Immunology in Tumor and Transplant

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Abbreviations

APC	Antigen presenting cell
CTLA4	Cytotoxic T-lymphocyte-associated antigen 4
HLA	Human leukocyte antigen
MHC	Major histocompatibility complex
PD1	Programmed death 1

In this chapter on the immunology of cancer and transplantation, we successively expose the steps and actors of the immune response, the current concepts of immunotherapy in cancer, and the global principles of the immunosuppressive treatment in transplantation.

Immune System and Immune Response

The function of the immune system is to protect the individual, mainly against infections. This function is indispensable to life, needs: to be able to distinguish antigens from the "self" and the "non-self" (so-called alloantigens), and to have many effectors to start a defensive response. This recognition system is very specific; it has to be able to detect and destroy a wide variety of aggressors (bacteria, virus, tumoral cell ...) but also to prevent an immune response against the cells belonging to its own organism [1, 2]. Thus, a transplanted graft will be recognized as not belonging to the "self" and will therefore be considered as an aggression. An immune reaction, called the allogeneic response, will therefore be started against the graft. The purpose of the immunosuppressive therapy is to avoid the rejection of the graft. The same immune response occurs against the cancer cells, but cancer cells are able to escape the immune system and induce tolerance. The new immunotherapies aim to block these escape ways and allow the immune system to target and destroy cancer cells.

The Allogenic Response

The immune response schematically comprises three steps: recognition of alloantigens, activation of effector T cells and destruction of the "non-self" (Fig. 11.1).

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C. R. Chapple et al. (eds.), *Urologic Principles and Practice*, Springer Specialist Surgery Series,
https://doi.org/10.1007/978-3-030-28599-9_11

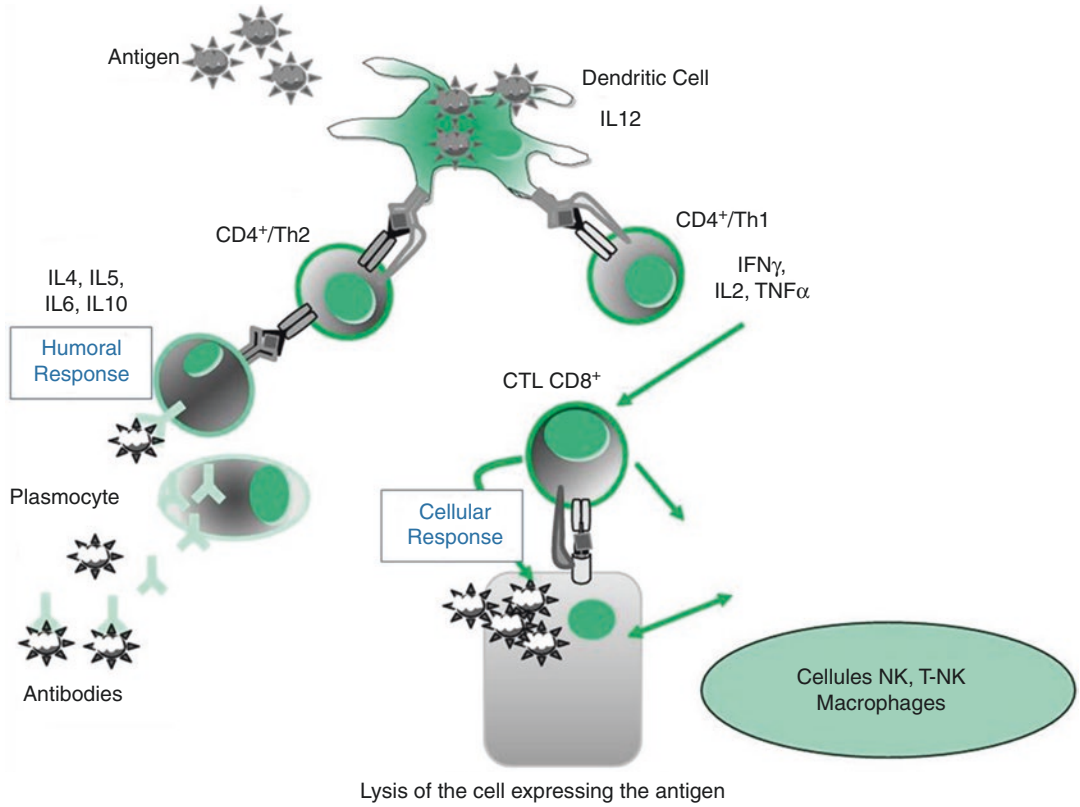

Fig. 11.1 The immune response. The immune response includes: • the alloantigen recognition: the alloantigen is presented by the Major Histocompatibility Complex (MHC) on the surface of the Antigen-Presenting Cell (APC), • the activation of the T lymphocyte, • destruction of the cell expressing the targeted antigen by humoral (antibodies) and/or cellular response (Cytotoxic lymphocyte)

Alloantigen Recognition

The Major Histocompatibility Complex (MHC)

The central element that gives to the immune system the ability to recognize the "non-self", is the Major Histocompatibility Complex (MHC), also called Human Leukocyte Antigen (HLA) system. Its existence and role in the immune response in transplantation was discovered by Jean Dausset who was awarded for this by a Nobel Prize in 1980 [3, 4]. The role of the HLA is to present antigens to the lymphocytes.

The loci coding for the HLA are located on the short arm of the chromosome 6. There are six loci defining two types of HLA: HLA class I which is expressed on the surface of nucleated cells, and HLA class II that is specifically expressed on the surface of Antigen Presenting Cells (APCs). The HLA system has two features. It is codominant, which means each individual expresses the alleles of the two chromosomes for each of the six loci. Thus, the HLA genotype of an individual comprises 12 different HLA molecules: HLA-A, HLA-B and HLA-C coding for the HLA type I, and HLA-DP, HLA-DQ, HLA-DR coding for the HLA type II. HLA is polymorphic, which mean each HLA locus can express many different molecules.

This polymorphism and codominant characters explain the huge variety of HLAs. In transplantation, the major loci involved in the alloimmune response are the HLA-A, HLA-B, HLA-DR and DQ loci. The impact of the HLA-C, HLA-DP loci appears to be lower and they are not taken into account routinely.

Presentation of the Antigen

The two main actors of the allo-antigen recognition are the APCs and the lymphocytes T CD4+. APC expose on their surface the complex antigen + MHC. The lymphocytes T CD4+ screen the APC and detect whether the antigen belongs to the "self" or the "non-self" [5]. The immune response starts with the presentation of an alloantigen to the immune cells. Antigens (from the tumor cell or from the donor in the case of a transplant) are caught by the APCs, mainly the dendritic cells. The dendritic cell binds these antigens to the MHC, exposes the complex MHC-Antigen on their cell surfaces and then migrate to the surrounding lymph node where the lymphocytes are concentrated [1, 2].

Activation of Lymphocytes

The activation of the naive T lymphocyte requires three signals, which are essential for the ongoing of the allogenic response.

First Signal

The first signal arises with the recognition of the antigen (tumor or donor) presented on the MHC on the APCs, by the T Cell Receptor (TCR) of a naive T lymphocyte. The TCR that is expressed on the surface of T lymphocytes, binds to the MHC-antigen complex and induces an intracellular signal that initiates the activation and the proliferation of the antigen-specific lymphocytes [1, 2].

Second Signal

The second signal, also called "co-stimulation signal" consists in reinforcing the link between the lymphocytes and the APC by other surface molecules (CD 40, 154, CD 28 on the lymphocytes, CD 40, CD80, CD86 on the APCs) [6]. This second signal results in the huge synthesis of interleukin-2 (IL-2), the main cytokine involved in the proliferation of lymphocytes, by the lymphocyte itself (Fig. 11.1).

Third Signal

The third signal, also called "proliferative signal" starts with the binding of Interleukin 2 to its receptor (IL-2R), leading to the proliferation of lymphocytes by a clonal expansion and the secretion of other cytokines and chemokines. The binding of IL-2 to its receptor (IL-2R or CD25) results in the activation of the Pi3 K pathway and Akt kinase that activates the mTOR protein involved in mitosis and controls the cell cycle [7]. The activated T cell proliferation will result in the recruitment of immune effector cells, such as T CD8, T NK, B lymphocytess or macrophages which will participate in the immune response (antitumor immunity or graft rejection). The recruitment of leucocytes is triggered by wide range of cytokines. Depending on the type of cytokines that are released, two ways of immune response are activated: the cellular and the humoral immunities. In the cellular immunity profile, Lymphocyte T helper type 1 secrete IL-2, Interferon-g (IFN-g) and tumor necrosis factor (TNF) which lead to the activation and recruitment of cytotoxic T lymphocytes and macrophages [8]. In the humoral immunity profile, Lymphocyte T helper type 2 leads to the secretion of antibodies via the IL-4, IL-5 and IL-10 [7, 8].

Mechanisms of "Non-self" Destruction

Activated T CD4 T are the pivot of the immune response against the "non-self". The activated lymphocyte expresses on its surface molecules of MHC and secretes cytokines, both leading to the recruitment of other immune cells targeting the "non-self" antigen. The activated CD4 T lymphocyte stimulates the proliferation of cytotoxic lymphocyte T CD8 and lymphocyte B. Lymphocytes T CD8 have a direct cytotoxic action on the cell expressing the targeted antigen, by a cytotoxic secretion of perforin and granzyme. T CD8 cells also promote the over-expression of Fas-L (Fas ligand) which induces apoptosis. On the other side, lymphocytes B are also activated by the lymphocyte T CD4 and get differentiated into plasmocytes secreting high affinity antigen antibodies. The accumulation of antibodies on the surface on the targeted cells lead to the activation of the complement and their destruction. Some of

these B-cells, called memory B cells, have a long shelf life and are also able of an immediate proliferation and a permanent secretion of anti-HLA antibodies [1, 2].

Tumor Immunology

Every day in our body, some cells manage to escape the mechanisms of apoptosis and engage in malignant differentiation. The immune system is able to detect and eliminate the cells engaged in the process of malignant differentiation before they multiply and evolve into a tumor. Tumor cells however can develop "escape ways" that are the targets of new immunotherapies.

The Immune Response in Cancer

As any other cell, the tumor cell constantly releases antigens into its environment. In the same way as it was previously described, these tumor antigens are captured by APCs dendritic cells. After capturing these tumor antigens, the dendritic cells expose the antigen on their membrane and migrate to the surrounding lymph nodes where stand the T lymphocytes. The activation signal between the dendritic cell and the T lymphocyte specific for the antigen is mediated by two surface molecules: B7 and CD28 (Fig. 11.2). Once activated, the lymphocytes proliferate, migrate to the tumor and release the antibodies that will lyse the tumor cells.

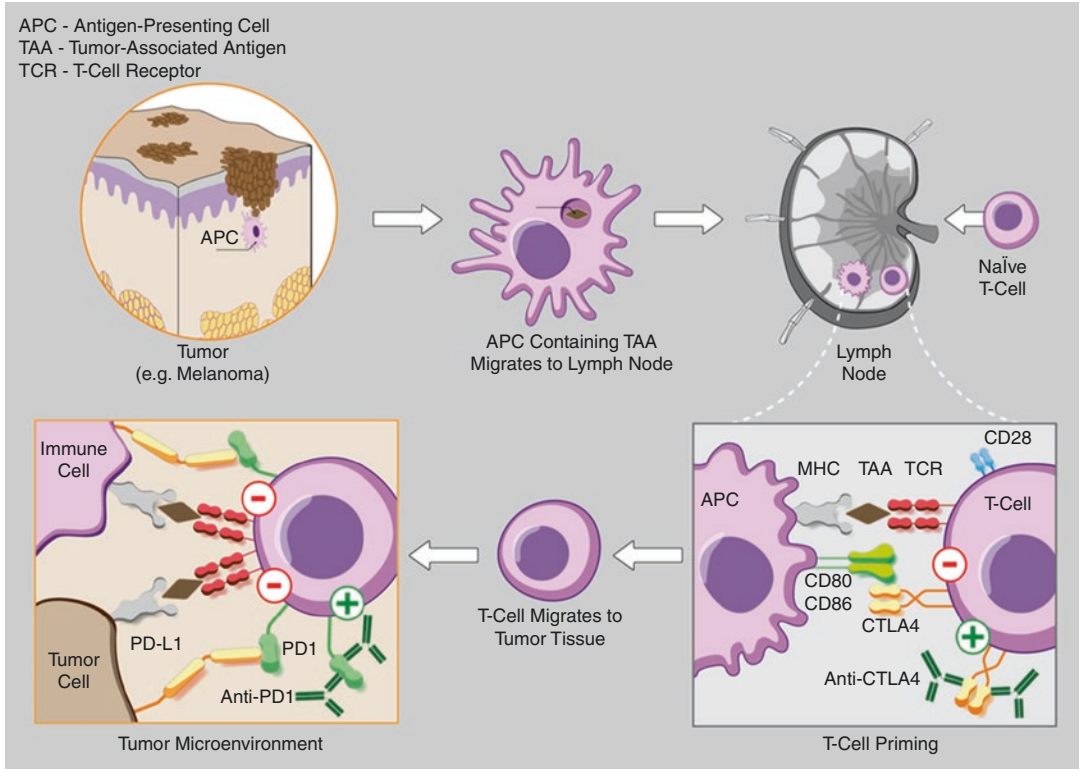

Fig. 11.2 PD1 and CTLA4 echapatory pathways to the antitumoral immune response. The cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) immune checkpoints are negative regulators of T-cell immune function. Inhibition of these targets, result in an increased activation of the immune system. CTLA-4 is thought to regulate T-cell proliferation early in an immune response, primarily in lymph nodes, whereas PD-1 suppresses T cells later in an immune response, primarily in peripheral tissues

In the same way the tumor cell can thwart the physiological mechanisms of apoptosis, it can also develop ways to escape the anti-tumoral immunity. Two pathways capable of "slowing down" the anti-tumor response have been identified:

- Cytotoxic T-Lymphocyte-Associated antigen 4 (CTLA4) which binds B7 and blocks the acceleration phase of the immune reaction in the peritumoral lymph node,
- Programmed Death 1 (PD1) which is expressed by activated T cells. The binding of the PD1 receptor localized on the T lymphocyte to its ligand PDL1, sends an inactivation signal to the lymphocyte and slows down the anti-tumor immune reaction [9, 10] (Fig. 11.2).

These immune checkpoints and their ligands are the targets of the new immunotherapies developped for the treatment of cancer.

Immunotherapy in Urological Oncology

Immunotherapy is not a new concept in oncology [11]. By the mid-1980s, interleukin 2, a cytokine that stimulated T-cell proliferation, was used at high doses in oncology. The principle was to globally stimulate the immune system especially the anti-tumoral immunity [12]. Although the response rate was low, some patients had durable responses. This first immunotherapy found an indication in metastatic melanoma and kidney cancer. Other immunotherapy approaches exist, such as anti-tumor vaccination or endovascular BCG therapy [13].

The principle of "modern" immunotherapy is no longer to stimulate the immune system globally (as was the case with interleukins and interferon alpha), but to block the echapatory mechanisms that the tumor develops to escape the anti-tumoral immune system (PD1, CTLA4 and their ligands).

Compared to the first generation of immunotherapy and classical chemotherapy, the "modern" immunotherapy has several advantages. The CTLA4 and PD1 immune checkpoints are ubiquitous and common to all kind of tumors, so that immunotherapy based on one or the other of its pathways is potentially effective on all types of cancer. This property is all the more interesting for chemo-refractory cancer, such as cancer of the kidney, lung, bladder or melanoma. Immunotherapy can be prescribed in monotherapy, which limits the risk of toxicity and restrict the types of toxicities, while on the opposite several molecules adding their own toxicities are combined in "standard" chemotherapies. The administration of immunotherapy usually consists in a single 60 min intravenous infusion every 2–3 weeks. So far the studies reported show that the side effects of immunotherapy were less severe than conventional chemotherapy, and most importantly, that complete and lasting responses could occur [14].

The predictive factors of response to immune checkpoints inhibitors that have been identified so far are mainly intratumoral factors: the PD1 mutation rate and the T lymphocyte infiltration rate [15, 16]. These three parameters allowed identification of two tumor profiles: the "hot" tumor (high load of PD1 mutation associated with a major intratumoral lymphocytes infiltration) characterized by a high level of antigenicity and a high sensitivity to immunotherapy, and in contrast the so-called "cold" tumors predicting a weak response to immunotherapy. One solution to potentially improve the response rate to the immunotherapy would be to transform "cold" tumors into "hot" tumors by increasing their level of antigenicity with a combination of immunotherapy, chemotherapy, radiotherapy or even oncolytic virus [17, 18].

Immunology of Transplant

The Immune Response in Renal Transplantation

There are two types of immune response: innate immunity and acquired/adaptive immunity [25, 26]. The innate immunity is not specific to any alloantigen, and constitutes the first step in the

immune response. Owing to the surgical stress of the kidney removal/transplantation and ischemia reperfusion, many pro-inflammatory cytokines (IL-1, IL-12) are released into the bloodstream of the recipient resulting in the recruitment of many immune cells (macrophages, polynuclear cells, natural killer cells) to the graft. This mechanism is named "Homing" [27]. Due to ischemia/reperfusion lesions, the endothelial cells of the graft vessels produce numerous adhesion molecules such as LFA-1 (CD11a, leukocyte factor antigen) or ICAM-1 (CD154, intracellular) that catch circulating leukocytes also expressing adhesion ligands. By a process of extravasation, the leucocytes of the recipient leave the vessels, infiltrate the graft following a gradient of chemokines and cytokines. Due to release of pro-inflammatory cytokines, APCs are activated and strongly express on their surface the major histocompatibility complex (MHC) of class I, as well as the co-stimulation molecules (CD80, CD86) essential to the development of the acquired/adaptive immune response. At this step, all the actors of the allogenic response are present in the graft which becomes the target of an adaptive immune response.

Because of the important polymorphism of the MHC, T lymphocytes of the recipient have the ability to recognize the MHC on the APCs of the donor being in the graft and then initiate an immune response with respect to the donor cells carrying this MHC. This mechanism is called direct presentation. This mode of presentation is particularly involved in the mechanism of acute rejection. The recipient's APCs may also present antigens lost by donor cells to the recipient's T-cell. These antigens are lost by the donor cells, are catched by the APCs which expose them on the MHC. This mechanism of antigen recognition, called indirect presentation, is the main activation pathway for T CD4 cells and is particularly involved in the mechanism of chronic rejection [28].

Humoral Hyperacute Rejection

This rejection occurs within minutes or hours after the transplantation. The graft quickly becomes cyanotic, purplish, oedemated and doesn't product any diuresis. Histologically, it consists in a massive intravascular thrombosis of the renal capillaries. Major cell necrosis and complete renal infarction occur within a few hours [29]. Immunologically, the hyper-acute rejection is related to the presence of Donor Specific Antibodies (DSA) in the recipient [8, 30]. These DSA bind to the endothelial cells of the graft and activate the complement cascade resulting in the secretion of coagulation factors leading to intravascular thrombi. The immunohistochemical analysis of the graft shows the presence of immunoglobulin and complement C3 fragments in the capillaries. The hyperacute rejection has now almost disappeared because of the pre-transplant immunological evaluations of the donor and the recipient, particularly by thepre-transplant regular and systematic search for anti-HLA antibodies and by carrying out the "Cross match" test before the transplantation.

Acute Rejection

Acute rejection occurs in the first few weeks of the transplantation. There are two types of acute rejection: the cellular and the humoral acute rejection. The cellular acute rejection is induced by T cells while humoral acute rejection is mediated by B cells, IgG immunoglobulins and complement [30]. Cellular acute rejection is the most common. It is characterized by an infiltration of the graft by leukocytes and monocytes in the tubules and glomeruli. The diagnosis relies on a biopsy of the graft. The histological lesions are evaluated according to the classification of BANFF which combines morphological lesions (inflammatory infiltrate in peri-tubular capillaries, thrombosis of the glomerular capillaries), immunohistological criteria (markings for the C4d fragment positive) and serological criteria (circulating antidonneur antibody) [31].

Chronic Rejection

The term "chronic rejection" is now replaced by "chronic allograft nephropathy" (CAN). CAN is defined by a progressive loss of the graft function resulting in a decrease of the glomerular filtration rate and the occurrence of a proteinuria. It is the first cause of transplant loss, generally leading to

a new transplant or a return to dialysis. Chronic allograft nephropathy is due to immunological factors (chronic rejection) and non-immunological factors (immunosuppressive toxicity). The diagnosis is histological and is based on the biopsy of the transplant: thickening of the intima of the capillaries, proliferation of myofibromatous cells derived from the differentiation of endothelial cells (epithelio-mesenchymal transition) induiced by ischemia reperfusion injury. There are also tubular atrophy lesions associated with interstitial fibrosis, and an infiltration by lymphocytes and plasmocytes [8, 31]. An histological classification, the Chronic Allograft Disease Index (CADI) has been developed and was integrated into various evaluation scores of the renal function on the long-term [32–34].

Immunotherapy in Renal Transplantation

Anti-Lymphocytes T Treatments

Monoclonal Antibodies Anti-Lymphocytes T

Monoclonal anti-Lymphocytes T antibodies (anti-CD3 and anti-CD52) are mainly used in acute rejection. They induce a rapid depletion of lymphocytes populations. Side effects are related to the significant release of inflammatory cytokines called *cytokine release syndrome*, and consist in lymphopenia, fever, sweat and pulmonary edema.

Calcineurin Inhibitor: Ciclosporin and Tacrolimus

Calcineurin Inhibitors (CNIs) revolutionized transplantation in the early 1980s. It is still the main immunosuppressive therapy in renal transplantation despite many side effects: nephrotoxicity, hepatotoxicity, neurotoxicity, infections and increased risk of cancer [35]. Calcineurin inhibitors inhibit the expression of cytokine genes in the lymphocyte T, especially the gene coding for interleukin 2 [5]. The main side effect of CNIs is nephrotoxicity, requiring a regular monitoring of blood rates of anticalcineurin, especially as many treatments may change their absorption and pharmacological characteristics. In the long term, anti-calcineurins are also involved in the occurrence of allograft nephropathy responsible for graft loss.

Other treatments involved in the inhibition of T lymphocytes are:

- Monoclonal antibodies competitively binding to the receptors involved in the CPA-Lymphocyte T interaction (anti-CD80, CD86, CD40)
- The anti-receptor antibodies of interleukin 2.

Anti Lymphocyte B Treatment

The anti-CD20 Monoclonal Antibody (Rituximab) is a CD20-targeted monoclonal antibody which induces a profound depletion of B lymphocytes by induction of apoptosis, complement-dependent cytotoxicity and antibodies [19,63]. Currently its current indications are, the desensitization for immunized recipients in compatible ABO transplantation. In this indication, anti-CD20 are administred in combination with polyvalent immunoglobulins. The second indication is in incompatible ABO transplantation, as an alternative to splenectomy [36, 37, 37–39].

Steroids

Corticosteroids have been used early in the history of kidney transplantation, for their immunomodulation potential. Steroids inhibit the secretion of cytokines, induce a depletion and an apoptosis of T lymphocytes, block the Th1 differentiation and alter the functions of macrophages. However, the numerous side effects related to their long-term use (diabetes, hormonal changes, infections, osteoporosis, behavioral disorders and delayed healing) tend to limit their use in kidney transplantation [40, 41].

Inhibitors of Purine Bases (Azathioprine and Mycophenolate Mofetil)

In the alloimmune response, cell proliferation is an essential step that requires the synthesis of purine nucleotides. Purine bases inhibitors (PBI) are active on the enzymes involved in the synthe-

sis of purine mucleotides during mitosis and therefore have an antiproliferative action. In contrast to CNIs, PBI have no nephrotoxicity and do not induce metabolic disorders. Their main side effects are cytopenia and diarrhea [42].

mTOR Inhibitors

The best-known inhibitor of mTOR is rapamycin, from which mTOR's name derives. mTOR pathway has an important regulatory function in cell growth and proliferation. Rapamycin was originally applied as an immunosuppressant and has been in use since around 2000 to prevent kidney graft rejection [19]. Sirolimus also exhibits immunosuppressive effects via inhibition of B cell and T cell proliferation [19, 43]. The main side effects are hyperlipidemia, thrombocytopenia and arthralgia.

While it is tempting to presume that it can promote tumor development via immunosuppression, actually Sirolimus has been proven to inhibit cancer cell proliferation through the same mechanism that is responsible for immunosuppression; the PI3K/AKT/mTOR pathway being also crucial in the production of the Vascular Endothelial Growth Factor (VEGF) which plays a key role in the growth and neo-angiogenesis of kidney cancer. Both Temsirolimus and Everolimus mTOR inhibitors were approved by the US Food and Drug Administration for metastatic renal cell carcinoma in 2007 and 2008 respectively [20, 21].

Considering the incidence of most cancers increases substantially after kidney transplantation, and cancer is one of the main cause of death for the recipient (after cardiovascular events), the use of mTOR in kidney transplantation for both graft rejection and reducing the risk of malignancy has been explored in several studies [22, 23]. The results are contradictory, although it seems that Sirolimus in kidney transplantation is associated with a reduction in the risk of malignancy and non-melanoma skin cancer [24]. The benefit seems most pronounced when patients are converted from an established immunosuppressive regimen to Sirolimus. Given the increased risk of mortality, however, the use of this drug does not seem warranted for most patients with kidney transplant.

Immunosuppression Strategies and Current Trends

The immunosuppression in renal transplantation consists in an initial "*induction therapy*", that has the objective to prevent the acute rejection. It is then relayed by a *"maintenance therapy"* to contain the allogenic response of the immune system on the long term [5, 7, 44]. The main limits to the maintenance treatment are the risk of nephrotoxicity, de novo neoplasia and cardiovascular events. The conventional maintenance immunosuppression regimen usually combines calcineurin inhibitor, purine bases inhibitor and steroids. Current trends are a growing use of tacrolimus instead of ciclosporin, of mycophenolic acid instead of azathioprine and especially an early withdrawal of steroids [45, 46]. With regard to maintenance treatment, the current challenge is to limit the use of calcineurin inhibitors by replacing them, if possible, by a m-TOR inhibitor to prevent the occurrence of chronic allograft nephropathy.

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