tumour doubling time of more than 60 days [49].

Radiotherapy

Melanoma is a relatively radioresistant tumour, but radiotherapy has an established role in the immunocompetent patient in certain clinical scenarios:

a. Palliation of painful, fungating, or bleeding deposits
b. Definitive management of spinal cord compression when surgical decompression is not appropriate, or as "adjuvant" or a second stage of treatment after decompressive laminectomy.
c. Intracranial disease, where metastatectomy is not thought to be appropriate, may be treated by whole-brain radiotherapy [50], by stereotactic "radiosurgery" (also called "gamma knife" at some centres, and not a surgical procedure as such), or by a combination of the two. Stereotactic radiotherapy delivers high-dose-rate

radiation to a limited target volume (usually in a single session) and is generally recommended for metastases that are less than 3 cm in diameter. Precise guidelines vary among institutions [51, 52].

Drug Therapy 1. Chemotherapy

The reference drug by which all treatment for malignant melanoma is judged is dacarbazine, a tetrazine alkylating agent that is administered intravenously and which produces measurable reduction in melanoma burden in about 20% of the immunocompetent population. Many clinicians would probably elect to use temozolomide, which is metabolised to the same active moiety as dacarbazine, but which is an oral preparation that has the added advantage of crossing the blood–brain barrier [53–55]. There is no evidence base on which to recommend the use of these drugs in OTR, but personal experience suggests that they are safe in such patients. It is important to recognise that treatment is designed, first and foremost, to improve symptoms and that there is no overall survival benefit to the use of chemotherapy.

Drug Therapy 2. Immunotherapy

Immunotherapeutic strategies in the management of melanoma can be divided into cytokine therapy (predominantly interferon-α and interleukin-2) and vaccine therapy. There is little evidence for the use of either in the immunocompromised patient.

Interferon-α at standard doses (3–5 MU subcutaneously thrice-weekly) has a response rate of around 15% in immunocompetent patients with metastatic disease [56]. There are no case series of this treatment being used in OTR. Individual case reports suggest activity is seen, although at the expense of allograft rejection [57].

Interleukin-2 (IL-2) has shown its greatest promise when used in high doses to treat patients with unresectable metastatic disease confined to skin, subcutaneous tissues, and lymph nodes (stage M1a). An overall response rate in excess of 50% is seen in this patient group, although the toxicity of the treatment means that patients are highly selected [58]. The potential for immune-mediated toxicity and the requirement for adequate cardiac, hepatic, and renal function would probably preclude the use of high-dose IL-2 in most OTR.

Vaccine therapy of all types demands the ability of the recipient to mount a cellular immune response, and consequently this is not a logical treatment for OTR. There are no published reports of vaccine-based approaches in the organ transplant population.

Biochemotherapy

The publication of several randomised phase III trials has shown that biochemotherapy (the combination of chemotherapy and cytokines) has no survival benefit when compared to chemotherapy alone [59, 60]. That it has a higher response rate is unde-

niable (together with its greater toxicity), but there are no reports of its use in OTR and there are unlikely, now, to be any formal trials.

Newer Agents

The era of molecular drug development continues to generate new drugs whose role in melanoma has yet to be defined. There are some features of these agents, however, that enable a degree of cautious speculation.

Humanised anti-CTLA4 monoclonal antibodies (MDX-010, ticilumimab, ipilumimab) have shown activity in phase II trials [61], but response seems to be strongly associated with autoimmune side effects ("immune breakout events"). Such an approach would be undesirable in an OTR if retention of the graft were necessary. Where loss of the graft is not an issue, however, it would be of interest to establish whether anti-CTLA4 therapy combined with immunosuppressant withdrawal resulted in enhanced antitumour activity.

Tyrosine kinase inhibitors acting on the B-RAF signalling pathway have produced disease stabilisation in pretreated patients, and one of these drugs, sorafenib, has been studied in combination with chemotherapy in two phase III trials. Reported so far only in abstract form, the combination of sorafenib with dacarbazine as first-line therapy led to improved response rate and progression-free survival [62]. Mature results are needed, but this may be an important step forward. It may be that the combination of sorafenib with an mTOR inhibitor may lead to even more substantial improvement in outcomes. There is no reason to suppose that such a regimen would be tolerated any less well by OTR.

Rare Skin Cancers

Angiosarcoma of the Scalp

This tumour has a poor prognosis even when management of the primary is optimal. It is sensitive to lipsomal doxorubicin [63], and this should probably be the first line of treatment in OTR as the drug is not cardiotoxic and not dependent on renal function. Caution should be exercised in liver transplant recipients, as with any anthracycline, and paclitaxel would be an evidence-based alternative [64].

Dermatofibrosarcoma Protuberans (DFSP)

Reports of distant metastases in DFSP are few: the Milan group reported just 7 such patients (from a population of 218) over 20 years [65]. It certainly appears that isolated distant metastases should always be considered for metastatectomy, and that patients whose tumours express c-*kit* should be offered imatinib in the first instance if there is no surgical option [66, 67].

Eccrine Porocarcinoma

Plunkett et al. reported on the use of Docetaxel in a renal transplant patient who developed eccrine porocarcinoma of the breast. This patient had been on triple-therapy immunosuppression with cyclosporin, azathioprine, and prednisolone. The first therapeutic drug manoeuvre after initial wide local excision was to reduce her immunosuppression to cyclosporin alone, and this maintained her in a disease-free state for 5 months. Response to the Docetaxel used at relapse was also documented [68].

Metastatic eccrine porocarcinoma is a demoralising disease to have to treat, and responses to any of the drugs documented in case reports is far from guaranteed. Most articles describe the use of taxanes or interferon-α, either alone or in combination [69–72]. There have also been some responses to fluoropyrimidines [70, 71], and the author has had one patient respond to capectabine (S. Nicholson, unpublished). Retinoids also may have a role [69, 70, 73]. The author has treated two patients with thalidomide, neither responding. There are no reports of mTOR inhibitors being active in this disease. The combination of an oral fluoropyrimidine and acitretin would certainly have much to commend it in the OTR population, although the evidence base is, as ever, lacking.

Merkel Cell Carcinoma

The literature on the use of chemotherapy in the management of metastatic Merkel cell carcinoma is more than one might have reason to expect, given its rarity. This evidence base does not, unfortunately, extend to treating metastatic disease in OTR. That being said, the wide range of cytotoxic agents with activity against this tumour does enable rational combinations to be identified: personal experience has shown that vincristine plus etoposide* [a regimen validated in small cell lung cancer [74], albeit with higher doses of etoposide] is particularly useful in renal transplant patients, yielding good clinical responses without compromising allograft function and with an acceptable toxicity profile.

A brief list of agents of proven activity in Merkel cell cancer (MCC) is given below, together with some attributes likely to be restrictive in the OTR group. Single agents and combination regimens can be selected based on standard criteria and experience in other tumours.

Cutaneous Lymphoma

A full discussion of the management of the many varieties of primary cutaneous lymphoma is beyond the scope of this chapter and has been reviewed (with guidelines) elsewhere. The concept of transplant-associated lymphoma being largely driven by Epstein–Barr virus in the presence of immunosuppression is widely accepted, as is the need for a reduction or change in immunosuppressive drugs [78, 79]. This approach is unlikely to compromise graft function where the treatment

Individual drugs	Category	Attributes and limitations
Adriamycin (doxorubicin)	Anthracycline	Cardiotoxic; hepatic excretion
Carboplatin	Platinum derivative	Renal excretion; not nephrotoxic in standard doses, but myelotoxicity and neuropathy likely to be exacerbated where there are low clearances
Cisplatin	Platinum derivative	Nephrotoxic
Cyclophosphamide	Alkylating agent	Renal excretion and potentially nephrotoxic
Etoposide	Topoisomerase inhibitor	Neutropenia; well tolerated in transplant population
Vincristine	Vinca alkaloid	Peripheral neuropathy; well tolerated in transplant population
Published regimens in MCC	Drugs	
CAV [75]	Cyclophosphamide, adriamycin, vincristine	Contraindicated in OTR
EP [75, 76]	Etoposide + cisplatin [carboplatin may be substituted [77]]	Contraindicated in renal transplant recipients

*Author's recommended regimen is vincristine 1.4 mg/m^2 iv day 1 (maximum dose, 2 mg), etoposide 100 mg po bid days 1–3 (can be extended to days 1–5 according to response and tolerance) repeated on a 21-day cycle.

of the lymphoma is, itself, immunosuppressive, for example, the CHOP-rituximab regimen for primary cutaneous large B-cell lymphoma of the leg. Such conventional B-cell lymphoma treatment is in stark contrast to standard first-line treatment of advanced cutaneous T-cell lymphoma (CTCL), typically a combination of PUVA and interferon-α [80, 81]. Certainly PUVA plus acitretin would be a preferable first line in such patients, even though it is probably inferior to the interferon-α regimen on the evidence of clinical trials [82, 83]. Bexarotene should certainly be considered on the basis of its activity in the nontransplant CTCL population and a case report of activity in post-transplant T-cell lymphoma [84, 85].

It will come as no surprise that, here as elsewhere, conversion to mTOR inhibitors offers not just a reduction in other immunosuppressive drugs, but also de novo antitumour activity, the magnitude of which has yet to be established [86–88].

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