Neoadjuvant Immunotherapy Treatment of Localized Genitourinary Cancers

Multidisciplinary Management

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Editors
Andrea Necchi
Medical Oncology and Urology
Vita-Salute San Raffaele University
Milano, Milano, Italy

Philippe E. Spiess
Department of Genitourinary Oncology
University of South Florida
Tampa, FL, USA

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Foreword

The modern management of genitourinary cancers implies a close relationship between specialists throughout the clinical stages. This multimodal perspective implies the ability of healthcare providers and treating physicians to provide urologic patients with dedicated pathways within hospitals, along with a clinical research portfolio that best fits the peculiarities of each tumor entity.

Following a trend of therapeutic management initially proposed in other solid tumors, expanding the delivery of systemic treatments in early-stage genitourinary cancers, i.e., using newer systemic therapy options in patients with a potentially resectable tumor, may offer to clinical investigators a myriad of research opportunities.

As such, urologists and treating physicians are necessarily becoming more familiar with the molecular pathogenesis of genitourinary tumors and how it relates to diagnosis, prognosis, and treatment.

Neoadjuvant Immunotherapy Developments in Genitourinary Tumors focuses on very innovative and peculiar aspects of urologic cancers science, pathology, molecular genetics, and cutting-edge treatment in relation to the administration of preoperative immunotherapies at resectable stages. This book provides a deep dive into the current understanding of the biology of tumor response to immunotherapy by providing insights into the comparison of pre-therapy and post-therapy tumor samples analyzed within clinical trials.

Furthermore, the authors shed light over the need for designing novel clinical trial designs that align the molecular underpinnings and patient's needs. A special focus is also provided to the quite novel topic of surgical safety assessment of immunotherapy, on which the community of urologists is largely unprepared, and recommendations by the major urologic societies still lacking.

Moving forward, a "Clinical Trials Corner" chapter is also provided to the readers, in which the latest trials in progress are presented to help contextualizing the presented findings into the avenues of ongoing research.

Other timely topics include machine learning and artificial intelligence application to predict response to immunotherapy across genitourinary tumors.

Lastly, the textbook includes an overview of the next avenues of clinical research and presents the ultimate way of conceiving the neoadjuvant therapy paradigm, i.e., the administration of systemic immunotherapy (alone or in combination strategies) in organ-sparing strategies. This new paradigm is particularly well suited nowadays for the field of muscle-invasive bladder cancer, and initial data in this regard are presented.

The editors and contributing authors are a renowned group of urologists, clinical oncologists, pathologists, and physicians who are experts in their fields, across many different institutions, states, and even countries.

Professors Andrea Necchi and Philippe Spiess, both outstanding physician-scientists, represent a well-suited combination of physician scientists from different disciplines whose spirit is mirrored throughout the textbook they have originally conceived. I am quite confident you will find it informative and useful in your practice and inspiring for your clinical research.

Francesco Montorsi
Department of Urology, IRCCS San Raffaele Hospital
and Scientific Institute, Vita-Salute San Raffaele University
Milan, Italy

Preface

Moving the field forward in urologic oncology, under a renewed perspective, will naturally imply the involvement of representatives from academia, healthcare, industry, patient advocates, and regulatory organizations discussing the role of novel therapies inclusion into clinical and research pathways. This rapidly shifting scenario in the management of urologic patients deeply involves the use of multimodal treatments in curative settings. As such, the development of newer approaches in the perioperative setting of tumors, conventionally considered as "localized" or "potentially resectable," represents the most suited way to embrace the evolutionary road.

The developments in the use of immune-checkpoint inhibitors deserve a special focus in urologic oncology, based on the outstanding achievements that marked the pace of therapeutic improvements, primarily in kidney and bladder cancers. Other tumors, such as prostate cancer, representing the primary interest of urologic oncology research for several decades, are conversely suffering from delays due to the acquired understanding of their inferior sensitivity to immunotherapy approaches, thus making them exemplifying topics for translational research.

In parallel, editorial and educational activities should be able to tackle both the trajectory of this development and the anticipated needs of the treating physicians and research staff, when the use of immunotherapy compounds in neoadjuvant or adjuvant settings will likely be incorporated in routine practice.

This new book from Springer, *Neoadjuvant Immunotherapy Developments in Genitourinary Tumors*, is aimed in particular to tackle the most relevant issues of neoadjuvant immunotherapy administration in patients with urologic tumors. In this regard, the textbook is unique in the field and anticipates a new wave of editorial production in the near future.

Among the most relevant topics in this field, which are addressed within each one of the major urological cancer areas, we included the presentation of the primary efficacy results obtained within trials, the ongoing status of tumor biomarker development in association with immunotherapy response, and safety of neoadjuvant immunotherapy administration preceding radical surgery. An additional

selected topic of primary interest is represented by the new role for imaging assessment of urologic tumors.

The important debate underpinning the development of newer effective therapies administered preoperatively is certainly represented by the role of pathological response as a surrogate of survival in these patients.

As another primary aim of this textbook, an underlying flavor suggesting a raise of the bar of therapeutic success for the patients in this particular clinical setting, across the tumor types, may be acknowledged throughout the chapters.

We sincerely hope that this textbook will be suitable for your clinical practice, and will help you identify existing gaps, which will be filled by further refining the quality of our clinical research.

Milano, Italy — Andrea Necchi
Tampa, FL, USA — Philippe E. Spiess

Contents

Contributors

Mohamed E. Ahmed Department of Urology, Mayo Clinic, Rochester, MN, USA

Alessandra Alessi Nuclear Medicine Unit, Department of Radiology, Foundation IRCCS National Cancer Institute, Milan, Italy

Massimo Alfano Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Marco Bandini Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Francesco Barletta Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Giuseppe Basile Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Regina G. H. Beets-Tan Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Karin Birkenkamp-Demtröder Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

Peter Black Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Zuhir Bodalal Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Thierry Boellaard Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Alberto Briganti Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy

Teresa Bucho Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Giovanni Enrico Cacciamani The Catherine and Joseph Aresty Department of Urology, USC Institute of Urology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Giuseppina Calareso Department of Radiology, Foundation IRCCS National Cancer Institute, Milan, Italy

Umberto Capitanio Unit of Urology, IRCCS Ospedale San Raffaele, Milan, Italy

Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Milan, Italy

Jad Chahoud Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Monica Chatwal Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Emil Christensen Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

Lauren Folgosa Cooley Department of Urology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

Vito Cucchiara Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Aaron Dahmen Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Nick van Dijk Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Charles G. Drake Columbia Center for Translational Immunology, Columbia University Irving Medical Center, New York, NY, USA

Division of Hematology and Oncology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, USA

Janssen Research and Development, Spring House, PA, USA

Columbia University Medical Center, Herbert Irving Comprehensive Cancer Center, Division of Hematology/Oncology, New York, NY, USA

Lars Dyrskjøt Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark

Nicola Fossati Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy

Andrea Gallina Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Giorgio Gandaglia Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy

Daniel J. George Division of Medical Oncology, Department of Medicine, Duke University, Durham, NC, USA

Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA

Ewan A. Gibb Decipher Biosciences Inc., Vancouver, BC, Canada

Veracyte, Inc., Vancouver, BC, Canada

Shilpa Gupta Department of Hematology and Oncology, Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Rohit K. Jain Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Tuomas Jalanko Department of Urology, Helsinki University Hospital, Helsinki, Finland

Joep J. de Jong Erasmus University Medical Center, Department of Urology, Rotterdam, The Netherlands

Vidhu B. Joshi Department of Urology, Mayo Clinic, Rochester, MN, USA

Georgia Sofia Karachaliou Division of Medical Oncology, Department of Medicine, Duke University, Durham, NC, USA

Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA

Deborah R. Kaye Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA

Division of Urology, Department of Surgery, Duke University, Durham, NC, USA

Alessandro Larcher Unit of Urology, Division of Experimental Oncology, URI – Urological Research Institute, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

Riccardo Leni Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy

Kevin Groot Lipman Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Technical Medicine, University of Twente, Enschede, The Netherlands

Roberta Lucianò Department of Pathology, IRCCS Ospedale San Raffaele, Milan, Italy

Jasnoor Malhotra Department of Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Laura Marandino Department of Medical Oncology, Oncology Institute of Southern Switzerland, EOC, Bellinzona, Switzerland

Alberto Martini Unit of Urology, University Vita-Salute, San Raffaele Scientific Institute, Milan, Italy

Division of Experimental Oncology, URI, Urological Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy

Unit of Urology, Division of Experimental Oncology, URI – Urological Research Institute, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

Elio Mazzone Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

Joshua J. Meeks Department of Urology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA

Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL, USA

Departments of Urology and Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Antonella Messina Department of Radiology, Foundation IRCCS National Cancer Institute, Milan, Italy

Luis Meza Department of Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Francesco Montorsi Unit of Urology, University Vita-Salute, San Raffaele Scientific Institute, Milan, Italy

Division of Experimental Oncology, URI, Urological Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy

Department of Urology, San Raffaele Hospital and Scientific Institute, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy

Marco Moschini Department of Urology, San Raffaele Hospital and Scientific Institute, Milan, Italy

Valentina Murdica Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Andrea Necchi Vita-Salute San Raffaele University, Milan, Italy

Department of Medical Oncology, IRCCS Ospedale San Raffaele, Milan, Italy

Sumanta Kumar Pal Department of Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA

Filippo Pederzoli Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy

Praful Ravi Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

A. Gordon Robertson Dxige Research Inc., Courtenay, BC, Canada

Giuseppe Rosiello Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy

Jeffrey S. Ross Foundation Medicine Inc., Cambridge, MA, USA

Mathieu Roumiguie Department of Urology, CHU Toulouse/IUCT-O, Toulouse, France

Nicholas Salgia University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA

Andrea Salonia Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy

Simone Scuderi Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy

William Paul Skelton IV Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Susan F. Slovin Genitourinary Oncology Service, Department of Medicine, Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Guru P. Sonpavde Department of Medicine, Dana Farber Cancer Institute, Boston, MA, USA

Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

Dana-Farber Cancer Institute, Boston, MA, USA

Philippe E. Spiess Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Department of GU Oncology, Moffitt Cancer Center, Tampa, FL, USA

Stefano Trebeschi Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Selam Waktola Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Ivar Wamelink Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Technical Medicine, University of Twente, Enschede, The Netherlands

Tian Zhang Division of Medical Oncology, Department of Medicine, Duke University, Durham, NC, USA

Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA

Part I
Immune Checkpoint Inhibitors in the Preoperative Setting of Urothelial Bladder Cancer

Chapter 1
Background: State-of-the-Art and Ongoing Developments

Marco Moschini, Laura Marandino, and Francesco Montorsi

Bladder cancer (BCa) is the second most common genitourinary malignancy, with 81,400 new cases and 17,980 deaths estimated in 2020 in the United States [1]. Radical cystectomy (RC) with bilateral pelvic lymph node dissection is considered the standard treatment for recurrent high-grade non-muscle-invasive bladder cancer (NMIBC) and localized muscle-invasive bladder cancer (MIBC). Although surgical technique remained substantially unchanged in the last decades, the robotic approach recently surpassed the standard open surgery in many referral centers in Europe and the United States [2]. Similarly for the prostate cancer, no differences in survival outcomes have been recorded comparing open, laparoscopic, or robotic techniques [3], while only limited benefit in reducing length of stay and the need of perioperative transfusion favored the robotic approach [4, 5]. The decision on which technique reserve to RC candidates should therefore reserve on the bases of surgical and surgeon expertise [6, 7]. Concomitant bilateral pelvic lymph node dissection is a fundamental part of the procedure, improving survival and increasing the staging of RC candidates [8]. On the other hand, recently a randomized trial failed to prove any survival benefit comparing extended lymph node dissection to standard template [9]. However, it has to be mentioned that an important portion of patients included in the trial was found with no lymph node metastases and some of those

M. Moschini
Department of Urology, San Raffaele Hospital and Scientific Institute, Milan, Italy

L. Marandino
Department of Medical Oncology, Oncology Institute of Southern Switzerland, EOC, Bellinzona, Switzerland

F. Montorsi (✉)
Department of Urology, San Raffaele Hospital and Scientific Institute, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy
e-mail: montorsi.francesco@hsr.it

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were high-risk non-muscle-invasive bladder cancer patients, bringing to the question that maybe selecting higher-risk patients would have taken to different results. In this context it appears rationale to reserve an extended lymph node dissection only to patients at major risk of harboring node metastases in the extended or super-extended pattern [10].

A recent international multistakeholder effort [6, 7] evaluated the most burning topics regarding the management and future development of BCa. In this sense, a special role is played by the development of new therapeutic strategies including immunotherapy. Considering systemic therapies, cisplatin-based neoadjuvant chemotherapy (NAC) represents the standard of care in cT2-cT4a cN0 cM0 bladder cancer patients who are eligible to receive cisplatin [11]. NAC demonstrated a pathologic complete response (pCR) rate of approximately 20–35%, a pathologic downstaging to non-muscle-invasive disease in approximately 50% of cases, and an absolute improvement in overall survival (OS) of 8% at 5 years [12–14]. However, only a small portion of RC candidates receives neoadjuvant cisplatin-based chemotherapy in the real world as a consequence to fear of complications, disbelief from potential benefit, and fear in delay of the radical surgery delivery [15]. Moreover, nearly 50% of patients are ineligible to receive cisplatin according to Galsky criteria [16], and for these patients, the standard of care is still represented by RC alone, with poor survival outcomes.

Novel therapies are being evaluated in the preoperative setting, with immune checkpoint inhibitors being the most advanced in clinical development. The role of immunotherapy is known since the introduction, approximately 40 years ago, of intravesical BCG for NMIBC. From May 2016, five anti-programmed cell death-1/ligand-1 (PD-1/PD-L1) inhibitors – atezolizumab, durvalumab, avelumab, nivolumab, and pembrolizumab – have been approved by the US Food and Drug Administration (FDA) for patients with locally advanced or metastatic urothelial cancer that had recurred following platinum-based chemotherapy, and 3 of them, atezolizumab, nivolumab, and pembrolizumab, have also received European Medicines Agency (EMA) approval. Of note, only pembrolizumab has succeeded in showing an improvement in overall survival (OS) in this setting, while the phase 3 randomized controlled trial (RCT) of atezolizumab showed unexpected negative results [17, 18]. Atezolizumab and pembrolizumab have also received accelerated approval by the FDA and EMA for the first-line treatment of cisplatin-ineligible patients whose tumors express programmed death-ligand 1 (PD-L1). In addition, avelumab was recently approved for maintenance treatment of patients who have not progressed to first-line platinum-based chemotherapy for advanced disease, based on the results of JAVELIN Bladder 100 trial, that showed an improvement in OS in favor of avelumab versus (vs.) best supportive care [19].

Moving to nonmetastatic disease, based on the results of the phase 2 KEYNOTE-057 study, pembrolizumab was approved by the FDA in Bacillus Calmette-Guerin (BCG)-unresponsive, high-risk, NMIBC with carcinoma in situ (CIS) [20]. The PURE-01 (NCT02736266), a phase 2 study conducted in two centers in Italy, was the largest trial evaluating a single-agent neoadjuvant immunotherapy in MIBC (clinical [c]T2-T3bN0; cT2-T4a after protocol amendment in March 2018). Patients were enrolled independently of cisplatin eligibility and

received three cycles of pembrolizumab every 3 weeks before RC. Early findings reported pathologic complete responses (pT0) in 42% of patients, with a manageable safety profile. Updated results confirmed the activity of pembrolizumab with a pT0 rate of 37% and pathologic downstaging to non-muscle-invasive disease in 55% of patients in a less selected population (n = 114) after protocol amendment including also patients with variant histologies [21]. The surgical safety results revealed high-grade complications (defined as Clavien-Dindo ≥3a) in only 34% of patients with no perioperative mortality at 90 days [22]. The study also showed encouraging event-free survival (EFS) with 12- and 24-month EFS rate of 84.5% (95% confidence interval [CI], 78.5–90.9) and 71.7% (CI 62.7–82), respectively. Another phase 2 trial, the ABACUS study (NCT02662309), which was a multicenter European trial, evaluated single-agent immunotherapy [23]. Patients with cT2-T4a MIBC (n = 95), ineligible or refusing NAC, received two cycles of neoadjuvant atezolizumab. The pCR rate was 31% in the entire population and 17% in patients with cT3-T4 disease; 1-year relapse-free survival (RFS) was 79% (95% CI: 67–87%). Grade 3–4 Common Terminology Criteria for Adverse Events (CTCAE) occurred in 11% of patients. Following the interesting results of trials evaluating single-agent immune checkpoint inhibitors, immunotherapy and chemo-immunotherapy combinations started being investigated in the neoadjuvant context. Preoperative immunotherapy with anti-PD1 plus anti-CTLA4 antibodies, nivolumab plus ipilimumab, was tested in NABUCCO (NCT03387761) trial, a single-arm feasibility trial, that enrolled 24 patients with stage III urothelial cancer (cT3-4aN0M0 or T1-4aN1-3M0) ineligible for cisplatin or refusing NAC [24]. The primary endpoint was the feasibility to resect within 12 weeks from treatment start. All patients underwent resection and 96% of them within 12 weeks. Eleven patients (46%) obtained pCR and 58% had downstaging to NMIBC. Of note, the treatment was complicated by grade 3–4 immune-related adverse events in 55% of patients. Another recent study tested an immunotherapy combination with durvalumab-tremelimumab in 28 patients with cT2-T4a MIBC [25]. The pCR was 37.5% and downstaging was 58%; grade ≥ 3 immune-related adverse events were observed in 21% of patients. The same combination was tested versus chemotherapy in patients with "hot tumors," as classified by TIS (tumor inflammation signature) score, in the DUTRENEO trial, showing a pCR of 34.8%. However, the IFN gamma signature used for the selection of patients failed to select patients more likely to benefit from immunotherapy versus chemotherapy [26]. GU14-188 trial evaluated the combination of neoadjuvant gemcitabine (3 cycles) with pembrolizumab (5 cycles) prior to RC in cisplatin-ineligible patients (n = 40) [27]. The study showed a downstaging to non-muscle-invasive disease (primary endpoint) in 52% of patients and a pCR of 45%. One-year RFS was 67%. Two trials, HOG (n = 36) [28] and BLASST (n = 41) [29], evaluated in cisplatin-eligible patients a combination of cisplatin plus gemcitabine (CG) regimen with pembrolizumab (HOG) or nivolumab (BLASST) before RC. BLASST trial enrolled patients with cT2-T4a, N ≤ 1, while in HOG trial cN+ were not allowed. In these trials, the rates of pCR were 44% (HOG) and 34% (BLASST), while pathological downstaging was obtained in 61% and 66%, respectively.

Following the promising results of these studies, several phase 3 clinical trials testing perioperative immunotherapy, eventually combined with NAC according to cisplatin eligibility, started recruitment in the setting of MIBC (NCT03661320; NCT03732677; NCT03924856; NCT03924895). Of note, to date chemo-immunotherapy or immunotherapy combinations have shown disappointing results in first-line metastatic disease [30, 31], with only one study – IMvigor-130 – showing a benefit in PFS over chemotherapy alone [32]. It has recently been announced that adjuvant nivolumab showed superior disease-free survival (DFS) vs. placebo in the phase 3 CheckMate-274 trial. However, whether this result will change the development of treatment strategies in the neoadjuvant setting is yet to be clarified. Of note, the above mentioned ongoing phase 3 trials include a postoperative phase with immunotherapy after NAC and RC.

Table 1.1 summarizes the main results of trials conducted in the neoadjuvant setting in MIBC.

Improving clinical tumor staging is still an unmet clinical need in MIBC. Contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) represent the standard radiological techniques to stage patients with MIBC, even if their accuracy limitations are well known. The addition of immunotherapy in the neoadjuvant context could further enhance the complexity of staging and response evaluation since it is known that immunotherapy can lead to inflammatory reactions [33]. The PURE-01 trial included a large imaging program focused on staging and response evaluation, including multi-parametric MRI of the bladder and fluorodeoxyglucose [18F] positron emission tomography (PET/CT) in addition to standard thorax abdomen CT. Interestingly, patients defined as radiological responders to multi-parametric MRI of the bladder had a >90% chance to obtain a pT ≤1N0 response after pembrolizumab [34].

If many open questions remain before moving immunotherapy in MIBC from clinical trials to clinical practice, such as the validity of pCR as an intermediate endpoint, it appears clear that the study of predictive biomarkers should proceed concurrently with the planning and the conduction of new clinical trials. In this context, MIBC represents a unique platform to test biomarkers. Trials evaluating immunotherapy in MIBC reported conflicting results regarding potential immune biomarkers. PD-L1 expression and tumor mutational burden (TMB) are among the most studied biomarkers for immune checkpoint inhibitors. In the PURE-01 trial, PD-L1 expression according to combine positive score (CPS) was suggested as a potential predictive factor of response to pembrolizumab, and it was associated with longer EFS [21, 35–37]. On the contrary, in the ABACUS trial, there was no significant correlation between PD-L1 expression on immune cells or tumor cells and outcomes [38]. The results of ABACUS are in line with those of another trial testing neoadjuvant durvalumab-tremelimumab [25], while in the recently published NABUCCO trial [24], complete response (CR) rate was 73% in PD-L1-positive tumors (CPS >10%) vs. 33% in PD-L1-negative tumors ($p = 0.15$). Also data regarding TMB are different among the various studies. A significant association between high TMB analyzed as a continuous variable and pCR or downstaging was found in the PURE-01 trial, while in the ABACUS trial there was a lack of correlation

Table 1.1 Trials of immunotherapy in muscle-invasive bladder cancer

	PURE-01 [21]	ABACUS [38]	NABUCCO [24]	HOG GU 14-188 [27, 28]		BLASST [29]	DUTRENEO [26]	MDACC [25]
Treatment	Pembrolizumab	Atezolizumab	Nivolumab/ ipilimumab	Pembrolizumab-gemcitabine/ cisplatin	Pembrolizumab-gemcitabine	Nivolumab-gemcitabine/ cisplatin	Durvalumab/ tremelimumab	Durvalumab/ tremelimumab
Sample size	114	88	24	43	37	41	23	28
cT2-stage	54% (CT+mpMRI)	73%	0	47%	43%	90%	78%	43%
cN+ state	0	0	42%	0	0	3%	8.7%	0
pT0N0 rate	37%	31%	46%	44.4%	45.2%	34%	34.8%	37.5%
pT ≤ 1N0 rate	55%		58%	61.1%	51.6%	66%	56.5%	58%
1-year RFS	91% [EFS 87%]	79%	92%	2-y 66%	67%	n.a.	n.a.	82.8%
Biomarkers	PD-L1+ (TMB) Immune gene signatures	Pre-existing T-cell activation (CD8/ GZMB, tGE8-high)	PD-L1+ DDR GAs TLS signature	Not reported	Not reported	Immune gene signatures	Preselected with 18-gene IFNγ signature	TLS signature

Abbreviations: *c* clinical, *p* pathologic, *RFS* relapse-free survival, *EFS* event-free survival, *n.a.* not available, *CT*, computed tomography scan, *mpMRI* multi-parametric magnetic resonance imaging, *PDL1* Programmed death-ligand 1, *TMB* tumour mutational burden, *GZMB* granzyme B, *tGE8* eight-gene cytotoxic T cell transcriptional signature, *DDR* DNA damage response, *GAs* gene alterations, *TLS* tertiary lymphoid structure

between TMB with a cutoff set at the median value (10.1 mut/Mb) and response to treatment [38]. Trials testing immune biomarkers in MIBC used different methods and assays; therefore a comparison within studies is even more difficult considering a lack of standardization in biomarker evaluation. More details regarding biomarkers will be presented in next chapters. Gene expression-based immune signatures represent an interesting chapter in this context, since molecular subtypes of bladder urothelial cancer may be associated with different sensitivity to immune checkpoint inhibitors. In PURE-01, basal-type tumors, which showed the highest CPS, high Immune190 scores, and high immune gene expression, had the highest pathological response rates to pembrolizumab. Of note, higher RNA-based immune signature scores were significantly associated with pCR [39].

Precision medicine is rapidly changing the treatment of patients affected by cancer, and its role is under debate in virtually every type of tumor. The entrance of precision medicine in the management of urothelial cancer was marked by the encouraging results in the advance disease of erdafitinib, a potent pan-FGFR tyrosine kinase inhibitor, in patients with *FGFR3* mutations or fusions. However, in urothelial cancer, and especially in MIBC, precision medicine has still a long way to go. TCGA project [40] and several other studies have enhanced our knowledge of MIBC biology and have identified different potential actionable gene alterations. Umbrella studies may represent a useful platform for the development of targeted treatment strategies and for biomarker discovery. Interestingly, a multi-arm trial testing various neoadjuvant treatment on the basis of the presence/absence of FRFR3 gene alterations is going to start recruitment for cisplatin-ineligible/refusing RC patients (Optimus trial, NCT04586244).

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Chapter 2
Clinical Case Debate: Neoadjuvant Checkpoint Inhibition Versus Standard Chemotherapy

Marco Bandini, Giuseppe Basile, and Andrea Gallina

Introduction

Neoadjuvant chemotherapy (NAC) prior to radical cystectomy (RC) is advocated by international guidelines as the gold standard for treating patients with muscle-invasive bladder cancer (MIBC) [1–4]. The primary effect of NAC is to control tumor progression by acting on occult micrometastases present at the time of RC and possibly reduce tumor volume before surgery. Accordingly, randomized data showed that NAC yielded a pathological complete response (pCR, i.e., ypT0N0) in up to 40% of MIBC and tumor downstaging to non-muscle-invasive disease (i.e., ypT1-is-a) in approximately 50% of cases, entailing a small (5% absolute improvement at 5 years) nonetheless significant overall survival (OS) benefit compared with RC alone [5, 6].

Based on the positive results from randomized phase III trials [1, 7, 8], combination of methotrexate, vinblastine, doxorubicin, and cisplatin (i.e., MVAC) became the standard of care in patients with MIBC eligible for RC [1], in spite of the well-known side effects associated with such treatment [1, 9]. By time, other cisplatin-based regimes were proposed in order to achieve better tumor control with reduced side effects. For example, dose-dense MVAC (ddMVAC) and gemcitabine plus cisplatin (GC) [6, 10–12] gained important consideration over the years, overtaking MVAC as the preferred options in MIBC patients. Nevertheless, nearly half of patients with MIBC are ineligible to cisplatin-based NAC (ddMVAC or GC) due to poor renal function (GFR < 50–60 ml/min), poor performance status (PS) (ECOG-PS ≥2), severe (grade ≥ 2) neuropathy, or heart failure (NYHA-class-III/IV) [13, 14].

M. Bandini · G. Basile · A. Gallina (✉)
Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy
e-mail: gallina.andrea@hsr.it

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Barriers in the use of standard cisplatin-based chemotherapy (CHT) regimens upfront RC progressively resulted in its underutilization in clinical practice. Accordingly, real-world data reported a use of NAC in only 20% of all patients referred to RC [15–17].

The need for a reliable and more tolerable alternative to neoadjuvant CHT boosted the consideration of immune checkpoint inhibitors (ICIs). The idea to rely on immunotherapy (IO) as alternative to CHT came from the encouraging results in the advanced and metastatic settings, where IO showed lower side effects, higher tolerability, and superior oncologic efficacy. Atezolizumab was the first ICI approved by the US Food and Drug Administration (FDA) for locally advanced or metastatic urothelial carcinoma (UC) progressed during or after platin CHT [18]. Similarly, also pembrolizumab received FDA approval after the positive findings of the KEYNOTE-045 [19]. The study showed longer median OS in metastatic bladder cancer (BC) patients who progressed after systemic CHT and were treated with pembrolizumab vs. CHT (10.3 vs. 7.4 months). The latter study revealed also that the effect of IO was heavily influenced by the expression of tumor's biomarkers. Specifically, in patients expressing combined positive score (CPS) $\geq$ 10% (i.e., the percentage of programmed cell death-ligand-1 (PD-L1) expressing tumor cells and infiltrating immune cells relative to the total number of tumor cells), the effect of pembrolizumab was greater (8.0 vs. 5.2 months) than in the overall population, aside from less grade 3 treatment-related adverse events (AEs) (16.5 vs. 50.2%) [19]. The concept of personalized medicine according with tumor-response biomarkers became crucial in the IO setting since other phase III RCTs enhanced different efficacy of ICIs according, for example, with PD-L1 expression. In particular, the KEYNOTE-361 [20], the IMvigor-130 [21], and subsequently the DANUBE [22] trials proved no significant OS improvement for patients with low expression of PD-L1, where on the contrary, platin-based CHT reached better survival outcomes. Based on these evidences, the FDA amended its recommendations on the administration of pembrolizumab and atezolizumab to patients who are not eligible for cisplatin CHT and who have high expression of PD-L1 (CPS $\geq$ 10 for pembrolizumab and PD-L1 stained tumor-infiltrating immune cells covering $\geq$5% of the tumor area for atezolizumab) or are not eligible for any platin CHT regardless of the level of PD-L1 expression [23].

Based on the experience gained from the locally advanced and metastatic BC settings, several phase II trials exploring the feasibility and effectiveness of ICIs alone, combined or in association with CHT, were launched in the neoadjuvant setting. Preliminary results from these trials were encouraging and provocative but limited to the evaluation of the local tumor response [i.e., rates of ypT0N0 achieved (pCR)] after RC. To date, IO remains an option only in the metastatic setting, as a second-line therapy. Nevertheless, results from the aforementioned phase II studies are challenging this axiom giving a plea for assessing the use of ICIs also in the neoadjuvant setting. Within this chapter, we discussed three clinical cases that may

explain how, when, and why neoadjuvant IO could affirm its value and become a valid alternative to NAC. These cases will focus particularly on patients in whom the use of NAC is limited by significant toxicity or scarce efficacy. In these scenarios, neoadjuvant immunotherapy may warrant further considerations.

Case 1: A Case of an Old Woman with MIBC and Concomitant Chronic Kidney Disease

A 73-year-old woman came at our uro-oncology clinic with a diagnosis of a pT2 MIBC following transurethral resection of the bladder performed at another center but confirmed after internal pathologic revision. According with the anamnestic evaluation, the patient reported a well-controlled hypothyroidism status, mild hypertension, and a condition of poorly controlled diabetes conditioning severe (grade ≥ 2) neuropathy. The body mass index (BMI) was 32 kg/m^2. As a part of the preoperative evaluation, the patient underwent total body CT scan and lab tests which revealed the presence of a bladder tumor causing left hydroureteronephrosis with concomitant raise of creatinine serum level. Creatinine clearance was 43 mL/min. The presence of chronic kidney disease and her general status made the patient ineligible for platin-based NAC. Thus, she was referred directly to RC. The pathologic report enhanced a pure urothelial tumor extended to the peri-vesical fat (pT3) with one pelvic lymph node involved (pN1).

Case 2: A Case of a Man Suffering from Low Urinary Tract Symptoms with Incidental Diagnosis of UC with Variant Histology

A 78-year-old man with indwelling catheter due to progressive worsening of low urinary tract symptoms (LUTS) and urinary retention reported a persistent hematuria. Ultrasound imaging and cystoscopy revealed the presence of a suspicious area for BC; therefore the patient received transurethral resection of the bladder. The pathological report of the removed bladder tissue revealed the presence of a pT2 MIBC with mixed UC and pure squamous cell carcinoma (SCC) involving more than 50% of the specimen. Besides the hypertension not affecting cardiac dysfunction, the patient had no further comorbidities, with an ECOG-PS <2. The patient was referred for three cycles of neoadjuvant GC and then RC. Pathologic report revealed a locally advanced unresponsive tumor (pT3b) with predominant squamous differentiation and metastatic invasion of the regional lymph nodes (pN2).

Case 3: A Case of a Young Woman with MIBC Showing High PD-L1 Expression and Claudin-Low Molecular Subtype Pattern

A 54-year-old woman diagnosed with pure UC of the bladder (cT2N0) was enrolled in a phase II trial testing neoadjuvant IO regardless of cisplatin eligibility. Based on the trial protocol, TURB specimen underwent comprehensive genomic profiling. Analyses reported high PD-L1 expression (90%), tumor mutational burden (TMB) of 21 mut/Mb, and a claudin-low molecular subgroup pattern according to the consensus classification and The Cancer Genome Atlas (TCGA). The patient received three courses of pembrolizumab 200 mg, and then she was restaged with preoperative multi-parametric magnetic resonance imaging (mpMRI). MpMRI revealed no evidence of morphologic or active disease. Pathology after RC showed a complete tumor eradication with no evidence of residual disease (ypT0N0).

The reported cases summarize three different clinical scenarios where the standard-of-care cisplatin-based CHT is contraindicated (case 1), unlikely to be associated with effective response (case 2), or possibly inferior to IO in reaching adequate tumor control (case 3). According with findings from phase II trials, alone or combined ICI treatment has proved to be a reliable alternative to CHT especially when the latter is contraindicated or tumor characteristics lean forward a more tailored approach. Several studies examining neoadjuvant IO in MIBC have been designed [24–30]. Of those, five have reported preliminary findings [24–26, 28, 30].

The PURE-01 [24] is a single-arm study which evaluated pembrolizumab (three doses every 3 weeks) as neoadjuvant therapy in patients with pure or predominant UC ($T \leq 3bN0$) regardless of cisplatin eligibility. Initial results from a preliminary cohort of 50 patients showed a 42% pCR, with downstaging to $pT < 2$ achieved in 54% of patients enrolled. Based on these encouraging findings, the study was amended also accepting patients with cT4a tumor and the presence of predominant variant histology (VH). A subsequent update with 114 patients confirmed the efficacy of neoadjuvant pembrolizumab with ypT0N0 response reached in 37% of enrolled patients and 41% downstaged [31]. Recently, the authors released also preliminary survival data reporting event-free survival (EFS) and recurrence-free survival (RFS) rates at 24 months. Specifically, 24-month EFS was 71.7% (95% CI, 62.7–82) and 24-month RFS was 78.3% (95% CI, 68.9–89). The study showed favorable results across all the different pathological response subgroups, except for ypN+ patients who presented the lowest RFS with 39.3% (95% CI, 19.2–80.5) at 24 months. Of no less importance was the surgical safety of RC after neoadjuvant pembrolizumab treatment, with 34% high-grade complications reported, which was not different from that experienced after NAC [32]. The PURE-01 preliminary survival results recapitulated the clinical findings of another trial testing single ICI treatment. The ABACUS trial [25] is a phase II study assessing neoadjuvant atezolizumab in patients with muscle-invasive UC of the bladder who were not eligible for neoadjuvant CHT [16]. The study recruited 88 cisplatin-ineligible patients treated with two courses of atezolizumab prior to RC and showed an overall pCR rate of

31% (95% CI, 21–41) and a 12-month RFS of 79% (95% CI, 67–87). Perioperative safety of RC after IO was also confirmed with grade 3/4 Clavien-Dindo (C-D) complications occurring in 10% of patients and no perioperative deaths. Encouraging results supporting neoadjuvant IO came also in the locally advance setting. The NABUCCO [26] study tested two doses of ipilimumab and two doses of nivolumab, followed by resection in stage III UC in patients who were either cisplatin ineligible or refused CHT. Twenty-four patients were enrolled in this study, and 46% (95% CI, 26–67%) reached pCR while 58% (95% CI, 37–77%) had no residual invasive tumor after treatment (pCR or pTisN0/pTaN0). Interestingly, pCR was reached in 40% (95% CI, 12–73%) of patients with clinically node-positive disease (cT2-4aN1-3). Of note, the trial reported a significant higher proportion of grade 3/4 C-D immune-related AE (54%) compared to other neoadjuvant IO trials. This was probably a consequence of the combined regimen of two immunotherapy agents. Remaining on the setting of combined ICI agents given preoperatively, the DUTRENEO [27] and MDACC trials [28] were developed. While the former prospectively enrolled patients using a tumor inflammation signature (TIS) score, showing a 34.8% pCR in the intention-to-treat (ITT) population, the latter reported a pCR of 37.5% and downstaging to pT1 or less in 58% of patients who completed surgery, with 21% grade ≥ 3 immune-related AEs. Finally, similar results have been achieved also in the HOG [29] and BLASST-1 [30] trials, testing a combination regimen of GC together with pembrolizumab (HOG) or nivolumab (BLASST-1) before RC, in which the rates of pCR were 44% and 34%, respectively. Results from all these phase II trials are supporting the hypothesis that neoadjuvant IO either alone or in combination with CHT can achieve similar local disease control compared with standard-of-care NAC. With this in mind, we can affirm that patients who are ineligible to CHT, like the woman of our first case report, may be considered as future candidates to IO, if phase III study will not refute these findings.

Beside this aspect, it is also important to emphasize that IO started to show promising results also in specific settings where CHT had previously failed. The efficacy of NAC on VH of UC, either in its pure or mixed form, is still an open question due to the paucity of available data and disheartening results. Specifically, available scientific evidences suggest that patients whose tumor is predominantly characterized by VH usually display an aggressive clinical course and a poor response to conventional CHT [33], except for the neuroendocrine variant [34]. In particular, within patients treated with NAC and subsequent RC, pure or mixed SCC exhibited the worse survival among all the other VHs, when compared with pure UC [35]. Further proof of this was found in the largest cohort of SCC, with no OS benefit derived from NAC compared to RC alone [36]. Based on available and mostly retrospective evidences, the European Association of Urology (EAU) reported that it is unclear if patients with SCC histology can benefit from NAC, suggesting instead primary radical treatment [4]. The second discussed clinical case summarizes exactly this scenario, where a patient with predominant SCC MIBC received NAC with no apparent benefit. In contraposition with CHT studies, the interim results from the PURE-01 study [31] showed that SCC and lymphoepithelioma-like (LEL) tumors were associated with a remarkable

sensitivity to IO. Specifically, 86% of SCC patients achieved a pT ≤ 1 response, and 67% LEL patients achieved a pCR. Conversely, the authors of the PURE-01 study reported a substantially lower activity of pembrolizumab in patients with other than SCC or LEL VH, with pCR rate of 16% (95% CI, 3.4–40) and tumor downstaging rate of 42% (95% CI, 21–67). Although these preliminary findings are promising, future trials are needed to confirm the effect of IO in the VH setting and move forward our knowledge on the field.

Last but not least, the third discussed clinical case focused on a patient that received ICI treatment and reported an outstanding response reaching complete eradication at RC. Interestingly, the patient presented with specific overexpressed biomarkers that are known to trigger IO efficacy. In particular, the patients expressed elevated TMB, high PD-L1 expression, and a claudin-low molecular subtype. In this regard, it is worthy to remind that pembrolizumab has been approved by the FDA for adult and pediatric patients with tumor mutational burden (TMB) ≥10 [37]. Several evidences in urothelial and non-urothelial tumor have proved that TMB can be a reliable marker of IO susceptibility, and thus it can be advocated as predictor of tumor response. Besides that, the overexpression of PD-L1 by tumor cells has been also recognized as possible marker of IO efficacy. Since the introduction of ICIs targeting PD-1 or PD-L1, a great deal of interest was shown in understanding whether checkpoint protein expression could be used as a prognostic and predictive marker for MIBC. Early studies showed PD-L1 expression in UC was associated with a higher tumor grade [38], worse clinical outcomes, and decreased survival [39]. PD-L1 status was inconstantly associated with tumor response. Indeed, while in the IMvigor210 trial [18], a higher PD-L1 expression was associated with a better response rate; the CheckMate 275 findings showed a response to nivolumab irrespective of PD-L1 expression [40]. Correlation between PD-L1 and tumor response has been also evaluated in the neoadjuvant setting. A subgroup analysis of the PURE-01 trial showed that PD-L1 was strongly associated with EFS [41], appearing to be the most robust predictor of ypT0N0 response [42]. Authors highlighted also that high level of preexisting immune infiltration was a predictor of favorable pCR to neoadjuvant pembrolizumab but not to platin-based NAC [43]. Conversely, within the ABACUS trial [25], TMB and PD-L1 expression did not predict outcomes in patients treated with atezolizumab. Instead, it emerged that responding tumors showed predominant expression of genes related to tissue repair after treatment and high CD8+ immune cell infiltration. Conversely, stromal factors such as transforming growth factor-β (TGF-β) signaling and fibroblast activation protein were linked to atezolizumab unresponsiveness [25]. The NABUCCO trial [26] partly confirmed the results of the PURE-01 study, reporting a CR rate of 73% (95% CI, 45–92%) in tumor expressing PD-L1 CPS > 10% compared to 33% (95% CI, 7–70%) in PD-L1-negative tumors, highlighting also a higher TMB in tumors achieving pCR. TGF-β expression appeared instead to be associated with no response, as previously evaluated in the ABACUS. Conversely, using quantitative multiplex immunofluorescence, authors observed no correlation between baseline CD8+ cell infiltration and response to combined ICIs. Interesting findings on this

topic were reported also by DUTRENEO trial [27]. According to TIS score, measuring a preexisting but suppressed adaptive immune response within tumors, patients were classified as "hot" (high TIS score) or "cold" (low TIS score), and particularly, tumors with a higher TIS score showed better clinical response to anti-PD-1 blockade [44]. Although results from these phase II studies are somehow conflicting, several explanations can be identified. Firstly, PD-L1 expression by immunohistochemistry assays was differently evaluated among the reported trials. Secondly the cutoffs used to define high and low expression were not uniform in the aforementioned studies. Third, the small sample size and the lack of a control arm in each trial do not allow to accept or refute the hypothesis that the examined tumor biomarkers are distinguished between patients with high and low likelihood of response to neoadjuvant IO. Finally, the patient of the clinical case number 3 harbored a tumor with claudin-low molecular subtype, in accordance with genomic subtype classification (GCS). The molecular subtypes denote a specific genomic signature that helps to classify patients with UC according to their biological aggressiveness and response to treatments [45]. Based on the expression of microRNAs, [46–48], the first evidence supporting a prognostic role of molecular subtyping emerged once again from the advanced setting. In the IMvigor210 which tested atezolizumab in 316 patients with locally advanced or metastatic tumor, luminal II subtype tumors showed higher susceptibility to IO [18]. Similarly, in the CheckMate 275 study testing nivolumab in 270 metastatic UC patients, basal I tumors had higher objective response rate [40]. The prognostic impact of molecular subtypes was explored also in the PURE-01 study where claudin-low subtypes exhibited the best response rate and survival outcomes [41, 43]. This suggests that stratification of BC based on molecular subtype could be an effective strategy for therapeutic regimen allocation.

Taken together, molecular subtyping, TMB, and biomarkers analysis emerged as potentially useful tools for selecting patients who are likely to benefit the most from neoadjuvant IO. Unfortunately, stronger evidences are required before biomarker analyses can be implemented to every day clinical practice in patients receiving neoadjuvant treatment upfront RC.

Where Are We and Where Are We Heading?

The clinical cases here presented allow us to highlight the possible future issues regarding IO in BC treatment, especially when considering well-selected patients. So far, despite the surprising results in terms of efficacy and safety which have been reported by several clinical trials [25–31], even for long-term outcomes [41], IO is still bound to be a second choice in the therapeutic armamentarium for patients with MIBC and only within trial environment. Currently, aside from NAC-ineligible patients, there are several new therapeutic conditions in which IO could be considered as the principal therapeutic weapon. Indeed, although some of the clinical cases discussed (case 2 and case 3) reported patients eligible for NAC, because of

the tumor characteristics, they could be an example of who may benefit the most from ICI treatment. Particularly, patients with high PD-L1 expression, high TMB, and which belong to defined clusters of molecular subtypes are those expressing the best pCR and survival outcomes. The phase II studies that are still in progress and especially the phase III trials testing ICIs alone, combined, or in association with CHT will allow us to better understand the real field of application of IO for BC patients. Further results on single or combined ICI treatment will become available from ongoing phase II trials testing atezolizumab (NCT02451423), pembrolizumab plus epacadostat (NCT03832673), avelumab (NCT03674424), nivolumab (NCT03520491) (NCT02845323), and durvalumab plus olaparib (NCT03534492). Moreover, several randomized phase III trials are either currently open or soon-to-be opened. Many are testing perioperative pembrolizumab plus RC versus RC alone in cisplatin-ineligible patients (NCT03924895) or pembrolizumab plus CHT in cisplatin-eligible patients (NCT03924856) (NCT02365766) (NCT02690558). Others are randomizing patients to durvalumab in combination with CHT vs. cisplatin-based CHT alone (NCT03732677), durvalumab/tremelimumab in association with ddMVAC (NCT03549715), or nivolumab with/without linrodostat mesylate alongside CHT or CHT alone (NCT03661320).

In addition to MIBC patients, in which the use of IO has been investigated for many years, several ongoing trials are testing ICIs also in non-muscle-invasive bladder cancer (NMIBC), where available therapeutic algorithm is outdated and often inefficient. Although NMIBC usually carries a favorable prognosis, there is a high risk of disease recurrence and progression to MIBC, especially for T1G3 patients with concomitant CIS [49]. The standard of care for NMIBC after transurethral tumor resection is represented by adjuvant intravesical chemotherapy or Bacillus Calmette-Guerin (BCG) instillation, which allows to reduce both recurrence and progression rates [50]. However, up to 19% of patients may not be able to complete BCG maintenance course due to adverse side effects such as BCG-induced cystitis, fever, or general malaise. Moreover, due to interruptions in BCG supply, global BCG shortage is a challenge for many healthcare systems in the last years [51, 52]. Unfortunately, a considerable part of high-risk NMIBC patients become "BCG-unresponsive" with high probability of disease progression to MIBC and subsequent worsened survival outcomes. Patients with these characteristics are unlikely to benefit from additional BCG therapy, and other available bladder-sparing treatments are limited and without evidence of effectiveness. Therefore, RC is still the recommended treatment for patients who have failed BCG. Nevertheless, RC represents a risk-bearing therapeutic option with a high perioperative complication rate and mortality, especially in frail patients [53]. In this context, many phase I–III clinical trials evaluating systemic IO are now enrolling patients with NMIBC [54]. The pivotal study of systemic IO in NMIBC was the KEYNOTE-057 [55], a phase II trial of pembrolizumab monotherapy for high-risk NMIBC patients who refused or were unsuited for RC. Authors reported a 3-month CR rate of 41.2%, with a durable response up to 16.2 months. The unexpected results of this study led FDA to approve pembrolizumab as a new standard therapeutic option for NMIBC patients with carcinoma in situ (CIS). Following the enthusiastic findings of the

KEYNOTE-057, several trials testing IO are recruiting patients with NMIBC. KEYNOTE-676 (NCT03711032) is testing pembrolizumab in patients with high-risk NMIBC after an induction course of BCG. ALBAN, a phase III trial (NCT03799835), is enrolling patients to be treated with atezolizumab, while POTOMAC (NCT03528694) will randomize patients in three arms: durvalumab/BCG (induction and maintenance) vs. durvalumab/BCG (induction only) vs. BCG alone (induction and maintenance). With a similar design, the NCT04165317 trial will evaluate the efficacy of sasanlimab (PF-06801591) with different regimens of BCG therapy. As a new horizon for clinical research, these trials may also be helpful to show the timing of ICI initiation, evaluating IO not only in BCG-unresponsive patients but also in "BCG-naïve" patients with high-risk features. In this context, the definition of "early bladder cancer" has been proposed to define selected high-risk NMIBC and limited-stage MIBC [56] who can benefit from IO treatment.

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Chapter 3
Pathologic Features of Response to Neoadjuvant Therapies in Muscle-Invasive Bladder Cancer: More than Meets the Eye

Filippo Pederzoli, Roberta Lucianò, Ewan A. Gibb, Jeffrey S. Ross, and Andrea Necchi

Introduction

The tumor microenvironment is a complex and heterogenous arena in which tumor cells interact with many different cellular (e.g., fibroblasts, immune cells, endothelial cells, etc.) and acellular (e.g., extracellular matrix, cytokines, chemokines, etc.) players. As the fulcrum of the tumor microenvironment, the neoplastic cells are able to create complex signaling networks to take advantage of their "neighborhood" and repurpose it for their own growth and survival benefit [1]. It is now clear that a comprehensive analysis of the tumor microenvironment and of the complex interactions that take place in and around its location is critical to better understand cancer biology and the current and future mechanisms of anticancer therapies.

F. Pederzoli
Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy

R. Lucianò
Department of Pathology, IRCCS Ospedale San Raffaele, Milan, Italy

E. A. Gibb
Decipher Biosciences Inc., Vancouver, BC, Canada

J. S. Ross (✉)
Foundation Medicine Inc., Cambridge, MA, USA
e-mail: jross@foundationmedicine.com

A. Necchi
Vita-Salute San Raffaele University, Milan, Italy

Department of Medical Oncology, IRCCS Ospedale San Raffaele, Milan, Italy

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Other than sustaining tumor growth and progression, the tumor microenvironment plays a role in the response to anticancer therapies. For example, DNA damaging agents (e.g., doxorubicin) can impact fibroblasts at the tumor microenvironment, pushing them into senescence [2]. In this altered state, fibroblasts start producing a storm of cytokines and growth factors, including transforming growth factor (TGF)-beta and vascular endothelial growth factor (VEGF), which eventually enable cancer cell survival after the exposure to chemotherapy [3]. Fibroblasts may also influence response to immune checkpoint blockade. For instance, fibroblasts expressing fibroblast activation protein-alpha promote an immunosuppressive environment by recruiting myeloid cells and inhibiting T-cell activity, resulting in decreased efficacy of immunotherapeutic compounds [4]. The introduction of molecular and genomic tests in clinical trials has pushed forward our understanding of the complex signaling in the tumor microenvironment, and the routine implementation of these analyses in the near future would further establish personalized cancer care as the standard of care [5–7].

Complementary to molecular and genomic analyses [8–10], traditional histologic evaluation of the tumor microenvironment still represents an important starting point for the evaluation of the efficacy of neoadjuvant therapies. This importance of the histologic evaluation of surgical specimens after neoadjuvant therapy is also highlighted by the position statement of the Food and Drug Administration (FDA) about the role of pathologic complete response (pCR) as surrogate endpoint in clinical trials. Based on the results of the ad hoc working group promoted by the FDA and known as Collaborative Trials in Neoadjuvant Breast Cancer (CTneoBC), pCR was defined either as "the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected […] specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0/Tis ypN0 in the current AJCC staging system)" or, more stringently, as "the absence of residual invasive and in situ cancer on hematoxylin and eosin evaluation of the complete resected […] specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0 ypN0 in the current AJCC staging system) [11]." Besides the concept of pCR, several histopathological features have been investigated over the last years as potential biomarkers of therapy response. In this chapter, we aim to review the available studies about the pathologic features after neoadjuvant chemotherapy (NAC) in muscle-invasive bladder cancer (MIBC). Moreover, we will provide an overview of the specific histological changes associated with response to neoadjuvant immunotherapy in different malignancies, offering some insights from MIBC patients enrolled in the PURE-01 trial [12].

Morphologic Determinants of Response to Neoadjuvant Chemotherapy in MIBC

In the neoadjuvant chemotherapy (NAC) setting, several pathologic features have been described as potential markers of complete pathologic response at surgical resection in colorectal carcinoma [13, 14] and non-small cell lung cancer [15, 16].

Table 3.1 Definition of the tumor regression grades (TRGs) as applied to bladder specimens after neoadjuvant chemotherapy [17]

TRG 1	Complete response: absence of histologically identifiable residual cancer cells and extensive fibrosis of the tumor bed
TRG 2	Strong response: predominant fibrosis of the tumor bed and residual cancer cells occupying <50% of the evaluated area
TRG 3	Weak/no response: predominant residual cancer cells outgrowing tumor bed fibrosis (≥50% of the evaluated area occupied by cancer cells) or absence of regressive changes

In bladder cancer, few reports have analyzed the histopathological features associated with response to NAC in bladder cancer. In 2014, Fleischmann et al. [17] reported the first application in MIBC of the tumor regression grades (TRGs), a system to histologically quantify the extent of tumor response to chemotherapy, already in use in other malignancies as a prognostic biomarker [18, 19]. The 3-tier TRG classification (Table 3.1) evaluates the extension of the residual tumor area compared to the size of the original tumor bed, identified as areas of fibrotic changes in the deep layers of the bladder wall. Together with the fibrotic reaction, associated morphological features observed in bladder specimens were edema of the tissues (infrequent), focal accumulations of macrophages (frequent), inflammatory infiltrates with lymphocytes and granulocytes (frequent), large areas of necrosis (infrequent, probably due to the long time between the initiation of chemotherapy and the radical cystectomy), and cytoplasmic vacuolation in residual neoplastic cells (rare). The group also proposed to apply a similar grading system to the regressive changes in metastatic lymph nodes. Based on these findings, the researchers also defined the concept of "dominant TRG," which is the higher TRG between the primary bladder lesion and metastatic lymph nodes. In their published cohort of 56 patients with locally advanced or node-positive disease treated by neoadjuvant platinum-based chemotherapy, Fleischmann and colleagues reported a significant correlation between the TRG system and other traditional histopathologic features of negative outcomes, including the ypT and ypN stages and the greatest diameter of residual primary tumor. Moreover, the dominant TRG emerged as an independent risk factor in multivariable survival analyses (hazard ratio (HR) 4.0, 95% confidence interval (95%CI) 1.1–14.9, $p = 0.035$). Another important finding in their study is that 50% of patients showed different TRGs in the bladder and in the lymph nodes, highlighting that NAC may have discordant effects in different tumor lesions. This observation highlights the importance of including an evaluation of the regional lymph nodes in posttreatment pathologic scores, avoiding including only the evaluation of the primary bladder lesion. Moreover, performing a transurethral resection of bladder tumors (TURB) to assess the response to NAC could be unreliable in a large proportion of treated patients, raising a word of caution about this approach in bladder-sparing protocols [20, 21]. Lastly, the authors evaluated paired pre-NAC TURB and post-NAC cystectomy specimens to correlate morphologic characteristics of the pre-therapy tumor with post-therapy characteristics and therapy response. Only the number of mitotic figures per high-power field was higher in the TURB specimens of responders (dominant TRG1; median 4, $p < 0.03$) vs. partial/

Table 3.2 Categories of response to neoadjuvant chemotherapy according to the TNM and TRG systems in muscle-invasive bladder cancer [23]

Major response	Absence of muscle-invasive disease and lymph node involvement (≤ypT1N0)
Partial response	Residual disease ≥ypT2N0-3 with TRG 2
No response	Residual disease ≥ypT2N0-3 and TRG 3

Abbreviations: *TRG* tumor regression grade

nonresponders (dominant TRG2/TRG3; median 2). This finding was in line with the report by Grossman et al. [22] using a group of 42 patients treated with NAC in the SWOG-8710 trial.

The same group recently led the multicenter retrospective validation of their TRG system on a final cohort of 389 cT2-4aN0-3M0 bladder urothelial carcinoma patients [23]. The TRG system was easily implemented by all pathologists at their local institutions, and interobserver agreement between pathologists was substantial (Cohen's kappa coefficient = 0.82). Of note, higher TRG scores were associated with the presence of glandular or squamous differentiation of urothelial carcinoma, with 26 of the 38 cases harboring those variant histologies classified as TRG3. Using a combination of TNM and TRG staging, response to NAC was stratified into three classes (i.e., major response, partial response, and no response; Table 3.2). Those novel classes of response showed significantly different overall survival, with a survival probability at 2 years of >90% for major responders, about 80% for partial responders, and less than 60% for nonresponders. Moreover, multivariate analyses confirmed a significant association between survival and classes or response (for partial response, HR 3.44, 95%CI 1.74–6.81, $p < 0.001$; for no response, HR 5.75, 95%CI 3.36–9.84, $p < 0.001$).

The prognostic value of the TRG system was not confirmed by another study. In their cohort of 165 patients, Brimo et al. [24] reported that TRG was significantly associated with disease progression (defined as presence of metastatic disease and/or recurrence) and survival only at univariable analysis. However, it is worth mentioning that Brimo et al. included patients with variant histologies (24% of the total cohort) and did not perform central review of the cases or did not test interobserver agreement.

Compared to other malignancies such as rectal and esophageal carcinomas, in which it has clear predictive and prognostic value, evaluating TRG in urothelial bladder carcinoma poses the challenge of discriminating NAC-related changes from previous TURB procedure-related tissue reactions. Wang et al. [25] evaluated the spectrum of morphological changes related to TURB alone or from NAC in cystectomy specimens, finding substantial overlapping between the two groups. However, patients receiving NAC showed more hyalinization of the bladder wall and less inflammation or foreign body-type reaction compared to TURB only patients. The inseparable contribution of TURB to the pathologic response after NAC was also evaluated by Brant et al. [26], who estimated it to be around 40%. While it is undoubtedly clear that the TURB has an effect on the bladder wall that may mask

the regression effects of NAC to some extent, the TRG seems to be a potentially reliable marker of NAC response in MIBC. Further multicenter, prospective studies are awaited to validate its predictive value and reproducibility.

Peculiar Histological Features of Response to Neoadjuvant Immunotherapy

The number of clinical trials testing neoadjuvant immunotherapy protocols is rising across the whole spectrum of oncology. Therefore, surrogate biomarkers of therapy success, like pCR, have been extensively used to design informative and feasible trials, rather than long-term survival data, which would become available in the next 5 years and more. Therefore, there has been growing interest to assess the morphologic changes associated with response to neoadjuvant immunotherapy, with the aim to refine the prognostic reliability of pCR and to design specific histology-based scoring systems to predict immune checkpoint inhibitor's therapeutic efficacy. Initially, some scoring systems developed for neoadjuvant chemotherapy were used to assess also post-immunotherapy specimens. However, translating the histological findings associated with successful chemotherapy to immunotherapy is not straightforward, as the mechanisms behind antitumor activity is different between chemotherapy and immunotherapy [27, 28]. Therefore, there is a clear need for immunotherapy-specific pathology-based scoring systems that could be reproducible among different malignancies and among different pathologists.

To this end, Cottrell et al. [29] performed a comprehensive analysis of the resection specimens from 20 patients affected by non-small cell lung cancer and treated with neoadjuvant nivolumab. In the tumor bed (i.e., the area occupied by residual tumor cells or by the regression changes associated with response), patients (9/20) who experienced a major pathologic response (i.e., ≤10% residual viable tumor in the posttreatment resection specimen) showed some distinctive features (Fig. 3.1):

- Tissue repair-like reaction, including proliferative fibrosis (i.e., high fibroblast nuclei/collagen ratio; proliferating fibroblasts, activated by the pro-inflammatory local microenvironment) and neovascularization
- Local activation of the immune system, including high infiltration of lymphocytes and presence of tertiary lymphoid structures [30] (TLS; i.e., de novo formation of ectopic lymphoid-like structures with spatial organization of the different cell populations) and plasma cell aggregates
- Cell death, like cholesterol clefts (i.e., crystal-like accumulation of cholesterol)

Of note, while some of those morphological features were also described after neoadjuvant chemotherapy, presence of TLS/plasma cells and tissue repair-like reaction appeared to be specific of immunotherapy, suggesting a different activation of the tumor microenvironment in response to immunotherapeutic agents. Based on the identification of specific histological characteristics of successful

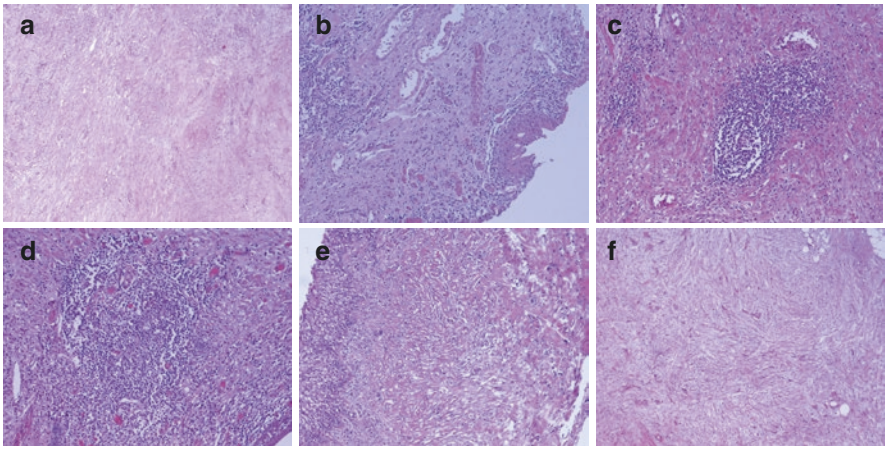

Fig. 3.1 Examples from the PURE-01 trials of pathologic features after neoadjuvant immunotherapy for muscle-invasive bladder cancer. (**a**) Tumor bed; (**b**) neovascularization; (**c**, **d**) follicular structures in the bladder wall; (**e**, **f**) fibrotic remodeling of the bladder stroma

immune-mediated tumor clearance, the same authors developed a scoring system called "immune-related pathologic response criteria (irPRC)" to standardize the assessment of post-immunotherapy specimens and with the potential to test it as a surrogate endpoint of successful therapy and recurrence-free survival. Quantitative irPRC was measured by the following formula: % immune-related residual viable tumor = total viable tumor area/total tumor bed area (including regression bed). Moreover, the authors suggested that the same scoring approach can be applied to lymph nodes thanks to the specificity of the immune-mediated regression, representing a major improvement compared to other scoring systems based only on the evaluation of the primary tumor lesion. Moreover, the author also evaluated pre-/posttreatment paired specimens (n = 17), but they did not find any pretreatment morphological features associated with response.

Similarly, Tetzlaff et al. [31] described the histopathologic changes observed in post-immunotherapy melanoma specimens. In patients treated with neoadjuvant checkpoint inhibitors, pathologic features of response included the infiltration of the tumor bed by different immune cells (tumor-infiltrating lymphocytes, plasma cells), lymphoid aggregates, and the presence of a reactive, wound healing-like stroma. Moreover, tumoral necrosis and melanosis were observed to different extents, especially in patients treated with neoadjuvant BRAF/MEK inhibitors. Whereas the cut-off to define the response classes was slightly different from the study by Cottrell et al., Tetzlaff et al. confirmed the abovementioned histologic findings associated with successful immunotherapy, suggesting their potential applicability in different tumor types.

Based on these premises, Stein and colleagues [32] recently reported a pan-tumor pathologic scoring system to be used after treatment with anti-PD-1/PD-L1 compounds. They reviewed >250 cases of patients treated with anti-PD-1/PD-L1 blockade for 11 different tumor types, plus other 98 cases of patients treated by combinatory protocols including anti-PD-1/PD-L1 compounds. They observed that the previously described histological features of response to immunotherapy (i.e., wound healing-like fibrosis, immune infiltration/activation, cell death) were consistently present among all tumor types and that the same features were identifiable not only at the primary tumor site but also in the lymph nodes – as previously shown by Cottrell et al. [29] – or distant metastasis. Reproducibility of the scoring system among pathologists was very high (intraclass correlation coefficient 0.982, using 10% scoring intervals). Moreover, the authors reported that the features of immune-related pathologic response can be observed also in specimens from patients treated with drug combination protocols including anti-PD-1/PD-L1 blockade, offering the opportunity to test the prognostic value of the immune-specific pathologic scoring system also in these patients.

Although there is still much to be done, the possibility that the abovementioned pathologic scoring systems will enter clinical practice soon is real and welcomed [33]. The development and implementation of such systems, characterized by reproducibility among observers and across tumor types and based on H&E images, is critical to assess and compare the rising number of clinical trials testing neoadjuvant anti-PD-1/PD-L1 blockade alone or in combination with other drugs, especially while we are waiting for mature long-term survival data. Moreover, the introduction of novel molecular subtyping (Figs. 3.2 and 3.3) and genetic testing associated with the traditional histology evaluation will move the field forward toward a personalized, multidisciplinary molecular pathology approach.

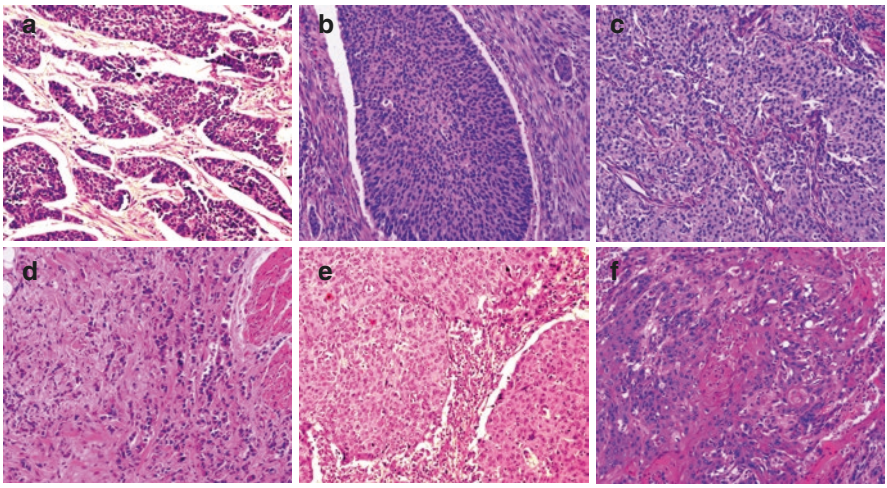

Fig. 3.2 Examples of the histological appearance of luminal (**a**, **d**), basal (**b**, **e**), and scar-like (**c**, **f**) cases from the PURE-01 trial

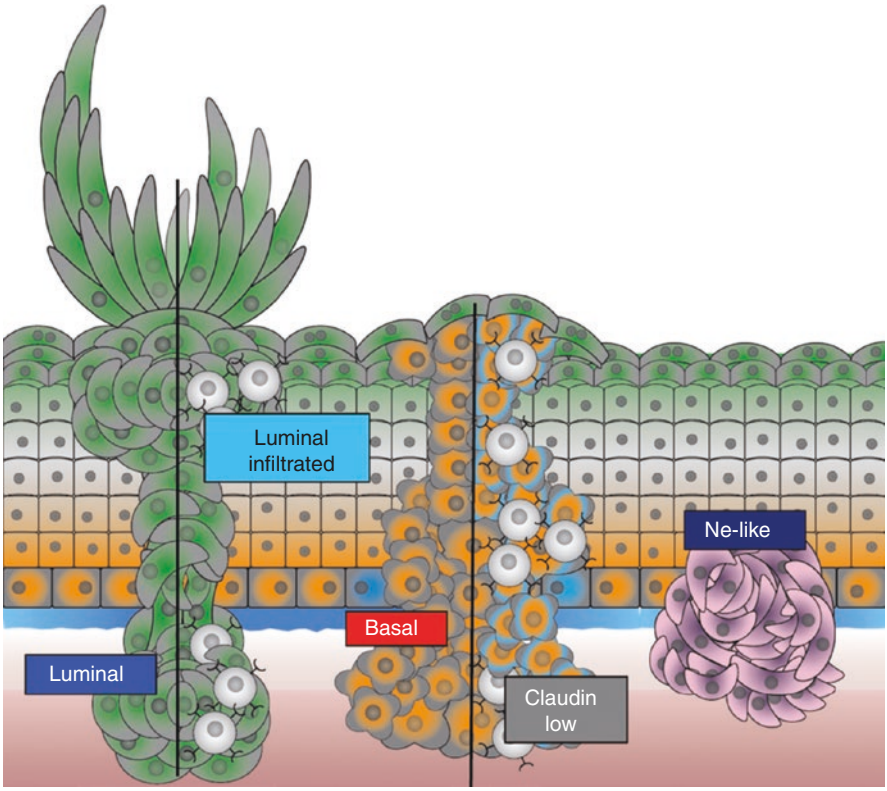

Fig. 3.3 Schematic representation of the novel molecular clusters in muscle-invasive urothelial carcinoma

Conclusions

While we are waiting for the era of the next-generation oncology, when genomic profiling of all potentially life-threatening tumors and MIBC in particular will be available for all patients, it is of paramount importance to refine the validation and predictive accuracy of prognostic biomarkers that will be used to determine a reliable risk stratification of MIBC patients prior to treatment initiation. Development of such tools will become more and more important in the next several years, when more single agents and combinations of agents (i.e., anti-PD-1/PD-L1 molecules, chemotherapy, targeted therapy compounds, etc.) would become standard options in all perioperative settings. Therefore, the future validation of histology-based scoring systems to evaluate the success of neoadjuvant treatments for MIBC patients in large, multicenter, prospective studies is greatly anticipated.

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Chapter 4
Biomarkers Predicting Outcomes Before and After Neoadjuvant Immune Checkpoint Inhibition Therapy for Muscle-Invasive Bladder Cancer

Joep J. de Jong and Ewan A. Gibb

Introduction

Muscle-invasive bladder cancer (MIBC) is an aggressive disease with limited treatment options. For the approximate 25–30% of patients who present with MIBC, the recommended treatment option is neoadjuvant cisplatin-based chemotherapy (NAC) followed by pelvic lymph node dissection and removal of the bladder (radical cystectomy; RC) [1, 2]. Despite this aggressive treatment regimen, the 5-year overall survival rate is only approximately 55% from the time of surgery, highlighting an unmet clinical need for better patient stratification and improved therapeutic intervention [3]. Patients who are ineligible for NAC are recommended to proceed to immediate cystectomy [4], where the outcomes for these patients are poor [1, 5]. For these reasons, there is a significant unmet need for improved patient stratification and additional treatment options for both cisplatin-eligible and cisplatin-ineligible patients.

Immune checkpoint blockade (ICB) has emerged as a promising therapy for metastatic urothelial carcinoma, with several checkpoint inhibitor drugs been approved in the second-line setting for patients who have progressed with cisplatin-based chemotherapy [6–10]. The application of IBC in earlier disease stages is also being investigated, with atezolizumab and pembrolizumab having received approval for use in the first-line setting for patients who are cisplatin ineligible and are PD-L1 positive [11]. The higher mutational rates in primary bladder cancer tumors [12,

J. J. de Jong
Erasmus University Medical Center, Department of Urology, Rotterdam, The Netherlands
e-mail: j.j.dejong@erasmusmc.nl

E. A. Gibb (✉)
Veracyte, Inc., Vancouver, BC, Canada
e-mail: ewan.gibb@veracyte.com

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	PURE-01	ABACUS	NABUCCO	HOG GU14-188		BLASST-1	DUTRENEO	MDACC
Treatment	**Pembrolizumab**	**Atezolizumab**	**Ipilimumab > Ipi/Nivolumab > Nivo**	**Pembro-GEM/CIS**	**Pembro-GEM**	**Nivolumab-GEM/CIS**	**Durva/Treme**	**Durva/Treme**
Reference	[18]	[19]	[16]	[21]	[20]	[22]	[23]	[17]
Sample size	114	88	24	43	37	41	23	28
cT2-stage	54% (CT+mpMRI)	73%	0	47%	43.2	90%	78.2%	43%
cN+ stage	0 (but 6% PET+)	0	42%	0	0	3%	8.7%	0
pT0N0 rate	**37%**	**31%**	**46%**	**44.4%**	**45.2%**	**34%**	**34.8%**	**37.5%**
pT≤N0 rate	55%		58%	61.1%	51.6%	66%	56.5%	58%
1-y RFS	91% (85-98) [EFS: 87%] [x]	79% (95% CI: 67-87)	92%	2-y: 66%	67%	n.a.	n.a.	82.8%
Biomarkers	PD-L1+ TMB Immune-gene signatures Molecular subtypes	PD-L1+ TMB Immune-gene signatures Molecular subtypes	PD-L1+ DDR GA TLS signature	None	None	Immune-gene signatures Molecular subtypes	Pre-selected with 18-gene IFN-g signature	TLS signature
RC tissue	Yes	Yes	Yes	No	No	No	No	Yes

Fig. 4.1 Clinical trials involving checkpoint inhibitors in the neoadjuvant setting

13], coupled with significant immune infiltration levels [14, 15], make checkpoint inhibitors a rational and attractive avenue of neoadjuvant therapy.

There are numerous ongoing clinical trials evaluating the use of checkpoint inhibitors in the neoadjuvant setting (Fig. 4.1). These clinical trials have showed pathological response rates comparable to platinum-based chemotherapy ranging between 31% and 46% [16–23]. Unfortunately, these lower positive responses mean most patients are not receiving benefit from neoadjuvant checkpoint therapy, further emphasizing the need to improve our understanding of the mechanisms driving treatment response. Moreover, treatment decisions are becoming increasingly complex as novel immune and targeted therapies are developed and approved, where they provide compelling alternatives to current standard of care therapies, such as chemotherapy, radiation therapy, and radical cystectomy. With these challenges in mind, the development and application of biomarkers will be instrumental to improving patient selection, ultimately driving immune therapy toward standard of care.

Unfortunately, many of the clinical trials to date have limited biomarker-associated data or the data is generated using inconsistent methodologies, making a direct comparison between trials challenging. The data available for post-treatment radical cystectomy specimens is also limited, leaving a rather large knowledge gap around the impact of immune therapy on tumor biology and resistance mechanisms. One advantage of the neoadjuvant setting is access to untreated primary tumor tissue, which can facilitate biomarker discovery for key endpoints including pathological response and patient outcomes [24]. Moreover, access to matched patient tumor tissue post-treatment can provide key data to facilitate the characterization of resistance mechanisms. Finally, detailed characterization of post-treatment tissues can provide a foundation to begin to inform adjuvant treatment decisions. Although studies comparing tumor tissue pre- and post-treatment are limited, the available data is intriguing, with the emerging trends for each of these trials providing key insights into neoadjuvant checkpoint therapy.

In this chapter, we discuss the molecular characterization of MIBC tumors treated with systemic IBC, focusing on biomarker discovery opportunities where tumor tissue was profiled before and after therapy. We will focus our discussion on four major clinical trials including ABACUS (atezolizumab), PURE-01 (pembrolizumab), NABUCCO (ipilimumab), and MDACC (neoadjuvant durvalumab plus tremelimumab) [16–19], illustrating the similarities from the perspective of biomarkers in MIBC.

The ABACUS Trial: Neoadjuvant Atezolizumab

The ABACUS trial is a single-arm, phase 2 study that investigated the use of two cycles of neoadjuvant atezolizumab (anti-PD-L1) for operable MIBC (cT2-4aN0M0), including patients who were ineligible for cisplatin-based chemotherapy [19]. Of the 95 patients recruited, 88 were assessable for the primary endpoint which was complete pathological response at radical cystectomy surgery. With 27 out of 88 patients (31%) achieving a complete pathological response, the trial met the primary endpoint and showed a 1-year relapse-free survival of 79%, with a median follow-up of 13.1 months. For this study, tumor tissue was profiled from untreated TURBT samples and matched post-atezolizumab radical cystectomy samples (ClinicalTrials.gov Identifier: NCT02662309).

Immunohistochemistry

Baseline PD-L1 positivity was assessed on immunohistochemistry (IHC), using SP142 assay at the ≥5% immune cell staining threshold. For pretreated tumor tissue, PD-L1 staining intensity (immune cells or tumor) was not significantly correlated with outcome, suggesting initial immune infiltration of primary tumor tissue does not predict long-term therapeutic benefit. However, PanCK-CD8 IHC analysis revealed that high baseline presence of intraepithelial CD8+ cells was significantly associated with a complete pathologic response rate of 40%. High levels of immune infiltration appeared to facilitate regression at the primary tumor site, resulting in effective local control as measured by pathological response, but these correlates may be less meaningful for predicting the metastatic potential and ultimately patient outcome.

The PanCK-CD8 scoring method allowed for a granular assessment of the immune infiltration in the pretreatment tumor tissue, resulting in the classification of the ABACUS tumors into three distinct CD8+ immune phenotypes, including desert, excluded, and inflamed [25]. The desert phenotype indicates little to no immune staining positivity, coupled with resistance to atezolizumab. The excluded phenotype is characterized by stromal infiltration, where immune cells accumulate on the tumor periphery but have not penetrated the tumor tissue. Finally, the inflamed phenotype indicates a high level of immune infiltration within the tumor tissue. In

the metastatic MIBC setting, earlier studies have reported the inflamed phenotype was associated with response to atezolizumab [25]. The ABACUS study, despite a high occurrence of inflamed tumors, did not report an improved response rate for this phenotype. However, dual CD8 and granzyme B (GZMB) staining was positively associated with response, suggesting the quality of the immune infiltration, beyond CD8 expression, is relevant when determining outcomes within the inflamed immune phenotype. A correlation with response was not seen for the excluded or desert immune phenotypes.

Comparing TURBT tissue to treated RC tissue, significantly increased levels of CD8, PD-L1, and FAP were observed, indicating increased immune activity in the treated tumor tissue. Moreover, dynamic changes to the immune phenotypes occurred with therapy, with five patients changing from excluded to inflamed and four from an inflamed to excluded phenotype.

RNA-Based Molecular Biomarkers

The ABACUS study also conducted RNA-seq gene expression analysis to explore several response categories. Global gene expression patterns were compared before and after treatment in patients with stable disease, finding higher immune signature scores and immune gene expression in posttreated tissues. These data were consistent with the IHC results, where increased staining for immune markers was observed post-therapy. The RNA-seq expression data was also analyzed in the context of a predefined eight-gene (*IFNG*, *CXCL9*, *CD8A*, *GZMA*, *GZMB*, *CXCL10*, *PRF1*, and *TBX21*) cytotoxic T-cell transcriptional signature (tGE8), revealing a significant increase in signature scores for responders, compared to patients with stable disease and/or relapse. As with the IHC data, higher levels of immune infiltration in pretreated tissue quantified by RNA-based immune gene signatures correlated with response to atezolizumab.

With respect to RNA-based molecular subtyping, the ABACUS study used the 2012 Lund taxonomy to classify the pre- and post-tumors into five molecular subtypes UroA, GU, Inf, UroB, and SCCL [26]. While in the metastatic setting the Lund subtypes were found to correlate with outcome after treatment with atezolizumab, this was not the case in the neoadjuvant setting pretreatment [19]. After treatment, the majority (14/15, 93%) of responding tumors were classified as the "infiltrated" molecular subtype. Importantly, treated tumors classified as infiltrated had increased levels of angiogenesis, stromal and immune infiltration, and with decreased cellular proliferation [19]. These expression-based patterns may potentially reflect reorganization of the tumor microenvironment, as responding tumors also showed upregulation of extracellular matrix and collagen formation signatures. It is tempting to speculate these features reflect tumor "scarring" or wound healing, a concept which will be explored in detail in the PURE-01 study section (1.3). Importantly, higher angiogenesis signature scores suggest anti-angiogenesis drugs

(VEGFR inhibitors) may be a promising candidate for the adjuvant treatment post-atezolizumab.

DNA-Based Molecular Biomarkers

Biomarkers, including tumor mutational burden (TMB) and DNA damage repair (DDR) gene alterations, have previously been reported to correlate with outcomes in the metastatic setting of bladder cancer [27]. However, in the ABACUS study, associations with pathologic response were not observed for neoadjuvant atezolizumab [19]. Exploratory analyses found that responding tumors had increased DNA amplification levels for the 11q13.3 locus, which includes the *FGFR3*, *FGF19*, and *CCND1* genes. With the recent approval of fibroblast growth factor (FGF)-targeted therapy for bladder cancer, these results may imply the justification for future trials combining atezolizumab with additional targeted therapy [28]. However, FGF pathway activity has not been consistently linked to increased response rates with checkpoint inhibitors, as discussed below.

When comparing TMB and DNA mutations pre- and post-atezolizumab, consistent DNA alterations were not revealed, suggesting an absence of clonal evolution within the relatively short time span of neoadjuvant atezolizumab therapy. This contrasts with data from the neoadjuvant chemotherapy setting, where pressure from platinum-based therapy induced rapid clonal evolution [29]. Considering these results together, the data from the ABACUS study suggest that entire populations of tumor subclones are eliminated with therapy, while with chemotherapy, additional mutations are induced, facilitating a rapid tumor evolution and the emergence of new dominant subclones. However, the time frames between each treatment differ greatly, with 5.6 weeks between atezolizumab and surgery compared to the approximate 12 weeks required to complete chemotherapy. There may be insufficient time to account for all but the most aggressive tumor regrowth in the former time span.

Integrating Biomarkers to Refine the Molecular Diagnosis in the ABACUS Study

In the metastatic MIBC setting, biomarkers like TMB, DDR gene alterations, and PD-L1 staining have all shown promising utility for predicting benefit from atezolizumab [27]. However, these biomarkers did not correlate with treatment response in the context of neoadjuvant atezolizumab in the ABACUS study [19]. Nonetheless, preliminary biomarker data suggests that quantifying preexisting immune infiltration, using either RNA-based signatures or IHC staining, holds promise for predicting pathological response after neoadjuvant checkpoint inhibition with atezolizumab. However, simply quantifying the levels of immune infiltrates may be insufficient, as

the type or quality of immune infiltrate may improve response prediction even further, particularly for the inflamed phenotype, suggesting an important avenue of biomarker discovery. Notably, when comparing pre- and post-atezolizumab tumor tissues, an increase in angiogenesis and stromal gene expression for responding tumors was revealed, suggesting a scarring or wound-healing phenotype in a significant proportion of cases, which has significant implications for targeted adjuvant therapies. Taken together, future standardization for the application of these promising biomarkers is warranted, in order to validate them as response predictors in the context of neoadjuvant anti-PD-L1 treatment.

The PURE-01 Trial: Neoadjuvant Pembrolizumab

In the PURE-01 trial (single arm, phase 2), 143 cisplatin-eligible MIBC patients (cT2-T4aN0M0) were enrolled and treated with 3 cycles of neoadjuvant pembrolizumab (anti-PD-1) [30, 31]. Of the 143 treated patients, 55 (38.5%) showed a complete pathologic response (ypT0N0) at radical cystectomy. The median follow-up of 23 months, corresponded with 12- and 24-month event-free survival rates of 84.5% and 71.7%, respectively [30] (ClinicalTrials.gov Identifier: NCT02736266).

Immunohistochemistry

PD-L1 positivity was determined by IHC using the 22C3 assay and the combined positive score (CPS), defined as the percentage (≥10%) of PD-L1-expressing tumor and infiltrating immune cells relative to the total number of tumor cells. Within the PURE-01 cohort, PD-L1 positivity was significantly associated with complete pathologic response at radical cystectomy [31], which directly contrasts the ABACUS data. However, while both studies investigate neoadjuvant checkpoint inhibitors, there are numerous differences which confound direct comparisons, including differences in the assay (SP142 vs 22C3), treatment (anti-PD-L1 vs anti-PD-1) or patient inclusion (cisplatin ineligible vs eligible). Notably, CD8 IHC analysis for matched pre- and post-pembrolizumab samples in PURE-01 revealed an increase of CD8+ cells infiltrating the tumor stroma, consistent with observations from the ABACUS study where a 78% increase in median values for intraepithelial CD8 expression was noted. However, unlike ABACUS, PURE-01 did not report an association of pretreatment CD8 IHC positivity with pathologic response.

RNA-Based Molecular Biomarkers

In the PURE-01 study, gene expression data was analyzed for 84 TURBT specimens collected pre-therapy using whole-transcriptome microarrays [18]. Molecular subtyping was used to classify the PURE-01 cohort into subtypes according to the

consensus, TCGA and GSC (genomic subtyping classifier) models, with the latter consisting of a luminal, luminal-infiltrated, basal, claudin-low, and neuroendocrine-like molecular subtypes [32, 33]. Pathological response to pembrolizumab was not significantly associated with molecular subtypes, which was consistent with previous studies for atezolizumab and NAC, where molecular subtypes did not predict response [19, 33]. Notably, basal-like tumors did have favorable response rates averaging around 65%, differing slightly with each subtyping model. Downstaging was observed for 17/26 (65.4%) for both the TCGA and consensus basal squamous subtypes and for 7/11 (63.3%) for the GSC claudin-low subtype [18]. The basal squamous and claudin-low subtypes differ in that the basal squamous encompasses all basal tumors, regardless of immune or stromal infiltration, while the claudin-low are more restrictive, encompassing basal tumors with higher immune infiltration and activity.

Integrating immune-associated IHC data with the molecular subtypes revealed that PD-L1 combined positive score on IHC was higher for basal-type tumors compared to the other subtypes. These data were consistent with immune gene signatures, including the generalized immune190 signature, which also showed higher scores for basal-type tumors. Moreover, basal-type tumors had higher gene expression for select immune-associated genes, including *CD274*, *PDCD1*, and *CD8A*. The immune190 signature and three additional immune hallmark signatures, IFNγ, IFNα, and inflammatory response, were all significantly associated with pathological complete response after receipt of pembrolizumab. These data are in alignment with the observations from the ABACUS trial, which found that preexisting immune infiltrates were key for predicting response to therapy.

There were several notable associations of molecular subtypes and immune gene signatures with patient outcome in PURE-01. In the first gene expression profiling study for PURE-01, the GSC claudin-low subtype had exceptional recurrence-free survival, with 0/11 events in 24 months [18]. In contrast, with NAC, the claudin-low patients had high rates of disease progression, suggesting neoadjuvant pembrolizumab is a highly favorable treatment option for these patients. When additional outcome data became available, the event-free survival rates remained extremely favorable, with only a single event in 14 patients over 24 months [30]. The basal-type tumors from the TCGA and consensus model were not significant for either RFS or EFS, in the initial or expanded study, respectively. However, when the basal-type tumors were subset according to the median immune190 signature scores, a significant association with RFS was identified [18]. These data suggest that immune infiltration and intrinsic subtype may both play a role in predicting long-term benefit from pembrolizumab.

The PURE-01 study also profiled 26 radical cystectomy samples collected post-pembrolizumab [34]. These samples were compared to a cohort of post-chemotherapy radical cystectomy samples (n = 133) and to a cohort of radical cystectomy samples without systemic therapy (n = 94). The gene expression profiles for these samples had several consistencies with each other but also with the ABACUS study. First, in all three cohorts, regardless of systemic therapy, there were numerous cases classified as stroma-rich by the consensus model [15]. As this subtype is defined by high stromal and immune cell infiltration, these data have

interesting parallels to the enrichment for the "infiltrated" subtype in the post-atezolizumab RC samples from the ABACUS study. The PURE-01 RC study defined these tumors as "scar-like," represented by high stromal marker expression, higher angiogenesis activity, and lower levels of proliferation, which is directly in alignment with the reports from ABACUS. Second, after NAC or surgery alone, there was a good representation of basal and luminal tumors, which contrasted with the post-pembrolizumab subtypes. In PURE-01, there was a poor representation of basal-type tumors (4/26) compared to luminal tumors (9/26). Moreover, the scar-like tumors post-pembrolizumab also showed expression of many luminal markers, differing from the other two cohorts. Given luminal tumors tend to be immune desert phenotype, it is tempting to speculate that the resistant tumors collected post-pembrolizumab represent an intrinsic resistance mechanism of luminal tumors, represented by a higher ratio of luminal tumors post-pembrolizumab. However, the sample sizes in the PURE-01 study are small, so these observations require further data to corroborate this hypothesis.

DNA-Based Molecular Biomarkers

The interim study results for PURE-01 reported that patients with higher tumor mutational burden (TMB) had a significant pathologic response to pembrolizumab [JCO ref]. As additional study data became available, however, follow-up reports found TMB was neither significantly associated with complete response ($p = 0.06$) [18] nor did TMB appear to significantly predict event-free survival outcomes on multivariable analyses ($p = 0.2$) [30].

The PURE-01 study also investigated the mutational status in the exons of 395 cancer-associated genes and select introns from 31 genes that are frequently rearranged in cancer. Of these, only *PBRM1* mutations were found to have a significant association with complete response, although this was no longer significant after multiple hypothesis testing. Furthermore, the observed associations between DDR and *RB1* gene alterations were weakened after multivariable adjustments for TMB. These data suggest that mutational status at either the individual gene level or whole genome level is not a significant predictor of response to pembrolizumab.

The ABACUS study found that several genes involved in the fibroblast growth factor (FGF) pathway were amplified in responding tumors [19]. Interestingly, increased FGF pathway activity tends to be associated with tumors of the luminal subtype, which are also typically immune-depleted [13, 15, 33] and therefore would not be predicted to have good response to therapy [10]. To attempt to reconcile these discrepancies, multiple fibroblast growth factor receptor-3 (*FGFR3*) genomic alterations (GA) were investigated as candidates for predicting response to pembrolizumab in PURE-01 [35]. In this study, *FGFR3* mutations, gene expression, and pathway activity were all investigated. In addition, using a molecular signature based on long noncoding RNA expression, a subgroup of luminal tumors with

excellent prognosis and amplified FGFR3 activity was identified and evaluated. Only higher *FGFR3* gene expression was found to have a significant association with lower rates of complete response, but this is likely balanced by multiple clinical and biological factors. Further study is warranted, and until such time, it was not recommended to exclude patients with *FGFR3*-altered tumors from neoadjuvant immune checkpoint therapy.

Integrating Biomarkers to Refine the Molecular Diagnosis in the PURE-01 Study

The PURE-01 study was an important clinical trial moving pembrolizumab into the cisplatin-eligible neoadjuvant MIBC setting. As was observed with the ABACUS study, preexisting immune infiltration was a significant predictor of response to therapy, indicating immune infiltration is a common link to predict responses to atezolizumab or pembrolizumab. However, the nature of the intrinsic subtype may also be relevant as claudin-low tumors had exceptional outcomes with pembrolizumab. Splitting basal-like tumors by immune190 signature scores revealed similar outcomes, indicating basal features with high immune infiltration may have the most favorable response to pembrolizumab. Comparatively, traditional biomarkers used to predict response to immune therapy, such as TMB and PD-L1 IHC, were significantly associated with pathological response but not with outcome. These data would suggest that local control of the primary tumor may be informed using these tools, but more advanced RNA-based signatures would be preferred for predicting long-term benefit. Finally, the prevalence of luminal and scar-like tumors expressing luminal makers in the PURE-01 radical cystectomy cases suggests that luminal tumors may have an intrinsic resistance to pembrolizumab or that clones of the luminal subtype may be selected for during therapy.

The NABUCCO Trial: Neoadjuvant Ipilimumab with Nivolumab

The NABUCCO study evaluated a combination of two immune checkpoint inhibitors, ipilimumab (anti-CTLA4) and nivolumab (anti-PD1), in the neoadjuvant setting [16]. The study endpoint was the feasibility to resect within 12 weeks from initiation of treatment. This study was a single-arm, phase 1 feasibility trial including 24 patients with locoregionally advanced (cT2-4aN0-3M0) urothelial carcinoma. With all patients in the NABUCCO trial undergoing surgical resection, 23/24 patients received surgery within 12 weeks, therefore meeting the primary endpoint of the study. Of the 24 included patients, 11 (46%) had a pathological complete response, and 14 (58%) had no remaining invasive disease (pT0N0 or pTisN0/

pTaN0). The median postoperative follow-up was 8.3 months (ClinicalTrials.gov Identifier: NCT03387761).

Immunohistochemistry

Like the PURE-01 study, baseline PD-L1 IHC was performed using the 22C3 assay and the combined positive score (CPS). The pathological complete response rate was higher (73%) for PD-L1-positive tumors compared to PD-L1-negative tumors (33%), but this was not significant ($p = 0.15$). Quantitative multiplex immunofluorescence was used to analyze correlations between baseline CD8+ T-cell density and treatment response, as was done in the ABACUS trial. There was no correlation with combination anti-PD1 and anti-CTLA4 immunotherapy, suggesting this regimen has the potential to induce pathologic response, irrespective of preexisting CD8 levels. Multiplex immunofluorescence was also used to establish CD20+ B-cell counts, revealing stromal B-cell counts were significantly increased in nonresponding tumors compared to responding tumors. Of note, the presence of increased B cells in nonresponders was irrespective of preexisting CD8+ T-cell immunity. Finally, multiplex immunofluorescence was also used to quantify the dynamics of tertiary lymphoid structures (TLS). Although baseline TLS was not associated with treatment response, comparison of pre- and post-therapy tissue specimens did show an enrichment in TLS among tumors that responded to therapy. Further analysis of the TLS dynamics indicated that regulatory T cells were reduced in TLS upon treatment. Since TLS are ectopic lymphoid formations generally found in inflamed, infected, or tumoral tissues [36], these findings could potentially be in alignment with the scarring phenotypes observed on post-therapy specimens from the PURE-01 and ABACUS studies.

RNA-Based Molecular Biomarkers

Preexisting immunity was also assessed by transcriptomic signatures that also had been shown to have potential utility within the PURE-01 and ABACUS studies. However, neither the baseline IFN-γ, tumor inflammation, nor CD8+ T-cell effector (tGE8) signatures were associated with a complete response, in contrast to the observations from the ABACUS trial. Notably, the use of different immunotherapy drugs, differences in patient populations, and a lack of standardized biomarker platforms may contribute to these inconsistencies. Notably, a TGF-β gene expression signature was found to be associated with nonresponse to ipilimumab/nivolumab, which aligned with the proposed mechanism of resistance in the ABACUS study, where TGF-β-mediated T-cell inhibition was suggested to drive the immune excluded phenotype, which is resistant to atezolizumab [19]. Finally, hierarchical clustering analyses of differentially expressed genes between responders and

nonresponders revealed an upregulation for the expression of B-cell-related genes in patients with tumors that did not respond. Of note, expression of these "B-cell genes" positively correlated with B-cell counts on immunofluorescence, confirming the results of the differential gene expression analyses.

DNA-Based Molecular Biomarkers

Despite the small sample size of the NABUCCO trial, tumors achieving complete pathologic response had slightly higher levels of TMB on pretreatment tissue specimen, but this was not a significant difference ($p = 0.056$). Further analysis of mutations in a set of DDR genes revealed alterations in these genes were more frequently observed for responding tumors. As was observed for the PURE-01 trial, TMB and DDR gene alterations seem to represent promising biomarkers, based on these initial reports. However, thresholds for "high" TMB and DDR are not yet standardized. Importantly, these initial findings warrant further evaluation within an additional representative cohort, should any clinical utility be confirmed.

Integrating Biomarkers to Refine the Molecular Diagnosis in the NAMBUCCO Study

The NAMBUCCO study investigates the addition of anti-CTLA-4 to PD-1 blockade in the neoadjuvant setting for locoregionally advanced MIBC. Unlike the ABACUS and PURE-01, preexisting immune infiltration was not correlated with response. As this is a feasibility study, an expanded trial will be important in better understanding the underlying biology driving response to this combination therapy.

Neoadjuvant Durvalumab with Tremelimumab (MDACC)

The University of Texas MD Anderson Cancer Center (MDACC) initiated the first pilot combination trial of neoadjuvant durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4), recruiting a total of $N = 28$ patients with "high-risk," cisplatin-ineligible, operable muscle-invasive bladder cancer [17]. Of note, "high risk" was defined as having features including bulky tumors, variant histology, lymphovascular invasion, hydronephrosis, and/or high-grade upper tract disease. The primary study endpoint for MDACC was safety. Of the 28 patients recruited, 24 patients ultimately underwent cystectomy as per study protocol. From these 24 patients, 9 (37.5%) achieved a complete pathological response, and the relapse-free survival rate was 82.8% at 1 year (ClinicalTrials.gov Identifier: NCT02812420).

Immunohistochemistry

Like the ABACUS trial, PD-L1 immunohistochemistry within the MDACC trial was executed using the E1L3N assay, and like the ABACUS trial, PD-L1 expression did not correlate with treatment response. Multiplex immunofluorescence staining was also used to identify tertiary lymphoid structures (TLS) as in the NABUCCO trial. Although baseline TLS numbers were not associated with treatment response within the NABUCCO trial, the MDACC study results included a higher density of TLS in pretreatment tissue of responders, which corresponded with favorable outcomes. Further characterization of immune cell subsets revealed that responding tumors had significantly higher density of pretreatment B cells, CD4+ T cells, and CD8+ T cells, again suggesting utility for preexisting immune infiltration. Finally, comparing pre- and post-immunotherapy specimen showed an increase in ICOS+CD4+ T cells in posttreatment tumor tissues of responding tumors compared to nonresponders. These observations align with the concept of increased immune infiltration for responding tumors post-therapy, reinforcing the concept of tumor scarring which, at least in part, involves immune cell recruitment [37]. The scarring phenomenon has been proposed (albeit in different contexts) for all three of the previous studies.

RNA-Based Molecular Biomarkers

A four-gene TLS expression signature (*POU2AF1*, *LAMP3*, *CD79A*, and *MS4A1*) was found to be significantly higher in responding tumors in the MDACC study. However, the tGE8 signature scores were not found to be significantly different when comparing responders to nonresponders. Unfortunately, this signature has only demonstrated significance for predicting response to atezolizumab monotherapy (ABACUS) and lacks clear utility in either the NABUCCO or MDACC trials, suggesting this signature in the context of combination immunotherapy is of minimal utility.

DNA-Based Molecular Biomarkers

Whole exome sequencing for the MDACC trial found that neither TMB nor DDR GA correlated with response to therapy. This study also predefined *KRAS*, *PIK3CA*, *PBRM1*, *EFGR*, *NRAS*, *APC2*, and *FGFR* mutations as interesting targets for investigation, although none of these were found to have an association with response to therapy in context of this pilot study. Similarly, the ABACUS study explored the fibroblast growth factor (FGF) pathway as a potential region of interest, but neither

the PURE-01 nor MDACC trials found this pathway to be predictive of response to immune checkpoint blockade.

Integrating Biomarkers to Refine the Molecular Diagnosis in the MDACC Study

The MDACC study was similar to NABUCCO, combining an anti-CTLA4 inhibitor (tremelimumab) with an anti-PD-L1 inhibitor (durvalumab). Likewise, this study also included patients with more aggressive disease, with MDACC including patients with variant histology. Like ABACUS and PURE-01, response in MDACC appeared to have correlation with preexisting immune infiltration. However, these data contrast with the findings from NABUCCO, which used a similar combination of checkpoint inhibitors. Further study will be required to reconcile these differences and identify the clinical and biological features which may help predict response to combination anti-CTLA and anti-PD-L1 therapy.

Conclusions and Future Directions

There are several challenges associated with transitioning immune therapy into the neoadjuvant setting, many of which may be mitigated, at least in part, by biomarker-driven approaches [38]. First, the pathological response rates of IBC are comparable to NAC, averaging about 40% overall [39], which raises questions as to whether these patients may have received greater benefit from chemotherapy or radical cystectomy alone. As discussed for PURE-01, patients with a tumor of the claudin-low subtype had exceptional outcomes with pembrolizumab, but comparatively poor outcomes with NAC [18], providing compelling data that molecular subtypes may provide a mechanism to stratify patients to the treatment which would provide the greatest benefit. One caveat to this finding was that the claudin-low tumors did show a significant association with pathological response, which may contradict the data generated in the NAC setting, where complete response was associated with improved survival [2].

This may be an issue associated with radiological tumor assessment, as this has not been standardized for immune therapy in the neoadjuvant setting [38], although multiparametric magnetic resonance imaging has shown promise in this respect [40]. At this time, we have limited information on how immune therapy impacts the biology and phenotype (i.e., volume) of patient tumor, which may confound our ability to connect pathological response and outcomes. There may also be the added effect of the TURBT procedure which until recently has not been considered in molecular profiling studies.

In the PURE-01 study, three molecular subtypes were identified post-pembrolizumab, including luminal, basal, and "scar-like" tumors [34]. The latter, defined largely by higher expression of stromal markers, represented half of the PURE-01 RC samples. Notably, in the ABACUS study, responding tumors were predominantly found to be an infiltrated subtype (Lund 2012 model). Like the "scar-like" subtype, the infiltrated subtype is defined by high levels of stromal and immune infiltration, suggesting a commonality between these two classifications. This scar-like subtype has been suggested to be the result of the impact of TURBT, resulting in a wound healing at the tumor site [34, 41]. Given the high rates of the infiltrated subtype and scar-like in ABACUS and PURE-01, respectively, it is tempting to speculate that TURBT, rather than systemic immune therapy, has a greater impact on local tumor control, while systemic therapy offers metastatic control and improved outcomes. This may explain, at least in part, why certain molecular signatures (i.e., claudin-low subtype) were not significantly associated with response yet were significantly associated with patient outcome. The truth is likely somewhere in between, where local tumor control in terms of pathological response is achieved by a combination of surgery (TURBT) and systemic therapy.

In a recent post-chemotherapy study, four molecular subtypes were identified, including a scar-like subtype and a highly immune-enriched subtype which was not identified in the PURE-01 study [34, 41]. In the ABACUS study, the infiltrated subtype is considered immune-enriched but is also enriched with stromal type cells (i.e., myofibroblasts) [26], where the post-NAC immune subtype did not report stromal infiltration [41]. In both studies, amplified or increased immune activity was also reported for treated tissues in both checkpoint studies, suggesting that a generalized immune response is achieved with either neoadjuvant chemotherapy or immune therapy [18, 19]. In the ABACUS study, the infiltrated subtype was enriched post-pembrolizumab, while luminal (UroA, GU, UroB) and basal (SCCL) tumors were evenly represented [19]. In contrast, tumors collected post-pembrolizumab were enriched with scar-like and luminal subtypes, with basal tumors poorly represented [34]. An important caveat, when considering the molecular subtype cells generated for the ABACUS study, is most molecular subtyping models are trained on untreated tumor tissue (TURBT and/or RC), meaning accuracy of the subtypes on treated tissues is unknown. The subtypes for PURE-01 were generated using consensus clustering, meaning they are not "true" subtype cells by a classifier, but rather groups of tumors that have molecular features consistent with a representative subtype.

In general, preexisting immunity appears to be a reasonable metric to predict response to checkpoint inhibitors. In ABACUS, PURE-01 and MDACC patients with tumors that showed higher levels of immune infiltration, by a variety of assays, had improved responses compared to those with lower immune infiltration [16–19]. However, the NABUCCO trial did not find a correlation between preexisting immune infiltration and response [16]. This study included patients with more advanced disease, but the small numbers in the trial preclude determining how this may affect any correlation between treatment and response. One of the limitations of using "high immune infiltration" as a method to stratify patients to treatment is

the lack of standardization of a threshold or cut point for determining "high." Another limitation is the range of assays used across the various studies described in this study. To best enable comparison across studies, future trials would ideally include several standardized metrics (i.e., median, quartiles, etc.) and platforms. Another potential option is to standardize the use of molecular subtypes for studies involving gene expression analysis. One advantage of molecular subtypes is the models tend to be categorical, stratifying patients into one of several different subtypes. Several of these molecular subtypes are characterized by higher immune infiltration (i.e., basal or claudin-low) or by a lack of infiltrates (i.e., luminal), suggesting good utility for predicting outcomes, as was demonstrated in the PURE-01 study [18].

Taken together, the ABACUS, PURE-01, NAMBUCCO, and MDACC study, while different in some respects, all provide key biomarker data to further our understanding of which tumor features may be driving response to therapy in the neoadjuvant setting. A commonality among these trials is baseline immunity appears to be predictive of response, except for NABUCCO. However, as an independent biomarker, immune infiltration scores would not facilitate stratification of patients to immune therapy or chemotherapy, which remains the standard of care. Here, molecular subtyping may have greater utility, as different subtypes have now been reported to have varying response to both chemotherapy and immune therapy. After treatment with neoadjuvant immune therapy, there appears to be an enrichment of stromal infiltration, whether defined as an infiltrated subtype [26], scar-like subtype, or increased TLS. Unfortunately, there is no clear pattern for which tumors may adopt such a profile, further emphasizing a need to profile tumor tissue post-therapy. Another advantage of this approach is that the character of the tumor post-therapy may also help to inform adjuvant treatment decisions.

Biomarker development in the neoadjuvant immune checkpoint setting is ongoing, as are the clinical trials in this setting. The data accumulated to date are promising and suggest that selection of patients using biomarkers is highly feasible and may ultimately facilitate the adoption of neoadjuvant immune therapy as a new standard of care.

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Chapter 5
The Role of Circulating Tumor DNA Analyses

Emil Christensen, Karin Birkenkamp-Demtröder, and Lars Dyrskjøt

Circulating cell-free DNA

All cells shed DNA into circulation (cell-free DNA; cfDNA) based on various processes like apoptosis, necrosis, or active release [1, 2]. A fraction of the cfDNA may be circulating tumor DNA (ctDNA) that represents the DNA released from cancer cells, likely associated with cancer cell turnover [3]. DNA entering the blood is degraded, and only DNA fragments protected by, e.g., nucleosomes or DNA-binding chromatin-remodeling proteins, remain long enough in the circulation to enable detection. Accordingly when circulating DNA fragments are aligned to the genome, they form a pattern reflecting the chromatin state of the tissue of origin [4]. ctDNA is found in circulation in often minute amounts mixed with DNA fragments released from normal cells, making robust detection of ctDNA an essential technical challenge to overcome prior to clinical implementation. With a half-life of approximately 2 hours [5], ctDNA provides a direct window into the growing cancer and can provide information about both the presence of cancer and potential therapeutic targets. Detection of ctDNA has been shown to be highly associated with clinical parameters like tumor stage [6], tumor size [7], and metastatic status [8]. Furthermore, as ctDNA is extracted from blood samples, it is easily accessible for serial sampling, e.g. during treatment.

E. Christensen · K. Birkenkamp-Demtröder · L. Dyrskjøt (✉)
Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark
e-mail: echs@clin.au.dk; kbdr@clin.au.dk; lars@clin.au.dk

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ctDNA in Early Detection of Invasive and Metastatic Disease

Bladder cancer is characterized as a highly heterogeneous disease. Studies have demonstrated heterogeneity at interpatient, intertumor, and intrapatient levels, reflecting potential challenges in single tumor tissue biopsy approaches for genomic characterization [9–12]. ctDNA inherently has the ability to represent tumor DNA from all lesions and could more effectively represent the entirety of the mutational spectrum – especially for metastatic lesions that are the primary target of therapy [7, 13]. Importantly, ctDNA detection has been demonstrated to precede radiographic imaging-based detection of metastatic disease. Studies of patients with muscle-invasive bladder cancer (MIBC) have identified lead times in ctDNA-based recurrence detection of approx. 100 days [8, 14]. Similar lead times, however with numerous instances of several months, have been demonstrated in, e.g., lung cancer and colorectal cancer [7, 15, 16]. ctDNA-based recurrence detection could therefore aid clinicians in initiating treatment at an earlier time point, which could prove crucial to prolonging patient survival. The clinical value of earlier treatment based on ctDNA detection is currently being investigated in the TOMBOLA trial (NCT04138628). Detection of invasive disease using ctDNA measurements has also been demonstrated at earlier disease stages before cystectomy using both urine and plasma monitoring [17, 18].

ctDNA in Monitoring Treatment Response

The accessibility and thereby potential for frequent longitudinal sampling make ctDNA a compelling biomarker for evaluating treatment efficacy. A recent study with 27 patients treated with durvalumab analyzed ctDNA status before initiation of treatment and after 6 weeks of treatment. Patients with response to treatment demonstrated significantly lower ctDNA levels after 6 weeks of treatment, whereas patients with stable or progressing disease demonstrated no significant change in ctDNA level. Patients with an increase in ctDNA level had a median progression-free survival of 1.63 months, while patients with a reduction in ctDNA level had a median progression-free survival of 13.8 months [19]. Furthermore, in a trial where patients received both pembrolizumab and radiotherapy for metastatic urothelial carcinoma, treatment response was also associated with a ctDNA fraction decline [20].

ctDNA-focused studies on MIBC have demonstrated a reduction in ctDNA during treatment with chemotherapy in both plasma [14] and urine samples [21]. A recent study by Christensen et al. identified ctDNA clearance to be associated with response to chemotherapy and ctDNA persistence to be associated with a lack of response. Interestingly, the association between the dynamics of ctDNA during treatment and disease recurrence was more pronounced than the association between pathological downstaging status at cystectomy and disease recurrence thereby indicating ctDNA analysis might more accurately reflect treatment response [8].

ctDNA level before initiation of treatment with immune checkpoint inhibitor (ICI) has been associated with outcome in non-small cell lung cancer (NSCLC), and the relative ctDNA level after only one infusion with ICI was also associated with outcome [22]. In addition, a reduction in ctDNA during treatment with ICI has been associated with improved outcome and elevated response rates in both NSCLC and gastric cancer [23, 24]. A recent study analyzed ctDNA after chemoradiation in patients with NSCLC and demonstrated a lack of ctDNA to be associated with a low progression rate. Interestingly, a subset of patients were subsequently treated with ICI, and for ctDNA positive patients, a clearance of ctDNA during treatment with ICI was associated with outcome [25]. Treatment with ICI has demonstrated remarkable long-term efficacy in some patients; a recent study by Hellmann et al. analyzed ctDNA status in long-term responders to PD-L1 treatment and identified a 93% ($n = 25$) disease-free rate in the ctDNA-negative patients and a 0% ($n = 4$) disease-free rate in the ctDNA-positive patients.

Tumor mutation burden (TMB) has been extensively described as a promising predictive biomarker for immunotherapy response – also in bladder cancer [26], however tumor heterogeneity might complicate precise assessment of the tumor mutation burden. Evaluation of TMB using ctDNA may have technical limitations due to the degraded nature of the DNA, but if this limitation could be overcome and standard laboratory and bioinformatics methods are developed, ctDNA-based TMB measures could aid in selecting patients with a high likelihood of response to immunotherapy in the future. A study by Wang et al. indeed found ctDNA-based TMB to be associated with progression-free survival and response rate for patients with NSCLC treated with ICI [27].

Collectively, these observations reflect an association between ctDNA and tumor burden – and ctDNA may harbor predictive value related to TMB measurements. ctDNA could therefore become an integral part of treatment monitoring as a surrogate endpoint for patients. Furthermore, the results outlined above indicate ctDNA could become a powerful marker for selecting patients for treatment and for assessing the risk of recurrence after completion of treatment. If validated in further studies, the expanding application of ICI in treating patients with MIBC could be accompanied by ctDNA analysis for selecting patients for treatment and for monitoring treatment efficacy.

Technical Considerations

The amount of cfDNA in the blood circulation depends on clearance by the kidney, degradation kinetics, as well as other physiological processes [2]. Elevated cfDNA levels complicate ctDNA detection, and it is therefore important to limit cell lysis after blood draw, and blood samples should consequently be either drawn in dedicated cfDNA conservation tubes or plasma should be isolated within 1.5–2 hours and snap frozen at −80C. High cfDNA levels immediately post-surgery, probably caused by surgical trauma, have recently been observed in patients with bladder and

colorectal cancer which complicates ctDNA detection in this important setting. Identification of mutated DNA fragments at very low frequency may require large blood volumes.

A range of technological platforms exists for efficient and accurate detection of ctDNA. To date, the most sensitive and specific approaches involve a tumor-guided analysis where detection of specific ctDNA mutations is guided by the somatic mutations identified in the primary tumor [7, 28]. In this way false-positive ctDNA tests due to mutations from, e.g., clonal hematopoiesis and other premalignant lesions are avoided. Droplet digital PCR (ddPCR) is a highly sensitive and specific method that has a sensitivity down to 1/10,000 – however, the disadvantage is that it is difficult to multiplex assays, and hence only few mutations can be assessed per DNA sample [29]. Deep targeted sequencing of patient-specific mutations has also shown high sensitivity, and here the advantage is that more DNA mutations can be interrogated per DNA sample, which may increase sensitivity. Non-patient-specific cancer panels have been reported to also yield high sensitivity, but there is a risk that no mutations can be detected for the individual patient using this approach [30, 31]. Furthermore, deep sequencing on larger panels containing multiple probes for tiling frequently mutated genes is associated with significant costs. Finally, whole exome and whole genome sequencing (WGS) may be applied in cases where ctDNA levels are high during treatment for metastatic disease. Larger panel sequencing (exome or smaller) or whole genome approaches pave the way for monitoring novel genomic variants that could be associated with treatment resistance, and hence new therapeutic approaches may be applied based on this [32].

Future Aspects

The majority of ctDNA-based studies to date employ tumor-informed approaches that necessitate tumor analysis prior to ctDNA analysis. This serves the purpose of only investigating molecular markers that are specific to a given patient's tumor and facilitates easier detection of clinical relevant mutations in circulation. However, novel mutations arising, e.g., during treatment are not possible to assess using this approach. Furthermore, the rarity of ctDNA in many samples creates a sampling issue, as a random draw of blood might not always contain DNA fragments with mutations at the investigated genomic sites. To circumvent sampling issues and avoid having to sample large volumes of blood, recent studies have employed WGS-based approaches to detect ctDNA. A study by Zviran et al. demonstrated a method for integrating mutation signal and copy number signal across the genome to accurately detect ctDNA in both melanoma and colorectal cancer patients [33]. Importantly, the identification of tumor-derived mutation signals at few specific genomic sites in most cases requires deep sequencing, which makes WGS too costly. However, the integration of signal across the genome enables the detection of signal while requiring only a modest sequencing depth. WGS-based approaches further enable the integration of DNA fragment size, which has been found to be smaller in

fragments originating from tumor cells [34]. Christiano et al. integrated fragment size data with mutation data and detected ctDNA in 91% of patients with a wide range of cancers without utilizing prior tumor information [35].

Besides investigating cfDNA, future studies may benefit from integrating biological information from other molecular layers such as the T-cell receptor alpha repertoire of naïve and memory CD8+ T cells. The T-cell receptor (TCR) repertoire provides a window into the cellular adaptive immune response. In the context of cancer, determining the repertoire within a tumor can give important insights into the evolution of the T-cell anticancer response and has the potential to identify specific personalized biomarkers for tracking host responses during cancer therapy, including immunotherapy. This has, e.g., been applied in early-stage breast cancer, where TCR DNA sequencing of serially collected peripheral blood samples and tumor tissue was used as a biomarker for T-cell responses to therapy showing an increase of TCR DNA sequences after cryoablation + ipilimumab combination therapy [36].

Finally, the application of ctDNA analysis to guide treatment decisions needs to be validated in clinical trials to demonstrate clinical value in terms of improved survival and quality of life and reduced costs from optimizing treatment to relevant patient groups only. Several studies on bladder and other cancers are currently ongoing to address this.

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Chapter 6
The Value of Tumor Sample Analyses Before and After Checkpoint Inhibition: Contextualizing the Treatment-Induced Changes in Gene Expression

Lauren Folgosa Cooley, A. Gordon Robertson, and Joshua J. Meeks

Introduction

Neoadjuvant cisplatin-based chemotherapy (NAC) prior to radical cystectomy is supported by multiple randomized trials based on improved cancer-specific and overall survival for muscle-invasive bladder cancer (MIBC) [1–5]. Yet, less than half of MIBCs are treated with NAC prior to cystectomy, as ~50% of MIBCs are cisplatin ineligible, and many patients defer chemotherapy until after surgery [6, 7]. Immune checkpoint inhibitors are revolutionizing the treatment landscape of urothelial carcinoma (UC) and have recently shown promising long-term efficacy and patient tolerability in the neoadjuvant setting. Agents targeting the programmed cell death-1 (PD-1) receptor/PD-1 ligand (PD-L1) checkpoint pathway are currently approved for first- and second-line metastatic UC patients who are unfit for or non-responsive to platinum-containing chemotherapy. In this review, we will focus

L. F. Cooley
Department of Urology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

A. G. Robertson
Dxige Research Inc., Courtenay, BC, Canada

J. J. Meeks (✉)
Department of Urology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA

Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA

Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL, USA

Departments of Urology and Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
e-mail: joshua.meeks@northwestern.edu

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mainly on genomic and immunologic biomarkers assessed in three recent neoadjuvant immunotherapy trials: PURE-01 (NCT02736266), ABACUS (NCT02662309), and NABUCCO (NCT03387761) [8–14]. The neoadjuvant paradigm is unique in allowing analysis of both pre- and posttreatment tissue, which facilitates biomarker analysis. We discuss this and contextualize the molecular- and immunologic-based biomarkers investigated in these trials to predict response and outcomes to neoadjuvant immunotherapy in nonmetastatic MIBC patients.

PURE-01, ABACUS, and NABUCCO

There have been two major single-arm phase II trials (PURE-01 and ABACUS) and one single-arm feasibility trial (NABUCCO) of neoadjuvant immunotherapy prior to radical cystectomy (RC). The drug, dosing, inclusion criteria, and results differ between each trial. Each trial has both pre- and posttreatment tumors available for RNA expression and DNA analysis (Table 6.1).

PURE-01

PURE-01 is a phase II trial of neoadjuvant pembrolizumab prior to RC in newly diagnosed patients with cT2-T3bN1M0 MIBC who had not received prior chemo- or immunotherapy [11, 12]. From 2017 to 2019, 114 patients were recruited from

Table 6.1 Neoadjuvant immunotherapy trials in MIBC

	Pembrolizumab	Atezolizumab	Nivolumab + Ipilimumab
Study name	PURE-01	ABACUS	NABUCCO
Study phase	II	II	I
No. patients	114	95	24
Cisplatin eligibility	92% eligible	100% ineligible or refused	54% ineligible 46% refused
Prevalence of PD-L1 positivity in whole cohort	67/114 (59%)	39/95 (41%)	15/24 (63%)
PD-L1 positivity cutoff values	PD-L1 CPS ≥10%	PD-L1 CPS >10	≥5% IC staining
PD-L1 biomarker	Dako 22C3	Ventana SP142	Dako 22C3
Biomarkers	PD-L1 expression DNA analysis	PD-L1 expression DNA analysis RNA analysis Immunohistochemistry	PD-L1 expression DNA analysis RNA analysis Immunohistochemistry Multiplex immunofluorescence

CPS combined positivity score (tumor PD-L1 + immune cell PD-L1), *IC* immune cell

two centers in Milan, Italy, of which 92% were cisplatin eligible [11, 12]. Inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status 0–2, predominant UC histology, and a glomerular filtration rate of ≥20 mL/min. The cohort had a median age of 66 (IQR 60–71) and was predominantly male (87%) and prior or current smokers (72%). Most patients presented with de novo MIBC (82%) and did not have prior Bacillus Calmette-Guerin (BCG) exposure (88%). Many patients, 50/114 (44%), were cT3 prior to surgery, and all patients had residual cancer at the time of neoadjuvant immunotherapy. Following transurethral resection of the bladder tumor (TURBT), patients received 3 cycles of 200 mg pembrolizumab intravenously every 3 weeks, with the trial-specified goal of performing RC within 3 weeks of completion (the median time to RC for the initial 50 patients accrued was 22 days, and the interquartile range (IQR) was 15–30 days). The primary endpoint was pathologic complete response (pT0); secondary endpoints were pathologic downstaging, safety, and biomarker analysis (PD-L1). Patients that did not have an observable response to neoadjuvant immunotherapy underwent chemotherapy prior to surgery (7/114 patients, 6.1%). Biomarker analysis was performed on pretreatment (TURBT) and posttreatment RC tumor specimens [11, 12].

ABACUS

ABACUS is an open-label, multicenter, single-arm phase II trial of neoadjuvant atezolizumab in 95 T2-T4aN0M0 UC patients who were ineligible or refused NAC and planned to undergo RC [13]. Additional eligibility criteria included ECOG 0–1, residual disease post-TURBT, adequate fitness for RC, no nodal or metastatic disease on imaging, and adequate hematologic and end-organ function within 4 weeks of first treatment. Patients received 2 cycles of atezolizumab (1200 mg every 3 weeks) with a median of 5.6 weeks between starting atezolizumab and RC. The ABACUS cohort had a median age of 72 years and was predominantly male (85%), current or prior smokers (78%), and without a prior history of non-muscle-invasive disease (85%) or BCG exposure (88%). The primary endpoint was pathologic complete response. Secondary endpoints included pathologic response in PD-L1-positive tumors, safety analysis, response rate, and relapse-free survival.

NABUCCO

NABUCCO is a single-arm feasibility trial in 24 patients with stage III UC who received dual checkpoint blockade consisting of two doses of ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4)) (3 mg/kg on day 1 and 22) and two doses of nivolumab (anti-PD-1) (1 mg/kg on day 22 and 43) prior to RC [14]. Patients were either ineligible for (54%) or refused (46%) NAC. The NABUCCO cohort was predominantly male (75%) with a median age of 65 years.

The primary endpoint was centered around safety of immunotherapy. All patients tolerated immunotherapy and no surgeries were delayed longer than 12 weeks. Secondary endpoints were pathologic complete response (ypT0N0). An exploratory analysis was reported for immune-related predictors of response. Dual checkpoint blockade resulted in grade 3–4 immune-related adverse events in 55% of patients, and only one patient had a delay in time to RC by 4 weeks.

Tumor Factors

Tumor PD-L1 Expression

PD-1 is upregulated on CD8+ T cells in response to acute or chronic antigen exposure [15, 16]. The interaction of PD-1 on CD8+ T cells and PD-L1 on tumor cells or other immune cells leads to T cell inactivation and loss of T cell proliferative capacity, negatively affecting T cell-mediated antitumor responsiveness [15, 16]. This suggested that inhibiting the PD-1/PD-L1 pathway could boost T cell-mediated antitumor immunity and that tumors with higher PD-L1 expression may be the most susceptible to this blockade. However, using PD-L1 as a biomarker of responsiveness to PD-1/PD-L1 checkpoint blockade has proved to be more complex than anticipated, with outcomes varying depending on the agent used, prior systemic therapy status, stage of disease, and companion biomarker utilized in analysis.

The PD-L1 biomarker for pembrolizumab is Dako 22C3, which measures PD-L1-positive immune cells and tumor cells by immunohistochemistry (IHC) [17]. The combined positive score (CPS) separates PD-L1-positive tumors at a cutoff of 10%. In untreated patients with metastatic UC, the biomarker-enriched population from Keynote-361 (NAC vs pembrolizumab monotherapy) had an HR of 1.01 for PD-L1-positive tumors [9, 18]. In PURE-01, however, PD-L1 CPS $\geq$ 10% related directly to pT0 status at the time of RC: 19 of 35 patients (54.3%) with PD-L1 CPS $\geq$ 10% compared to only 2 of 15 patients (13.3%) with PD-L1 CPS < 10% achieved pathologic complete response ($p = 0.011$) [11]. Of note, pembrolizumab did not significantly induce PD-L1 expression when comparing pre- and post-cystectomy tumor specimens [11].

For atezolizumab, the PD-L1 IHC biomarker is the Ventana SP142, measured only in immune cells [17]. PD-L1 status on immune cells is scored as IC0 < 1%, IC1 = 1 to <5%, IC2 = 5 to <10%, and IC3 $\geq$ 10%, with a score $\geq$ 5% being considered PD-L1 positive [13]. In first-line metastatic UC (IMvigor130), the atezolizumab monotherapy group B had a similar percentage of IC2/3 tumors (88 of 362, 24%) as the placebo plus NAC group C (91 of 400, 23%) [10]. Median overall survival was non-estimable for group B, compared to 17.8 months for group C (HR

0.68, 95% CI 0.43–1.08). Furthermore, there was no difference in objective response rate in IC2/3 subgroups between groups B and C (39% vs 37%) [10]. In the ABACUS trial (atezolizumab), 40% of the cohort was PD-L1-positive tumors; these tumors had a pathologic complete response rate of 37.1% (95% CI 21.5–55.1), compared to 24.5% (95% CI 13.3–38.9) for PD-L1-negative tumors ($p = 0.21$) [13]. Two cycles of neoadjuvant atezolizumab resulted in increased PD-L1 expression comparing TURBT to posttreatment RC samples ($p < 0.001$). However, increased expression of PD-L1 in TURBT did not distinguish responders from nonresponders [13]. Furthermore, there was no correlation between PD-L1 expression and one-year relapse-free survival (PD-L1 positive, 75%, 95% CI 53–87, vs whole cohort, 79%, 95% CI 67–87). Collectively, this data suggests that post-immunotherapy, PD-L1 expression increases, regardless of pathologic response, disease-free survival, or companion antibody.

To increase the modest improvement in response to anti-PD1/PD-L1 antibodies compared to chemotherapy, the addition of an anti-CTLA4 antibody was hypothesized to increase the systemic response of PD-L1 low-expressing tumors in metastatic UC. Phase III data from DANUBE, which combined durvalumab (anti-PD-1) plus tremelimumab (anti-CTLA4), in locally advanced and metastatic UC, demonstrated promising results in PD-L1-positive tumors, with an objective response rate of 29% and a median overall survival of 18.9 months, compared to 22% and 9.5 months in the overall cohort [19]. In NABUCCO (nivolumab plus ipilimumab), the PD-L1 biomarker used was 22C3. The tumor PD-L1 positivity rate (PD-L1 CPS >10%) was 63% ($n = 15$) [14]. Complete pathologic response in PD-L1-positive tumors was 73% (95% CI 42–99) compared to 33% (95% CI 7–70) in PD-L1-negative tumors ($p = 0.15$) [14]. While a trend was noted for greater response in PD-L-positive tumors, a larger sample size will be needed to draw robust conclusions for response based on biomarker status. Furthermore, the role of PD-L1 as a biomarker for combination blockade is still under investigation in the neoadjuvant (NCT03387761) and advanced UC (NCT03682068 and NCT03036098) settings.

There are two notable challenges for interpreting PD-L1 expression and its relationship to patient survival in the above trials. First, the tumors analyzed had prior TURBT and in some cases BCG exposure, which may alter the interpretation of PD-L1 expression [8, 11, 20, 21]. Second, the criteria, subjective quantification, and reproducibility of PD-L1 staining vary across studies, depending on the companion biomarker. PD-L1 positivity can range between 25 and 55%, depending on the study and the biomarker used [21–23]. These potential confounders make interpretation of PD-L1 as a predictive biomarker for neoadjuvant immunotherapy challenging. While not currently utilized in UC, PD-L1 gene expression by RNA (RNA-seq or RT-PCR), rather than immunohistochemistry, may be an alternative method that is more easily compared and replicated across study centers [24, 25]. To date, the post-immunotherapy prognostic value of PD-L1 positivity has not been evaluated.

Expression Subtyping

MIBCs can be clustered into subsets based on RNA expression patterns that resemble breast cancer subtypes [26]. MIBC subtyping has evolved from a system with two subtypes that included basal or luminal tumors (UNC) to three (MDA), four (TCGA 2014), five (TCGA 2017), and more [26–31]. While subtyping is prognostic for untreated tumors, there does not appear to be a tumor subtype that is predictive of response to immunotherapy. In an evaluation of IMvigor210 cohort, the Lund genomically unstable (GU) subtype had the best response to atezolizumab, with more than 60% with stable or improved response [32]. An independent evaluation of the same cohort identified a clinical response in 8 of 11 neuroendocrine tumors using a TCGA 2017 classifier [32]. Evaluating bladder cancers at earlier stages (such MIBC) suggests different responses by expression subtype. In the PURE01 cohort, basal subtypes had an improved pathologic response that did not reach statistical significance [12]. In ABACUS, tumor subtype evaluation identified no significant differences in response by subtype, but after atezolizumab treatment, the frequency of luminal-infiltrated tumors increased in pathologic-responsive tumors (14/15 complete responders) [13]. Alternatively, 7/16 resistant tumors were basal subtype post-immunotherapy, suggesting that basal tumors in ABACUS may be more resistant to immunotherapy [13]. Collectively, a comparison of pre- and post-tumor subtyping suggests major subtype switching occurs with immunotherapy, with few pure luminal tumors remaining after immunotherapy. It will be important to determine if posttreatment subtyping is correlated with disease-free survival or response to therapy at the time of metastasis.

Immune and Stomal Factors Within the Tumor Microenvironment

Immune Tumor Microenvironment (TME)

In solid tumors, response to immunotherapy is shaped by the composition of the tumor microenvironment (TME). Based on the TME originally investigated in melanoma, three states or architectures have been described: inflamed, immune excluded, and immune desert [33]. Inflamed tumors are infiltrated with CD8+ T cells and also have increased interferon gamma (IFNγ) signaling, PD-L1 expression, and the presence of pro-inflammatory immune cell subsets. Immune-excluded tumors have a physical barrier of stromal cells between the tumor and immune cells. While excluded tumors have CD8+ T cells, these T cells are spatially separated from the tumor and express markers of inhibited T cell activity or exhaustion. Immune populations in excluded tumors are predominated by regulatory cells (e.g., regulatory T cells, myeloid-derived suppressor cells, and M2 macrophages). Immune-desert tumors have more prominent stromal components, without immune

cell or CD8+ T cell infiltration within the vicinity of the tumor [33]. Based on prior evaluation of TME phenotypes from the IMvigor210 cohort, the ABACUS cohort stratified response status by immune TME [13]. No pathologic responders were identified in the immune-desert phenotype ($n = 6$), with 4/6 having stable disease and 2/6 experiencing relapse. Responders to immunotherapy ($n = 24$) were either excluded ($n = 8$) or inflamed ($n = 16$) phenotypes, with inflamed tumors further subdivided into CD8+ GranzymeB low (2 of 16) and CD8+ GranzymeB high (14 of 16). However, inflamed tumors were still found among patients who experienced relapse ($n = 14$) but were predominantly CD8+ GranzymeB low (7/14) vs CD8+ GranzymeB high (3/14). Furthermore, gene expression related to cell cycle and proliferation was associated with relapse ($p = 0.02$) [13]. Further study of tumors from PURE-01 and NABUCCO cohorts could provide important insights into how TMEs change with immunotherapy treatment. The immune TME was not evaluated in PURE01 or NABUCCO.

CD8+ T Cell Infiltration

The effector immune cell of the adaptive immune system is the cytotoxic, CD8+ T cell. Pathologic response in ABACUS correlated with pretreatment CD8+ infiltration, with responders found to have greater than median numbers of CD8+ cells (17/42, 40.5%) compared to tumors with less than the median CD8+ T cell infiltration (8/41, 19.5%, $p = 0.04$) [13]. After treatment with atezolizumab, the median CD8+ increased by 78% (pre- vs post-CD8+ infiltrate, $p = 0.004$). Among responders, CD8+ T cells increased more than nonresponders ($p = 0.001$) [13]. In PURE-01, a similar increase in CD8+ immune cells after treatment was described but was not correlated to pathologic response [11]. In NABUCCO, baseline intratumoral CD8+ T cell level by multiplex immunofluorescence was not correlated to pathologic response (complete responder vs non-complete responder, $p = 0.65$) [14]. One hypothesis from NABUCCO is that adding a CTLA-4 inhibitor to a PD-1 inhibitor can induce a complete pathologic response, irrespective of the baseline CD8+ T cell immunity. Collectively, findings of all three studies suggest that neoadjuvant immunotherapy can alter the TME, and, while preexisting CD8+ immunity appears to be associated with response to anti-PD-1/PD-L1 monotherapy, NABUCCO suggests that low baseline CD8+ T cell levels can potentially be overcome by adding anti-CTLA-4 [14].

Immune Signatures

A surrogate of immune cell infiltration is a composite immune RNA signature that describes multiple populations of innate and adaptive immune cells. An example is Immune190,a bladder-specific immune signature which includes IFN γ, IFNα, and

inflammatory responsiveness [34]. PURE-01 assessed four immune signatures, and all four correlated significantly with pathologic response: Immune190 (HR 1.51, 95% CI 1.09–2.17, $p = 0.02$), inflammatory (HR 1.23, 95% CI 1.05–1.46, $p = 0.01$), IFNγ (HR 1.11, 95% CI 1.04–1.19, $p = 0.004$), and IFNα (HR 1.07, 95% CI 1.02–1.13, $p = 0.006$) [34]. Tumors with higher Immune190 showed improved 2-year progression-free survival (PFS) compared to those with lower scores, although this did not reach significance (2-year PFS 93% vs 79%, $p = 0.15$) [34].

In NABUCCO, 18 other composite immune signatures of tumor inflammation were used, including TIGIT, CD27, CD8A, PD-L2, LAD3, PD-L1, CXCR6, CMKLR1, NKG7, CCL5, PSMB10, IDO1, CXCL9, HLA-DQA1, CD276, STAT1, HLA-DRB1, and HLA-E, as well as a CD8+ effector cell function [32, 35]. Overall, baseline IFNγ ($p = 0.67$), tumor inflammation ($p = 0.87$), and CD8+ T cell effector signatures ($p = 0.21$) did not differ between complete and non-complete responders [14]. The study's single-arm trial design did not allow assessing whether CTLA-4 expression is associated with pathologic response. Combining CD8+ infiltrate and immune signatures may aid in the prediction of response to neoadjuvant checkpoint therapy. Evaluation of posttreatment tumors has not been performed to determine if immune signatures after checkpoint therapy are associated with recurrence or survival.

Stromal-Related Factors: TGF-β and Epithelial-Mesenchymal Transition (EMT)

Within the TME, the stroma plays an active role in regulation of immune cell function and tumor growth [36]. The fibroblast, or cancer-associated fibroblast, is the primary cell type of the stroma, with other cell types including endothelial cells [36]. While less is known of the stroma in UC, a critical regulator of the stromal compartment is TGF-β. TGF-β is a multifunctional cytokine that inhibits adaptive immune function, induces regulatory functions of CD4+ T cells, and induces stromal (fibroblast) proliferation [37]. In metastatic UC, TGF-β expression has been correlated with active exclusion of CD8+ T cells, the immune-excluded phenotype, and poor response to atezolizumab [32]. Specifically, mean TGFB1 expression was significantly higher in patients with stable and progressive disease ($n = 230$) compared to complete or partial responders ($n = 68$) ($p = 0.00011$), and lower TGFB1 expression was associated with better overall survival (median overall survival lowest to highest TGF-β quartile (Q), Q1 ~ 14 months, Q2 ~ 11 months, Q3 ~ 6 months, Q4 ~ 8 months; $p = 0.0096$, likelihood ratio test) [32]. Furthermore, in an EMT6 mouse model of immune-excluded mammary carcinoma, Mariathasan et al. noted that combined PD-L1 and TGF-β blockade, but neither alone, caused significant reduction of tumor burden and an increase in tumor-infiltrating CD8+ T cells [32]. In ABACUS, there was no difference in pretreatment TGF-β gene expression between responders and patients with stable disease. Of tumors that relapsed ($n = 15$), 2/15 were immune excluded and had higher TGF-β z-scores, while 13/15

were inflamed and had low TGF-β z-scores (p value not significant) [13]. Thus, as in the metastatic setting, immune-excluded tumors were associated with increased TGF-β expression [13, 32]. Unfortunately, more robust insight was difficult given the small sample size. Overcoming the immunosuppressive signaling of TGF-β is a major challenge for UC, and pretreatment identification of tumors with high TGF-β expression may guide biomarker-directed approaches.

Epithelial-mesenchymal transition (EMT) is the process by which tumor epithelial cells assume a more mesenchymal phenotype, which confers an enhanced ability for invasion and metastasis [38]. EMT is thought to be regulated by stromal components and has been evaluated in advanced UC. Utilizing tumors from CheckMate 275, a positive correlation was demonstrated using CD8+ T cell infiltration and an EMT/stromal-related gene signature using a 200-gene panel that included *FLNA*, *EMP3*, *CALD1*, *FN1*, *FOXC2*, *LOX*, *FBN1*, and *TNC* (Spearman's $\rho = 0.32$, $p < 1 \times 10^{-4}$) [38]. While the EMT-stromal signature alone was not predictive of outcomes, inclusion of a CD8+ signature identified tumors with improved overall survival (OS), PFS, or objective response. Specifically, there was an improvement in PFS and OS, with a concomitant increase in CD8+ T cell infiltration and decrease in an EMT/stromal-related gene signature [38]. Further investigation of the EMT-related gene signatures may identify possible targets for combination therapies in stromal-enriched tumors.

Conclusion

The treatment landscape of MIBC is evolving rapidly, given data from phase II and III clinical trials that compare chemotherapy and immunotherapy. While analysis of pre- and posttreatment TME phenotypes has provided insight into immune changes occurring in response to systemic therapy, further investigation is needed, especially of posttreatment samples. Understanding treatment-induced TME changes in response to neoadjuvant therapy may help inform treatment decisions in the adjuvant and metastatic settings as well.FundingJ.J.M. is supported by Jesse Brown VA Medical Center, Chicago, IL (BX003692), and the Polsky Urologic Cancer Institute of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University at Northwestern Memorial Hospital.

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Chapter 7
Ongoing Trial and Clinical Trial Endpoint Debate: The Role of Pathologic Response as a Surrogate of Survival Endpoints

Praful Ravi and Guru P. Sonpavde

Introduction

Bladder cancer is among the ten commonest cancers worldwide, with nearly 550,000 cancers diagnosed in 2018 [1]. Approximately 30% of patients present with muscle-invasive bladder cancer (MIBC) while 10–20% of patients with non-muscle-invasive progress to MIBC [2]. Historically, the approach to treatment of MIBC was upfront radical cystectomy (RC), which is associated with a 20–50% risk of recurrence after 5 years, implying the presence of micrometastatic disease in a large proportion of patients [3]. As a result, efforts were made to study neoadjuvant cisplatin-based chemotherapy (NAC) prior to RC [4, 5]. The updated results of a landmark meta-analysis in 2005 demonstrated a significant overall survival (OS) benefit with NAC compared to RC alone, with an absolute benefit of 5% at 5 years [6]. Therefore, the current standard of care of MIBC is NAC followed by RC in cisplatin-eligible patients who are fit for RC, while patients who are ineligible for cisplatin are recommended for upfront RC [7]. In patients who are not fit for RC or wish to preserve the bladder, trimodality therapy (TMT) with transurethral resection of bladder tumor (TURBT) followed by chemoradiotherapy is usually favored and can lead to good long-term outcomes [8], although there are no head-to-head data comparing TMT to NAC and RC in fit patients.

P. Ravi
Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

G. P. Sonpavde (✉)
Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

Dana-Farber Cancer Institute, Boston, MA, USA
e-mail: gurup_sonpavde@dfci.harvard.edu

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The past few years have seen major advances in systemic therapy for advanced urothelial cancer, in particular with immune checkpoint inhibitors – anti-PD-1 and anti-PD-L1 – with avelumab [9], durvalumab [10], nivolumab [11], atezolizumab [12], and pembrolizumab [13] all approved after platinum-based chemotherapy. The hallmark of these agents is the durability of responses – while the median OS increment with pembrolizumab versus chemotherapy was modest (10.1 months vs. 7.3 months [hazard ratio 0.70; $P < 0.001$]) and responses were seen in only ~20% of patients, the median duration of response exceeded 2 years [14]. The use of maintenance avelumab in patients who have disease stability or response after platinum-based chemotherapy has recently demonstrated an OS benefit and will increasingly be adopted in clinical practice [15]. Given the relative success of immunotherapy in advanced disease, interest is naturally turning to whether these agents may provide benefit in the neoadjuvant setting. The focus of this chapter is to outline the emerging data for neoadjuvant immunotherapy in MIBC and to explore questions regarding the appropriate endpoint for use in the trials to date and how the field may evolve in the future.

Pathologic Endpoints in MIBC

The advantage of testing therapies in the neoadjuvant setting is the availability of tissue pre- and post-therapy (i.e., from biopsy and surgery, respectively) to evaluate the effectiveness of therapy. Typically, this is assessed by the depth of pathologic response, with an absence of any residual tumor termed a pathologic complete response (pCR). In MIBC, pCR is defined as ypT0N0 disease, whereas the term "pathologic response" is often used to denote downstaging to non-muscle-invasive disease (<ypT2N0).

The prognostic relevance of pCR was first demonstrated in the context of a prospective trial in a post hoc analysis of the SWOG 8710 trial of neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) [5]. Among the 154 patients assigned to the MVAC arm, 46 (30%) had a pCR, and attainment of a pCR was strongly prognostic at the individual patient level (median OS 13.6 years) compared to either a pathologic response (but not pCR, median OS 10.6 years) or lack of a pathologic response (i.e., persistent muscle-invasive or greater disease, median OS 3.7 years) [16]. Moreover, those with pathologically involved lymph nodes exhibited a median OS of only 2.4 years. Subsequently, a trial-level meta-analysis of 13 prospective and retrospective studies encompassing 886 patients and utilizing a variety of cisplatin-based neoadjuvant regimens was undertaken. In this analysis, the pooled pCR rate at RC was 29%, and patients achieving a pCR had a 51% and 26% absolute improvement in the risk of recurrence (RR = 0.19) and death (RR = 0.45), with 4 patients needing to be treated with NAC to prevent 1 death [17]. These results confirmed the prognostic importance of achieving a pCR although it was a trial-level meta-analysis and therefore could not assess the independent impact of pCR after accounting for other prognostic factors in a multivariate model.

Furthermore, it could not evaluate true surrogacy of pCR for OS since individual patient data were not available, and the magnitude of increment in pCR rate at the trial level that translates to improved OS is unclear.

More recently, retrospective studies have attempted to discern the prognostic impact of the depth of pathologic response (i.e., ypT0, ypTis, ypTa, ypT1) and whether differences may be teased apart within these subcategories. In an analysis of 464 patients from 19 institutions with <ypT2N0 disease after NAC, no difference in OS was seen between the subgroup of patients with ypT0N0 disease (n = 257) and those with ypTa/Tis/T1N0 disease (n = 207) [18]. A study of 625 patients with <ypT2N0 after NAC from 10 institutions has also recently been presented and was able to identify significant differences in outcomes based on depth of response. Patients with ypT0N0 and ypTisN0 disease at RC had a similarly low risk of recurrence (~10% at 5 years), whereas those with ypTa or ypT1 disease (with or without concomitant Tis) had notably higher recurrence rates (~25–30% at 5 years), with these differences translating into significant differences in overall survival between the two groups (ypT0/Tis vs. ypTa/T1) [19].

Neoadjuvant Immunotherapy in MIBC

Since the approval of immunotherapy in advanced urothelial carcinoma, the past few years have seen several studies that are bringing these agents into the neoadjuvant space, either alone or in combination with chemotherapy. The aim of these studies is to improve upon the pCR rates seen with the two most commonly used NAC regimens – dose-dense MVAC and gemcitabine-cisplatin (GC) – which are associated with similar pCR rates in the order of 25–40% [16, 20–23]. Moreover, these agents are also being studied in patients who are cisplatin-ineligible in a chemotherapy-free approach [24], given that such patients account for up to 50% of all MIBC patients.

Single-Agent Immunotherapy

Ipilimumab

The first data on neoadjuvant immunotherapy in bladder cancer came from two small proof-of-concept studies performed at MD Anderson in the late 2000s. The first study enrolled six patients who received two doses of ipilimumab (anti-CTLA-4) at 3 mg/kg given 3 weeks apart before undergoing RC [25]. The primary aim of this study was to examine immunologic biomarkers in blood and tissue pre- and post-therapy, and the authors found that neoadjuvant ipilimumab led to increased expression of inducible costimatulor (ICOS) on CD4 T cells from blood and tissue, which produced IFN-γ and were able to identify the NY-ESO-1 tumor antigen. A

subsequent study from the same group added a further six patients treated with a higher dose of ipilimumab (10 mg/kg every 3 weeks for two doses) prior to RC [26]. All patients had localized (T1-2N0) disease, and two patients experienced a delay with RC due to an immune-related adverse event (both diarrhea). There were no perioperative complications as a result of therapy. Eight of the 11 patients who ultimately underwent RC had pathologic downstaging after neoadjuvant ipilimumab, though this may have also been as a result of TURBT. These early studies with neoadjuvant ipilimumab confirmed that it was feasible to deliver preoperative immunotherapy and that such therapy was generally safe and did not appear to compromise surgery. They also provided preliminary signs of efficacy, although assessment of pCR was not reported in either study.

Pembrolizumab

The anti-PD-1 agent pembrolizumab has been evaluated as neoadjuvant therapy in the PURE-01 study in MIBC patients with cT2-T4aN0 disease who were enrolled regardless of cisplatin eligibility, with patients with predominant variant histology also included [27, 28]. The most recent update from the study included 143 patients, the majority (71%) with pure urothelial histology and the remainder with variant histology (including predominant variant histology) [29]. One hundred thirty-five patients (94%) ultimately underwent RC after three cycles of pembrolizumab, with no delay to RC occurring as a result of pembrolizumab-related adverse effects. Thirteen patients underwent additional systemic therapy after neoadjuvant pembrolizumab, with nine of these patients undergoing RC. Overall, a pCR was seen in 55 patients (39%), and the overall pathologic response rate (downstaging to <ypT2N0) was 56%. The rate of pCR appeared to be lower in patients with variant histology (32%) and was particularly low in those with predominantly variant histology (3 of 19, 16%). Neoadjuvant pembrolizumab was safe with an overall low rate of adverse events (~5% grade 3), and perioperative complications did not appear increased as a result of therapy [28].

The most recent update of the PURE-01 study also provided early data on longer-term outcomes after RC [29]. Overall 2-year event-free survival (EFS) was 72%, and a strong impact of depth of pathologic response on EFS was noted: 2-year EFS was 96%, 75%, 79%, and 39% in patients with a pCR, ypTa/T1/TisN0, ypT2-4N0, and ypN+ disease, respectively. These data, while still somewhat immature with a median follow-up of only 2 years, are similar to those seen after traditional NAC and serve to validate the strong prognostic impact of pCR – and, to a lesser extent, pathologic response – on long-term outcomes after RC.

Exploratory biomarker analyses from PURE-01 have provided some insights into which patients may experience a better outcome from neoadjuvant pembrolizumab. A higher tumor mutational burden (TMB) was correlated with achieving a

pCR, with a cutoff of TMB ≥15 seeming to provide the best predictive value [27]. However, TMB was not associated with EFS, but a higher CPS score was an independent predictor of EFS, although the magnitude of its impact was low (HR = 0.98, $p = 0.02$). Transcriptomic analyses using the Decipher classifier also suggested that "claudin-low" tumors appeared to have the best outcomes after neoadjuvant therapy and RC and "neuroendocrine-like" tumors had the poorest outcomes [29].

Atezolizumab

The anti-PD-L1 agent atezolizumab was evaluated in the phase 2 ABACUS trial as neoadjuvant therapy in patients with cT2-T4aN0M0 MIBC who either refused or were ineligible for cisplatin-based chemotherapy [30]. A total of 95 patients were enrolled and were planned to receive 2 cycles of atezolizumab prior to RC; 87 patients (92%) underwent RC, with 3 patients being unable to undergo RC due to a treatment-related adverse event (pneumonia, myocardial infarction, and decline in performance status). Atezolizumab was relatively safe, with fatigue being the commonest side effect and transaminitis being the most frequent grade 3 adverse event (in 4%). Surgical complications were seen in 62% of patients overall, with 17% having Clavien-Dindo grade 3–4 complications, most commonly wound dehiscence. One patient had a postoperative complication resulting in death.

The rate of pCR in the overall population was 31%, though it was lower (17%) in patients with T3 or T4 disease at baseline. Exploratory analyses suggested that the rate of pCR was not significantly different among patients whose tumors were PD-L1 positive (37%), had a high presence of intraepithelial CD8 T cells (40%), or had a TMB of ≥10 (31%). Data on longer-term outcomes from the study were recently presented, with 2-year relapse-free survival (RFS) of 77% and 2-year OS of 82% in patients who underwent RC [31]. Outcomes stratified by depth of pathologic response have not been reported in full, but only one patient with a pCR had recurred after a minimum follow-up of 2 years, which is in line with longer-term data from the PURE-01 study [29].

Durvalumab

The feasibility of durvalumab, a PD-L1 inhibitor, as neoadjuvant therapy in cisplatin-ineligible patients was evaluated in the BLASST-2 trial. Ten patients were enrolled and received 3 doses of durvalumab 750 mg every 2 weeks prior to RC, with all patients undergoing RC. Among eight patients with sufficient follow-up, one had a grade 3 adverse event (anemia), and a pathologic response was seen in two patients (25%), with one (12.5%) having a pCR [32].

Combination Immunotherapy

Two trials assessing combinations of anti-PD-(L)1 therapy with CTLA-4 blockade have recently been reported, with the aim of these single-arm studies being to see whether this is safe and feasible. The NABUCCO trial evaluated 2 doses of ipilimumab (anti-CTLA-4, days 1 and 22) and nivolumab (anti-PD-1, days 22 and 43) prior to RC in 24 patients with cT2-T4aN0-1, who were cisplatin-ineligible or cisplatin-refusing [33]. Forty percent of patients had N+ disease, and the vast majority (88%) had an ECOG performance status of 0. All 24 patients had surgical resection, with 23 of 24 having RC within 12 weeks of starting therapy, which was the primary endpoint. One patient had RC delayed by 4 weeks as a result of immune-related hemolysis. Overall, the addition of an anti-CTLA-4 agent seemed to increase treatment-related toxicity, with 55% of patients having at least one grade 3 or 4 adverse event (most commonly elevated lipase). Twenty-five percent of patients were only able to complete two of the planned three cycles of therapy as a result of an immune-related adverse event. The rate of pCR was 46%, while an additional 13% of patients had no residual invasive disease (ypTis or ypTa), and this appeared to be greater among patients whose tumors were PD-L1 positive (73% vs. 33%). While no relationship between pathologic response and the degree of immune infiltration was seen, responding patients were enriched for induction of tertiary lymphoid structures (TLS), a phenomenon that is known to be correlated with response to immunotherapy [34, 35].

A similar feasibility study assessed the combination of durvalumab (anti-PD-L1) with tremelimumab (anti-CTLA-4) in 28 patients with high-risk urothelial carcinoma (as defined by T3 or T4 disease, presence of variant histology, lymphovascular invasion, hydronephrosis, or high-grade upper tract disease) who were ineligible for cisplatin [36]. All but one patient (96%) had T2 or higher disease, and two patients (7%) had upper tract disease; 25% of patients had a component of variant histology. Patients received two doses of durvalumab (1500 mg) and tremelimumab (75 mg) every 4 weeks prior to surgery, while a second as-yet-unreported cohort received 300 mg of tremelimumab. Overall, 21% of patients had a grade 3 or higher adverse event (most frequently elevated lipase). Four patients did not complete RC on protocol, with an immune-related adverse event leading to delay in two patients. The rate of pCR among the 24 patients completing surgery on protocol was 38%, while the rate of overall pathologic response (<ypT2N0) was 58%. Four of the seven patients (57%) with variant histology achieved a pCR. One-year OS was 89%, and 1-year RFS among the 24 patients undergoing RC was 83%, with three patients relapsing and two deaths as a result of non-cancer-related causes. Exploratory analyses again failed to identify immune infiltration as a predictor of response, though the baseline density of TLS correlated with response.

Combination of Immunotherapy with Chemotherapy or Other Agents

Urothelial carcinoma is a generally chemotherapy-sensitive disease and a logical question is whether addition of other agents to a platinum backbone may improve outcomes. This has been tested in the frontline metastatic disease setting, where the addition of atezolizumab [37] or pembrolizumab [38] to platinum-based chemotherapy has not shown a survival benefit in phase 3 randomized trials, and a similar paradigm is also being evaluated in the neoadjuvant setting using GC as the backbone.

Results from a Hoosier Cancer Research Network (HCRN) trial combining pembrolizumab (200 mg q3 weeks × 5 doses) with four cycles of GC have been presented [39, 40]. Forty-three patients were enrolled, and the majority was able to complete all planned neoadjuvant therapy. Thirty-six patients underwent RC with a median time from last dose of therapy to RC of 5 weeks; one patient was unable to undergo RC as a result of an adverse event (grade 4 thrombocytopenic purpura), and there was one postoperative death as a result of mesenteric ischemia. Forty-four percent of patients had a pCR, while 61% of patients had <ypT2N0 disease at RC. Two-year RFS was 66%, while 4-year OS was 82%.

The HCRN study also included a cohort of 37 cisplatin-ineligible patients who received 5 doses of pembrolizumab together with 3 cycles of gemcitabine [41]. Interim results from this cohort showed that this combination was feasible, with most patients completing all doses of therapy. Three patients (8%) progressed prior to RC, and while 36% of patients had a non-hematologic grade 3 or 4 adverse event, this did not preclude RC in the vast majority (86%) of cases; 4 patients had an immune-related adverse event (pneumonitis in 2, colitis and LFT elevation in 1). The rate of pCR (45%) was similar to the cisplatin-eligible cohort, and the overall pathologic response rate was 52%. While follow-up was relatively short (median 11 months), the 12-month RFS rate was 67%.

The addition of nivolumab (360 mg q3 weeks × 4 doses) to GC has been evaluated in 41 patients in the BLASST-1 phase 2 trial [42]. The combination appeared relatively safe (20% grade 3–4 adverse events, majority from GC, and 3 immune-related adverse events) and did not appear to delay RC. In the rate of pathologic response, the primary endpoint was 66%, with 34% having a pCR. Similarly, the SAKK 06/17 study evaluated four cycles of neoadjuvant durvalumab and GC prior to RC, with patients also receiving adjuvant durvalumab for a total of 1 year [43]. In a preplanned analysis encompassing surgical outcomes from the first 34 (of a planned 61) patients, all patients completed the planned neoadjuvant therapy. Twenty-four percent had a grade 3 or 4 adverse event ascribed to durvalumab, with 88% proceeding to RC as planned. Thirty-three percent of patients had a pCR and the overall pathologic response rate was 60%.

The combination of durvalumab with olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, has also been tested in the neoadjuvant setting given preclinical evidence of possible synergism between immune checkpoint blockade and PARP

inhibition [44]. Twenty-nine patients with cT2-T4a MIBC were treated with two cycles of durvalumab (1500 mg q4 weeks) and olaparib 300 mg twice daily for 8 weeks [45]. Results from the 26 patients who completed RC showed a pCR rate of 50%; however, 21% of patients had pathologic upstaging of disease at RC, while 10% had disease progression on therapy and prior to RC [46].

The only randomized study evaluating immunotherapy in the neoadjuvant setting is the phase 2 DUTRENEO trial, which assessed cisplatin-eligible patients with cT2-T4aN0-1 MIBC and classified their tumors as "hot" or "cold" based on a tumor-immune RNA signature [47]. Patients with "hot" tumors were randomized to durvalumab 1500 mg and tremelimumab 75 mg every 4 weeks for 3 cycles or to standard dose-dense MVAC or GC chemotherapy. Patients with "cold" tumors received chemotherapy. A total of 61 patients were enrolled, with 45 having "hot" tumors and 16 having "cold" tumors. The rate of pCR in these patients was similar in patients receiving immunotherapy doublet (8 of 23, 35%) or NAC (8 of 22, 36%), while 69% of patients with "cold" tumors had a pCR with NAC. The authors concluded that the immune signature was therefore unable to prospectively identify patients more likely to benefit from neoadjuvant immunotherapy, in line with correlative work from other neoadjuvant immunotherapy trials [27, 30, 33, 36].

Future Directions

A summary of results from trials evaluating immunotherapy (as single agent or in combination) as neoadjuvant therapy for MIBC is presented in Table 7.1. It can be seen that single-agent anti-PD-(L)1 therapy is generally feasible to deliver in the neoadjuvant setting and does not appear to harm the likelihood of proceeding to RC in this potentially curative patient population. The rate of pCR is in the order of 30–40% [28, 30], which is similar to that seen with cisplatin-based NAC, and there is preliminary evidence that the prognostic value of pCR seen with NAC is also seen with neoadjuvant immunotherapy [29, 31]. Notably, the results of CheckMate 274 bode well for the potential of neoadjuvant checkpoint inhibitors [48]; CheckMate 274 is a pivotal phase 3 trial evaluating adjuvant nivolumab after RC in patients with high-risk, muscle-invasive urothelial carcinoma that has met its co-primary endpoints of improving disease-free survival (DFS) versus placebo in all patients as well as in those whose tumor cells expressed PD-L1 $\geq 1\%$.

With the caveats of comparing across trials of modest size, dual checkpoint blockade (CTLA-4 and PD-(L)1) appears to be associated with a higher pCR rate at a cost of greater toxicity [33, 36]. The addition of anti-PD-(L)1 therapy to traditional GC chemotherapy appears feasible and potentially confers an increment in pCR, although randomized trials are required to provide definitive data [39, 42, 43].

These early results have prompted the initiation of several randomized phase 3 trials evaluating immunotherapy in both cisplatin-eligible and cisplatin-ineligible patients (Table 7.2), with pCR and EFS being the co-primary endpoints for these trials. The benefit of using pCR as a surrogate endpoint (for OS) is that it enables

Table 7.1 Summary of neoadjuvant immunotherapy trials in MIBC

Therapy	N	Population	% Underwent RC	% Progressed pre-RC	Grade ≥ 3 adverse events, %	pCR, %	Survival outcomes
Ipilimumab [26]	12	cT1-T2N0	92	8	33	36	83% disease-free at median f/u of 20mths
Pembrolizumab [27–29]	143	cT2-T4aN0, VH allowed	94	8	7	39	2-year EFS 72% overall 2-year RFS 96% if pCR
Atezolizumab [30, 31]	95	cT2-T4aN0, cis-ineligible	92	16	11	31	2-year RFS 77% overall 2-year RFS 96% if pCR
Durvalumab [32]	10	cT2-T4aN0, cis-ineligible	100	0	12.5	12.5	NR
Ipilimumab + nivolumab [33]	24	cT2-T4aN0-3, cis-ineligible	100	0	55	46	2-year RFS ~90% 2-year RFS 100% if pCR
Durvalumab + tremelimumab [36]	28	High-risk UC, cis-ineligible	88	13	21	38	1-year RFS 83%
Durvalumab + tremelimumab [47]	23	cT2-T4aN0-1, "hot" tumor	96	4	22	35	NR
Durvalumab + olaparib [45, 46]	29	cT2-T4a	90	10	3	50	NR
Pembrolizumab + GC [39, 40]	43	cT2-T4aN0	88	2	30 (nonheme), 60 (heme)	44	2-year RFS 66% overall
Pembrolizumab + gemcitabine [41]	37	cT2-T4aN0, cis-ineligible	92	8	36 (nonheme), 44 (heme), 11 (irAE)	45	1-year RFS 67% overall
Nivolumab + GC [42]	41	cT2-3aN0-1	98	NR	20	34	NR

(continued)

Table 7.1 (continued)

Therapy	N	Population	% Underwent RC	% Progressed pre-RC	Grade ≥ 3 adverse events, %	pCR, %	Survival outcomes
Durvalumab + GC [43]	34	cT2-T4aN0-1, VH allowed	88	NR	24	33	NR

Abbreviations: *VH* variant histology, *RC* radical cystectomy, *MIBC* muscle-invasive bladder cancer, *pCR* pathologic complete response, *EFS* event-free survival, *RFS* relapse-free survival, *GC* gemcitabine/cisplatin, *UC* urothelial carcinoma, *irAE* immune-related adverse event, *NR* not reported

Table 7.2 Ongoing phase 3 trials involving neoadjuvant immunotherapy

Trial	N	Population	Regimen/design	Primary endpoint(s)
NCT04209114	540	cT2-4a N0, cisplatin-ineligible	N/A nivolumab vs. N/A nivolumab + NKTR-2104 vs. RC	pCR and EFS
NCT03924895, EV-303/ KEYNOTE-905	836	cT2-T4a N0-1, cisplatin-ineligible	N/A pembrolizumab vs. N/A EV + pembrolizumab vs. RC	pCR and EFS (overall and PD-L1 positive)
NCT03732677, NIAGARA	1050	cT2-T4a N0-1, cisplatin-eligible	N/A durvalumab + Neo GC vs. Neo GC	pCR and EFS
NCT03924856, KEYNOTE-866	790	cT2-T4a N0-1, cisplatin-eligible	N/A pembrolizumab + Neo GC vs. Neo GC	pCR and EFS (overall and PD-L1 positive)
NCT03661320, ENERGIZE	1200	cT2-T4a N0, cisplatin-eligible	N/A nivolumab + N/A linrodostat + Neo GC vs. N/A nivolumab + Neo GC vs. Neo GC	pCR and EFS

Abbreviations: *N/A* neoadjuvant and adjuvant, *Neo* neoadjuvant, *GC* gemcitabine/cisplatin, *EV* enfortumab vedotin, *RC* radical cystectomy, *pCR* pathologic complete response, *EFS* event-free survival

earlier "read-out" of results and therefore accelerates the drug development and approval process. Notably, DFS has also been shown to correlate with OS at an individual patient level in patients with muscle-invasive urothelial carcinoma undergoing RC with or without perioperative chemotherapy [49, 50]. Moreover, the durability of benefit and responses to PD-(L)1 blockade in metastatic disease suggests that pCR and EFS are possibly likely to translate to improved OS in this setting.

Surrogate endpoints are widely used in oncology, including the use of pCR as an endpoint in neoadjuvant breast cancer trials [51] as well as metastasis-free survival (MFS) in localized prostate cancer [52]. It is worth noting that establishment of surrogacy requires analysis at both the individual and trial level [53]. The former refers to determining whether the surrogate outcome is prognostic for the true endpoint, whereas the latter requires a good correlation to be shown between the effect of an intervention on the surrogate outcome and that on the true endpoint. In the case of MIBC, there is clear evidence that pCR (or perhaps even pathologic response) meets the first criterion [16–19], but there is no data on whether the surrogacy of pCR for OS is true at the trial level.

Since all of the ongoing phase 3 trials evaluate both neoadjuvant and adjuvant immune checkpoint blockade, it will be difficult to determine whether any EFS or RFS benefit seen with immunotherapy is attributable to the neoadjuvant portion of therapy, adjuvant portion, or a combination of both. Therefore, there is all the more need for a collaborative effort to pool together individual patient data from the major NAC and neoadjuvant immunotherapy trials to date and formally test pCR (or other candidate intermediate endpoints) as a surrogate endpoint for OS in MIBC. This would allow the field to have confidence in the use of pCR as the appropriate surrogate endpoint in neoadjuvant studies that also utilize an adjuvant therapy component. It will also be important to establish whether EFS or RFS (co-primary endpoints in ongoing studies) is a surrogate for, or at least correlate with, OS at the individual patient level in the setting of immune checkpoint inhibitors.

In summary, the past few years have seen rapid advancement in drug development in urothelial carcinoma with the increasing use of immunotherapy in both the advanced and localized disease settings. Results from phase 2 studies in the neoadjuvant setting have shown that immunotherapy is likely safe and feasible, while there are preliminary signs of efficacy, particularly in cisplatin-ineligible patients, which is a clear unmet need. We await results from ongoing randomized phase 3 trials evaluating neoadjuvant immune checkpoint blockade monotherapy and in combination with chemotherapy. While pathologic response (or pCR) and EFS appear prognostic for survival in the setting of immunotherapy, we suggest that further work to study the surrogacy of these endpoints for OS is required before we can confidently accept their use as the primary endpoint in trials evaluating perioperative therapy for MIBC.

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Chapter 8
State-of-the-Art and Future Role of Molecular Biomarkers for Patient Selection

Tuomas Jalanko, Mathieu Roumiguie, and Peter Black

Introduction

Neoadjuvant cisplatin-based chemotherapy (NAC) followed by radical cystectomy remains the standard of care treatment for non-metastatic muscle-invasive bladder cancer (MIBC) [1]. However, in daily practice, more than 70% of patients undergo cystectomy without NAC. This is partly due to patient ineligibility for cisplatin (e.g., comorbidities or renal failure) and partly due to a lack of enthusiasm for NAC by the treating physicians based on the perceived balance between the risk of side effects and modest improvement in patient outcome [2]. A key to overcoming the underutilization of NAC will be the introduction of predictive biomarkers that will enable us to administer NAC to patients who are likely to benefit from the treatment, while foregoing NAC in those unlikely to benefit. Through molecular characterization of bladder cancer, various genes have been identified that correlate with cisplatin response (e.g., DNA repair pathway: *ERCC2*, *ATM1*). Bladder cancer molecular subtypes may also be important in identifying responders to NAC [3]. In one report, patients with basal tumors had a poor outcome with cystectomy alone but a markedly improved outcome with NAC, yet patients with luminal tumors had excellent overall survival whether or not they received NAC [4].

T. Jalanko
Department of Urology, Helsinki University Hospital, Helsinki, Finland
e-mail: tuomas.jalanko@helsinki.fi

M. Roumiguie
Department of Urology, CHU Toulouse/ IUCT-O, Toulouse, France

P. Black (✉)
Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada
e-mail: pblack@mail.ubc.ca

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Molecular classification has also been used to identify patients with metastatic urothelial carcinoma most likely to respond to immunotherapy, although the results have been inconsistent. In the CheckMate 275 study, the basal tumors, which have a strong immune-infiltrated signature, had a better response to nivolumab than the other subtypes [5], while in IMvigor 210, the genomically unstable subtype, according to the Lund classification, which is associated with a high rate of mutations, was associated with the highest response rate to atezolizumab [6].

In metastatic disease, some biomarkers such as PD-L1 expression by immunohistochemistry and CD8 T effector signature may be relevant in predicting response to immunotherapy, but the results have not been consistent enough to guide therapeutic decisions except for the use of PD-L1 expression to guide the use of first-line pembrolizumab or atezolizumab in cisplatin-ineligible, carboplatin-eligible patients [7, 8]. Indeed, PD-L1 expression, which is detected in 20–30% of bladder tumors, is a dynamic biomarker that may change during progression and metastasis, as well as under the selective pressure of systemic treatment. In addition, the immune system is better preserved in the early stage of the disease, allowing a greater expansion of T cells for immune response [9].

The next frontier for immune checkpoint therapy in bladder cancer is in the neoadjuvant, adjuvant, and combined perioperative settings. In five single-arm phase II trials of neoadjuvant immunotherapy alone or in combination with chemotherapy or a second immunotherapy (Table 8.1), a complete response was achieved in 31–46% of patients [10–14]. With the advent of multiple treatment options in the neoadjuvant setting, the importance of predictive markers to guide the selection of therapy in an individual patient will be even greater. Although the results from practice-changing phase III trials are still pending, there have already been multiple reports from the early phase trials investigating the role of biomarkers in predicting response to therapy (Table 8.2). Here we aim to summarize the results of the biomarker analysis performed in these neoadjuvant trials.

Study Selection

To date, five published phase II trials have evaluated the rate of pathological complete response (pCR) at radical cystectomy after neoadjuvant immunotherapy.

PURE 1 included 114 patients who were treated every 3 weeks prior to cystectomy with 3 cycles of pembrolizumab [6]. pCR was achieved in 39% of patients. Biomarker analysis included expression of programmed death ligand 1 (PD-L1) by immunochemistry (Dako22C3 pharmDx assay). Genomic sequencing (FoundationOne®) and gene expression profiling were also performed in this analysis [10, 15].

In the ABACUS trial, 88 patients received two cycles of atezolizumab prior to radical cystectomy, and 31% achieved pCR. Biomarker analysis included PD-L1 expression and other protein expression assays (PanCK, CD8, GZMB, and FAP), as well as RNA- and genome sequencing (Foundation One®) [11].

Table 8.1 Neoadjuvant immunotherapy trials in muscle invasive bladder cancer

Trials	Population	Treatment	pCR	pT ≤ 1N0	Biomarkers
PURE-01	*n* = 143 cT2-4aN0	Pembrolizumab (3 cycles / 3 weeks)	39%	56%	PD-L1 CPS Genome sequencing (FoundationOne assay = 395 + 31 genes) RNA-microarray
ABACUS	*n* = 88 cT2-4aN0 Cisplatin-ineligible	Atezolizumab (2 cycles)	31%	NR	Genome sequencing (FoundationOne assay = 395 + 31 genes) RNA-sequencing Pre-existing T cell activation (CD8, tGE8, GZMB) PD-L1 expression
NABUCCO	*n* = 24 cT3-4 or cT2-4aN1-3	Nivolumab + Ipilimumab (3 cycles sequential/ combination)	46%	58%	PD-L1 CPS Tumor immune cell infiltration (CD3, CD8, CD68, FoxP3, CD20, PanCK) Whole exome sequencing RNA microarray Tertiary lymphoid structures
BLASST-1	*n* = 41 cT2-4aN ≤ 1 Cisplatin-eligible	Gemcitabine/ Cisplatin + Nivolumab (4 cycles)	34%	66%	PD-L1 expression Tumor immune cell infiltration (CD3, CD8, CD56) Whole genome sequencing Molecular subtypes
MDACC	*n* = 28 cT2-4aN ≤ 1 Cisplatin-ineligible	Durvalumab + Tremelimumab (2 cycles / 4 weeks)	37.5%	58%	PD-L1 expression Tertiary lymphoid structures Whole exome sequencing RNA expression by NanoString Multiplex immunofluorescence

CPS combined positive score, *TMB* tumor mutational burden, *tGE8* predefined eight-gene cytotoxic T cell transcriptional signature, *GZMB* granzyme, *PD-L1* program death ligand 1, *pCR* pathological complete response, *MDACC* MD Anderson Cancer Center, *NR* not reported

In the NABUCCO trial, 24 patients were treated with the combination of ipilimumab (anti-CTLA-4; 3 mg/kg on day 1 and day 22) and nivolumab (1 mg/kg on day 22 and 3 mg/kg on day 43) followed by cystectomy. pCR was achieved in 46% of patients. Biomarker analysis included whole-exome sequencing of baseline tumor and local lymph node metastases, RNA sequencing, and multiplex immunofluorescence for immune cells before and after treatment [12].

In the BLASST trial, 41 patients were treated with four cycles of standard gemcitabine and cisplatin (a split dose of cisplatin allowed), but nivolumab was also

Table 8.2 Association between biomarkers and pathological complete response in neoadjuvant immunotherapy trials

Trials	PD-L1 antibody	PD-L1+ cut off	Biomarkers of treatment response						
			PD-L1+	CD8+	B Cells	tGE8	TLS	TMB	Others
PURE-01	Dako 22C3	CPS ≥10%	V					X	
ABACUS	Ventana SP142	>5% immune cells	X	V		V		X	GZMB
NABUCCO	Dako 22C3	CPS ≥10%	X	X	X	X	X	X	ICOS + CD4+
BLASST-1	Dako 22C3	CPS ≥ 10%	X						
MDACC	Cell Signaling 13684S	PD-L1+ density (number of positive cells mm^{-2})	X	V	V	X	V	X	TLS signature (POU2AF1, LAMP3, CD79A and MS4A1)

CPS combined positive score, *TMB* tumor mutation burden, *tGE8* predefined eight-gene cytotoxic T cell transcriptional signature, *TLS* tertiary lymphoid structure, *GZMB* granzyme B, *PD-L1* programmed death ligand 1, *X* no association, *V* biomarker associated with pCR

administered together with the day 8 dose of gemcitabine in each cycle. The pCR rate was 34%. Preliminary results of PD-L1 immunohistochemistry and RNA subtypes were also reported. Other analyses including whole-genome sequencing and additional IHC are still unpublished [14].

Gao et al. evaluated durvalumab in combination with tremelimumab (anti-CTLA-4) in 28 cisplatin-ineligible patients with MIBC and reported a pCR rate at 37.5%. The authors analyzed multiple potential biomarkers of the treatment response, including tertiary lymphoid structure, total mutational burden, and gene expression signatures. This trial from MD Anderson Cancer Center (MDACC) is referred to as MDACC in this chapter [13].

Genetic Alterations

Bladder cancer is known to have a high number of genetic alterations and significant molecular heterogeneity [16]. There are on average 7.7 somatic mutations per 1 Mb (million bases) in MIBC which is similar to lung cancer and melanoma. Together these cancers comprise the top three most mutated cancers [17]. Somatic genetic alterations in bladder cancer include base (A, T, C, G) substitutions, insertions or deletions, chromosomal rearrangements, and copy number variations. Some of the genetic alterations are synonymous and do not alter the amino acid sequence of the translated protein while others are non-synonymous leading to changes in the amino

acid sequence. Non-synonymous mutations can be deleterious as the structure of the protein is altered and this can lead to loss of function. Synonymous mutations on the other hand are benign as they do not alter the function of the protein.

The most common ways to identify genetic alterations are whole genome sequencing (WGS), whole exome sequencing (WES), and targeted sequencing using a gene panel. The sample is usually tissue obtained from the archived formalin-fixed, paraffin-embedded primary tumor. Metastatic lesions are only rarely biopsied, and analysis of these small samples can be limiting. Sequencing of circulating tumor DNA in plasma or cell-free DNA in urine has not been tested in this context [18]. While WES and WGS may be important in the research and discovery settings, targeted gene assays, also called comprehensive genomic profiling (CGP), may demonstrate highest clinical utility due to the focus on known cancer-related genes and more stream-lined bioinformatic analysis. These have been incorporated into many immunotherapy trials. A typical CPG assay can detect base substitutions, insertions, deletions, and copy number variations in around 400 cancer-related genes and a few dozen selected intronic segments, and can be used to estimate tumor mutational burden and to determine microsatellite instability (MSI). Currently, there are several FDA-approved commercial CGP-assays on the market (e.g., Foundation One CDx, MSK-IMPACT) and more are under development.

In addition to PD-1/PD-L1 protein expression, perhaps the most studied biomarker in cancer immunotherapy is the tumor mutational burden (TMB). High TMB can be caused by certain mutational signatures (e.g., APOBEC), MSI, and impaired DNA damage repair. TMB is usually defined as the number of somatic exonic mutations per 1 Mb. However, there is considerable variability in the literature on what type of mutations are included in TMB and how TMB is calculated, especially when using different sequencing platforms (e.g., WES vs. WGS vs. CPG). Some trials report a number of mutations per tumor. Some trials take into account both synonymous and non-synonymous mutations. This variability in the literature makes it difficult to compare TMB between studies. TMB is nevertheless an important factor in cancer immunotherapy as it is a surrogate marker for tumor neoantigen load. Tumor-specific neoantigens arise from somatic mutations and are presented on the tumor cell surface to be recognized by the immune cells. Some of these neoantigens are identified as "non-self" which can lead to T-cell activation and antitumor immune response [19]. This T-cell activity in the tumor microenvironment has been regarded to be a prerequisite for the immune checkpoint inhibitors to work efficaciously.

Multiple clinical trials, including some phase III randomized controlled trials, have shown an association between high TMB and clinical response to immune checkpoint inhibitors in various advanced and metastatic solid tumors including bladder cancer, where TMB has been shown to predict objective response rate, progression free, and overall survival [20–25]. In the neoadjuvant setting, there is considerably less available evidence in the literature. A small phase II trial in melanoma showed a trend toward better clinical benefit from neoadjuvant ipilimumab-nivolumab in patients with high TMB [26]. Another phase II trial in

non-small cell lung cancer did not reveal a difference in clinical response between those with high and low TMB [27].

In bladder cancer, the recently published five phase II trials did show a statistically significant association between high TMB and clinical response to neoadjuvant immunotherapy. In the PURE-01, ABACUS, and MDACC trials, pathologic response did not correlate significantly with TMB while in NABUCCO there was a trend toward a higher TMB on univariable analysis in tumors that responded completely to ipilimumab-nivolumab combination therapy compared to those that did not [10–13]. In PURE-01, TMB was initially found to predict pathologic response on multivariable analysis, but on the latest update from this study this association was no longer statistically significant [15, 28]. It is important to highlight that high TMB is a favorable prognostic marker in MIBC regardless of treatment, so assessment of the predictive value of TMB for immunotherapy will require comparison to a treatment arm managed without immunotherapy [29].

There are several possible explanations for the discrepancy between TMB and clinical response in neoadjuvant and advanced/metastatic trials in immunotherapy. The neoadjuvant studies were mostly small single-arm phase II trials that may not have been able to detect a difference. The methods used to detect TMB are variable in these trials. PURE-01 and ABACUS used a commercial CGP-assay, and NABUCCO and MDACC used WES. CGP-assays have been shown to detect TMB accurately and comparably to WES even though these assays target a smaller region (≈1 Mb) of the exome [30, 31]. However, the CGP-assay used in PURE-01 and ABACUS measured both synonymous and non-synonymous somatic mutations. Other studies have demonstrated that measuring clonal (mutations found in every tumor cell), non-synonymous mutations depicts neoantigen load and response to checkpoint inhibitors more accurately than measuring both synonymous and non-synonymous mutations [24].

Thirdly and most importantly, the clinical scenario is significantly different between neoadjuvant and metastatic immunotherapy trials. The tumors in the neoadjuvant trials are localized and biologically different from metastatic tumors. Patients have usually undergone several treatments before immunotherapy in the trials with metastatic patients while the tumors in neoadjuvant settings are treatment-naïve. Also, the time difference from sample acquisition to treatment is usually very different. The tissue samples in trials on metastatic disease have often been collected much earlier in the disease course, usually with one or more rounds of systemic therapy between sample collection and starting immunotherapy. In neoadjuvant trials, on the other hand, the sample has been obtained shortly before starting immunotherapy.

Interestingly, in PURE-01, tumors classified based on RNA expression as neuroendocrine-like had the highest TMB, but this did not correlate with better treatment outcome with pembrolizumab [10]. In the platinum-refractory metastatic setting, neuroendocrine-like tumors in IMvigor210 were observed to have an excellent response to atezolizumab [32]. One key difference between these results is the stringency of the classifier used to determine neuroendocrine-like gene expression [33].

As mentioned, MSI is one potential cause of high TMB. In MSI, an impaired DNA mismatch repair pathway leads to the accumulation of mutations in locations with repeated DNA sequences (known as microsatellites). MSI together with high neoantigen load and TMB have been associated with better response to checkpoint inhibitor therapy in a cohort comprising various metastatic cancers [22]. Pembrolizumab has been approved for the treatment of any locally advanced/metastatic solid tumor after exhaustion of standard treatment options if either of these markers (MSI or TMB) is elevated. Nonetheless, in neoadjuvant trials on bladder and other solid cancers, an association between MSI and pathological response has yet to be reported.

There are several specific gene alterations characteristic of bladder cancer including mutations in the *TERT* promoter, *p53*, *Rb1*, and *FGFR3*. Other distinctive alterations are seen in DNA damage repair and chromatin-modifying genes [29]. In terms of clinical response to immune checkpoint inhibitors, several gene mutations have been implicated in analyses performed on patients with metastatic tumors. *PBRM1* is a gene encoding a protein that belongs to SWI/SNF-B (PBAF) chromatin-remodeling complex. Mutations in *PBRM1* have been found to be enriched in responding tumors on various immunotherapy studies [24, 34, 35]. Other interesting gene mutations that have been previously associated with immunotherapy response are *PIK3CA* and *KRAS* alterations [24]. However, none of these gene mutations have been established as biomarkers of response in the neoadjuvant trials in bladder or other tumors.

Impaired DNA damage repair (DDR) can lead to high TMB and genetic instability. In bladder cancer, mutations in the DDR pathway genes (e.g., *ERCC2*, *ATM*, *FANCC*) have been linked to clinical response to both NAC in localized disease [36–38] and immunotherapy in metastatic urothelial carcinoma [39]. In PURE-01 and ABACUS, DDR pathway alterations did not correlate with clinical outcome after treatment with single-agent PD-(L)-1 inhibitor [10, 11]. However, in the NABUCCO trial, DDR mutations were enriched in responders to dual checkpoint blockade. Interestingly, the tumors in the NABUCCO trial were more advanced (cT3-4a or cN1-3) when compared to PURE-01 and ABACUS (cT2-4aN0).

Some genetic alterations have been associated with resistance to immunotherapy. *PTEN* is a well-known tumor suppressor and regulator of cell cycle. Homozygous deletion of *PTEN* has been linked to resistance to immune checkpoint inhibitors in preclinical and clinical studies [24, 40, 41]. The biological mechanism behind this resistance is believed to be increased expression of immunosuppressive cytokines and hence decreased T-cell infiltration in the tumor [40]. *PTEN* has yet to be tested as a marker of response to neoadjuvant immunotherapy in bladder cancer.

The fibroblast growth factor receptor (FGFR) inhibitor erdafitinib has been recently approved by the FDA for use in platinum-refractory advanced and metastatic urothelial carcinoma. Typically, FGFR alterations are enriched in the luminal subtype of MIBC (see below), which is also known to have decreased T-cell infiltration and low immune marker expression. Previous studies have raised questions whether FGFR-pathway alterations could be markers of resistance to immunotherapy due to these molecular correlations [42], but the clinical evidence

suggests this is not the case in the second-line metastatic setting [43]. The authors of both the PURE-01 and ABACUS trials demonstrated that FGFR3 alterations (mutations and fusions) did not correlate with response to neoadjuvant pembrolizumab or atezolizumab, respectively [11, 44]. In the ABACUS trial, *FGF3*, *FGF4*, and *FGF19* gene amplifications were enriched in the pretreatment tissue of responders [11]. These are ligands of the FGFRs, and this exploratory analysis suggests that FGFR signaling could be relevant in the response to immunotherapy, which requires further study.

In bladder cancer, two thirds of single base substitutions are caused by APOBEC-mediated mutagenesis. The APOBEC mutational signature is associated with tobacco exposure. APOBEC is a family of cytidine deaminases involved in RNA editing that can cause mutations when dysregulated. APOBEC dysregulation is one of the causes of high mutational burden in bladder cancer and therefore an interesting biomarker candidate in cancer immunotherapy. Not surprisingly, APOBEC signature has been linked to better clinical response to checkpoint inhibitors in metastatic solid tumors [24], but it has not yet been studied in the neoadjuvant trials.

Although numerous genetic alterations have been proposed as potential markers of response to immunotherapy, none to date has been adequately studied to demonstrate utility in predicting response to neoadjuvant immunotherapy for bladder cancer. Nonetheless, it is premature to draw any definitive conclusions which will be dependent on randomized trials. Furthermore, it may yet be shown that certain genetic alterations could be useful in a panel that combines genomic, transcriptomic, and proteomic markers to predict clinical outcome in the future.

Gene Expression Signatures and Molecular Subtypes

The parallel emergence of the molecular classification of MIBC and immunotherapy in metastatic urothelial cancer has led to the identification of tumor subtypes that would benefit most from immune checkpoint inhibition (Fig. 8.1). The IMvigor210 trial reported a greater response to atezolizumab in TGCA cluster II (luminal tumors with immune and stroma infiltration), while basal tumors (TCGA cluster III) achieved a better response to nivolumab than tumors in the other subtypes in Checkmate 275 [5, 45]. A later analysis of IMvigor210 suggested that the genomically unstable subtype (a subset of luminal tumors with high mutation rates) in the Lund tumor taxonomy may correlate best with a favorable response [6]. These results are all derived from single-arm phase II trials, as none of the larger phase III trials with comparator arms in the first- or second-line setting has reported results by molecular subtype. These findings may not be applicable in the neoadjuvant setting owing to the dynamic change of the tumor transcriptome under the pressure of the different treatments [46]. As described above, molecular analysis in patients undergoing second- or subsequent line therapy for locally advanced or metastatic urothelial carcinoma is often performed on archived specimens of the primary tumor prior to any treatment, while in the neoadjuvant setting

the same samples are used without any intervening treatment and with little elapsed time.

The difficulties in determining the predictive value of a biomarker in single-arm trials is particularly relevant to the discussion of molecular subtypes as putative predictive markers of response to neoadjuvant immunotherapy. The prior report indicating that basal tumors may gain the most survival benefit from NAC was dependent on the comparison of overall survival between molecular subtypes when treated with radical cystectomy alone, and when treated with NAC followed by cystectomy [4]. If analysis is performed only in the cohort treated with NAC, there is no convincing difference between subtypes. However, since the basal subtype does poorly without NAC and quite well with NAC, and there is little difference in survival in the other subtypes with and without NAC, it is apparent that the basal subtype predicts response to NAC. It is also important to highlight that molecular subtypes in the prior report did not detect a difference in pCR rate but only a survival difference between subtypes after NAC. With these findings in mind, it is not surprising that the reported single-arm phase II trials do not provide definitive evidence of a predictive effect of molecular subtypes.

In PURE-01 (neoadjuvant pembrolizumab), Necchi et al. reported the impact of molecular subtyping and immune signatures on pathological complete response (pCR) and progression-free survival (PFS) and compared these to the previously reported NAC cohort. For both NAC and neoadjuvant chemotherapy, pathologic response was not clearly linked with molecular subtype. There was a suggestion that luminal unstable (equivalent to genomically unstable) and basal-squamous tumors from the consensus model [3] and claudin-low tumors from the University of North Carolina model [47] had higher rates of pathologic downstaging to non-muscle invasive disease after treatment with pembrolizumab with rates of 68.4%, 65.4%, and 63.6% respectively, compared to 53.6% for the overall cohort. In ABACUS, there was no clear link between subtype according to the Lund taxonomy and pathologic response.

The basal tumors (across subtyping models) in PURE-01 demonstrated the highest PD-L1 expression and the highest immune gene expression. Four previously described hallmark immune signatures were investigated, including the immune190 score and IFNg, IFNa, and inflammatory response signatures. These were all more highly expressed in basal, stroma-rich, and claudin-low tumors, and all were significantly associated with pathological response to pembrolizumab on multivariable analysis. These signatures were not associated with response to NAC. In addition, TMB did not differ across subtypes and TMB did not predict response to pembrolizumab.

The relationship of subtyping to PFS was difficult to interpret for the reasons cited above, and due to small sample size within the different subtypes. When comparing the PURE-01 cohort to the NAC cohort, there was a suggestion that claudin-low tumors and luminal infiltrated tumors (equivalent to the TCGA cluster II tumors), both rich in immune infiltrate, had better PFS than the same subsets treated with NAC. This is reinforced by preliminary analysis of a small subset of patients in BLASST-1 [14]. In PURE-01, there was a trend toward improved PFS in patients

with the highest quartile immune190 score compared to the lowest quartile (2-year PFS 93% vs 79%, $p = 0.15$) [10]. Intriguingly, the same patients had a worse prognosis in the NAC cohort, suggesting a differential response to different neoadjuvant therapies based on immune gene expression signature.

The authors of the ABACUS trial focused on a predefined eight gene RNA expression signature (IFNG, CXCL9, CD8A, GZMA, GZMB, CXCL10, PRF1, and TBX21) that reflects interferon signaling and the presence of CD8+ effector T cells. This signature was significantly higher in complete responders compared to non-responders who were subclassified into those with stable disease (presumably reflecting residual MIBC after neoadjuvant atezolizumab that did not relapse during follow-up) and those who relapsed [11]. The ABACUS results also recapitulated results from IMvigor210, in that a TGF-β-induced gene signature correlated with an immune-excluded phenotype, suggesting that TGF-β may be an important regulator of response to neoadjuvant immunotherapy [6]. The same TGF-β response signature predicted poor response to dual checkpoint blockade in NABUCCO. However, there was no correlation between pathologic response and baseline interferon gamma, tumor infiltration, or CD8+ T cell effector signatures in the NABUCCO trial, which the authors interpreted as meaning that the addition of anti-CTLA-4 to PD-1 blockade can induce pCR in tumors irrespective of pre-existing CD8+ T cell immunity [12].

Overall, these results suggest that benefit from neoadjuvant immunotherapy may correlate with molecular subtypes, but this appears to be driven primarily by immune infiltration, which is recognized as a key component of the subtyping models (Fig. 8.1).

Figure 8.1 Biological characterization of 404 patients from the publicly available TCGA 2017 cohort according to gene expression and mutation status and using the consensus and TCGA subtype classification. (Taken from [48] with permission from Springer)

Immunohistochemical Immune Profiles

A well-described biomarker of non-responsiveness to immune checkpoint inhibition is the absence of lymphocytes within the tumor microenvironment. Indeed, in a tumor devoid of immune cell, often referred to as a "cold tumor," a therapy that acts by unleashing activated T cells cannot elicit an immune response if there are no T cells present [49]. This is reflected in the descriptions above of the RNA-based immune signatures. Other analyses have focused on immunohistochemical markers of immune activity in primary tumors.

CD8+ immune cells in tumors are considered a marker of pre-existing T cell immunity. Borrowing from the metastatic setting where the presence of intratumoral CD8+ cells was associated with better objective response [6], ABACUS also showed that tumors with greater than the median number of intraepithelial CD8+ cells demonstrated an increased pCR rate (40% vs 20%; $p < 0.05$) compared to

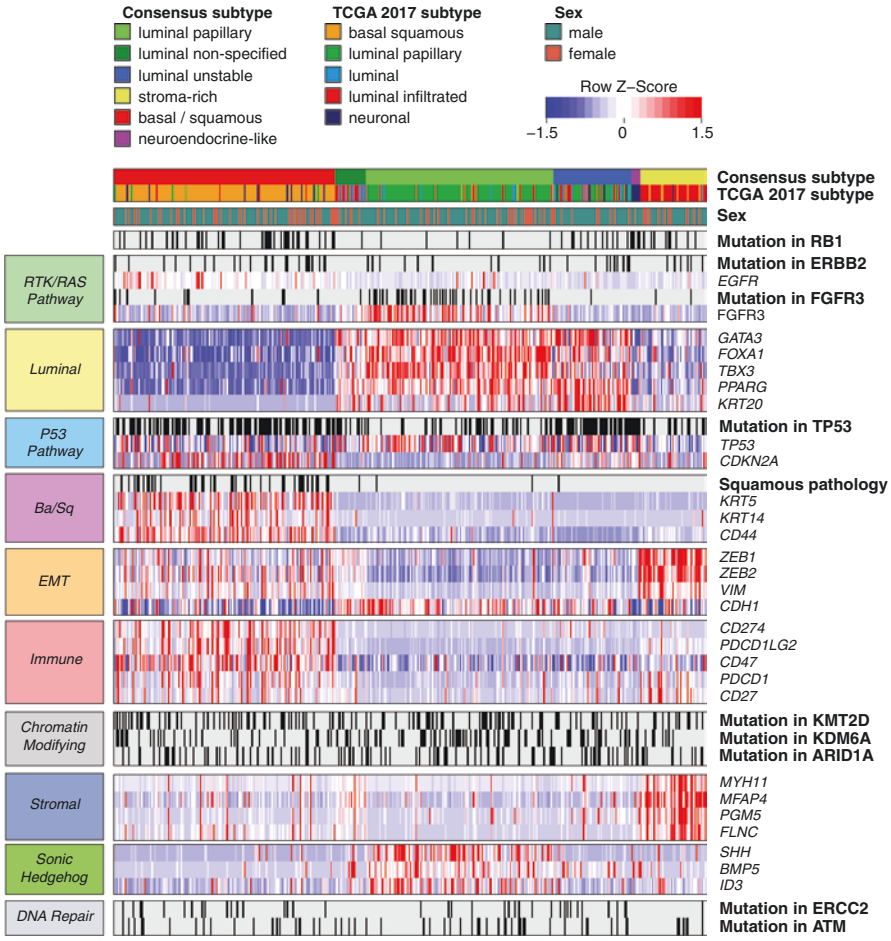

Fig. 8.1 Biological characterizationBiomarkersgene expression signatureBiomarkersmolecular subtype of 404 patients from the publicly available TCGA 2017 cohort according to gene expression and mutation status and using the consensus and TCGA subtype classification

tumors with less than the median number of CD8+ cells [11]. The authors distinguished three different tumor CD8+ immune phenotypes, referred to as desert (no CD8+ cells in microenvironment), excluded (CD8+ immune cells around the periphery of the tumor), and inflamed (CD8+ immune cells in the tumor). The T cell inflamed phenotype was much more common in the neoadjuvantly treated tumors compared to a prior report in metastatic urothelial carcinoma, which may have prevented the ability of the authors to correlate this phenotype to pathological response in the neoadjuvant setting as they had previously done in the metastatic setting [6]. To carry the analysis of the tumor immune infiltrate further, the investigators of ABACUS focused on the quality of immune infiltrate using granzyme B (GZMB) immunostaining, a surrogate marker of CD8+ lymphocyte activity. The results showed a higher level of dual GZMB+/CD8+ expression in responding tumors compared to those that relapsed [11].

The importance of immune cell infiltration was also observed in the MDACC trial. The authors reported a higher density of B, CD4+, and CD8+ T cells in responding tumors compared to non-responding tumors. The authors focused on tertiary lymphoid structures (TLS) as a specific type of tumor immune infiltration. TLS are ectopic lymphoid formations that exhibit the characteristics of lymph nodes structures including T cell, dendritic cell and proliferating B cells, and high endothelial venules (HEV). Tumor infiltration by TLS has been associated with a greater prognosis in some solid cancers (e.g., lung carcinoma, melanoma, colorectal carcinoma) [50]. Gao et al. showed that a higher density of TLS in the pretreatment tumor correlated with a longer overall and recurrence-free survival. This corresponded also with the observation of higher expression of a four-gene TLS signature (*POU2AF1*, *LAMP3*, *CD79A*, and *MS4A1*) in responding tumors compared to responding tumors [13].

As alluded to above, the authors of the NABUCCO trial did not observe a correlation between baseline CD8+ immune cell infiltration and pathological response to dual immune checkpoint blockade. They also failed to show a correlation between the number of TLS at baseline and response to therapy, although treatment analysis revealed enrichment of TLS on therapy in complete responders. T-reg infiltration in TLS was reduced after therapy. The NABUCCO investigators also analyzed ICOS+CD4+ T cells as a specific biomarker of anti-CTL4 treatment and found a greater increase in tumors from responders than non-responders [12].

Future Role of Biomarkers

Currently, there are no validated biomarkers for checkpoint blockade either in the neoadjuvant or metastatic setting, although several biomarkers are associated with clinical outcomes. As clinical trial data expand and our understanding of correlative biology evolves, these promising biomarkers and other novel markers may ultimately be proven to have utility in selecting patients for neoadjuvant immunotherapy in the clinic. The essential next step is to test the biomarkers discussed here in the ongoing prospective randomized trials which will provide comparative data from other treatment arms.

Established biomarkers continue to evolve. For example, the burden of insertion and deletion events (also known as indels) may be complementary or superior to TMB [51, 52]. In parallel to this progress, novel biomarkers are likely also to develop in the near future. Circulating tumor (ct)DNA, circulating tumor cells (CTC), and extracellular vesicle (EV) analysis could become particularly relevant in an effort to overcome spatial molecular heterogeneity in the primary bladder tumor [53–58]. It is not uncommon that the genomic landscape diverges between different parts of the primary tumor and between the primary tumor and lymph node metastasis [59, 60]. The biomarker data in neoadjuvant setting is obtained from individual tissue chips from transurethral bladder tumor resection. Circulating markers, together referred to as a liquid biopsy, can provide a summary evaluation of different clones in the primary locoregional disease. ctDNA, for example, allows

us to identify genetic alterations and mutational load that may be more representative of the entire tumor than any one resection chip from the primary tumor [61]. A drop in ctDNA levels can also act as an early response marker after initiation of immunotherapy [62]. CTCs and EVs expand this analysis to include proteins and RNA.

Peripheral immune markers are likely to take on a more important predictive role in the future. Mass cytometry to assess peripheral immune cells and T cell receptor sequencing to determine T cell diversity are untapped biomarker opportunities in the neoadjuvant disease state [63–65]. High-plasma interleukin-8 (pIL-8) levels and IL8 gene expression in peripheral blood mononuclear cells have been linked to decreased efficacy of atezolizumab in patients with metastatic urothelial and renal cell carcinoma, and dynamic changes on therapy may also predict response [66]. This is thought to relate to high IL8 expression in myeloid cells that suppress tumor antigen presentation. Similar analyses will be necessary for the neoadjuvant therapy trials.

Another domain of active research and rapid discovery is the role of the gut microbiome in regulating response to immunotherapy [67]. This promises not only to provide a biomarker to predict response but also to lead to a therapeutic intervention to enhance response. Moreover, the urinary microbiome remains relatively understudied in this context [68].

The future of biomarkers to predict response to neoadjuvant immunotherapy depends of course first on the demonstration in phase III trials that immunotherapy is efficacious. It may end up being efficacious only in a subset of patients, making biomarkers critical from the beginning as a companion diagnostic. Furthermore, as more diverse neoadjuvant therapies are tested, including especially combination therapies, and as the number of options increases, the need for biomarkers to select the optimal therapy for individual patients will increase. Clinical trials dedicated to the validation of predictive markers have been conducted or launched to guide optimal neoadjuvant chemotherapy [69] and the subsequent need for radical cystectomy (e.g. Alliance A031701 NCT03609216), and similar trials will be needed for neoadjuvant immunotherapy.

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Chapter 9
The Role of Immunotherapy as Bladder-Sparing Solution for Muscle-Invasive and Non-muscle-Invasive Bladder Cancer: Current Status and Future Perspectives

Giuseppe Basile, Giovanni Enrico Cacciamani, Simone Scuderi, Francesco Barletta, Vito Cucchiara, and Elio Mazzone

Introduction

Radical cystectomy (RC) with bilateral pelvic lymph node dissection (PLND) is the standard of care for patients with localized or locally advanced muscle-invasive bladder cancer (MIBC) [1]. Moreover, according to the European Association of Urology (EAU) guidelines, RC is the only recommended option in case of BCG-unresponsive or recurrent non-muscle-invasive bladder cancer (NMIBC) with high-risk features [2]. However, although RC is an effective treatment with a 60% 5-year overall survival (OS) [1–3], it represents a risk-bearing surgery with a high perioperative complication rate and mortality [4], especially in frail or elderly patients. Moreover, a non-negligible proportion of patients may be unfit to undergo RC due to poor preoperative performance status [5]. It is also of note that many patients treated with RC and urinary diversion often report a progressive decline in their quality of life (QoL) [6]. Thus, bladder-sparing solutions are urgently needed to fulfill the gap of request of this increasing number of patients who aim to postpone or avoid RC without impairing their survival. However, the diffusion of organ-preserving strategies for bladder cancer (BCa) treatment is still arduous due to the

G. Basile · S. Scuderi · F. Barletta · V. Cucchiara · E. Mazzone (✉)
Unit of Urology, Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Vita-Salute San Raffaele University, Milan, Italy
e-mail: Mazzone.elio@hsr.it

G. E. Cacciamani
The Catherine and Joseph Aresty Department of Urology, USC Institute of Urology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

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lack of evidence from large series or randomized controlled trials (RCTs) supporting their use. Several bladder-preservation options have been proposed, such as maximal transurethral resection of bladder tumor (TURBT) alone, partial cystectomy, radiotherapy (RT), or chemotherapy (CHT) alone. Nevertheless, single-modality treatments result in inferior oncological outcomes compared with RC for MIBC [1]. In this context, the concept of personalized medicine has become crucial to guide physicians toward the best therapeutical choice, and a multidisciplinary approach is mandatory. Consequently, the main alternative to RC that can be proposed to selected patients is represented by the trimodal therapy (TMT). The rationale of TMT is to achieve local tumor control by TURBT and RT, while adding systemic CHT or other radiosensitizers to improve local radiation effect and control on micrometastases spread [1]. Recently, the field of BCa treatment has been revolutionized by the encouraging results of immune checkpoint inhibitor (ICI) trials in metastatic and bladder-confined disease, offering novel opportunities as adjuvant and neoadjuvant strategies [7, 8]. Hence, immunotherapy gained momentum, and several ongoing clinical trials testing ICIs are making their way also as bladder-sparing solutions with the aim to improve BCa control together with RT alone or chemoradiotherapy (CRT) combination.

Bladder-Sparing Techniques for MIBC: Where Do We Stand?

Bladder-sparing strategies have recently emerged as a valid treatment option for patients with BCa. However, there are no completed RCTs or large prospective series comparing the outcomes of bladder-sparing strategies with RC. Therefore, current evidence mostly derives from retrospective studies or small series. Several are the barriers of bladder-sparing therapy spread in clinical practice which have limited the possibility to offer these alternative solutions to patients in routine clinical practice. For instance, the multicenter phase III SPARE trial was recently designed to randomize patients with MIBC to bladder preservation treatment or RC after neoadjuvant CHT response assessment [9]. The study failed to accrue patients and resulted in premature closure due to clinician and patient preferences for radical treatment. Moreover, there is a perception that bladder-sparing treatments result in an inferior survival compared to RC [10]. To date, there are two conditions in which multimodal-sparing strategies could be proposed: (1) patients fit for RC who aim to maintain their bladder and QoL or (2) elderly patients who are unfit for RC [1]. For the former group, patient selection is mandatory; for the latter, less stringent criteria can be applied [1, 5, 11]. To date, despite the optimal candidates for bladder preservation therapy including patients without nodal involvement [12], tumor-associated hydronephrosis, extensive CIS, poor bladder function, or tumor invasion into the prostate, there are still no agreed selection criteria to identify patients who can benefit the most from bladder-sparing treatments compared to RC [11, 13].

Trimodal Therapy as Alternative to RC for MIBC

Several RC series reported pathological complete response (pCR) rates in up to 18.9% of patients without neoadjuvant CHT treatment [3, 14], suggesting that a radical TURBT-only approach could be curative in selected patients. However, about 20% of MIBC have nodal involvement at final pathology [14]. Therefore, the staging ability and the potential therapeutic effect of an extended pelvic lymph node dissection (ePLND) are missed in a TURBT-only approach. Consequently, to partially compensate the role of ePLND, TMT consisting of maximal TURBT, external-beam RT, and concurrent CHT represents the best alternative treatment compared to RC [1]. There are two scheduled combination treatment following maximal TURBT: split course chemoradiation and single course chemoradiation [1, 15, 16]. For both regimens, bladder biopsies should be performed to detect nonresponders who may be treated with salvage cystectomy [1]. Platinum-based CHT protocols are the most used as radiosensitising drugs, despite a non-negligible proportion of patients being ineligible due to impaired renal function or poor performance status [17]. Consequently, alternative CHT regimens based on MMC/5-FU [18] or low-dose gemcitabine [19] showed acceptable toxicity and good efficacy. Recent systematic reviews and meta-analysis reported data on the effectiveness of bladder-sparing therapy vs. RC plus ePLND. In terms of OS and progression-free survival (PFS), no significant difference was found between the two groups (OS, HR, 1.06; 95% CI 0.85–1.31), (PFS, HR, 1.11; 95% CI 0.63–1.95) [20]. However, when considering studies including only patients who had surgically unresectable disease, 4-year OS was poor and ranged from 30% to 42%, while in operable patients, the 5-year OS was 50%, ranging from 36% to 74% [15].

Bladder-Sparing Technique and Neoadjuvant Immunotherapy for MIBC: New Perspectives

Despite TMT showing a comparable efficacy to RC in appropriately selected patients, the 5-year OS of nonmetastatic BCa remains around 50% [21]. Thus, therapeutic innovations are urgently needed in the treatment of MIBC, in case of patients unfit for RC or patients who aim to preserve their bladder without impairing their survival. Since the introduction of systemic immunotherapy in BCa treatment, many drugs targeting PD-1 or PD-L1 have been approved as second-line options for metastatic disease progressed after platinum-based CHT [22–26]. Moreover, interim results of ongoing studies, both in the neoadjuvant and adjuvant setting, are continually reshaping the classical concept that ICIs can be administered exclusively as a second-line therapy or in the context of clinical trials [27–30]. Notwithstanding, based on reports of phase III trials [31–33], the Food and Drug Administration (FDA) recently amended the use of pembrolizumab and atezolizumab in patients

with high expression of PD-L1 [7]. In the context of ICI delivery, CRT has shown both immunostimulatory and immunosuppressive properties through modulation of tumor microenvironment [21], leading to immunogenic cell death and an increase in immune biomarkers [21]. Thus, the rationale of CRT and immunotherapy combination is to synergize the efficacy of these systemic therapies to both increase eradication of local tumor and distant micrometastases. Given this hypothesis, despite a precise tumor biomarker's assessment and patient selection which is probably needed to evaluate who can benefit the most from combination therapies, several clinical trials were launched also in the context of bladder-sparing treatments, both as concurrent and sequential treatments.

Clinical Trials Testing Immunotherapy with Concurrent Chemoradiotherapy

Several ongoing clinical trials are testing the effect of CRT or RT only with concurrent systemic ICI delivery as bladder-sparing option. To date, only few studies reported results, and they are mainly based on small cohorts of patients of phase I/II trials. Atezolizumab has been tested in a phase I trial in combination with hypofractionated RT and gemcitabine in patients with localized MIBC. Despite results being available only on eight patients, the study was closed prematurely due to unacceptable grade 3 gastrointestinal adverse events (AEs), also after atezolizumab dose reduction [34]. Conversely, encouraging results were reported by the NCT02662062 phase II trial [35] testing pembrolizumab plus CRT in a cohort of patients who either wished to attempt bladder preservation or were ineligible for RC. Interim results showed that nine out of ten patients achieved CR after combination treatment and were free from metastasis at 6 months. Moreover, combination of pembrolizumab plus CRT appeared to be safe, despite four out of ten patients experienced grade 3–4 non-urinary AEs within 12 weeks of completing treatment. Following these results, the PLUMMB (NCT02560636) trial also reported on the safety of RT and pembrolizumab combination in patients with metastatic or locally advanced MIBC. However, the authors advise caution in combining RT and pembrolizumab, particularly when RT is given at high dose per fraction for pelvic tumors [36]. In this context of chemotherapy and/or RC ineligible patient population, other clinical trials released interim results. NCT02891161 is a phase I/II trial of concurrent durvalumab and RT followed by adjuvant durvalumab. After completion of durvalumab plus RT course, combination therapy was generally well tolerated and appeared to be effective with a clinical CR of 71.4% [37]. Finally, a phase II trial (NCT03421652) testing nivolumab and RT-only combination treatment reported partial results [38]. Concurrent nivolumab and RT showed to be tolerable in terms of AEs, and 6 out of 14 patients were complete responders after treatment at 12 months. Conversely, four patients had residual T1 disease or carcinoma in situ, and four had disease progression. Of note, PD-L1 combined

positive score (CPS) was <1% in all nonresponders except one patient with CPS of 5%. A summary of clinical trials testing combination therapy of immunotherapy plus chemo- or radiotherapy is reported in Table 9.1.

Table 9.1 Clinical trials testing immunotherapy with concurrent chemoradiotherapy

Study	Phase	Intervention	Eligibility	Primary endpoint	Main findings
NCT03775265 (SWOG/ NRG-1806)	III	Atezolizumab	T2–T4a MIBC	BI-EFS	Recruiting 475 patients
NCT04241185 (MK-3475– 992/ KEYNOTE-992)	III	Pembrolizumab	T2–T4 N0M0 MIBC	2-year BI-DFS	Recruiting 636 patients
NCT02621151 (MK3475)	II	Pembrolizumab	T2–T4a N0M0 MIBC	2-year BI-DFS	Recruiting 54 patients
NCT03617913	II	Avelumab	T2–T4a N0M0 MIBC	CR	Recruiting 27 patients
NCT02662062 (PCR-MI)	II	Pembrolizumab	T2–T4a N0M0 MIBC	Grade 3–4 AE	Recruiting interim results: 9/10 patients achieved CR at 6 months
NCT03620435	II	Atezolizumab	T2–T4 N0M0 MIBC	DLT in stage 1, safety (grade ≥ 3 AEs)	Closed 25 patients
NCT03844256 (CRMI)	I/II	Nivolumab	T2–T4a N0–1, M0 MIBC	AEs, DLT, DFS, DFS rate	Recruiting 50 patients
NCT04216290 (INSPIRE)	I	Durvalumab	Any T, N1–2, M0 MIBC	CR	Recruiting 114 patients
NCT03421652 (NUTRA)	II	Nivolumab	T2–T4b N0/+, M0 MIBC	PFS	Recruiting interim results: 6/14 patients achieved CR, 4 had residual T1 disease or carcinoma in situ, 4 had disease progression at 12 months
NCT03747419	II	Avelumab	T2–T4, N0M0 MIBC	CR	Recruiting 24 patients
NCT03702179 (IMMUNO PRESERVE)	II	Durvalumab + tremelimumab	Patients with localized MIBC treated with bladder preservation intent	Pathological response (≤ cT1c)	Recruiting 32 patients

(continued)

Table 9.1 (continued)

Study	Phase	Intervention	Eligibility	Primary endpoint	Main findings
NCT02891161 (DUART)	I/II	Durvalumab	T2–4 N0–2, M0 MIBC	DLT, PFS, disease control rate	Active, not recruiting 42 patients Interim results: 15/21 patients achieved clinical CR at 12 months
NCT02560636 (PLUMMB)	I	Pembrolizumab	T2-4, N0-3, M0-1	MTD	Active, not recruiting 34 patients The study met the protocol-defined definition of DLT
NCT03993249	II	Nivolumab	T2-4a N0 M0 MIBC	2-year locoregional control rate	Recruiting 78 patients

Table 9.2 Clinical trials testing chemoradiotherapy with sequential immunotherapy

Study	Phase	Intervention	Eligibility	Primary endpoint	Main findings
NCT03697850	II	CT + RT + Atezolizumab	T2-3 N0 M0	DFS	Recruiting 77
NCT03768570	II	CT + RT + Durvalumab	T2–T4 N0 M0	CR	Recruiting 76
NCT03171025 (NEXT)	II	CT + RT + Nivolumab	T2-4a N0-1 M0	EFS	Recruiting 28

Clinical Trials Testing Chemoradiotherapy with Sequential Immunotherapy

With the aim to reduce the high rates of AEs reported by studies testing concurrent administration of CRT and immunotherapy, several ongoing clinical trials are focusing their attention on the role and possible oncological efficacy of the sequential administration of CRT followed by systemic immunotherapy. In this context, NCT03768570 is a phase II trial testing the administration of durvalumab after completion of TMT. Similarly, NCT03171025 is a phase II trial testing adjuvant nivolumab after CRT in localized MIBC. Conversely, NCT03697850 is a phase II trial testing atezolizumab after CRT in patient's ineligible for RC. A summary of clinical trials testing sequential therapy of immunotherapy plus chemo- or radiotherapy is reported in Table 9.2.

Clinical Trials Testing Immunotherapy Delivery as Bladder-Sparing Solution in Molecularly Selected Patients

Recently, in the era of precision medicine, a great emphasis was placed on BCa molecular features to guide treatment selection. Particularly, urothelial carcinoma has been categorized into molecular subgroups using next-generation sequencing and transcriptomic platform, which showed different patterns of response to immunotherapy. Moreover, tumor mutational burden (TMB) and PD-L1 expression determined by CPS represent another emerging field of interest with the aim to predict response to ICIs. In the context of neoadjuvant ICI delivery for MIBC treatment before RC, many trials reported promising results. The PURE-01 study showed that PD-L1 CPS was the only biomarker associated with CR (OR 1.02, CI, 1.01–1.04) and that only high levels of TMB were associated with CR regardless CPS levels [30]. Conversely, the ABACUS trial reported that TMB and PD-L1 expression did not predict outcomes in patients treated using atezolizumab [27]. Finally, the NABUCCO trial partially confirmed these results, reporting a CR rate of 73% in PD-L1 CPS > 10% tumors compared to 33% CR rate in those patients with PD-L1-negative tumors [29]. Recently, next-generation sequencing allowed also to evaluate the role of DNA damage repair (DDR) genes in BCa. DDR gene alterations appeared to be associated with sensitivity to cisplatin chemotherapy, immunotherapy, and RT in MIBC [26, 39]. Moreover, DDR gene alterations were also associated with a higher TMB compared with tumors with intact DDR genes [40]. In this context, the NCT03558087 phase II trial is enrolling patients with BCa opting to avoid RC, whose tumors harbor deleterious DDR gene alterations and/or high TMB level to be treated with gemcitabine, cisplatin, plus nivolumab. Moreover, with the aim to increase the number of patients who could benefit from bladder preservation treatment, the NCT04506554 phase II trial is testing accelerated methotrexate/vinblastine/adriamycin/cisplatin (AMVAC) combined with nivolumab in selected patients with prespecified tumor mutations in the neoadjuvant setting. To date, data on genomic profiling are emerging, and findings are still discordant, likely due to heterogeneity in treatment types and duration, disease state, and type of ICI among the populations under evaluation. Nevertheless, molecular features, TMB, and biomarker analysis emerged as potentially useful tools to identify patients who may benefit the most from neoadjuvant CHT or ICI treatment, also in the bladder-sparing context. However, to facilitate the adoption of new molecular and biomarkers analyses in BCa treatment, further studies are needed. A summary of clinical trials testing immunotherapy with or without chemotherapy in molecularly selected patients is reported in Table 9.3.

Table 9.3 Clinical trials testing immunotherapy delivery as bladder-sparing solution in molecularly selected patients

Study	Phase	Intervention	Eligibility	Primary endpoint	Main findings
NCT03558087	II	Gemcitabine + cisplatin + nivolumab	T2-4a N0 M0 (DDR-GA +/− TMB-H)	CR	Recruiting 76 patients
NCT04506554 (RETAIN-2)	II	Nivo + AMVAC	T2-3 N0 M0 (ATM, RB1, ERCC2)	MFS	Recruiting 71 patients

BCG, Bacillus Calmette-Guerin, *CIS* carcinoma in situ, *CR* complete response, *DFS* disease-free survival, *MFS* metastases-free survival, *DLT* dose-limiting toxicities, *DOCR* duration of complete response, *EFS* event-free survival, *EBRT* external-beam radiation therapy, *AMVAC* accelerated methotrexate/vinblastine/adriamycin/cisplatin, *MTD* maximum tolerated dose, *MAD* maximum administered dose, *NMIBC* non-muscle-invasive bladder cancer, *RC* radical cystectomy, *RFS* recurrence-free survival, *BI-EFS* bladder-intact event-free survival, *BI-DFS* bladder-intact disease-free survival, *AE* adverse events

Bladder-Sparing and Neoadjuvant Immunotherapy in High-Risk NMIBC

The standard of care for NMIBC is represented by TURBT with a risk-adapted adjuvant intravesical CHT or immunotherapy, which allows to reduce both tumor recurrence and progression rates [2]. Particularly, Bacillus Calmette-Guerin (BCG) is the standard adjuvant treatment for high-risk patients. Nevertheless, a considerable part is doomed to fail or may not be able to complete maintenance course due to adverse side effects. These patients are classified as BCG failure. Patients experiencing BCG failure are unlikely to benefit from additional BCG therapy, and other available bladder-sparing treatments are currently limited; thus the recommended treatment is RC [2]. For patients unfit or unwilling to undergo RC, there is a growing interest in salvage bladder-sparing therapeutical options. Even in the absence of RCTs comparing salvage treatments and RC, novel options have been proposed thanks to the diffusion of ICI agents [41]. The pivotal phase II trial KEYNOTE-057 [42] tested the efficacy and safety of pembrolizumab in patients with high-risk BCG-unresponsive NMIBC who were ineligible for or declined RC. Updated results after 3 years of follow-up reported a 3-month CR rate of 40.6% (95% CI, 30.7–51.1). Of these, 33.3% remained complete responders for longer than 18 months, and 23.1% remained complete responders for longer than 24 months. Among patients who achieved CR, none progressed to MIBC. Conversely, 40 patients (41.7%) underwent RC after discontinuation of pembrolizumab. Among these, 35 had no pathologic upstaging to MIBC, while 3 were upstaged. Pembrolizumab administration showed to be safe, with grade 3/4 immune-related AEs occurring in 3% of patients.

Role of Imaging and Radiomics in Predicting Response to Neoadjuvant Chemotherapy for Bladder-Sparing Approaches

Recent advances in the radiological field have assessed the impact of imaging and radiomics in predicting the response to the NAC in MIBC [43]. Although significant limitations occur from low imaging resolution [44], bladder magnetic resonance imaging (MRI) and multiparametric (mpMRI) features have shown promising results. To standardize the image feature reporting, Panebianco et al. proposed a consensus criterion (vesical imaging-reporting and data system, VI-RADS) combined into a score that relies on morphological characteristics of the tumor, added to T2-weighted imaging, DWI, and DCE features (Fig. 1 [45]).

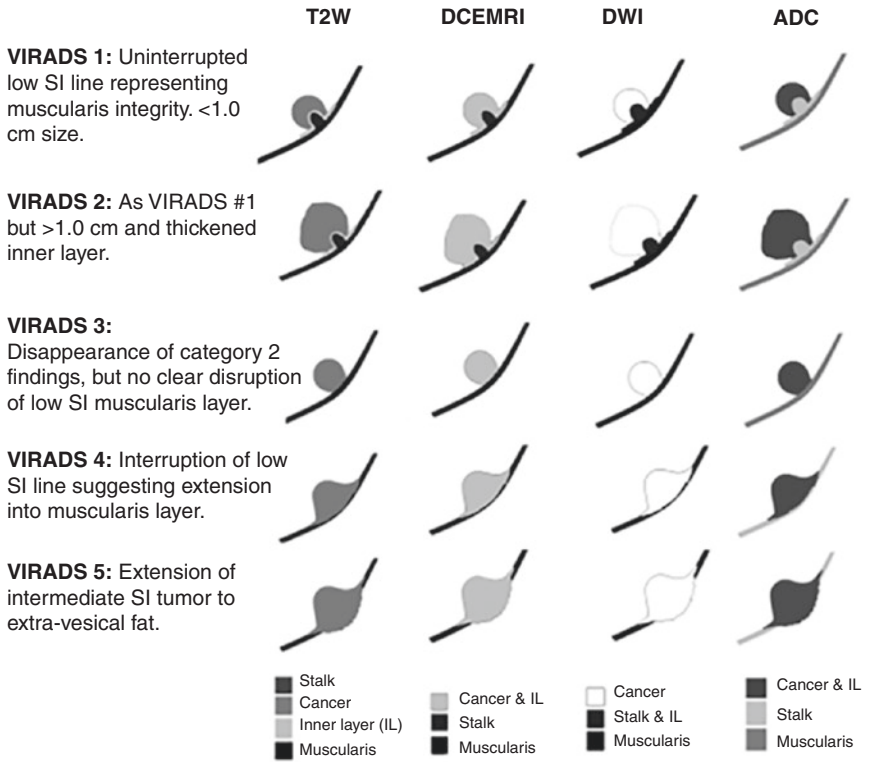

Figure 1 Schematic illustration of mpMRI appearances of VI-RADS scores 1–5 using T2, DCE-MRI, DWI, and ADC. ADC, apparent diffusion coefficient; DCE, dynamic contrast enhancement; DWI, diffusion-weighted imaging; MRI, magnetic resonance imaging; mpMRI, multiparametric MRI; SI, signal intensity [45]. (From Panebianco et al. [45])

Considering the absence of radiation, mpMRI could represent a safe tool to evaluate the patients prior to, during, and following treatment to determine response to NAC (difference in tumor volume and the change in pT classification), for expediting radical treatment or for assessing response to bladder-sparing approaches [45].

Yoshida et al. investigated the role of diffusion-weighted (DWI) MRI in predicting sensitivity to NAC in muscle-invasive bladder cancer. MIBC patients underwent induction chemoradiation (CRT) consisting of radiotherapy to the small pelvis with two cycles of cisplatin (20 mg/day for 5 days), followed by surgical treatment. They found that the apparent diffusion coefficient (ADC), which provides functional information of tumors, such as disorganization of tumor tissue and the high cellularity, was the significant and independent predictor of CRT sensitivity [46].

Nguyen et al. assessed the impact of k-means clustering of dynamic contrast-enhanced (DCE)-MRI pharmacokinetic parameters in predicting chemotherapeutic response in MIBC patients at the mid-cycle time point. The authors found that complex microcirculatory significant changes within an MIBC could be assessed using the k-means clustering of the two DCE-MRI pharmacokinetic parameters to enable early prediction of the tumor's NAC response [47].

Necchi et al. evaluated the potential of bladder mpMRI to predict the complete pathological response to NAC. The authors assessed patients with bladder mpMRI before and after pembrolizumab therapy (PURE-01 study) prior to RC. The imaging protocol consists of triplanar T2-weighted fast spin-echo sequences and DWIs in transverse planes at different b-value [48]. The promising results in terms of acceptable inter-reader variability (k values = 0.5–0.76) represent the foundation for the proposal of a radiological NAC response definition for predicting the response to pembrolizumab.

Radiomics is an emerging field of quantitative imaging with a variety of applications in clinical practice and research, particularly oncology. This deep-learning model-based technique has shown potential applications in oncology since it provides a noninvasive characterization of the tumor, using a panel of quantifiable tumor metrics (radiomics signature) which are extracted from multimodality medical images including computed tomography (CT), positron emission tomography (PET), MRI, and ultrasonography (US) [49]. Specifically for BC, radiomics showed promising potential in BC detection, staging, grading, and response to therapy [50]. Cha et al. assessed the utility of deep-learning convolutional neural network (DL-CNN) based on CT bladder cancer segmentation to evaluate the response to NAC. In this pilot study, using changes in tumor volumes, the World Health Organization (WHO) criteria, and the response evaluation criteria in solid tumors (RECIST) for defining the NAC response, the DL-CNN was trained to identify the patterns in the regions that were inside and outside of the bladder lesion. They found that the receiver operating

characteristic curve (AUC) for volume changes was 0.73 ± 0.06, showing that DL-CNN can rely on accurate BC for calculating tumor size change in response to NAC [51].

Although these AI-based approaches in clinical practice are disadvantaged by the deficiency of familiarity among radiologists [50, 52] and by the restricted accessibility of efficient and standardized systems of feature extraction and data sharing, radiomics improve the physician's armamentarium in personalizing a tailored-fit patient management [52].

Conclusions

Despite TMT bladder-sparing strategies demonstrating similar oncological outcomes compared to RC especially for patients eligible for surgery, high-level evidence is still needed to improve their diffusion which may facilitate the implementation of novel neoadjuvant approaches based on the use of ICI. To date, the available results of clinical trials testing the association of CRT with concurrent or sequential immunotherapy are still fragmented. However, despite it appearing to be effective in terms of early tumor response, the rate of adverse events currently recorded is not negligible. Moreover, type of treatment association, different populations included, and different measure of response to treatment make the interpretation of the results arduous. Moreover, the many ongoing clinical trials on the subject will help answer many practical questions related to combination therapy, such as scheduling and dose. Undoubtedly, phase II/III clinical trials will show the real role and possible applications of combination therapy as a bladder-sparing solution, especially in well-selected cohorts of BCa patients.

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Part II
Management of High-Risk Prostate Cancer in the Perioperative Setting

Chapter 10
An Introduction on Immunotherapy in Prostate Cancer

Giorgio Gandaglia, Riccardo Leni, and Alberto Briganti

Introduction

Significant advances have been achieved over the last few years in the understanding of the relationship between the immune system and different types of cancer, where it has been proposed that the immune system might be able to recognize and eliminate tumoral cells and, therefore, prevent the onset and progression of malignant diseases. As such, immunotherapy emerged as a promising therapeutic approach for a number of different tumors. For example, blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) axis with immune checkpoint inhibitors (ICIs) has revolutionized cancer care in tumors with dismal prognosis such as metastatic melanoma and metastatic non-small cell lung cancer (NSCLC). The aim of immunotherapeutic approaches would be to specifically target malignant cells expressing tumor-specific antigens and to spare at the same time healthy tissues. This, in turn, would confer advantages compared to therapeutic approaches indistinctly directed to the entire gland such as surgery and radiotherapy or systemic therapies with their short- and long-term side effects. This paradigm shift brought the whole landmark of oncological treatments toward a new era of immuno-oncology.

The potential efficacy of immunotherapy with ICIs relies upon tissue inflammatory characteristics such as tumor-infiltrating lymphocytes (TILs) and tissue somatic mutations. The current definition of immunologically "hot" tumors encompasses a spectrum of factors, where concentration of TILs and high tumor mutational burden (TMB) are undoubtedly the most relevant. Evidence of tumors expressing such

G. Gandaglia (✉) · R. Leni · A. Briganti
Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy
e-mail: gandaglia.giorgio@hsr.it

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characteristics led to the design of clinical trials with immunotherapy in a variety of cancers such as melanoma, NSCLC, colorectal cancer (CRC) with microsatellite instability (MSI), and head and neck squamous cell carcinoma (HNSCC). In these settings, we observed a widespread diffusion of immunotherapy. When considering genitourinary (GU) cancers, an increasing evidence of a potential role of immune infiltration is currently available for many diseases to date. For example, bladder cancer (BCa) and renal cell carcinoma (RCC) are considered the most immunologically active GU tumors, and a survival advantage of immunotherapy has been demonstrated in different settings. Moreover, immunotherapy with ICIs is currently under evaluation in many trials for metastatic renal cell carcinoma (mRCC), muscle-invasive bladder cancer (MIBC), as well as rare GU tumors such as metastatic penile squamous cell carcinoma (mPSCC) and upper tract urothelial carcinoma (UTUC) with promising results.

Prostate cancer (PCa) represents the most frequent and prevalent GU cancer, being the second most common solid tumor worldwide with approximately 1.5 million of new cases diagnosed in the year 2020 alone. A potential role of immunotherapy in the field of metastatic castration-resistant PCa (mCRPC) has been firstly proposed more than 10 years ago, when the administration of autologous active cellular immunotherapy with sipuleucel-T has been shown to increase overall survival (OS) in this setting [1]. On the other hand, PCa itself has not entered the "hall of fame" of immunologically "hot" tumors, and early available evidence of PCa infiltration by TILs suggests that this disease should be considered rather a "cold" tumor, where a scant immune infiltration is associated to a relatively low frequency of somatic mutations and inflammatory cytokines [2]. Nonetheless, PCa tissue expresses multiple tumor-associated antigens (TAAs) which might represent a target for the adaptive immune system. Moreover, the relatively prolonged natural history of PCa itself might provide an adequate time frame for the development of an antitumor immune response. As such, PCa recently attracted a lot of interest as a suitable target for immunotherapeutic intervention. Of note, immunotherapy in the field of PCa should be differentiated between "active" and "passive" therapeutic approaches. While "passive" treatments include the delivery of monoclonal antibodies with a high specificity for tumor antigens, "active" approaches include the administration of tumor vaccines. The main research in the field of PCa is currently focused on monoclonal antibodies and, in particular, ICIs.

Evidence of Immunotherapy Activity in Patients with Advanced Prostate Cancer

Immune checkpoint inhibitors (ICIs) represent a class of monoclonal antibodies with the ability to bind to immune checkpoint receptors and to prevent the inactivation of T-cell function. Among the others, the most commonly used ICIs are represented by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed

death-1 (PD-1), and programmed death-ligand 1 (PD-L1). Antibodies against these ICIs have been shown to induce potent antitumor immune responses in a variety of cancers. When considering PCa patients, these molecules have been mainly tested in the setting of mCRPC disease.

Pembrolizumab, an anti-PD-1 monoclonal antibody, was evaluated in 23 heavily pretreated patients with mCRPC in the phase Ib Keynote-028 trial. A median duration of response of 13.5 months was reported, along with a 60.9% of treatment-related adverse events (TRAEs) [3]. The results from the Keynote-199 phase II trial of pembrolizumab in pretreated patients with mCRPC showed promising results. The strongest benefit was reported in bone-predominant disease, where a 14.1 months OS was reported along with 22% disease-control rate [4]. Evidence of suboptimal activity of ipilimumab, an anti-CTLA-4 monoclonal antibody, was shown by a nonsignificant difference in overall survival (OS) in two phase III multicenter trials in patients with metastatic castration-resistant PCa (mCRPC) [5, 6]. Similarly, the IMbassador250 phase III multicenter trial tested the combination of enzalutamide plus atezolizumab, an anti-PD-L1 monoclonal antibody. This trial was designed on the rationale of converting an unfavorable immunologic milieu to a more favorable one after the administration of androgen blockade. Such strategy of turning an immunologically "cold" tumor into its "hot" counterpart has been extensively tested across different cancers with heterogeneous results [7]. Moreover, recent evidence showed a downregulation of PD-1/PD-L1 axis checkpoints in PCa, which might explain the failure of single-agent immunotherapy strategy explored in previous trials [8]. The phase III CheckMate 650 trial was designed on the rationale of a compensatory upregulation of the PD-1/PD-L1 axis a consequence of CTLA-4 inhibition in PCa cells. The combination of nivolumab, an anti PD-1 monoclonal antibody, with ipilimumab showed an encouraging 5.5 vs 3.8 months in median progression-free survival (PFS) and 19 vs 15.2 months in median OS in patients with mCRPC [9]. Another trial was designed to revert immune suppression in PCa through the combination of ICIs and tyrosine kinase inhibitors (TKIs). The COSMIC-021 is a multinational phase 1b basket trial currently evaluating atezolizumab combination in solid malignancies. Preliminary results of the COSMIC-021 Cohort 6 showed an objective response rate (ORR) of 32% in 44 patients with mCRPC treated with atezolizumab plus cabozantinib, a TKI targeting various pathways associated with tumor immune suppression [10]. Additionally, novel potential biomarkers of immune sensitivity include genomic alterations in homologous recombination defect (HRD) genes (23%), Fanconi anemia (FANCA) genes (5%), cyclin-dependent kinase (CDK) 12 (6%), and mismatch repair (MMR) genes (4%) [11]. Patients harboring these genomic alterations might represent a distinct subset of PCa patients prone to increased sensitivity to ICIs [12]. This prompts to a comprehensive molecular and histopathological selection of the best PCa candidate to receive ICI [13]. The most informative means of obtaining adequate biomarker information is comprehensive genomic profiling (CGP). Status of single-nucleotide variants (SNVs), loss of heterozygosity (LOH), microsatellite instability (MSI), and tumor mutational burden (TMB) can be obtained at once. Routine CGP was able to identify actionable alteration to tailor-targeted therapy and immunotherapy in more

than 50% of cases from a prospective evaluation of 3476 clinically advanced prostate tumors [11]. To date, no clear benefit of immunotherapy in the treatment strategy of mCRPC has been proven, the role of ICIs is largely elusive, and more results are needed to formulate solid conclusions to tailor management decisions. Moreover, ICIs are associated by a significant toxicity profile that needs a comprehensive evaluation considering the already significant burden of symptoms of these patients [14].

Ongoing Trials with Immunotherapy in Patients with Advanced Prostate Cancer

Previous knowledge of published trials has tailored a better design for further ongoing trials. The added value of combination therapy is the key factor of actual trials in patients with advanced PCa. Pretreated patients in cohort A of the phase Ib/II Keynote-365 trial will receive pembrolizumab 200 mg IV every 3 weeks and olaparib 400 mg PO twice daily after progression from hormonal therapy. Preliminary results at a median follow-up of 14 months from 84 patients showed a PSA response rate (PSA decline ≥50%) of 9% with 35% of high-grade TRAEs [15]. The phase III Keynote-641 trial is designed on the rationale of a synergic action, in mCRPC, between pembrolizumab and enzalutamide previously showed in the early-phase Keynote-365 and further-phase II studies [15, 16]. The Keynote-641 began in 2019 and has an expected accrual of 1200 patients randomized to enzalutamide 160 mg/day plus pembrolizumab 200 mg IV every 3 weeks versus enzalutamide and placebo [17]. The phase III Keynote-921 trial was designed on the basis of promising results of the administration of docetaxel and pembrolizumab in the early phase Keynote-365 trial after enzalutamide and abiraterone. An estimate of 1000 patients will be randomized from 2019 and will be treated with docetaxel 75 mg/m2 IV every 3 weeks plus prednisone and pembrolizumab 200 mg IV every 3 weeks vs. docetaxel plus prednisone and placebo [18]. The evidence of single-agent pembrolizumab and olaparib activity in pretreated patients with mCRPC tailored the design of the phase III Keylynk-010 trial. Patients with progression after taxane chemotherapy will be stratified according to previous therapy and will be randomized to receive pembrolizumab plus olaparib versus abiraterone plus prednisone versus enzalutamide [19]. Novel immunotherapies in advanced PCa rely upon bispecific T-cell engagers (BiTEs), among which blinatumomab, first studied in hematologic malignancies, is the only approved drug. BiTEs target tumor antigens and T-cells inducing a CD19- and CD3-mediated activation of patients T-cells promoting release of tumor-killing cytokines [20]. Preclinical evidence showed activity of AMG 160, a prostate-specific membrane antigen (PSMA), and CD3 BiTE in selective killing of PSMA-positive PCa cells in vitro. Clinical efficacy of BiTEs is currently being evaluated in the phase I AMG 160 study, in which patients with mCRPC are treated with AMG 160 IV every 2 weeks. This trial encompasses an early phase of dose exploration of AMG 160, followed by a combination therapy with AMG

Table 10.1 Ongoing trials with immunotherapy in mCRPC

Trial	Phase	Year of start	Accrual, pts	Treatments	Primary endpoint
Keynote-365 [15] NCT02861573	Ib/II	2016	1000	*Cohort A* Pembro+Olaparib *Cohort B* Pembro+TXT	PSA response rate (PSA decline ≥50%)
Keynote-641 [17] NCT03834493	III	2019	1200	Pembro+Enza vs Enza+placebo	OS, rPFS
Keynote-921 [18] NCT03834506	III	2019	1000	Pembro+TXT + PDN vs TXT + PDN + placebo	OS, rPFS
Keylynk-010 [22] NCT03834519	III	2019	780	Pembro+Olaparib vs Abi/Enza+PDN	OS, rPFS
AMG 160 [21] NCT03792841	I	2019	288	AMG 160 Pembro	Safety and tolerability

Pembro pembrolizumab, *TXT* docetaxel, *Enza* enzalutamide, *Abi* abiraterone, *OS* overall survival, *rPFS* radiological progression-free survival, *PDN* prednisone

160 and pembrolizumab. Preliminary results in 43 patients with mCRPC treated only with AMG 160 are encouraging. As expected, cytokine-release syndrome (CRS) was observed in almost 90% of patients, with high-grade CRS in 25.6%. Antidrug antibodies (ADAs) have been observed in 20% of patients. No patients discontinued AMG 160 for TRAEs. Ratios between 10% and 30% of response were observed; in particular, 27% of patients had confirmed PSA reduction ≥30%, with 3 patients with undetectable circulating tumor cells (CTCs) after treatment [21] (Table 10.1).

Further Perspectives of Immunotherapy for Patients with Localized Prostate Cancer

Of note, the majority of research efforts in ICIs for PCa is in the metastatic or mCRPC setting, where currently available therapies still offer limited efficacy in terms of survival benefit. Clinically localized or non-metastatic locally advanced PCa is mostly treated either with radical prostatectomy (RP), active surveillance (AS), or radiotherapy (RT); only a minority of patients need neoadjuvant or adjuvant therapies, mainly due to adverse pathological features (AP) after RP. However, a stage migration toward more aggressive forms of PCa (mainly high-risk Gleason ≥8) induced by the recommendations against PSA screening by the US Preventive Services Task Force (USPSTF) in 2012 might have exploited the need for effective perioperative therapies for high-risk PCa [23]. To date, adjuvant immunotherapy is not approved for the management of high-risk PCa. Similarly, although phase I/II

trials are ongoing, no published data are available in the neoadjuvant setting for high-risk localized PCa. Ongoing trials that will provide novel insights into the potential role of immunotherapy in PCa include patients treated with the administration of three cycles of neoadjuvant pembrolizumab (PEM-PRO and PICT-01), pembrolizumab and enzalutamide (NCT03753243), atezolizumab plus tocilizumab (NCT03821246) or plus PROSTVAC (AtezoVax, NCT04020094), or PROSTVAC plus ipilimumab (NCT02506114). Studies in the neoadjuvant setting will undoubtedly open toward the possibility of better understanding the biology of the disease and the potential role of immunotherapy with a comprehensive pathology assessment, especially in the lymph nodes and the PCa inflammatory milieu. This, in turn, would allow for understanding the relationship between immune checkpoint blockade and tissue biomarkers. The combination of administration of neoadjuvant ICIs with novel in vivo imaging technique such as ^{68}Ga-PSMA PET/CT will give the unmet possibility of early evaluation of immunotherapy action perioperatively. Combining tissue biomarker analyses with in vivo imaging will help in defining what patients will benefit most from ICIs both in the neoadjuvant and in the advanced PCa setting.

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Chapter 11
Preoperative Immunotherapy for Prostate Cancer: From Bench to Bedside

Charles G. Drake

Introduction: The Rationale for Neoadjuvant Immunotherapy in Prostate Cancer

A seminal study by Liu and colleagues directly compared preoperative to postoperative immunotherapy in a number of immunocompetent, syngeneic murine models [1]. These data showed that treating animals harboring multifocal metastatic disease with a sequential combination of systemic immunotherapy followed by surgery was able to induce long-term regression in the majority of animals treated, despite the fact that surgery alone was completely ineffectual in this metastatic setting. Although the immunobiology underlying this therapeutic regimen has yet to be fully elucidated, a basic understanding of the mechanisms underlying the activity of immune checkpoint blockade (ICB) using anti-PD-(L)1 agents provides two possible explanations: first is the notion that ICB functions by reversing the exhausted phenotype of tumor-infiltrating CD8 T cells, allowing them to acquire effector function and mediate tumor cell destruction. If that is indeed the case, then in the postsurgical setting, the majority of those cells is expected to be absent, removed along with the primary tumor. Second is the alternative, but not necessarily mutually

C. G. Drake (✉)
Columbia Center for Translational Immunology, Columbia University Irving Medical Center, New York, NY, USA

Division of Hematology and Oncology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY, USA

Janssen Research and Development, Spring House, PA, USA

Columbia University Medical Center, Herbert Irving Comprehensive Cancer Center, Division of Hematology/Oncology, New York, NY, USA
e-mail: cgd2139@columbia.edu

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exclusive, hypothesis that ICB primarily affects the "priming" phase of T cell activation, i.e., the initial recognition of a tumor antigen by its cognate T cell in the draining lymph node. During priming, the interaction of PD-1 on the antigen-specific T cell and PD-L1 on dendritic cells in the lymph node affects initial T cell activation and subsequent programming, attenuating an antitumor response, and ICB blocks that attenuating signal. Given that an adequate lymph node dissection is part and parcel of good cancer surgery, it also stands to reason that, if ICB functions in the priming phase, the absence of tumor draining lymph nodes in the postoperative setting could also lessen the impact of ICB. Other mechanisms may be involved as well, some data suggest that resection of the primary tumor lesion promotes the conversion of activated T cells to a more long-lived memory phenotype [1]. The immunobiology of preoperative immunotherapy, as well as its application to lung cancer and melanoma, has recently been reviewed in depth [2]; we will thus restrict our focus here to preclinical and clinical data relevant to prostate cancer.

Prostate cancer is the second most common cause of cancer-related mortality in men, and definitive local therapy represents the only treatment modality with the potential for cure [3]. Despite advances in surgical approaches, patients with high-risk localized prostate cancer have a significant likelihood of disease recurrence following definitive local therapy [4]. To date, no neoadjuvant therapy preceding prostatectomy has demonstrated sufficient efficacy to warrant regulatory approval. Although the development of the cancer vaccine sipuleucel-T for castration-resistant prostate cancer (CRPC) demonstrated the efficacy of immunotherapy in prostate cancer, immune checkpoint blockade has not yielded significant rates of response in patients with advanced disease [5], except perhaps when used in combination regimens [6, 7]. One significant challenge to inducing antitumor immunity in prostate cancer is the non-inflamed tumor microenvironment (TME) [8]. Further, prostate tumors generally have a low mutational burden as well as low PD-L1 expression; these factors predict a lack of response to immunotherapy in other tumor types [9, 10]. Finally, prostate tumors demonstrate multiple mechanisms of immune escape including defective antigen processing, decreased MHC class I expression, and infiltration with regulatory T cells (Tregs), myeloid-derived suppressor cells, and M2 macrophages [11–13].

The Immune Effects of Androgen Deprivation Therapy

Understanding the immune effects of androgen deprivation therapy (ADT) is important because ADT is a standard first-line treatment for recurrent or metastatic prostate cancer [14, 15]. In addition to its proapoptotic effects on cancer cells [16], androgen deprivation therapy drives immune infiltration into the prostate gland [17, 18]. This infiltrate is complex and includes potential antitumor effector cells such as CD8 T cells and M1 CD68+ macrophages [18]. Conversely, infiltrating B cells may serve to promote castration resistance [19]. Previously, we found that castration results in the de novo presentation of a prostate-restricted antigen in the tumor

draining lymph nodes and that the initial pro-inflammatory effects of ADT were sufficient to transiently mitigate T cell tolerance [20]. More recent data from our group [21] and others [22] showed that the immunological effects of ADT are likely far more complex, may evolve over time, and may depend on the therapeutic modality through which ADT is administered. For example, using a syngeneic preclinical model, Pu et al. showed that orchiectomy appears to be immunogenic, while ADT mediated by androgen receptor antagonists like flutamide compromised antitumor T cell responses in vivo [22]. Using the same syngeneic model, our group studied the evolution of the post-castration TME over time; we found that ADT was initially immunogenic, with T_H1 effector T cells predominant. Of note, similar acute effects were demonstrated in a second relevant (rat) model [23]. However, over time the T cell infiltrate was increasingly made up of suppressive, regulatory T cells (Treg); these cells express the canonical transcription factor *FoxP3* and are capable of down-modulating the activity of T cells, NK cells, and other antitumor effectors [21]. In addition, we found that the myeloid components of the TME also underwent repolarization, such that at later time points (approximately 4 weeks post-castration), the TME contained a preponderance of suppressive (M2) macrophages as well as myeloid-derived suppressor cells (MDSC) [24].

To test the ability of ADT to drive T cell infiltration into the prostate gland, we conducted a preoperative, randomized study to quantify the immunologic effects of ADT alone versus ADT combined with a cancer vaccine [25]. The trial was conducted in patients with high-risk localized prostate adenocarcinoma who were planned for radical prostatectomy. Consistent with the preclinical studies described above, both ADT and ADT plus vaccine led to significant increases in intratumoral CD8+ T cell infiltration as well as increased PD-L1 expression as compared to a cohort of untreated, matched control patients. However, the CD8+ T cell infiltrate was accompanied by a precisely proportional increase in regulatory T cells (Treg), suggesting that a process known as "adaptive Treg resistance" may dampen the immunogenicity of ADT. The effects of the vaccine therapy in this trial will be discussed below; the critical point here is that, in patients ADT likely drives T cell infiltration with regulatory T cells and that therapies directed against Treg may be required to optimize preoperative prostate cancer immunotherapy in ADT combination regimens. The other key point is that ADT results in upregulation of PD-L1 in the primary prostate tumor, potentially rendering anti-PD-(L)1 agents relatively more efficacious in this combination setting.

Blocking PD-(L)1 in Prostate Cancer

In contrast to bladder cancer and RCC, clinical results for PD-(L1) blockade in prostate cancer have been disappointing. For example, in the phase 1b trial of the anti-PD-1 agent nivolumab, there were no objective responses seen in the 17 CRPC patients enrolled [26]. The two prostate tumor samples that were collected both tested negative for PD-L1 expression. Other studies support the paucity of PD-L1

Table 11.1 Selected preoperative immunotherapy trials in prostate cancer

Therapy	Number	Phase	Trial ID	Initiation completion
Atezolizumab vs. atezolizumab + tocilizumab vs. atezolizumab + etrumadenant (sequential enrollment)	48	II	NCT03821246	January 2020 August 2022
Pembrolizumab + enzalutamide	32	II	NCT03753243	December 2018 April 2023
Nivolumab + Prostvac	29	II	NCT02933255	April 2017 August 2022
Enoblituzumab (anti-B7-H3)	32	II	NCT02923180	October 2016 October 2021
Daratumumab or JNJ-40346527 (FMS inhibitor)	33	II	NCT03177460	June 2017 June 2020
Non-fucosylated anti-CTLA-4 (BMS-986218) + ADT (degarelix acetate) vs. ADT alone	32	II	NCT04301414	March 2020

expression on prostate tumors, highlighting another challenge in treating the disease [27, 28]. More recently, a larger phase 2 study of the anti-PD-1 agent pembrolizumab in metastatic castrate-resistant prostate cancer (mCRPC) showed an underwhelming objective response rate of approximately 5% [5]. These results are consistent with our findings in preclinical models [21], where anti-PD-1 was ineffectual as a monotherapy. To augment the activity of ICB monotherapy, an ongoing trial at the University of San Francisco (L. Fong P.I.) is testing several novel combinations of anti-PD-L1 (atezolizumab) (Table 11.1). This 48-patient, sequentially enrolled, 3-arm trial is of interest because it includes a monotherapy arm (atezolizumab alone), an arm combining atezolizumab with anti-IL-6 (tocilizumab) to counteract the suppressive elements in the TME, and a third arm combining atezolizumab with etrumadenant, an oral inhibitor of the adenosine pathway likely important in maintaining the suppressive TME in prostate cancer. The primary endpoint here is the a pathological antitumor response assessed post-prostatectomy.

As above, a significant fraction of men with progressive metastatic disease are treated with next-generation androgen receptor antagonists; interestingly resistance to at least one of these agents (enzalutamide) is associated with increased PD-L1 expression [29]. These data suggest a potentially inflamed TME post-progression on ADT. In a phase 2 trial in which the anti-PD-1 antibody pembrolizumab was added to enzalutamide in patients progressing on enzalutamide, three of the first ten patients treated experienced a rapid PSA drop to $\leq$0.2 ng/mL. Two of the patients who had a biochemical response had measurable disease upon study entry; they both experienced a partial response [6]. Further follow-up of this cohort supported these data, leading to the notion that combining a next-generation hormonal therapy with anti-PD-(L)1 might be active in the setting of advanced prostate cancer [30]. While several ongoing trials are testing ADT + ICB combinations in the setting of metastatic disease, a relevant preoperative trial is also testing combination ADT/

immunotherapy (Table 11.1). This study, (NCT03753243, M. Garzotto P.I.), combines the next-generation hormonal therapy (NHT) enzalutamide with the anti-PD-1 agent pembrolizumab; men with high-risk disease will be treated for 16 weeks prior to definitive radical prostatectomy. The trial will enroll 32 patients with a primary endpoint of pathological complete response rate.

Cancer Vaccines

Cancer vaccines prime an immune system to recognize tumor-associated antigens and elicit a T cell response; they are generally comprised of an adjuvant that functions to activate antigen-presenting cells (APCs) like dendritic cells (DC) and a target protein or peptide known to be associated with a specific tumor type [31]. After subcutaneous or intradermal vaccine injection, antigen-loaded DC traffic to the draining lymph nodes where they present small peptide fragments of the target antigen to prime T cell recognition by specific CD8+ T cells, which are able to proliferate and lyse tumor cells presenting their target antigen. Currently the only therapeutic cancer vaccine approved by the US FDA is sipuleucel-T, which is used to treat mCRPC. This personalized immunotherapy product is manufactured by incubating a patient's extracted monocytes with recombinant fusion protein PA2024, which links PAP to GM-CSF and activates PAP-specific T cells. Activated antigen-presenting cells are then reinfused into the patient to elicit an antitumor immune response. The pivotal immunotherapy for prostate adenocarcinoma treatment (IMPACT) trial demonstrated an increased overall survival of 4.1 months for sipuleucel-T patients compared to placebo and led to FDA approval for the vaccine [32]. In a relevant preoperative trial, Fong et al. treated 14 prostate cancer patients with neoadjuvant sipuleucel-T [33]. Treatment resulted in an increased CD8 T cell infiltrate, and the infiltrating cells showed an antitumor, T_H1 effector phenotype. Consistent with the ADT results discussed above, the antitumor effects of infiltrating CD8 T cells appeared to be counteracted to some degree by upregulation of CTLA-4 and the immune checkpoint TIGIT.

A second vaccine approach, Prostvac-VF, uses a heterologous prime-boost strategy with sequential administration of a vaccinia virus (rV-PSA) then fowlpox virus (rF-PSA). These vaccine vectors include the costimulatory molecules ICAM-1, B7.1, and LFA-3 (TRICOM) as well as the target antigen PSA. One of the main challenges with poxvirus-based vaccines is their ability to elicit antibody responses; if given repeatedly, the antibody response to viral proteins attenuates a response to the encoded target antigen. The use of a fowlpox booster vector circumvents this challenge, allowing for repeated administration and increased T cell immunity [34]. This heterologous prime-boost strategy was tested in a phase 2 trial of mCRPC patients; post hoc retrospective analyses showed an 8.5-month increase in OS with a 44% reduction in death rate [35]. However, recent results of a 1200 patient randomized phase 3 trial of Prostvac-VF in combination with GM-CSF (NCT01322490) showed that treatment did not improve overall survival [36]. Those data led to the

early discontinuation of the trial. Relevant to this chapter, an ongoing trial at the NIH (NCT02933255) is testing the combination of Prostvac + ADT using enzalutamide. In addition to safety and tolerability, the primary endpoint for this 29-patient trial is to quantify posttreatment T cell changes in the prostate TME.

Another prostate cancer vaccine that has been unsuccessful in phase 3 trials is GVAX, a vaccine composed of whole tumor cells that have been genetically modified to secrete GM-CSF. The tumor cells provide the antigens for the vaccine; and GM-CSF serves to recruit antigen-presenting dendritic cells (DC). GVAX uses two prostate cancer cell lines: the hormone-sensitive LN-CaP and hormone refractory PC-3 cell lines which were derived from lymph node and bone metastases, respectively. Together, they express a number of prostate cancer-associated antigens [37]. Two phase 2 trials of GVAX prostate demonstrated PSA responses, with patients on higher dose levels showing the development of antibodies against proteins expressed in the vaccine. These results led to the initiation of two phase 3 trials, VITAL-1 and VITAL-2. VITAL-2 compared GVAX plus docetaxel to docetaxel plus prednisone but was terminated after early data showed a disproportionate number of deaths in the GVAX arm as compared to the standard treatment arm. The other trial, VITAL-1, was also terminated early after an early futility analysis revealed a low probability that the trial would meet its primary endpoint of improved survival. Relevant here, we conducted a randomized preoperative trial comparing ADT to the combination of ADT + GVAX prostate [25]. Both ADT and ADT + GVAX resulted in a statistically significant increase in T cell infiltration into prostate tumors as compared to untreated samples, with numerically greater increase in the vaccine group. However, consistent with our preclinical data, the increase in effector CD8 T cell infiltration was nearly exactly balanced by an increase in Treg infiltration, such that the ratio of CD8/Treg was markedly constant across groups. Using multivariate Cox regression, we found that there was a statistically significant increase in the time until PSA progression in the vaccine group, supporting the notion that preoperative immunotherapy may mediate some clinical benefit in prostate cancer, although larger confirmatory studies are clearly required for validation.

Additional Preoperative Immunotherapy Approaches for Prostate Cancer

PD-L1 (B7-H3)

As is by now well-accepted, PD-L1 (also known as B7-H1) plays an important role in maintaining T cell tolerance to tumors [38]. However, the B7 family includes multiple members, most notably B7-H3 (CD276), which was originally identified from a human dendritic cell-derived cDNA library [39]. Unlike B7-H1, B7-H3 is

rather broadly expressed in multiple tissue types including tumor epithelial cells. Because its receptor is currently unknown, the functional role of B7-H3 is not yet clear. On one hand, forced expression of B7-H3 in tumors promotes tumor regression in several models, and knockout of B7-H3 increases tumor growth in several mouse models [40, 41]. Those data suggest an antitumor effect for the B7-H3/receptor interaction. However, multiple clinical datasets argue otherwise. For example, an IHC series by Roth et al. showed that the majority of prostate tumors expressed B7-H3; however the intensity of expression varied [42]. Cancers exhibiting more aggressive phenotypes (larger tumors and those with extra-prostatic extension) expressed higher levels of the protein, and elevated levels of B7-H3 expression correlated with disease progression following surgery. These results were corroborated by several studies, including a series from Johns Hopkins evaluating several hundred cases in a comprehensive manner [44]. Based on the notion that elevated B7-H3 expression at the time of surgery is strongly prognostic for recurrence, Shenderov et al. initiated a preoperative trial of B7-H3 blockade using the monoclonal antibody MGA271 (NCT02923180, Table 11.1). This trial was originally designed to enroll 16 high-risk patients to evaluate the safety of preoperative B7-H3 blockade, with a secondary endpoint of increased CD8 T cell infiltration. Preliminary results reported in abstract format showed a statistically significant increase in CD8 T cell infiltration (as compared to matched control patients), as well as potential Gleason downgrading, so the trial was subsequently expanded to include an additional 16 patients to further characterize the posttreatment TME and to conduct a preliminary assessment of clinical outcome.

CD38

The prostate TME contains a number of suppressive cell types, including alternatively polarized macrophages (M2) and myeloid-derived suppressor cells MDSC (reviewed in [24]). Preclinical studies showed that depletion of MDSC enabled the activity of ICB in relevant models [43] and that secretion of the cytokine IL-23 from MDSC may play a role in the conversion of prostate cancer from androgen-sensitive to castration-resistant disease [45]. Clinically targeting MDSC, however, has proven more challenging, with agents aimed at inhibiting CSF-1R either ineffective or toxic in patients [46]. One clinically tractable target of interest is CD38, which is expressed on a discrete population of MDSC in RCC and other cancers [47] and which can be targeted clinically by the agent daratumumab. To test whether CD38 depletion affect the prostate TME, a preoperative trial at MD Anderson Cancer Center (NCT03177460, S. Subudhi P.I.) has completed enrollment; the key endpoints here are depletion of CD38-expressing suppressive cells and CD8 infiltration.

Treg Depletion

As discussed above, both cancer vaccines [25, 33] and ADT [21] have been show to increase Treg infiltration into prostate tumors. The optimal target through which to manipulate and/or deplete Treg remains unclear, especially in the clinical setting. However, multiple studies, including our own [48] showed that CTLA-4 is relatively overexpressed on the Treg that infiltrate multiple tumor types, including prostate cancer. Preclinical studies showed that an anti-CTLA-4 antibody optimized to mediate depletion is more active than a nondepleting version, both alone and in the context of combination treatment [21, 48]. A clinical homolog has been generated; BMS-986218 is an afucosylated version of anti-CTLA-4 (ipilimumab); defucosylation of a human IgG1 antibody increases the affinity to which it binds to the Fc receptor that mediates antibody-dependent cellular cytotoxicity (ADCC), FcγRIIIa. This agent was shown to be safe in a phase 1 dose escalation trial, and a recommended dose was determined. In an exciting ongoing trial, the Columbia group (M. Dallos P.I.) is testing whether the addition of this depleting anti-CTLA-4 can block the increase in Treg associated with preoperative ADT. This 32-patient trial (NCT04301414) will randomize 32 patients to either ADT alone or ADT combined with Treg depletion, with a primary endpoint of quantifying Treg and the CD8/Treg ratio in radical prostatectomy specimens.

Conclusions

Prostate cancer remains a tumor type that is challenging to treat effectively with immunotherapy. Early preclinical and clinical results suggested that ADT was pro-immunogenic, driving the hypothesis that combining ADT plus immunotherapy would prove synergistically effective in the clinic. The recent failure of a randomized phase 3 trial comparing ADT with enzalutamide to the combination of enzalutamide plus atezolizumab shows that the underlying immunology is likely more complex than initially appreciated. Indeed, ADT drives both pro- and antitumor immune effects, including the accumulation of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg). Ongoing preoperative trials are attacking these issues with both a push and pull, i.e., by either combining pro-inflammatory agents like cancer vaccines with immunotherapy or attempting to attenuate the suppressive elements in the TME by blocking the adenosine pathway or by depleting regulatory T cells. In the end, it might be that both approaches are required to render preoperative immunotherapy an important modality by which to increase the chances for surgical cure, particularly in high-risk patients.

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Conflict of Interest Disclosure Statement CGD is a co-inventor on patents licensed from JHU to BMS and Janssen; has served as a paid consultant to AZ Medimmune, Bayer, BMS, Pfizer, Janssen, Roche, Sanofi Aventis, Genentech, Genocea, Merck, Harpoon, Tizona, and Werewolf; and has received sponsored research funding to his institution from the Bristol Myers Squibb International Immuno-Oncology Network and Janssen. He is currently employed by Janssen Research, Springhouse, PA.

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Chapter 12
Clinical Case Debate: Immunotherapy Versus Alternative Therapies in the Neoadjuvant and Adjuvant Setting of Localized, High-Risk Prostate Cancer

Giorgio Gandaglia, Riccardo Leni, Giuseppe Rosiello, Nicola Fossati, and Alberto Briganti

Introduction

Although over the last decades PSA-based screening resulted in a stage migration phenomenon with an increase in the proportion of low-risk PCa at diagnosis [1], more than 30% non-metastatic PCa patients are currently diagnosed with high-risk disease (namely, PSA ≥20 ng/ml, biopsy grade group ≥4, and/or clinical stage ≥T2c) [2]. These individuals are typically at higher risk of recurrence and mortality, where more than 50% of them would eventually experience biochemical recurrence (BCR) during follow-up after radical prostatectomy (RP), which represents one of the most frequently adopted curative-intent treatments in this setting. For example, while the predicted 5-year BCR-free survival after RP for a 65-year-old man with preoperative PSA of 7 ng/mL, cT1c disease, and Gleason score 3 + 3 (i.e., low risk) is approximately 95%, the same patient with a PSA of 20 ng/mL, cT2a disease, and Gleason score 4 + 4 (i.e., high risk) has a risk of BCR at 5 years of 35%. The risk is even higher for men with more aggressive disease characteristics. In addition, the presence of high-risk features at diagnosis increases the risk of extracapsular extension and lymph node invasion (LNI), where up to 30% of high-risk patients harbor a node-positive disease at final pathology when treated with RP and extended pelvic lymph node dissection [3, 4]. While surgery alone can provide long-term recurrence-free survival rates in selected patients [5], this approach might be suboptimal in men with more aggressive disease characteristics [6]. Therefore, RP is often considered as one of the steps of a multimodal approach that includes radiation therapy or systemic treatments to improve oncologic control. Previous investigations

G. Gandaglia (✉) · R. Leni · G. Rosiello · N. Fossati · A. Briganti
Unit of Urology/Division of Oncology, IRCCS San Raffaele Hospital, Urological Research Institute, Vita-Salute San Raffaele University, Milan, Italy
e-mail: Gandaglia.giorgio@hsr.it

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assessed the role of neoadjuvant and adjuvant androgen deprivation therapy (ADT) in surgically managed PCa patients with aggressive disease features at diagnosis or at final pathology. While a randomized trial evaluating a cohort of patients with node-positive PCa and high disease burden demonstrated a benefit of the use of adjuvant ADT [7], the evidence is scarce regarding the impact of this approach in other settings [8]. Similarly, previous studies failed to show a benefit on strong oncologic endpoints (i.e., overall and cancer-specific survival) associated with the use of neoadjuvant ADT [9, 10]. As such, its use is currently discouraged by available guidelines, and hormonal therapies are considered in the adjuvant setting only in highly selected node-positive patients. On the other hand, several novel systemic therapies including immunotherapy have been introduced in recent years for the management of metastatic PCa patients, and their use has been proposed also in patients at an earlier disease stage, where clinical trials are assessing their safety and efficacy. We aimed at describing the current role of neoadjuvant and adjuvant systemic treatments in the management of high-risk PCa with a particular focus on the potential benefits associated with the use of immunotherapy.

Neoadjuvant Therapies in High-Risk Prostate Cancer

The use of ADT, CHT, and novel antiandrogen therapies before RP has been proposed in high-risk patients with the aim to determine a pathologic response (i.e., reducing the rate of adverse pathologic findings at surgery) and, thus, to improve the long-term outcomes allowing for a complete resection at the time of surgery. Over the last decades, several phase III randomized controlled trials compared neoadjuvant ADT to no treatment before RP and demonstrated a significant reduction in local disease extension measured as the presence of positive surgical margins (PSM), extracapsular extension (ECE), and lymph node invasion (LNI) at final pathology. Although neoadjuvant ADT is associated to pathologic response, no significant risk reduction in BCR-free survival and overall survival was observed. This has been summarized by systematic reviews and meta-analyses of available trials that used different agents (e.g., single antiandrogen or luteinizing hormone-releasing hormone (LHRH) agonists or combined androgen blockade) compared to no treatment before RP [9, 10]. Pooled analyses demonstrated that positive surgical margin (PSM) rates and ECE were lower in patients receiving neoadjuvant ADT compared to patients who received no treatment before RP. Similarly, LNI was significantly lower in patients receiving neoadjuvant ADT. However, no significant improvement of both BCR-free survival and OS was demonstrated. Other systemic therapies such as CHT have been proposed. However, their role in the neoadjuvant setting has been poorly addressed so far. Although several non-randomized phase I/II trials have proved the efficacy of docetaxel plus ADT in terms of feasibility, tolerability, and local tumor control rates, only one RCT (NCT00430183) evaluating docetaxel plus leuprolide or goserelin in HRPCa is ongoing, and the final results are expected in October 2030. A recent interim analysis of the Cancer and Leukemia

Group B (CALGB) 90203 RCT demonstrated an improved BCR-free survival, metastasis-free survival (MFS), and overall survival of CHT compared to RP alone, with, however, no significant difference in the 3-year BCR-free survival [11].

Since novel drugs, such as abiraterone, enzalutamide, and sipuleucel-T, demonstrated a significant overall survival improvement, both alone and in combination with ADT in the metastatic setting, their use has been proposed also in the neoadjuvant setting. However, limited evidence from phase I/II trials is currently available. The combination of abiraterone plus leuprolide demonstrated a significant reduction of intraprostatic androgen levels (dihydrotestosterone/testosterone) at RP, compared to leuprolide alone in a phase II study. However, the addition of abiraterone showed low incidence of complete response and minimal residual disease at final pathology compared to ADT alone, with residual ECE in the majority of patients [12]. In a recent phase III RCT [13], men with localized HRPCa were randomized to abiraterone plus prednisone plus LHRH agonists versus LHRH agonists alone followed by RP. No significant differences were observed in terms of pathologic results. However, a significant reduction in tumor volume at final pathology was observed in patients treated with abiraterone plus LHRH agonists, and lower tumor epithelium volume correlated with improved BCR-free survival beyond 4 years of follow-up.

The role of neoadjuvant ADT before RT was explored in four phase III RCTs [14–18]. In the Radiation Therapy Oncology Group (RTOG) 86-10 trial, men with non-metastatic PCa, including patients with clinical lymph node involvement or extraprostatic disease, were randomized to neoadjuvant ADT with goserelin and flutamide or no ADT prior to RT. At 10-year follow-up, the authors reported an improvement in BCR-free survival and cancer-specific mortality (CSM), but not in OS for ADT plus RT versus RT alone [14]. The Trans-Tasman Radiation Oncology Group (TTROG) 96-01 trial randomized men candidate to receive definitive RT in three arms: 3 months of goserelin + flutamide before RT, 6 months of goserelin + flutamide before RT, and RT alone. At 10 years, OS, CSS, and MFS were significantly higher in the 6-month arm, compared to RT alone, with no significant difference between the 3-month arm and RT alone. Moreover, 6 months of ADT prior to RT were also associated with longer BCR-free survival, metastasis-free survival, and OS, compared to RT alone. These results were demonstrated a tendency to achieve better treatment efficacy in high-risk PCa patients, such as clinical stage T2c-T4, GS > 7, and PSA > 20 ng/mL [16, 17]. The RTOG 94-08 randomized men with non-metastatic, node-negative PCa, up to cT2b, to 4 months of ADT (flutamide plus either goserelin or leuprolide) versus RT alone. ADT plus RT was significantly associated to lower CSM, BCR rates, and incidence of distant metastasis. In a subsequent post-hoc analysis, a greater clinical benefit at 10 years was observed in the high-risk group [18]. To address the lack of definitive evidence on duration of ADT before RT, as emerged from the results of the TTROG 96-01 trial, two phase III RCTs compared survival outcomes between short ADT course (2 or 3 months) and long ADT course (7 or 8 months) before RT. After an average follow-up of 8 years (6.6 and 9.4 years), no significant differences in BCR-free survival and OS were observed, prompting to consider 2 or 3 months of ADT adequate before RT [19].

Neoadjuvant chemo-hormonal therapy (CHT) before RT was studied in the Groupe d'Etude des Tumeurs Uro-Genitales (GETUG) 12 phase III RCT. Men with high-risk localized or locally advanced PCa, who received staging pelvic lymph node dissection (PLND), were selected for at least one clinical risk factor between clinical stage cT3-T4, Gleason score ≥8, PSA >20 ng/mL, and pN1 disease. Patients were randomized to ADT with goserelin plus docetaxel and estramustine versus ADT alone, and RT was administered at 3 months. Twelve-year BCR-free survival was improved in the CHT group compared to ADT alone (49.4% vs. 36.3%). Twelve-year clinical relapse-free survival, defined as incidence of metastases, proven local relapses, or deaths, was improved in the CHT group (58.8% vs. 50.5%). Neoadjuvant treatment with ADT plus docetaxel and estramustine was well-tolerated, and no significant differences in treatment-related toxicity were recorded [20].

Based on these evidences, the European Association of Urology (EAU) guidelines currently discourage the use of neoadjuvant ADT before RP, despite the use of neoadjuvant plus concomitant short-term ADT before RT is suggested in the intermediate- and high-risk settings [21]. The National Comprehensive Cancer Network (NCCN) guidelines discourage the use of neoadjuvant ADT in low-risk localized prostate cancer before RP, but accept ADT as both neoadjuvant, concurrent, and adjuvant therapy in association with external beam radiation therapy (EBRT). When final pathology is available (i.e., after RP), the use of ADT or CHT adds an undoubtedly significant opportunity for studying PCa biology and potential resistance patterns after neoadjuvant therapy.

Adjuvant Systemic Therapies in High-Risk Prostate Cancer

The administration of adjuvant therapies in PCa patients has been proposed with the aim of improving of disease recurrence-free survival and ultimately overall survival in patients with adverse pathologic features (i.e., PSM, ECE, SVI, and LNI) at RP who are typically considered at increased risk of recurrence. Several studies evaluated the role of additional systemic therapies after RP, and the majority of them focused on ADT. In particular, the ECOG 3886 was published in 1999 and evaluated the role of early ADT after RP in 98 men with node-positive PCa after RP and PLND. Men were randomly assigned to immediate ADT with either goserelin or bilateral orchiectomy, versus observation alone. At 10-year follow-up, men in the treatment arm had an increased OS and PFS compared to men in the observation arm [22]. Although this trial represents a level 1 evidence supporting the use of hormonal therapies in patients with LNI at RP, some limitations preclude its generalizability to the clinical practice. In particular, the study included a relatively small cohort of patients with high nodal burden diagnosed in the pre-PSA era. Therefore, the use of adjuvant ADT in node-positive patients is currently restricted to selected cases based also on the results of retrospective studies showing a potential benefit of observation or adjuvant RT + ADT in men with lower nodal burden [23, 24]. On the other hand, no studies demonstrated an improved survival associated with adjuvant

ADT in node-negative patients [7]. Therefore, the updated versions of the EAU and NCCN guidelines recommend against the use of ADT after RP in node-negative patients and encourage ADT, either alone or in association with RT, in patients with evidence of nodal disease after RP (Table 12.1) [21].

Table 12.1 Clinical guideline recommendations on the use of neoadjuvant and adjuvant therapies in high-risk PCa patients

Guideline	Neoadjuvant therapies	Adjuvant therapies
EAU-EANM-ESTRO-ESUR-SIOG 2020	Do not offer neoadjuvant ADT before RP (Strong)	*Candidate to RP* Do not prescribe adjuvant ADT in pN0 patients after RP (Strong) Discuss three management options with patients with pN+ disease after RP (Weak): 1. Adjuvant ADT 2. Adjuvant ADT + RT 3. Observation*
		Candidate to RT In patients with *high-risk localized* disease, use EBRT in combination with long-term ADT (2–3 years) (Strong) In patients with *high-risk localized* disease, use EBRT with brachytherapy boost, in combination with long-term ADT (2–3 years) (Weak) In patients with *locally advanced* cN0 disease, offer RT in combination with long-term ADT (Strong)
*Patients after eLND and <2 nodes with microscopic involvement and a PSA <0.1 ng/mL and absence of extranodal extension Strong…Weak = Strength of recommendation		
NCCN Version 3.2020	No indications	*Exp. patient survival >5 years or symptomatic and candidate to RP* pN0 and adverse pathology: EBRT ± ADT (Category 2B) pN+: ADT (Category 1) ± EBRT (Category 2B)
		Exp. patient survival >5 years or symptomatic and candidate to RT EBRT + ADT (1.5–3 years) ± docetaxel* (Category 1) EBRT + brachytherapy + ADT (1.5–3 years) (Category 1 for ADT)
		Exp. patient survival ≥5 years and asymptomatic: No indications
*For very high risk only (Category 1) Category 1 … Category 2B = NCCN Categories of Evidence and Consensus		

EAU European Association of Urology, *EANM* European Association of Nuclear Medicine, *ESTRO* European Society for Radiotherapy and Oncology, *ESUR* European Society of Urogenital Radiology, *SIOG* International Society of Geriatric Oncology, *NCCN* National Comprehensive Cancer Network, *ADT* Androgen deprivation therapy, *RP* Radical prostatectomy, *EBRT* External beam radiation therapy

Three phase III trials evaluated the role of adjuvant chemotherapy either alone or in association with ADT after RP. The TAX-3501 was a phase III RCT that compared leuprolide with docetaxel versus leuprolide alone in men treated with RP. This trial was concluded prematurely due to insufficient accrual to detect significant changes [25]. Similarly, the Southwest Oncology Group (SWOG) S9921 trial, designed to evaluate mitoxantrone combined with goserelin and bicalutamide, was closed after three patients in the mitoxantrone group were diagnosed with acute myelogenous leukemia [26]. The recent phase III Scandinavian Prostate Cancer Group (SPCG) 13 trial enrolled men with high-risk pT2 margin-positive or pT3a Gleason score ≥4 + 3, pT3b, or lymph node-positive disease Gleason score ≥3 + 4. Men were randomized to six cycles of adjuvant docetaxel without continuous prednisone versus observation. This study failed to show a clinical benefit of adjuvant docetaxel on BCR-free survival in this cohort of patients. Known limitations of this study include that some patients received RT before reaching the endpoint and not every patient received docetaxel by protocol [27]. Based on these observations, EAU guidelines encourage the use of adjuvant CHT only within clinical trials.

An increasing number of studies evaluating the most appropriate adjuvant therapy after RT are available. The RTOG 85-31 trial enrolled 977 patients with high-risk PCa, either node positive or T3-T4, who were treated with RT and subsequently randomized to goserelin, administrated during the last week of RT or afterward. After 10 years of follow-up, data from the RTOG 85-31 trial showed a significant lower incidence of distant metastases, CSM, and a significant improvement of OS in the goserelin arm compared to RT alone [28]. The EORTC 22863 phase III trial randomized 412 patients with high-risk, node-negative PCa to RT plus goserelin plus cyproterone for 3 years versus RT alone. Results, at 10 years of follow-up, from the EORTC 22863 trial showed a significant improvement of both OS, DFS, and DSS for the ADT group compared to RT alone [29]. These trials proved the net benefit, in terms of survival, of adding ADT after RT in patients with high-risk PCa, and further studies were designed to adjust the optimal duration of ADT. Long (2.5 years) versus short (6 months) adjuvant ADT (flutamide or bicalutamide) in 1113 patients with high-risk PCa previously treated with RT resulted in improved DFS, MFS, and OS compared to the short course [30]. The RTOG 92-02 trial evaluated the addition of 2 years ADT to patients treated with RT plus ADT and confirmed previous evident benefit in terms of DFS and MFS. In a subgroup post-hoc analysis in patients with high-risk disease (Gleason 8–10), OS was significantly improved [31]. To further analyze the benefit of long- versus short-term ADT, two phase III RCTs compared short ADT versus prolonged ADT. The TROG 03.04 study showed that long-course ADT plus zoledronic acid was more effected than the short course [32]. Similar results were obtained in the DART 01/05 phase III RCT that randomized men between RT plus goserelin plus flutamide or bicalutamide for 2 years and 4 months of treatment [33]. More recently, a pooled individual patient analysis of two RCTs demonstrated a significant benefit of adjuvant ADT after RT

compared to neoadjuvant ADT followed by RT in patients with localized PCa. Adjuvant ADT following RT was superior in terms of BCR, BCR-free survival, and incidence of metastases compared to the neoadjuvant approach. PCa-specific mortality (PCSM) was not significantly different between arms, but, when selecting only patients with HRPCa, a tendency of lower PCSM in the adjuvant arm was observed [34].

The role of CHT was explored in a prematurely closed phase III trial (RTOG 9902) that randomized 397 patients with high-risk PCa between RT plus 2 years of bicalutamide or flutamide plus leuprolide or goserelin and RT plus 2 years of adjuvant ADT plus paclitaxel, estramustine, and etoposide. The trial was terminated due to excess of thromboembolic events in the CHT arm, with no significant 10-year OS difference between groups [35]. The ongoing STAMPEDE trial includes the comparison between standard of care (SOC) and adjuvant therapies such as ADT with enzalutamide or abiraterone, docetaxel, and therapies with zoledronic acid, metformin, prednisolone, transdermal estradiol, and celecoxib. A survival benefit was observed when comparing docetaxel or abiraterone in adjunction to standard ADT, compared to ADT alone. However, in a post-hoc analysis on non-metastatic PCa, patients failed to confirm such advantage [36].

To date, in accordance with available data, EAU and NCCN guidelines suggest adding long-term ADT to patients with high-risk PCa treated with RT [21]. Moreover, the NCCN guidelines recommend the administration of docetaxel, in combination with RT plus ADT only in very-high-risk PCa.

Rationale for Immunotherapy in High-Risk Prostate Cancer Considered for Radical Prostatectomy

As highlighted above, no level 1 data suggesting a survival benefit associated with the use of ADT before surgery in PCa patients are currently available. Similarly, the evidence supporting a role for adjuvant systemic therapies after radical prostatectomy is limited. It should also be noted that the use of ADT is characterized by a non-negligible risk of side effects that have a profound effect on health-related quality of life [37, 38]. Therefore, there is an urgent need for novel systemic treatments administered in a multimodal setting to improve the outcomes of high-risk PCa patients undergoing RP + ePLND.

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. For example, the presence of CD8+ T cells and the ratio of CD8+ effector T cells/FoxP3+ regulatory T cells (Tregs) correlate with improved prognosis and long-term survival in solid malignancies, such

as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Although PCa has been historically considered as an immunologically "cold tumor," previous studies demonstrated that immunotherapy with an autologous active cellular immunotherapy (sipuleucel-T) might improve overall survival in men with metastatic disease [39]. Beside sipuleucel-T, a dendritic vaccine which was approved by the FDA for PCa in 2010, PROSTVAC (PSA-TRICOM), a PSA-targeted recombinant viral vaccine, has been developed and proposed in PCa patients. The TRICOM platform incorporates the co-stimulatory molecules B7.1, ICAM-1, and LFA-3. Presentation of these molecules to naïve T cells during antigen presentation favors type 1 helper T-cell responses, characterized by antigen-specific cytotoxic T-cell proliferation [40]. PROSTVAC-VF immunotherapy was well-tolerated and associated with a 44% reduction in the death rate and an 8.5-month improvement in median OS in men with mCRPC [41]. More recently, the advent of immune checkpoint inhibition (ICI) has revolutionized the therapeutic approach of different malignancies, and its role has been proposed also in PCa patients. Different programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), and anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) inhibitors have been included in disease-specific therapeutic pathways. To avoid immune-mediated antitumor activity, PCa cells have shown the ability to upregulate programmed death ligand-1 (PD-L1), the ligand of programmed death-1 (PD-1), a checkpoint inhibitor that reduces T-cell activity and promotes immune anergy. PD-1 is highly expressed on regulatory T cells (Tregs), and interaction between PD-L1 and PD-1 promotes Treg proliferation and suppression of T-cell antitumor activity [42]. Blocking the PD-1/PD-L1 axis can revert the tumor milieu to an antitumor immune cell phenotype and induce T-cell-mediated tumor suppression, as previously reported in other malignancies [43]. Historically, PCa was considered a non-immunogenic tumor. However, recent developments in the field of metastatic PCa showed efficacy of PD1/PD-L1 axis blockade, as well as CTLA-4 targeting, opening an unexplored field of interest also in patients with high-risk PCa eligible for surgical treatment [44].

The administration immune checkpoint inhibitors might lead to an antitumor immunity. This is particularly true if PD-L1 is expressed by tumor cells in the prostate and regional lymph nodes. In this context, it has been proposed that PD-L1 expression in PCa cells is proportional to Gleason score at final pathology, ranging from 50% in patients with favorable pathology (ISUP grade group $\leq$2) to 90% in patients with unfavorable pathology (ISUP grade group $\geq$3) [45, 46]. Moreover, there is evidence of PD-L1 expression in up to 15% of patients with LNI after RP. In a retrospective analysis of 51 patients with LNI after RP, a PD-L1 expression $\geq$1 was associated with shorter metastases-free survival (MFS) compared with no detectable PD-L1 with immunohistochemistry (IHC). In particular, patients with PD-L1 $\geq$1% had an almost 4-fold increased risk of developing metastases during

follow-up [47]. It should also be noted that low CD8 and higher PD-L1 expression might be associated with a shorter time to BCR and metastases compared to their counterparts with high CD8 and low PD-L1 [48]. PD-L1 can therefore be considered a potential marker for selecting a subset of patients with high-risk disease that are at increased risk of adverse oncologic outcomes after RP. Previous studies supported a role for the administration of PD-1/PD-L1 inhibitors in metastatic castration-resistant patients. In this context, the open-label phase II Keynote-199 trial is currently evaluating the efficacy of pembrolizumab in five cohorts of patients with mCRPC previously treated with docetaxel and ADT and is due to completion by the end of 2021. Initial results from cohorts 1, 2 (PD-L1-positive vs. PD-L1-negative patients with mCRPC), and 3 (bone-predominant disease, irrespective of PD-L1 status) showed promising results in terms of ORR, especially in the bone-predominant cohort [49]. Taken together, these observations highlight that targeting the PD-1/PD-L1 axis with ICIs may induce an antitumor immunity in selected PCa patients. This represented the basis for the design of trials aimed at assessing the role of neoadjuvant and adjuvant immunotherapy with both vaccines and ICIs.

Neoadjuvant immunotherapy with ICIs would hypothetically result in a pathologic response reducing the rate of LNI, tumor volume, and the risk of positive surgical margins at RP. More favorable pathologic outcomes would, in turn, have a beneficial effect on the risk of PSA persistence and early BCR, which are associated with an increased risk of cancer-specific mortality at long-term follow-up. Similarly, the administration of adjuvant ICIs or other forms of immunotherapy to selected patients with more adverse pathologic features (i.e., extraprostatic extension or lymph node metastases) might theoretically reduce the subsequent risk of experiencing metastases and of dying from the disease itself.

Trials in the neoadjuvant or adjuvant setting offer the unmatched possibility of sequential tissue biomarker research. The first obvious advantage of such approach is to identify biomarkers that could be used to select high-risk PCa patients more likely to benefit from the administration of immunotherapy in the perioperative period. Moreover, the availability of pathologic results might represent an early surrogate endpoint for stronger outcomes in the neoadjuvant setting, where the effect of the administration of neoadjuvant immunotherapy on tumor volume, risk of positive margins, and LNI after RP can be assessed. Finally, the use of advanced PCa imaging techniques such as 68Ga-PSMA PET/CT and multiparametric MRI will allow for an in vivo assessment of tumor progression and thus might theoretically identify those patients who are experiencing a response in terms of local and distant disease control. Analyses on blood and urine samples allow further circulating biomarker analyses, both tumor-related, such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs), and immune-related, such as interleukins, to better select potential subsets of patients that could benefit most from neoadjuvant or adjuvant immunotherapy.

Ongoing Trials Assessing the Role of Neoadjuvant and Adjuvant Immunotherapy in Prostate Cancer

When considering patients with localized disease, a recent study (NCT02153918) demonstrated that the administration of neoadjuvant PROSTVAC before surgery induced both tumor immune response (namely, increased CD4 T-cell infiltrate in tumor margins and CD8 T-cell infiltrate at the tumor core) and peripheral immune response [50]. A multi-center, open-label, randomized phase II trial of PROSTVAC or ipilimumab or the combination of PROSTVAC and ipilimumab as neoadjuvant therapy is currently enrolling patients with localized disease. Eligible patients will be randomized to PROSTVAC monotherapy (Arm A), ipilimumab monotherapy (Arm B), or combination therapy with both PROSTVAC and ipilimumab (Arm C), prior to RP. In all three arms, radical prostatectomy (RP) will occur 21 days, or 3 weeks, following final treatment administration of PROSTVAC or ipilimumab (NCT02506114). The primary outcome of the study is to assess the proportion of participants who demonstrate a positive response following neoadjuvant therapy as measured by the change from baseline in CD3+ T-cell infiltration within prostate tumor tissue by immunohistochemistry (IHC) assessment following treatment. Finally, an ongoing phase II clinical trial is currently assessing the role of PROSTVAC in high-risk patients treated with RP in the adjuvant setting (NCT02772562).

Robust evidence of the efficacy of PD1/PD-L1 axis blockade in the neoadjuvant setting is available in other genitourinary malignancies, such as muscle-invasive bladder cancer (MIBC) [51]. When considering PCa patients, several trials aimed at assessing the role of immunotherapy in the neoadjuvant or adjuvant settings are ongoing. The single-arm, phase II study of Neoadjuvant PEMbrolizumab Before Radical PROstatectomy in High-risk Prostate Cancer Patients (PEM-PRO, NCT04565496) has been designed to evaluate the efficacy of three cycles of neoadjuvant pembrolizumab to reduce the risk of LNI after RP. Disease progression will be monitored with multiparametric magnetic resonance imaging (mpMRI) and ^{68}Ga-PSMA PET/CT obtained prior to neoadjuvant therapy and before RP. Secondary endpoints are radiological progression, defined by tumor diameter at mpMRI; risk of positive surgical margins and pathologic response, defined as presence of minimal residual disease at final pathology; and rate of BCR. The PEM-PRO trial is a single-institution study and is due to start in 2021, with an expected enrollment period of 1 year. The expected accrual is 59 patients.

The PICT-01 phase II trial (NCT04009967) is currently recruiting patients with non-metastatic Gleason grade $\geq$ 8 PCa eligible for surgical treatment and positive ^{18}FDG PET/CT imaging (SUV max >4). Patients will receive three cycles of

neoadjuvant pembrolizumab, and ^{18}FDG PET/CT imaging will be obtained prior and after neoadjuvant treatment, for evaluating volume regression on imaging as primary endpoint. This trial expects 30 enrolled patients and is due to completion by the end of 2022.

An ongoing single-arm, single-stage open-label phase II trial aims at assessing the role of neoadjuvant immuno-hormonal therapy (i.e., pembrolizumab plus enzalutamide) in patients with high-risk PCa (NCT03753243). Treatment will be planned for a total of 14 to 16 weeks, where pembrolizumab will be administered every 3 weeks via IV infusion with a dose of 200 mg per infusion and enzalutamide will be given orally and dispensed to the patient on the date of their first infusion. A total of 32 patients will be enrolled, and the primary outcome is represented by pathologic complete response. Another phase II trial (NCT03821246) is evaluating atezolizumab in combination with tocilizumab, a monoclonal antibody targeting the interleukin-6 (IL-6) receptor in patients with high-risk prostate cancer before radical prostatectomy. The rationale of such a combination is based on the evidence of IL-6 expression in both PCa cells and tumor microenvironment, as well as on the association between IL-6 expression and PCa disease progression [52]. Therefore, targeting both the PD1/PD-L1 and IL-6 axis could increase the magnitude of the immune antitumor activity. The primary objective of this trial is to evaluate the impact of the atezolizumab combination on both the composition and function of tumor-infiltrating immune cells, the study started early in 2020 and is due to conclude in mid-2022, and 68 patients are expected to participate. The combination of ICI and vaccine therapy has been proposed in the perioperative period in high-risk PCa patients who will receive RP. In the AtezoVax study (NCT04020094), patients will be treated with an intraprostatic injection of MVA-BN-Brachyury and subcutaneous PROSTVAC therapy. MVA-BN-Brachyury is a replication-deficient, attenuated vaccinia virus (Ankara strain) expressing both a CD8+ T-cell epitope from the brachyury protein and a triad of T-cell co-stimulatory molecules (B7.1, ICAM-1, and LFA-3) which causes innate and then adaptive immune responses, antigen cascade, and improved T-cell trafficking to the tumor. T-cell-mediated tumor cell killing is dependent on specific T-cell recognition of a tumor target antigen, localization of those specific T cells to the tumor, and those T cells properly functioning within the tumor microenvironment. The hypothesis is that three primary issues comprise the major causes of most patients receiving no benefit from checkpoint inhibitor therapy or with vaccine monotherapy. These issues can be addressed with an active intratumoral virus administration approach combined with the use of a subcutaneously administered vaccine (PROSTVAC) to induce PSA-specific T-cell activation in combination with a checkpoint inhibitor. This would result in exposure of cancer-specific antigens and induce inflammation at the site of the cancer ultimately resulting in significant clinical antitumor effect (Table 12.2).

Table 12.2 Ongoing phase II trials of immunotherapy in the neoadjuvant setting in patients with high-risk prostate cancer

Study	Years of accrual	Expected number of patients	Key eligibility criteria	Drugs	Primary endpoint	Imaging assessment
PEM-PRO (NCT04565496)	2021–2024	59	PSA ≥20 ng/ml and/or ≥ cT3 at DRE and/or Bx GGG 4–5	Pembrolizumab 200 mg IV q3w	Reduction by 50% of the rate of LNI	^{68}Ga-PSMA PET/CT and mpMRI
PICT-01 (NCT04009967)	2020–2022	30	Bx GS ≥8 and (SUVmax) ≥4 at ^{18}FDG-PET/CT	Pembrolizumab 200 mg IV q3w	Tumor response rate based on the change in tumor volume as measured by ^{18}FDG PET/CT	^{18}FDG PET/CT
NCT03753243	2018–2023	32	PSA >20 ng/ml and/or ≥ cT3 at DRE and/or Bx GS 8–10	Pembrolizumab 200 mg IV q3w + enzalutamide 160 mg PO qid	No cancer detected on pathology examination of RP specimen	–
NCT03821246	2020–2022	68	Patients with high-risk non-metastatic PCa	Cohort A Atezolizumab 1200 mg IV Cohort B Atezolizumab 1200 mg IV + Tocilizumab 6 mg/kg IV	≥40% increase in CD3+ LyT at final pathology	–
AtezoVax (NCT04020094)	2019–2024	22	Unfavorable intermediate-risk, high-risk, or very-high-risk prostate cancer per NCCN guidelines	Atezolizumab 1200 mg IV q3w + PROSTVAC subq q3w + MVA-BN-Brachyury q3w	Change in CD8+ LyT at final pathology	mpMRI

Abbreviations: *PCa* Prostate cancer, *DRE* Digital rectal examination, *Bx* Biopsy, *GGG* Gleason grade group, *SUV* Standard uptake volume, *GS* Gleason score, *q3w* Every 3 weeks, *qid* Four times per day, *IV* Intravenous, *PO* Per os, *subq* Subcutaneous, *LNI* Lymph node invasion, *RP* Radical prostatectomy, *LyT* T lymphocytes (CD3+), *NCCN* National Comprehensive Cancer Network

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Chapter 13
Biomarkers of Immunotherapy Response and the Future Role of Targeted Therapies in Non-metastatic Prostate Cancer

Susan F. Slovin

Introduction

Immunotherapeutic approaches as a whole have been suboptimal in the treatment of metastatic castration-resistant prostate cancer (mCRPC), despite prostate cancer being the first solid tumor to have an approval for an autologous immune therapy, sipuleucel-T™, showing a survival benefit [1]. Checkpoint inhibitors have also had a lackluster track record in inducing responses in patients with mCRPC [2–5]. However, the proverbial "light at the end of the tunnel" and the most "actionable" function for their use was demonstrated by the agnostic approval of the anti-PD-1 checkpoint inhibitor, pembrolizumab [6], for the treatment of prostate patients with microsatellite instability-high (MSI^{hi}) and mismatch repair-deficient (MRD) phenotypes. The latter are only in 5–10% of patients with mCRPC. How to identify potential biomarkers of response has been predicated on preclinical data and endorses that the agent does impact biologically and/or radiographically on the disease and that the biomarker is reflective of a change in the biology of the cancer. The biomarker may be an immune product such as a soluble factor, i.e., cytokine, a peripheral blood lymphoid subset, or changes in a signaling pathway that underlies the immune function, or a product that is from the direct interrogation of the intratumoral or tumor microenvironment (TME). The immune cell populations in bone may be different than that within soft tissues such as the liver or lymph nodes; hence, more specific markers would be helpful in trying to make associations between the right biomarkers and biologic changes within the tumor. Of critical

S. F. Slovin (✉)
Genitourinary Oncology Service, Department of Medicine, Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan Kettering Cancer Center, New York, NY, USA
e-mail: slovins@mskcc.org

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importance is recognizing that the "response" to an immune agent may be different at different sites of disease, including the bone marrow, and that sensitive imaging may be needed to detect these differences. We now know that biomarkers can be extended to include radiographic biomarkers such as prostate-specific membrane antigen (PSMA) [7, 8] and six-transmembrane epithelial antigen of the prostate (STEAP) [9] or using novel radioligands that can demonstrate amino acid metabolic byproducts [10] or choline positivity [11]. More recently, genomic biomarkers such as BRCA 1, BRCA 2, and MSI^{hi}, along with immune profiling of the primary and metastatic tumor sites, are providing a unique signature profile that could potentially have clinical impact and may influence clinical trial designs.

Understanding the Immune Landscape in Prostate Cancer and the Quest for Novel Biomarkers

Unlike urothelial and renal cancers, prostate cancer does not have well-defined immune target molecules that are expressed on their surface such as programmed cell death protein 1 (PD-1)/PD-L1, fibroblast growth factor receptor 2 (FGFR2), and FGFR3 in addition to having robust clinical responses to a wide range of checkpoint inhibitors including atezolizumab, avelumab, durvalumab, nivolumab, and pembrolizumab. No direct correlation between under- and over-expression of these markers has been associated with clinical benefit [12]. However, despite the success of these agents and the focus on PD-L1 as an potential indicator immune impact, PD-L1 has remained an inconsistent biomarker in these malignancies.

While prostate cancer is considered to be immunologically "bland" or "cold," often cited as an "immune desert," multiple immunologic platforms from vaccines to chimeric antigen receptor (CAR) T cells to bi-specific T-cell engager (BiTE) given either alone or in combination with biologic agents and/or checkpoint inhibitors have failed to convert the prostate microenvironment from "cold" to "hot." For the rare cancer that is "inflamed," as inflammation [13] has been shown as a precursor in the transition to malignancy, only a subset of patients showed clinical responses from these interventions, largely associated with a T-cell-inflamed tumor microenvironment (Fig. 13.1). This type of phenotype is associated with the infiltration of $CD8^+$ T cells, CD8α/CD103-lineage dendritic cells (DCs), as well as high density of forkhead box P3 $(FoxP3)^+$ regulatory T cells (Tregs) that are associated with the efficacy of immune checkpoint blockade [14] (Fig. 13.2), while melanoma and other solid tumors have demonstrated clinical response to checkpoint inhibitors as demonstrated by infiltrating antigen-specific T cells [15] within the tumor cell, hence an inflamed microenvironment. This phenotype has been described as a T-cell-inflamed tumor microenvironment (TME) and can be used to predict responding and non-responding tumors. In fact, the observation of a correlation between $CD8^+$ T-cell presence and response to checkpoint blockade therapy has led to the adoption of T-cell presence or the presence of a T-cell gene signature as a

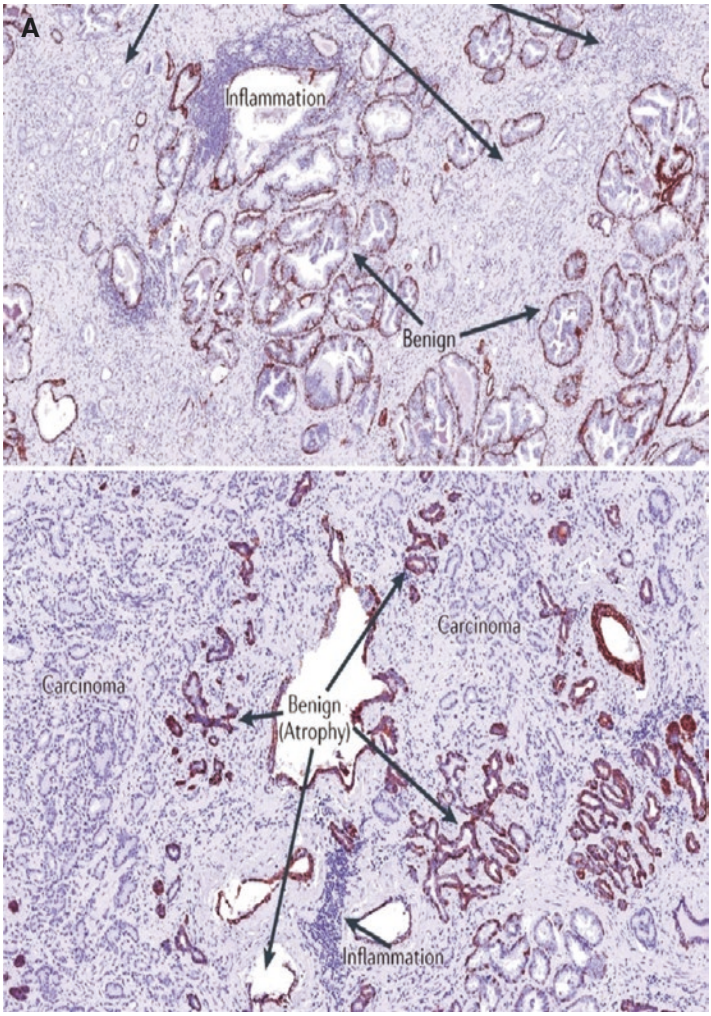

Fig. 13.1 (**A**) Immune distribution in normal and inflamed prostate tissue as part of the inflammatory microenvironment and microbiome. Immunohistochemistry of prostate cancer with areas of benign tissue, inflamed tissue, and carcinoma. The benign prostate glands are identified by positive CK903 stain (high molecular weight cytokeratin marker), indicating the presence of basal cells. Prostate carcinoma, which lacks basal cells, is identified by a lack of CK903 staining. Areas of inflammation are characterized by dense clusters of inflammatory cells in the stroma. Microphotograph is at 40× magnification [13]. Reproduced by permission of the publisher. (**B**) Evaluation of the changes incurred by Inflammatory processes and the impact and distribution of immune cells. (**a**) $CD8^+$ T cells are present but sparsely scattered in normal-appearing prostate and are often located within the epithelial compartment (arrows). By contrast, these cells are enriched in areas of chronic inflammation. (**b**) $CD20^+$ B cells are very scarce or absent in normal-appearing prostate but can be highly prevalent in inflamed areas. (**c**) $CD68^+$ macrophages are noted, and (**d**) tryptase-positive mast cells are sparsely distributed in normal-appearing prostate (arrows); their number can increase in inflamed prostate tissue [13]. Microphotograph is 200x magnification [13]. (Reproduced by permission of the publisher)

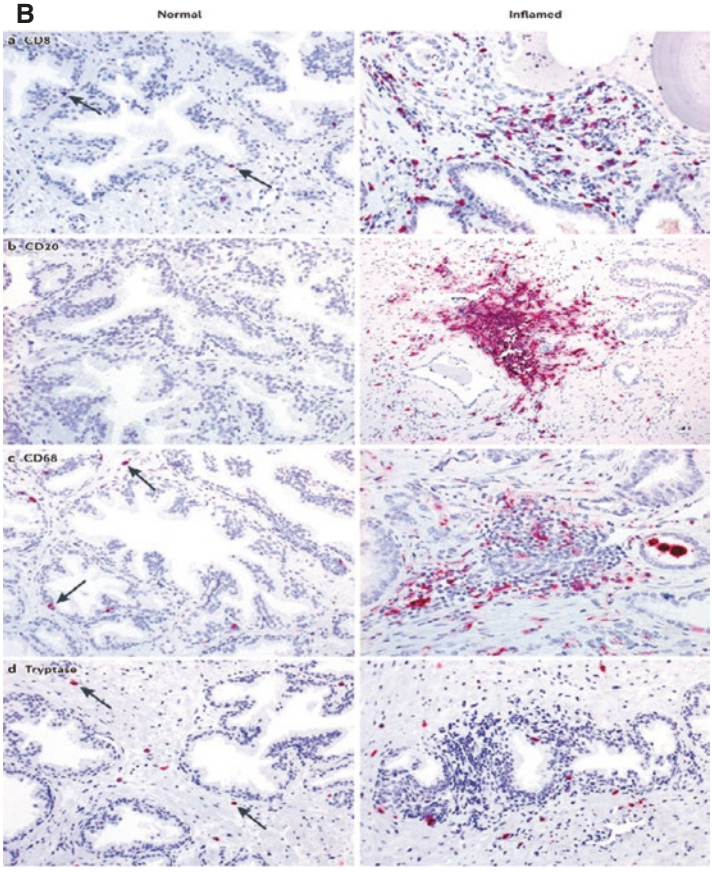

Fig. 13.1 (continued)

categorical biomarker for a response to checkpoint blockade therapy. Among the immune pathway regulators that have been associated with T-cell inflammation in the tumor microenvironment, the Wnt/β-catenin signaling remains a continued pathway of interest and is also the one that is fairly well-described. While there is tumor-intrinsic Wnt/β-catenin signaling activation that can be associated with weak T-cell infiltration in cancer, nevertheless, the concern that this pathway is involved in immune evasion makes it a continued focus of interest especially for the development of novel agents. Analysis of metastatic human cutaneous melanoma samples from The Cancer Genome Atlas (TCGA) revealed that patient samples that segregated into the non-T-cell-inflamed subset showed enrichment for tumor cell-intrinsic activated β-catenin signaling [16]. Similar findings may in fact be applicable to prostate cancer [16, 17].

Perhaps more challenging is trying to detect immune populations in the metastatic setting. Prostate cancer is a bone trophic disease with cells that can thoroughly

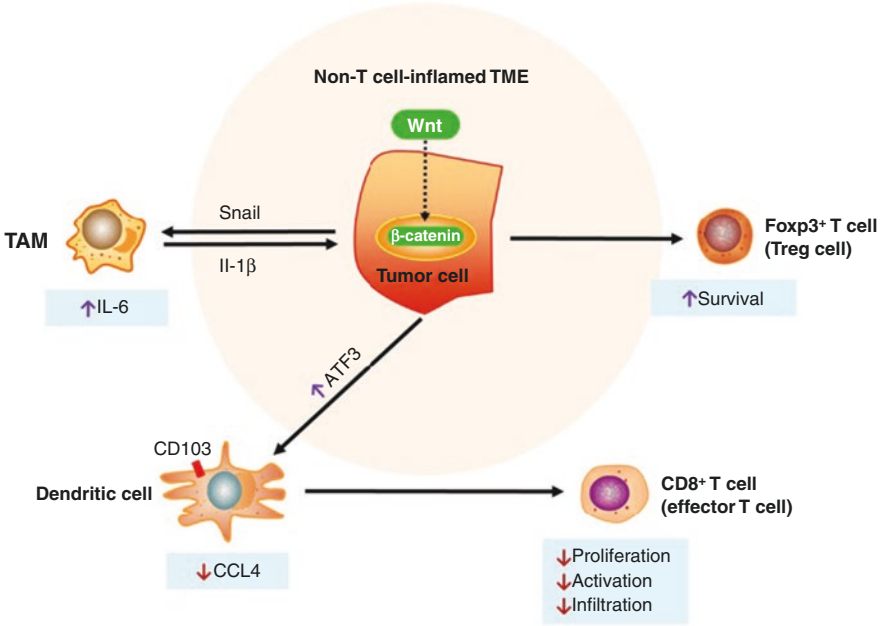

Fig. 13.2 Mechanisms of immune exclusion through the Wnt/beta-catenin pathway. (i) CCL4 production is inhibited in Batf3-lineage CD103$^+$ DCs via induction of the expression of the transcriptional repressor ATF3. This leads to the reduceion the initiation and infiltration of CD8$^+$ T cells. (ii) Increases in the interaction between Snail (a soluble factor and product of a Wnt-regulated gene) and TAMs, which in turn increases β-catenin activity via IL-1β. (iii) Enhanced Treg survival. (Modified from [14]). DC, dendritic cell; TAMs, tumor-associated macrophages; CCL4, C-C motif chemokine ligand 4; ATF3, activating transcription factor 3; TME, tumor microenvironment [14]. (Reproduced by permission of the publisher. Copyright © 2019 Li, Xiang, Li, Yin, Li, and Ke. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

infiltrate the bone marrow and prevent or suppress the entry and exit of immune cell populations. There are suggestions that this large volume of disease within the marrow could be immunostimulatory by providing increased antigenic stimulation; however, this volume could also be immunosuppressive due to the densely fibrotic stroma and immunosuppressive immune cell populations, namely, tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and Treg cells. This observation has been confirmed in metastatic pancreatic cancer [18]. There is upregulation of the glycolytic and aldose reductase pathways that create a metabolically hostile microenvironment in which T-cell function is profoundly suppressed [19]. T cells that infiltrate the tumor also have repressed mitochondrial activity and biogenesis that lead to loss of metabolic insufficiency, a state that cannot be rescued by PD-1 blockade therapy alone [20].

What Is Modulating the Immune Microenvironment?

While meta-analyses suggest that testosterone is overall immunosuppressive [21], there is still ambiguity depending on which aspects of immune function are studied and whether the impacts of testosterone on immune function are direct or indirect. There is ample evidence that androgens alter immune cell development and immune activation. A chronically inflamed tumor can lead to suppression of testosterone. Androgen-mediated suppression of immune reactivity and inflammation may lower the threshold toward malignancy [22]. On the other hand, low levels of circulating testosterone resulting from ADT can modulate prostate cancer and can be further influenced by the addition of radiation with concurrent ADT [23]. Given the importance of the androgen receptor (AR) in prostate cancer and the role of β-catenin as a transcriptional co-activator, a pertinent question is whether β-catenin regulates androgen receptor function or vice versa. Both are indeed the case, as prostate cancer cells contain a complex comprising β-catenin and the androgen receptor. Androgen deprivation therapy (ADT) also indirectly leads to the priming of tumor-specific adaptive immune responses [24], impairing immune cell infiltration, especially a T-cell subset, and the production of several inflammatory cytokines involved in the pathogenesis of numerous autoimmune diseases and in the regulation of tumor cell proliferation [24–29].

A retrospective analysis [30] of 844 CRPC patients who received AR-directed therapies, of whom 36 (4.3%) had autoimmune diseases and 47 (5.6%) second tumors as comorbidities, showed a direct relationship between the duration of the hormone-sensitive phase and increased risk of autoimmune diseases in prostate cancer patients. The authors concluded that CRPC patients with autoimmune alterations before starting AR-directed therapies may have worse prognosis. Chronic inflammation as has been previously noted plays a role not only with prostate cancer growth but also in the development of autoimmune diseases [30] potentially by inducing inflammation via a variety of cytokines including IL-1, IL-6, and IL-17 as has been previously reported with androgens also being accountable for altering T-cell immunity. Indeed, inflammation status, including also the release of inflammatory cytokines such as interleukin (IL)-1, IL-6, and IL-17, has been associated with the development and progression of prostate cancer [24, 25], and androgens have been reported to alter T-cell immunity [26–30]. Moreover, ADT has been revealed to reduce Th1 and Th17 responses and also the concentration of inflammatory cytokines involved in several autoimmune diseases, including IL-1β, IL-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ [24–27].

What Are the Potential Biomarkers That Can Reflect Changes in the Tumor Microenvironment and Can They Be Correlated with Clinical Response?

To date, biomarker discovery continues to be a priority to assess the impact of particular therapies, yet it remains challenging in prostate cancer [31–34]. Other than radiologic response, it is hard to determine whether or not there is a steadfast series of immune parameters that can reliably indicate that there is an "immune response" to an immunologic agent. Many panels that enlist T-cell subsets and ratios have been informative but not completely indicative that the cancer is reacting to the treatment, i.e., for example, the generation of high titer antibodies to a vaccine may indicate that there are antibodies to the immunogen, yet without a clinical correlation and change in the biology of the cancer, one cannot establish whether the immune therapy had impact on the cancer either acutely or by a delayed response. It is clear that current immunotherapies attempt to directly target the T cell but fail to overcome the multilayered immunosuppression (T cells, MDSCs, adenosine, cytokines).

As noted previously, there appears to be a de facto association between the presence and absence of PD-1/PD-L1 in some solid tumors but nothing that supports its role as a biomarker in prostate cancer despite the small percentage of men who may have a 5–10% response and have no DDR, BRCA, or MSI^{hi} mutations. The data by Mateo et al. [35] supports the sensitivity to PARP inhibitors in patients who harbor BRCA2 mutations; the presence of MSI^{hi} expressed in multiple cancers has been approved [6] for the agnostic use of pembrolizumab and indicates who may benefit from checkpoint inhibition. However, what is unclear is who overall may benefit from checkpoint inhibitors in the absence of these mutations, i.e., the general population. More recently, Sanchez-Magraner et al. [36] developed a real-time in vitro quantitative two-site labeling assay that showed immune checkpoint interaction by direct imaging to demonstrate that immunotherapy-treated patients with metastatic NSCLC with a low extent of PD-1/PD-L1 interaction show significantly worse outcome. The results of this assay may serve not only to screen patients for who may benefit from a checkpoint inhibitor but ultimately may be used as a potential biomarker of response to a particular checkpoint inhibitor in advance of the use of the particular drug.

CDK12 and Its Role as a Potential Biomarker for Lethal Prostate Cancer

CDK12 serves several functional domains: it regulates transcription and controls genomic stability via DNA damage response; CDK12 loss is associated with increased sensitivity to PARP inhibitors in ovarian cancer [37, 38]. Tumors with CDK12 loss have a higher level of genomic instability and gene-fused mutations,

resulting in an elevated neoantigen burden, the latter making it appealing for the use of checkpoint inhibitors [39, 40]. Interestingly, tumors with biallelic loss of CDK12 also were associated with higher levels of immune cell infiltration, along with the presence of increased TILs, and altered chemokine signaling. Wu et al. [41] looked at tumor samples that demonstrated biallelic loss of CDK12 and were the first to demonstrate the analytical value of neoantigen prediction from RNA-seq data, thereby demonstrating that tumors with this loss were more responsive clinically to checkpoint inhibitors.

Are There Relevant Biomarkers for Immune Response or Are There Inhibitory Biomarkers?

While demonstrating via immunohistochemistry the presence or absence of PD-1 and PD-L1 on immune and tumor cells and defining a quantitative cut point to precisely discriminate who will have a treatment response, it remains unclear whether there are soluble versus tumor-associated biomarkers that can be identified and reliably used to ascertain treatment response. Overall, it is well-recognized there is an impairment in immune cell activation that is potentially caused by tolerance and immunosuppression in cancer patients as demonstrated by the inhibitory actions of CTLA4, PD-1, PD-L1/2, and TGF-β. A more directed immune cell killing of tumors can be co-opted by the production of "decoy" molecules produced by the cancer cell. These molecules are against Fas and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced death pathways. In addition, immune cell killing of tumors can be weakened by the cancer cell production of decoy molecules against Fas and TRAIL-induced death pathways (i.e., decoy receptor 3 [DcR3] and decoy receptor 4 [DcR4 or TRAILR4]) [42, 43]. A recent study by Bou-Dargham et al. [44] used a series of computational methods to develop what they termed as an "immune evasion mechanism analysis" (IEMA). This was derived as a result of a series of combinatorial methods that included sequential bi-clustering, differential expression, immune cell typing, and machine learning to prostate cancer RNA-seq data obtained from The Cancer Genome Atlas (TCGA) [44, 45] (Fig. 13.3). IEMA was able to cluster prostate cancer patients into eight groups based on their patterns of immune gene expression with each of the eight clusters having a unique set of evasion mechanisms that were also found to be concurrently activated in cancer. Using a decision tree algorithm, they were able to detect biomarkers that could predict which patient populations would be most likely to respond to various immunotherapies [45]. A classification tree model was built to predict a patient's membership to a specific immune evasion cluster; overall accuracy of the tree was 77%. As seen in Fig. 13.4, the selected gene biomarkers and their expression cutoff values are shown; the biomarkers include CD48, SP140, KIRREL, RHOB, FBXO17, ANAPC1, EGFR, SOCS3, ALOX15, and UBR2. Particularly noteworthy in the

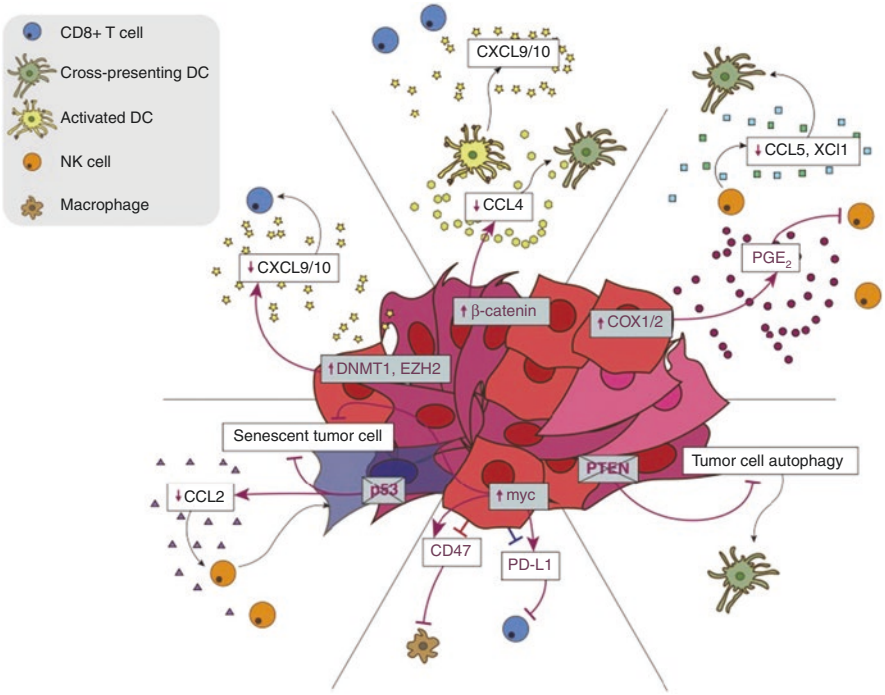

Fig. 13.3 Cross-talk among multiple signaling pathways involved in blunting T-cell activation and recruitment. Activated β-catenin signaling leads to a reduction of CCL4 production. CCL4 recruits cross-presenting $CD103^+$ DCs that are critical for cross-priming $CD8^+$ T cells. These DCs produce the T-cell chemoattractant CXCL9 and CXCL10 leading to a loss of T-cell recruitment. Elevated COX1/2 activity produces immunosuppressive PGE_2 that here is shown to blunt the recruitment and activity of NK cells, leading to a loss of CCL5 and XCL1, chemokines that attract $CD103^+$ DC, which lead to loss of T-cell priming and recruitment [45]. (Reproduced by permission of the publisher. Copyright @2020 Nguyen and Spranger by Rockefeller University Press. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

cluster was the absence of CTLA-4 and PD-1. The authors indicated that this approach may offer a more personalized approach to immunotherapy based on IEM but may foster a better understanding regarding why immunotherapy failures occur. For example, patients with upregulation of CTLA-4 or PD-1 expression on their prostate cancers (although infrequent) may have other pathways for immune evasion as shown by their clusters that not only had upregulated PD-1 expression but also had concurrent evasion mechanisms They also noted clusters with upregulated CTLA4 all possessed immunologic ignorance as an additional evasion mechanism, endorsing the observations of prior clinical trials that single agent targeting of CTLA4 or PD-1 alone will provide the desired antitumor response, treating prostate cancer [46].

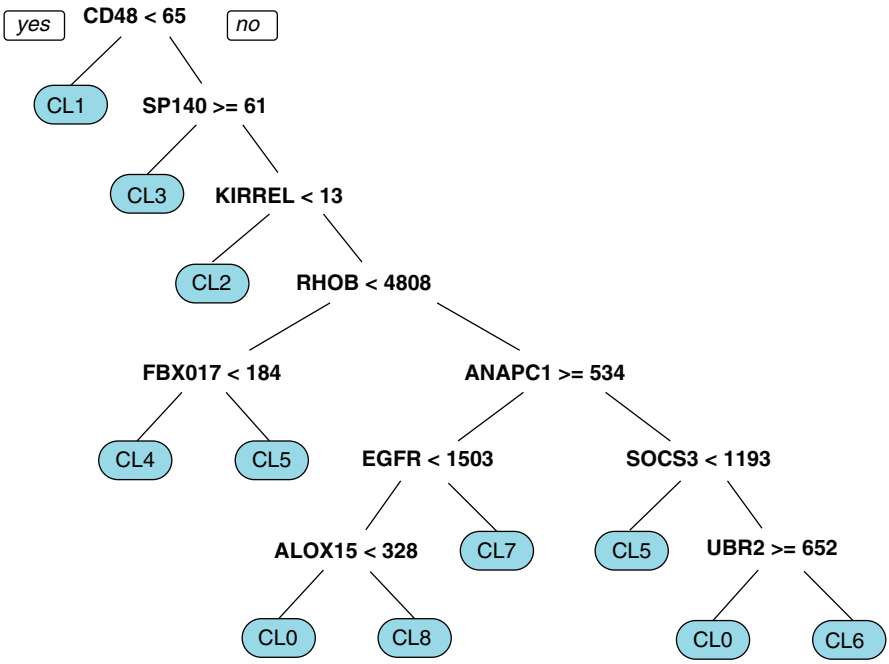

Fig. 13.4 Tree branches classifying ten predictive biomarkers of patients' immune evasion clusters (CL) and response to immunotherapy. Cluster of differentiation 48 (CD48), speckled 140 KDa (SP140), kin of IRRE like (KIRREL), Rho-related GTP-binding protein RhoB (RHOB), F-box protein 17 (FBXO17), anaphase-promoting complex subunit 1 (ANAPC1), epidermal growth factor receptor (EGFR), suppressor of cytokine signaling 3 (SOCS3), arachidonate 15-lipoxygenase (ALOX15), and ubiquitin protein ligase E3 component n-recognin 2 (UBR2). A noteworthy observation from the identified biomarkers is that even for the clusters that have upregulated CTLA4 and PD-1 expressions, these molecules are not the optimal biomarkers for the choice of anti-CTLA4 or anti-PD-1 treatments [44]. (Reproduced by permission of the publisher. Copyright @2020 Bou-Dargham, Sha, Sang, and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

How Do We Interpret the Impact of Current Immune Agents and By What Parameters?

Melanoma represents the prototypic example of a tumor to which an immune response results in a biologic effect. Early observations by Tumeh et al. [47] demonstrated that patients with melanoma often had pseudoprogression [48] or what appeared to be progressive disease on scans. However, when the tumor was biopsied, it was infiltrated by immune cells, and after a period of several weeks to months, the tumor shrunk. Unlike melanoma, prostate cancer is likely to be more "immunoevasive" as a result of immunomodulatory ligands as mentioned previously. In general, in order to assess immunologic impact, consideration needs to be

given to the drug's mechanism of action, its half-life, and whether or not it will generate a humoral and/or cellular responses. Parameters to be measured could include antibody avidity, B- and T-cell activation, lymphoproliferation, and cytokine responses among others with further interrogation into the T-cell specificity, functionality, clonality, or diversity [49]. These may act as surrogates to evaluate antitumor effect functionality. While functional testing is still heavily relied upon, tissue profiling continues to be of interest. Profiling consists of evaluating the immune cell populations that infiltrate the tumor, the "tumor-infiltrating lymphocytes" (TILs); however, they have inherent limitations with regard to sample size and ability to technically assess. There is a potential role for monitoring MDSCs whose function is to suppress adaptive and innate immunity via suppression of NK cells alone and in combination with T cells as well as via cytokines. MDSC proliferation has been thought to be an early event tumor progression via cytokine stimulation leading to recruitment of cells from the bone marrow as well as tumor sites. Thought to be a biomarker in its own right, there is a lack of specificity for this cellular population as it can increase in a number of benign inflammatory conditions. Is the lack of immune "response" due to the inability to present immunogenic antigens that native T cells can recognize? It has long been considered that immunosuppression may not be limited to the TME, the tumor itself, and that the stroma as well as circulating MDSCs may be responsible in creating dysfunctional immune responses.

Is there "tissue-specific immunity" and should all metastatic sites be biopsied and evaluated for an immune response? The divergence of responses at different tumor sites suggesting what can be termed as an "organ therapeutic approach" has been reported with checkpoint inhibitors, in particular, urothelial carcinoma, where the observation of tumors that metastasize to the liver may be much less responsive than those in the lymph node, ultimately resulting in complete responses at these sites. Balar et al. [50] demonstrated responses at the site of the primary tumor but without response in the liver [50, 51]. This may also be related to the overall immune populations harbored by certain sites such as the lymph node, skin, and lung as opposed to less infiltrated sites by immune populations such as the liver and bone. Should we rely on tumor DNA as an alternative biomarker understanding that there are limitations to this technology as well?

Conclusions

The identification of blood- and tissue-based biomarkers that can be associated with treatment response and can identify those patients who may benefit from a specific immune-based therapy is ongoing. Quantitation of cellular populations, i.e., B/T cells, MDSC, and intratumoral TIls, provides a window into the tumor microenvironment and may provide insight into how the immune system behaves systemically as well as intratumorally. Circulating tumor cells (CTCs) that express these immune markers as well as tumor DNA may offer potential as biomarkers as

indicated by recent work in the head and neck demonstrating that immune marker expression (PD-1/PD-L1) on CTCs correlates with the stage of the disease and may play a role as a prognostic biomarker [52]. However, it remains unclear what other immune markers are present on CTCs other than the PD family.

The Future of Targeted Therapies in Non-metastatic Prostate Cancer

To date, there remains a need for non-toxic interventions in patients with early-stage disease given that they are largely asymptomatic or minimally symptomatic. This is the precise niche for which sipuleucel-T [1] was developed and is still being used. Multiple papers have indicated that this autologous preparation induces dendritic cells or antigen-presenting cells (APCs), in addition to a peripheral immune response specific to the target (PAP) and/or immunizing (PA2024) antigen. Of note, it can stimulate systemic cytotoxic T-lymphocyte activity. In spite of these ancillary mechanisms, it has been postulated that the main mechanism is by mediating antigen spread (i.e., increased antibody responses to secondary or bystander proteins in addition to PAP and PA2024) [53]. Its real-world use [54] provides contemporary survival data that reaffirms its use as a beneficial agent in certain subsets of men. An unexpected survival benefit was also observed in patients treated with one or more agents post-treatment with sipuleucel-T. This included the androgen receptor signaling inhibitors abiraterone and enzalutamide, as well as other standards of care including docetaxel, cabazitaxel, and radium-223, respectively. Among these patients, 32.5% and 17.4% of the patients experienced 1- and 2-year treatment-free intervals, respectively [54].

Another target under intense investigation from both an immunologic and imaging standpoint is PSMA. ^{68}Gallium-PSMA PET imaging at two academic institutions in the USA has been recently approved for use in patients with biochemically relapsed prostate cancer following primary prostatectomy with the caveat that the PSA must be 0.2 or greater. Given its high sensitivity and specificity compared with choline and fluciclovine PET scans, respectively, this imaging modality provides insight into those patients who may or may not benefit from salvage radiation therapy. However, its use in serially monitoring patients with mCRPC undergoing therapies has still not been established nor has it been approved by insurance for reimbursement. PSMA has been a prominent immunologic target on prostate cancer cells given its uniqueness as a type II transmembrane protein. Agents against the internal and external domains, respectively, have been developed either as monoclonal antibodies or as small molecules, the latter having gained popularity given the lesser risks of developing human or murine anti-human antibodies by the patient. More recently, there are several ongoing trials using bi-specific antibodies (BiTEs) as well as CAR T cells that are focused on mCRPC although selection for

PSMA-positive patient via PSMA PET imaging to enrich the population has not as yet been routinely incorporated.

Attempts to target the tumor microenvironment at this stage of minimal tumor burden have included drugs that targeted integrins, as well as stromal-epithelial interactions. Of note, AR signaling in stromal cells has been shown to exhibit tumor-promoting effects. This also includes combinatorial approaches that may include sipuleucel-T with another biologic or perhaps bone-targeting agent with the plan to impact on the susceptible tumor cells within the TME, thereby changing how the TME changes biologically over time. The hedgehog (Hh) signaling pathway has also been tapped in this clinical space as it is involved in prostate development embryonically. Interestingly, the rate of tumor growth has been showed to be a function of the degree of Hh pathway activity. Other targeted pathways include FDF gene family, the Src family, TGF-B, and insulin-like growth factor signaling to which multiple inhibitors have been developed but have not been successful in interfering with the impact of these pathways on the stromal-epithelial cross-talk [55–57]. To date, single agents directed as inhibitors of these pathways have demonstrated some on-target effects but no direct antitumor effects.

The main target for non-metastatic prostate patients with early-stage hormone-sensitive or late-stage hormone refractory disease is the androgen receptor for which the androgen receptor signaling inhibitors play a pivotal role. While they are viewed as "targeted" therapies, they are "generational" with the first generation being abiraterone, a CYP1711 inhibitor. The ARSIs include the first-generation enzalutamide and second-generation apalutamide and darolutamide that exert their activity via binding to the ligand-binding domain of AR. As such, they displace the usual ligand, i.e., testosterone and dihydrotestosterone, and prevent the translocation of AR into the nucleus where it can initiate transcription. Apalutamide and enzalutamide have been approved in the early hormone-sensitive metastatic setting as well as the castrate non-metastatic setting along with darolutamide.

Currently, targeting mutations in AR, the ligand binding and amino-terminal domains, and AR splice variants [55, 57] as well as genomic alterations remains a top priority. Unique combinations of PARP inhibitors along with checkpoint inhibitors and other biologic agents are currently in clinical trials. More recently, the identification and subsequent role of the glucocorticoid receptor [58, 59] and its close association to AR have added another dimension to the treatment armamentarium of targeted drugs seeking to work in tandem with ARSIs such as enzalutamide, where there is currently an ongoing clinical trial seeking to determine whether patients who have failed enzalutamide and remain on drug can be "rescued" using a glucocorticoid inhibitor.

Biomarkers may ultimately become treatment specific as different immunologic, genomic, and targeted therapies emerge [60]. Efforts will continue to explore how to best identify those biomarkers that can play a pivotal role in understanding the impact of targeted therapies.

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Part III
Management of RCC in the Perioperative Setting

Chapter 14
Cytoreductive Nephrectomy in the Era of Targeted Therapy and Immunotherapy

Umberto Capitanio

In the case of recurrent metastatic disease, patients with renal cell carcinoma (RCC) are generally managed with systemic therapy, primarily using immunotherapy or agents targeting the vascular endothelial growth factor (VEGF) pathways [1]. However, in the case of metastatic disease at initial primary presentation, surgery is an option, as well. More specifically, cytoreductive nephrectomy (CN) was historically considered part of the multimodal approach in the case of upfront metastatic RCC (mRCC). Many different mechanisms were proposed to support the beneficial effect of a cytoreductive surgery even in the presence of distant metastases: (1) the removal of the potential source of new metastatic clones, (2) the enhancement of response to systemic therapies by removing potential interactions between the tumor and metastases, (3) palliation of symptoms, and (4) many others [2]. However, the role of CN changed several times in the last decades due to the introduction in clinical practice of several systemic therapies which became available over the years.

First Immunotherapy Era (Interleukin and Interferon)

Traditionally, when effective systemic therapies were limited, the goal of altering the biologic kinetics by reducing the local tumor burden led to the acceptance of performing CN even in the presence of metastases. When interferon and

U. Capitanio (✉)
Unit of Urology, IRCCS Ospedale San Raffaele, Milan, Italy

Division of Experimental Oncology, Urological Research Institute (URI), IRCCS Ospedale San Raffaele, Milan, Italy
e-mail: capitanio.umberto@hsr.it

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interleukins were the standards in the management of mRCC, two milestone randomized clinical trials were conducted and published: the SWOG 8949 trial and the EORTC 30947 trial.

The Southwest Oncology Group presented in 2001 the final results of a randomized controlled trial (SWOG 8949) in which 241 patients were randomly treated with IFN-α alone vs. CN plus IFN-α [3]. The authors demonstrated a statistically significant improvement in overall survival (OS) for patients treated with CN (11.1 months vs. 8.1 months; $p = 0.05$). OS benefit for CN was demonstrated in various subgroups of patients, as well.

Similarly, EORTC 30947 trial demonstrated that patients treated with CN plus IFN-α had a statistically significant benefit in time to progression (5 months vs. 3 months; $p = 0.04$) and median OS (17 months vs. 7 months; $p = 0.03$) [4] relative to patients treated with systemic therapy alone.

A subsequent combined analysis published in 2004 demonstrated a median survival of 13.6 months with CN versus 7.8 months for IFN-α alone, representing a 31% decrease in the risk of death ($p = 0.002$) [5]. Due to the publication of those two randomized clinical trials, CN has been considered the standard of care in the context of a multimodal therapy of mRCC patients for the subsequent two decades (2001–2018).

Tyrosine Kinase Inhibitors' Era

In the later years, mTOR inhibitors (everolimus and temsirolimus) and tyrosine kinase inhibitors (sunitinib, sorafenib, axitinib, and many others) were introduced in the management of mRCC [1]. With the advent of those targeted therapies, the role of CN has been then questioned, and several retrospective analyses were performed. A systematic review and meta-analysis published in 2016 of almost 40,000 patients found a statistically significant survival advantage for patients treated with CN plus targeted therapy vs. targeted therapy alone (HR 0.46; 95%CI 0.32–0.64; $p < 0.01$) [6]. More recently, a 2018 National Cancer Database analysis confirmed a survival advantage for patients who were treated with upfront CN compared with targeted therapy alone (median survival: 16.5 months vs. 9.2 months; HR 0.61; $p < 0.001$) [7]. However, all those data were retrieved from retrospective cohorts, limiting the level of evidence of supporting CN, also in the era of targeted therapies.

In 2018, the Clinical Trial to Assess the Importance of Nephrectomy (CARMENA trial; NCT0093033) was finally published by Mejean and colleagues. CARMENA trial is a phase III non-inferiority randomized trial investigating immediate CN followed by sunitinib vs. sunitinib alone in mRCC patients. Final results demonstrated that sunitinib alone was not inferior in terms of OS relative to CN followed by sunitinib [8]. The trial included 450 patients with intermediate- and high-risk mRCC of whom 226 were randomized to immediate CN followed by sunitinib and 224 to sunitinib alone. Median tumor burden resulted 140 mL in both arms, of which 80 mL for the primary renal tumor. The study did not reach the full accrual of

patients, and the study was prematurely closed. In an intention-to-treat analysis, median OS was 13.9 months in the CN plus sunitinib group vs. 18.4 months for sunitinib alone (HR: 0.89; 95%CI 0.71–1.10). For intermediate-risk patients, median OS was 19.0 months with CN vs. 23.4 months with sunitinib alone (HR: 0.92; 95%CI 0.60–1.24) and for poor risk 10.2 vs. 13.3 months, respectively (HR: 0.86; 95%CI 0.62–1.17). Median progression-free survival was 7 months for patients treated with CN plus sunitinib vs. 8 months treated with sunitinib alone (HR: 0.82; 95%CI 0.67–1.00). Interestingly, roughly 17% of patients in the sunitinib-only arm required a secondary CN due to acute symptoms or for a complete (or near-complete) response. Of the 226 patients assigned to undergo CN followed by sunitinib treatment, 16 (7.1%) did not undergo CN, and 40 (17.7%) did not receive sunitinib. In the sunitinib-only arm, 4.9% of the patients did not receive sunitinib, and 38 (17%) ultimately underwent CN within a median of 11 months due to onset of symptoms.

The Immediate Surgery or Surgery After Sunitinib Malate in Treating Patients with Metastatic Kidney Cancer trial (SURTIME, NCT01099423) randomly assigned patients to immediate CN followed by sunitinib versus a deferred surgery after three cycles of sunitinib. Inclusion criteria were a good performance status, the absence of central nervous system involvement, and a life expectancy longer than 3 months. Furthermore, patients had to have up to three surgical risk factors (metastasis-related symptoms, retroperitoneal or supradiaphragmatic lymphadenopathy, low serum albumin, organ function impairment, or stage cT3–cT4 disease) [9]. Of importance, these were not considered exclusion criteria in the CARMENA trial, which therefore included less healthy patients. The majority of patients were classified as intermediate risk, with only 13% identified as poor risk. Unfortunately, the trial accrued poorly ($n = 99$), and therefore results could be considered exploratory only. Although there was no difference in progression-free survival (42 vs. 43%), median OS was significantly better for patients in the deferred CN arm (HR, 0.57; 95% CI, 0.34–0.95; $p = 0.03$), which yielded a survival advantage of approximately 17 months (32 months vs. 15 months) relative to patients treated with upfront CN. Sunitinib was administered in 48 (98%) of 49 patients in the deferred CN cohort but only in 40 (80%) of 50 patients in the immediate CN cohort. Conversely, systemic progression before CN in the deferred CN arm lead in the avoiding of surgery in 29% of the patients. Furthermore, roughly 8% in the immediate CN arm did not receive nephrectomy because of rapid disease progression or refusal. Summarizing those data, 18% of patients did not receive the treatment initially assigned.

Unfortunately, both the CARMENA and SURTIME trials suffered in terms of accrual and of the fact that – with the implementation of immunotherapy – the landscape scenario of systemic therapies rapidly changed in the last years. These aspects made SURTIME and CARMENA not (or partially) applicable to contemporary RCC patients. Moreover, patients included in the SURTIME and CARMENA were mostly intermediate and high risk, limiting their applicability to patients with low-volume disease.

Today: The Second Immunotherapy Era

Novel immuno-oncology (IO) treatments have recently become the new backbone of systemic treatments. This has raised many questions about the role of CN in the current era. For instance, CN may play a synergistic role in immunotherapy [10]. Apart from the reduction of tumor volume which could limit the onset of new biological clones, CN can eliminate the immunological sink in which the primary tumor diverts circulating immune cells away from distant metastases, as well. Primary tumor has been demonstrated to have an immunosuppressive effect by actively secreting cytokines. Moreover, a rationale for neoadjuvant IO administration is also to prime the immune system prior to surgery, improving the tumor's antigenicity [10]. In this setting, three clinical trials, which all incorporate neoadjuvant IO prior to treatment of the primary tumor, will probably provide more insights about these specific aspects (PROSPER trial [NCT03055013], NORDIC-SUN trial [NCT03977571], and CYTOSHRINK trial [NCT04090710]).

Complications, Adverse Events, and Surgical Challenges of CN After Systemic Therapy

CN is per se associated with higher complications rates relative to patients treated with nephrectomy in the absence of nephrectomy [11, 12]. Roussel et al., for instance, aimed to evaluate morbidity associated with CN [11]. Data from 736 mRCC patients undergoing CN at 14 institutions were retrospectively recorded in the Registry for Metastatic RCC (REMARCC). Intraoperative complications were observed in 69 patients (10.9%). Two hundred seventeen patients (29.5%) encountered any grade complications. Perioperative mortality rate was 1.4%. Forty-one patients (11.5%) were readmitted within 30 d of surgery. Results were confirmed in subanalyses focusing solely on patients treated in the contemporary targeted therapy era. CN case load correlated with lower high-grade morbidity and highlighted the benefit of centralization of complex surgery [11]. Similarly, in a recent review of the literature, Larcher et al. reported high prevalence of complications in patients treated with CN relative to patients treated with radical nephrectomy in the absence of metastases (intraoperative complications rate 6–30%, major complication rates 3–29%, perioperative mortality 1–13%) [12].

In CARMENA trial, the authors reported perioperative results, including a 30-day postoperative mortality rate of 2%, after CN. Overall, 39% of the patients experienced at least one complication. In SURTIME trial, adverse events related to surgery in the immediate and deferred arms occurred in 52% and 53% of the population, respectively. Postoperative adverse event grades 3–4, 30-day readmission, and in-hospital mortality were 17%, 9%, and 2% in the immediate arm and 17.5%, 5%, and 2.5% in the deferred arm, respectively. A recent systemic review assessed perioperative surgical complication rates in patients treated with VEGF blockade

(bevacizumab, sorafenib, sunitinib, and pazopanib) and demonstrated no increase in surgical complications [13]. Nevertheless, given concerns especially for wound healing (7%) [14], VEGF inhibitors are usually stopped perioperatively.

Parallelly, it remains a concern that the benefits of surgery with immunotherapy could be counterbalanced by the iatrogenic inflammatory and metabolic events induced by surgical trauma. Significant fibrosis and desmoplastic reaction have been described in case series [10, 15]. The net effect is immunosuppression immediately after surgery but lasting for several weeks through the expansion of regulatory myeloid cells with increased PD-1/CTLA-4 expression, T cells, and impaired natural killer cell activity [15, 16]. Pignot et al. recently reported a multicentric cohort of patients ($n = 11$) surgically treated after IO. The mean operative blood loss was 909 ml, and 81.8% of cases were considered difficult by the surgeons. The 30-day postoperative complication rate was 55% (18% considering major complications) and 9% of surgery-related death [17], revealing a significant risk of such surgeries in IO setting. Being said that, immunotherapy is increasingly being used in the perioperative setting with little or no interruption in systemic therapy. For instance, in the ongoing PROSPER trial (NCT03055013), patients who receive nivolumab can proceed to surgery as soon as 1 week after receiving the therapy.

IO toxicities therapy should be monitored in relation with surgery, as well. Rate of immune-related adverse events is roughly 80%, and up to 35% of patients require high-dose corticosteroids [15, 16]. These toxicities and the potential need for corticosteroids must be considered in the perioperative setting, especially when surgery is planned after a long period of immune checkpoint inhibition. For instance, adrenal insufficiency and hypoxemia may not be related only to surgical stress or pulmonary embolism (surgical complications) but also be related to the IO regimen (immunotherapy iatrogenic effects). Moreover, immune-related adverse events usually require high-dose glucocorticoids which may impair surgical outcomes, as well, causing, for instance, hyperglycemia, fluid retention, and adrenal insufficiency [15, 16]. Risk for opportunistic infections is not insignificant, also. Finally, data is available suggesting that antibiotics may impair the efficacy of immunotherapy, particularly if surgery is offered early in the course of therapy [18]. Being aware of these toxicities and how they may present and mimic common surgical complications will be critical to obtain an efficient multidisciplinary care [19].

Role of CN for Symptom Control or Palliation

Regarding symptomatic mRCC patients, CN may have also a role for palliation, besides cancer control. Larcher et al. investigated the impact of CN on symptomatic improvement and perioperative morbidity and elucidated the trade-off between such benefit and harm. The proportions of any sign or symptom resolution and improvement after CN were 43% and 71%, respectively. The proportions of local sign or symptom resolution and improvement after CN were 91% and 95%, respectively. The risks of any complication and major complication were 37% and 10%,

respectively. Two out of three patients suffer from any sign or symptom, and one out of three suffers from local signs or symptoms. CN demonstrated a positive impact on symptomatic status [11].

Current Indication for CN and Future Insights

All the available high-level evidences regarding the effect and the indication for CN come from VEGF era. Nowadays, IO (alone or in combination) represents the standard of care for mRCC patients. Therefore, current indication for CN can be only derived from a critical analysis of the available literature, but it is likely of being dramatically modified in the next future, when ongoing multimodality IO trials will be reported.

Currently, upfront CN should not be considered as the standard of care in intermediate- and poor-risk mRCC patients. In those cases, upfront systemic therapy is the preferred option, and CN can be considered only in selected patients. The rationale for such an approach stems from the ability of systemic therapy to select non-responders who may not benefit from surgery. Conversely, CN is still the preferred option in low-risk and/or oligometastatic patients that could be managed by either active surveillance or local treatment (stereotactic radiation therapy or metastasectomy) offering a systemic treatment-free survival without compromising OS. Finally, patient selection for CN is sometimes indicated by the presence of local symptoms.

Further data coming from ongoing IO trials and retrospective population-based cohorts are needed to better understand how to select surgical candidates and how to decrease the morbidity and the complication rates associated with such a multidisciplinary approach.

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Chapter 15
Clinical Cases Debate: Neoadjuvant Versus Adjuvant Immunotherapy in Localized Renal Cell Carcinoma (RCC)

William Paul Skelton IV, Aaron Dahmen, Monica Chatwal, Rohit K. Jain, Jad Chahoud, and Philippe E. Spiess

Introduction

Survival and outcomes of patients diagnosed with renal cell carcinoma (RCC) vary greatly based on the stage of their disease. Approximately, 65% of patients with RCC are diagnosed with localized disease, 16% with locoregional disease, and 16% with metastatic disease [1]. Patients with localized disease have a significantly better 5-year survival rate of 93% compared to a dismal 12% among those with metastatic disease [1]. Though the frontline treatment for metastatic RCC is systemic therapy—largely based on vascular endothelial growth factor receptor tyrosine kinase inhibitors (VEGFR TKIs), immunotherapy (IO), and combination therapy with VEGFR TKIs and IO—localized and locally advanced RCC are primarily managed surgically with curative intent [2–5]. For these patients, radical nephrectomy and nephron-sparing surgery (partial nephrectomy) are accepted standard of care options. Surgical approach depends largely on the extent and position of the tumor along with other comorbid conditions (such as if the patient has a solitary kidney). However, many patients relapse either locally at the site of nephrectomy or at distant sites.

Recurrence risk is determined by pathological stage and Fuhrman nuclear grade as well as the patient's Eastern Cooperative Oncology Group (ECOG) Performance Status (PS). It is very important to identify which patients are at a high risk through different prognostic models and evaluate the role of adjuvant therapy to treat microscopic disease. The goal of neoadjuvant therapy prior to surgery, as discussed below,

W. P. Skelton IV · A. Dahmen · M. Chatwal · R. K. Jain · J. Chahoud · P. E. Spiess (✉)
Department of Genitourinary Oncology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA
e-mail: William.Skelton@moffitt.org; Philippe.Spiess@moffitt.org

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has been to downstage tumors among patients with a high risk of recurrence to optimize potential surgical options and long-term outcomes. The goal of adjuvant therapy is to decrease recurrence risk following surgical removal of the tumor, and adjuvant therapy represents a potential of great therapeutic benefit, as 30–40% of patients with localized RCC develop metastatic disease after nephrectomy, which confers dismal survival outcomes [6].

Neoadjuvant Therapy

A 71-year-old male with a history of prior right radical nephrectomy for clear cell RCC was found to have a contralateral solid renal mass measuring up to 4.8 cm. The patient underwent a renal biopsy, which was again consistent with clear cell RCC. Workup showed that he did not have metastatic disease, though the patient had mild renal insufficiency, with a creatinine level of 1.4 mg/dL. Because of the size of the mass, there was concern that a partial nephrectomy would pose a high risk of insufficient renal reserve, whether because of the size of surgical margins necessary or the need for completion nephrectomy. As the mass appeared indolent, growing from 2 to 4.8 cm over 60 months, the decision was made to proceed with neoadjuvant treatment with axitinib 5 mg twice daily. On 3-month restaging scans, there was a noted partial response from 4.8 to 3.5 cm, and he received a partial nephrectomy 4 months after the initiation of treatment (Fig. 15.1). The final

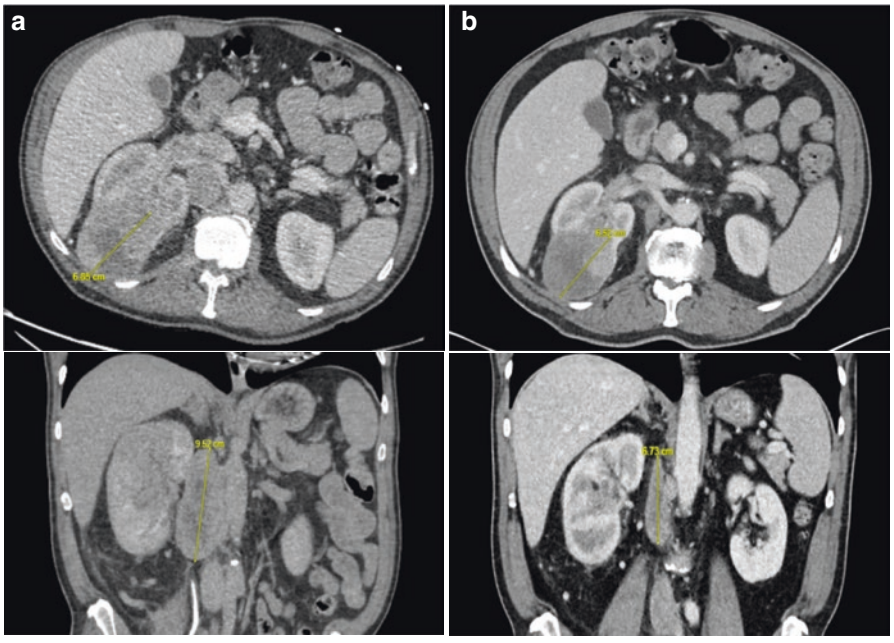

Fig. 15.1 Response of patient treated with neoadjuvant axitinib

pathology report was consistent with grade 2 pT1aNxMx clear cell RCC, and his postoperative creatinine level was 1.6 mg/dL. The patient tolerated treatment without significant adverse effects (AEs). This illustrates the potential benefit of neoadjuvant therapy to optimize surgical outcomes and prevent future recurrence.

Localized RCC is primarily managed surgically with either partial or radical nephrectomy, depending on the extent of disease. The goal of neoadjuvant therapy is to optimize surgical resection and potentially downstage the disease to allow for a less extensive surgery, especially among patients with T4 disease. This may make a patient a candidate for nephron-sparing surgery and thereby preserve more of the patient's renal function. It may also be used as a bridge to surgery among those patients for whom upfront surgical resection is not possible. For example, among patients with large disease burden or those who have RCC invading or extensively abutting adjacent organs, a response to neoadjuvant therapy can facilitate more complex surgical resections. Some also argue that upfront treatment with neoadjuvant therapy, when the burden of micrometastatic disease is at its lowest point, may portend better outcomes, decrease the likelihood of recurrence, and cure the patient [7].

Numerous trials have explored the role of neoadjuvant therapy in improving outcomes prior to surgical resection (Fig. 15.2). The bulk of evidence for neoadjuvant therapy comes from phase 2 trials examining different VEGF TKIs, though there is currently no standard of care. For patients treated with neoadjuvant therapy, it is important to monitor for AEs that may require dose reduction or discontinuation. It is also imperative to closely assess for any signs of disease progression that may change the disease stage or available treatment options. We now focus on examining the pivotal trials that have explored neoadjuvant therapy for patients with localized RCC.

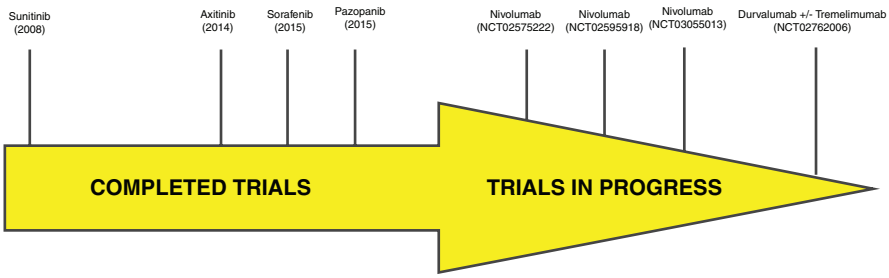

Fig. 15.2 Timeline of completed and ongoing clinical trials examining neoadjuvant therapy in locally advanced RCC

Sunitinib

Neoadjuvant therapy was first explored in a phase 2 trial examining sunitinib for unresectable advanced RCC [8]. Patients were eligible if they had RCC that was not suitable for nephrectomy, and patients with any histological subtype of RCC were eligible. Patients were treated with 50 mg of sunitinib daily for 4 weeks on a 6-week cycle and received a median of two cycles of sunitinib (range, 1–8 cycles). Of the 19 patients enrolled, none had a complete response (0%), three had a partial response (16%), seven had stable disease (37%), and nine had progressive disease (47%). Four patients (21%) underwent nephrectomy at a median 6-month follow-up. The most common AEs were fatigue (74%), dysgeusia (43%), hand-foot syndrome (32%), and diarrhea (31%). The dose of sunitinib was reduced for three patients to 37.5 mg daily because of toxicity and only one patient required discontinuation (5%) because of grade 3 hand-foot syndrome. Of note, this study included patients with both locally advanced and metastatic disease. Ultimately, the tumors of several patients with advanced RCC who received neoadjuvant sunitinib were downstaged, allowing the patient to undergo nephrectomy.

Axitinib

The next trial examining the role of neoadjuvant therapy among patients with locally advanced clear cell RCC was done several years later, in 2014 [9]. Unlike the sunitinib trial, this trial only enrolled patients with locally advanced disease and did not include patients who had metastases. In this phase 2 trial, which was conducted over 2 years from May 2011 to April 2013, patients with clear cell RCC received 12 weeks of 5 mg of axitinib twice daily, with the last dose 36 hours prior to nephrectomy. Of the 24 patients enrolled, 11 had a partial response, 13 had stable disease, and no patients had progressive disease. All patients were able to undergo surgical resection; 19 underwent radical nephrectomy and 5 underwent partial nephrectomy. The most common side effects were hypertension (79%), hoarseness (79%), fatigue (75%), mucositis (71%), hypothyroidism (71%), and hand-foot syndrome (63%). The most common grade 3 or higher AEs were hypertension (42%), transaminitis (8%), and abdominal pain (8%). This study showed that, among patients with advanced RCC, neoadjuvant axitinib was well tolerated and associated with a good response, making nephrectomy feasible.

Pazopanib

Rini and colleagues conducted a phase 2 trial examining the effects of neoadjuvant pazopanib among patients with localized RCC, with the goal of downsizing tumors and optimizing preservation of renal parenchyma [10]. Twenty-five patients with

localized clear cell RCC were enrolled and were given 800 mg of pazopanib daily for 8 to 16 weeks prior to surgery. Of the 13 patients who were not able to undergo partial nephrectomy at baseline, 6 were able to undergo a partial nephrectomy after treatment with pazopanib. Neoadjuvant pazopanib helped to downsize some patients' localized RCCs, allowing for partial nephrectomy among those who otherwise would have required radical nephrectomy.

Sorafenib

The use of neoadjuvant sorafenib among patients with high-risk RCC was explored by Zhang and colleagues in 2015 [11]. Patients were eligible if they had high-risk RCC, defined as (1) grade 2 or higher disease with inferior vena cava (IVC) thrombus, (2) tumor diameter >7 cm, (3) multiple tumors within a patient undergoing nephron-sparing surgery, (4) a tumor within a functional solitary kidney that was unsuitable to undergo nephron-sparing surgery, or (5) widespread metastatic RCC. This phase 2 trial took place over 6 years from April 2007 to October 2013. Of a total of 37 patients who received neoadjuvant sorafenib, 18 (48%) patients successfully received surgery and their characteristics were examined. The dose of sorafenib was 400 mg twice daily, and patients were treated for an average of 96 days (range, 30–278 days). Sorafenib was discontinued an average of 12 days prior to surgery (range, 7–30 days). The overall response rate (ORR) among patients who received neoadjuvant sorafenib was 94%; 4 patients had a partial response (22%) and 13 patients had stable disease (72%). The average tumor size decreased from 7.8 to 6.2 cm. Of note, there were also 5 patients with IVC tumor thrombus, and 4 of those patients had decreased tumor thrombus burden following neoadjuvant sorafenib. A total of 11 patients underwent radical nephrectomy, 5 patients underwent radical nephrectomy and IVC thromboembolectomy, and 2 patients underwent partial nephrectomy. This trial showed that neoadjuvant therapy can decrease primary tumor size, resulting in improved surgical outcomes. It may also reduce tumor thrombi and improve outcomes with thromboembolectomy.

Utility of Neoadjuvant Therapy

Phase 2 trials examining sunitinib, axitinib, pazopanib, and sorafenib among patients who were initially ineligible for nephrectomy have shown similar results regarding the reduction of tumor burden. This translates to expanded surgical options, including the potential of partial nephrectomy rather than radical nephrectomy. Despite these outcomes, however, the mainstay of treatment remains surgical resection, and neoadjuvant therapy with VEGFR TKIs has not been adopted as a standard practice. The role of IO in the neoadjuvant setting is currently being examined and remains under investigation. Compared to adjuvant IO trials, which are all

phase 3, trials focusing on neoadjuvant IO are mainly in earlier phases of clinical investigation.

RCC has a predilection for vascular invasion, often seen as an IVC thrombus. This can be seen in 10–25% of RCC cases [12]. The effect of neoadjuvant VEGF TKIs on IVC thrombi has been mixed [13]. Neoadjuvant sunitinib has shown potential benefit [14, 15], whereas neoadjuvant sorafenib has shown mixed results [11, 16]. There has been case-level evidence of neoadjuvant nivolumab/ipilimumab causing a complete response for patients with IVC thrombus [17].

Neoadjuvant Immunotherapy

A 60-year-old male presented with a large right renal mass, IVC thrombus, and a large retroperitoneal lymph node with anterior displacement of the IVC. The patient underwent confirmatory biopsy of the retroperitoneal node, which was consistent with clear cell RCC with focal rhabdoid features. Given the large renal mass, lymphadenopathy, and IVC thrombus, the decision was made to proceed with nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) every 3 weeks for 4 doses, followed by maintenance nivolumab of 240 mg every 2 weeks or 480 mg every 4 weeks preoperatively. Restaging scans after 4 cycles of combined nivolumab/ipilimumab demonstrated a 3 mm decrease in the primary renal mass and a significant decrease in the retroperitoneal lymph node from 9.5 to 6.7 cm (Fig. 15.3). The decision was

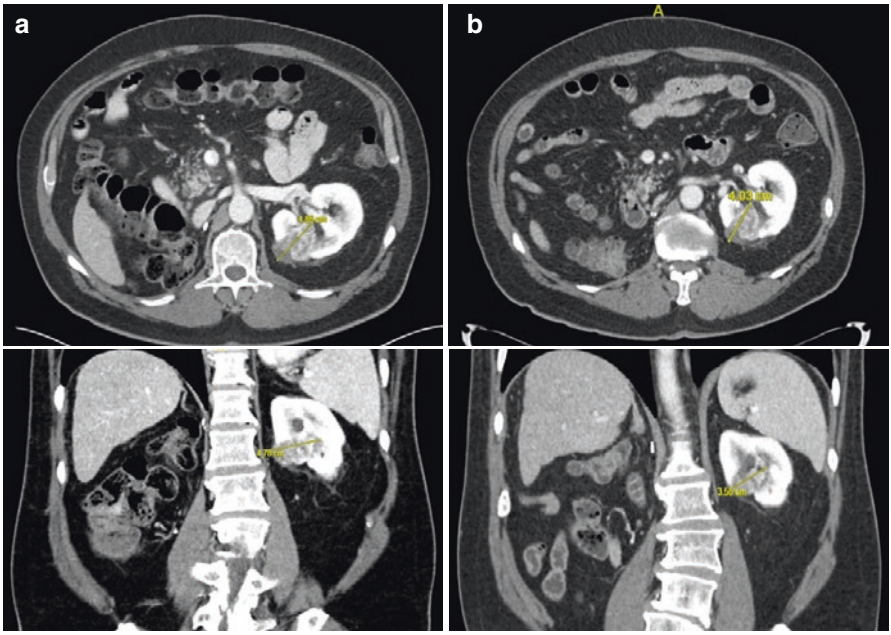

Fig. 15.3 Response of patient treated with neoadjuvant nivolumab + ipilimumab

Table 15.1 Ongoing clinical trials exploring neoadjuvant immunotherapy

Trial	Phase	Intervention	N	Primary endpoint	Estimated completion date
NCT02575222	Phase I	Nivolumab	17	Safety	June 2020
NCT02595918	Phase I	Nivolumab	29	Safety	April 2021
NCT03055013 (PROSPER)	Phase III	Nivolumab	805	Event-free survival	November 2023
NCT02762006	Phase Ib	Durvalumab +/− tremelimumab	29	Dose-limiting toxicity	November 2020

then made to proceed with right radical nephrectomy, IVC thrombectomy, right adrenalectomy, and retroperitoneal lymph node dissection. The final pathology report showed grade 4 clear cell RCC with focal rhabdoid features and extensive necrosis. The retroperitoneal node and thrombus were consistent with the primary tumor. This illustrates the potential benefit of IO to optimize surgical outcomes and prevent future recurrence.

Most active trials exploring neoadjuvant IO involve nivolumab (Table 15.1 and Fig. 15.2). The first phase 1 trial opened in February 2016 and is examining the effects of neoadjuvant nivolumab on nonmetastatic high-risk clear cell RCC. Patients were eligible if they had nonmetastatic high-risk clear cell RCC (T2a-T4N[any]M0 or T[any]N1M0), ECOG PS of 0 or 1, and adequate organ and marrow function and planned to have either a partial or radical nephrectomy. Seventeen patients were enrolled and received 3 mg/kg of nivolumab every 2 weeks for a total of 3 cycles prior to nephrectomy. The primary outcome was safety, with secondary outcomes including ORR, quality of life, metastasis-free survival, and overall survival (OS). This study finished enrollment in June 2020 and results are eagerly anticipated (NCT02575222).

A subsequent phase 1 study, which began enrolling patients in May 2016, is examining preoperative nivolumab among high-risk patients with RCC and includes patients with metastatic disease undergoing planned cytoreductive nephrectomy or metastasectomy. Patients are eligible if they have confirmed clear cell RCC; ECOG PS of 0 or 1; and adequate hematological, kidney, and liver function. This study is still actively recruiting (29 patients thus far), with an estimated accrual completion date of April 2021. Patients will receive nivolumab a total of 4 times on an every-other-week cycle at 8 weeks, 6 weeks, 4 weeks, and 2 weeks prior to surgery. The primary outcome is to determine if patients can receive at least 3 doses of nivolumab and undergo surgical resection without delay. Secondary outcomes include toxicity, ORR, and recurrence-free survival (NCT02595918).

The PROSPER trial (Perioperative Nivolumab vs. Observation in Patients with Renal Cell Carcinoma Undergoing Nephrectomy) is a phase 3 study comparing perioperative nivolumab to observation. The study began enrolling patients in February 2017 and is currently still recruiting, with an estimated enrollment goal of 805 patients. Patients randomized to the treatment arm will receive preoperative nivolumab every 2 weeks for 2 cycles, followed by partial or radical nephrectomy within 1–4 weeks. They then receive nivolumab every 2 weeks for 6 cycles, then monthly for 6 cycles or until toxicity or disease progression. The primary outcome

in this trial is event-free survival. OS and toxicity are secondary outcomes. The estimated completion is November 2023 (NCT03055013).

Durvalumab with or without tremelimumab is also currently being examined as neoadjuvant therapy for locally advanced RCC. A phase 1 trial opened in December 2016 and has finished accruing. Twenty-nine patients have been randomized to multiple cohorts, including durvalumab monotherapy, durvalumab + tremelimumab combination therapy as a neoadjuvant approach, and durvalumab + tremelimumab combination therapy as an adjuvant therapy within 4–6 weeks postnephrectomy. The primary outcome is toxicity, with secondary outcomes exploring postsurgical complications and ORR. This trial is estimated to be completed in November 2020 (NCT02762006).

Adjuvant Therapy

A 57-year-old male was diagnosed with a 10-cm left renal mass with renal vein involvement and associated para-aortic adenopathy during a workup for abdominal pain. He underwent upfront surgical management of his disease via an open left radical nephrectomy and adrenalectomy. His final pathology report was consistent with clear cell pT3NxMx RCC. Because of his high-risk pathology, he was then evaluated by the medical oncology team for further treatment, and after discussion, the decision was made to proceed with adjuvant sunitinib therapy. He received sunitinib for a total of 1 year and experienced grade 2 neutropenia and grade 1 hand-foot syndrome. He has since been followed up with and demonstrates no evidence of disease 1 year following treatment, with no long-term treatment-related adverse effects (TRAEs). This illustrates the potential benefit of adjuvant therapy to reduce the risk of recurrence among otherwise high-risk patients receiving upfront surgical management.

The role of adjuvant therapy has been the subject of numerous published and ongoing trials (Fig. 15.4) and remains controversial. Among patients with stage I disease, nephrectomy—partial or radical—is the mainstay of treatment, with active surveillance a consideration for select patients. For patients with stage II disease,

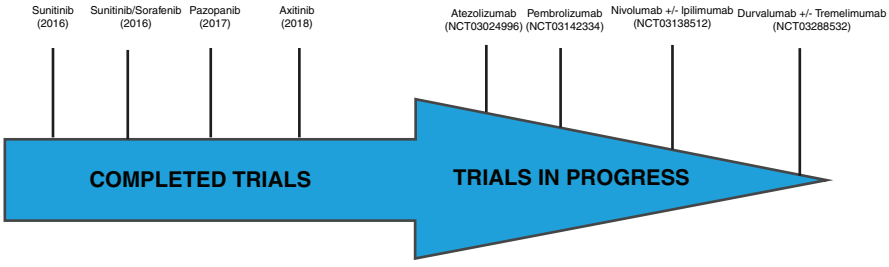

Fig. 15.4 Timeline of completed and ongoing clinical trials examining adjuvant therapy in locally advanced RCC

nephrectomy followed by surveillance is preferred. However, for patients with stage III disease, though nephrectomy remains the standard of care, multiple clinical trials have explored the role of adjuvant therapy compared to standard surveillance. Current challenges in this field include heterogenous inclusion criteria in clinical trials as well as a lack of clinically apparent radiographic disease to assess response outcomes.

ECOG-ACRIN E2805 ASSURE Trial

The first trial to examine the role of adjuvant therapy for localized RCC was the ECOG-ACRIN E2805 ASSURE (adjuvant sunitinib or sorafenib for high-risk non-metastatic renal cell carcinoma) trial [18]. Investigators examined whether VEGFR TKIs sorafenib or sunitinib conferred a survival advantage when used in the adjuvant setting for locoregional disease. This study was initiated based on prior data showing improved progression-free survival (PFS) among patients with metastatic RCC who were treated with sorafenib or sunitinib.

This study was conducted over a 4-year period from April 2006 to September 2010, and 1943 patients were enrolled from 226 centers in the USA and Canada. Eligibility criteria included high-risk clear cell or non–clear cell RCC with complete surgical resection within 12 weeks of trial enrollment. High risk was defined as having pT1b G3-4 N0 M0 to T(any) G(any) N+ (fully resected) M0 disease. Patients also needed to be treatment-naive; have good cardiac function (defined as left ventricular ejection fraction >50%); have an ECOG PS of 0–1; and have intact liver, kidney (CrCl > 30 mL/min), and hematological function. Exclusion criteria included uncontrolled hypertension, thyroid disease, or HIV infection.

The 1943 patients were randomized in a double-blind fashion to 1 of 3 groups: (a) 50 mg of sunitinib daily for 4 weeks of a 6-week cycle, (b) 400 mg of sorafenib twice daily, or (c) placebo. Because of toxicity, 3 years into the study (May 2009), the sunitinib starting dose was decreased to 37.5 mg daily for 4 weeks of a 6-week treatment cycle, and, if the medication was well tolerated after the first or second cycle, it was increased to the 50 mg dose. Patients were treated for a total of 54 weeks.

The primary endpoint of the study was disease-free survival (DFS), with secondary endpoints including OS and toxic effects. Median DFS was 70 months (5.8 years) for sunitinib, 73.4 months (6.1 years) for sorafenib, and 79.6 months (6.6 years) for placebo. Statistically, the DFS did not differ significantly between these groups. When sunitinib was compared to placebo, the hazard ratio (HR) was 1.02 (95% CI, 0.85–1.23; $P = 0.804$), and when sorafenib was compared to placebo, the HR was 0.97 (95% CI, 0.80–1.17; $P = 0.718$). The 5-year OS also did not differ significantly between groups, as it was 77.9% in the sunitinib group (95% CI, 74.1–81.9), 80.5% in the sorafenib group (95% CI, 76.8–84.2), and 80.3% in the placebo group (95% CI, 76.7–84.0).

Patients experienced many side effects in this trial, with a large cohort of patients withdrawing from the study because of treatment toxicity. The most common grade 3 or higher side effects in the sunitinib group were hypertension (17%), fatigue (17%), hand-foot syndrome (15%), and diarrhea (10%). For the sorafenib group, the most common grade 3 or higher side effects were hand-foot syndrome (33%), hypertension (16%), rash (15%), and diarrhea (9%). Prior to the dose decrease because of toxicity in May 2009, 3 years into the trial, 44% of patients on sunitinib, 45% of patients on sorafenib, and 11% of patients in the placebo arm withdrew from the study because of TRAEs. After the dose reduction, fewer patients withdrew due to TRAEs—34% of the sunitinib cohort, 30% of the sorafenib cohort, and 10% of the placebo arm. The number of patients who discontinued the trial because of TRAEs was significantly lower after dose reduction in the sunitinib ($P = 0.014$) and sorafenib groups ($P = 0.0001$) but not in the placebo group ($P = 0.696$).

The findings of the ECOG-ACRIN E2805 ASSURE trial suggest that there is no benefit (DFS or OS) from adjuvant sorafenib or sunitinib compared to placebo and that treatment with an adjuvant TKI can cause significant toxicity without additional benefit.

S-TRAC Trial

The next trial to examine the role of adjuvant therapy for localized RCC was the S-TRAC (Sunitinib as Adjuvant Treatment for Patients at High Risk of Recurrence of Renal Cell Carcinoma Following Nephrectomy) trial [19]. The aim of the trial was to determine if sunitinib conferred a survival advantage in the adjuvant setting after nephrectomy.

The trial was conducted over a 3.5-year period from September 2007 to April 2011, overlapping with the aforementioned ASSURE trial. A total of 615 patients at 99 centers across 21 countries were enrolled. Eligible patients were required to have locoregional RCC, defined as stage III disease or higher or regional lymph node metastases; successfully undergone nephrectomy with the absence of residual disease; and enrolled on the trial between 3 and 12 weeks after surgery. Patients also needed to have clear cell RCC, compared to ASSURE, in which both clear cell and non–clear cell subtypes were allowed. Other notable inclusion criteria included an ECOG PS of 0, 1, or 2, compared to ASSURE, which enrolled only patients with an ECOG PS of 0 or 1. Exclusion criteria included metastatic disease, histologically undifferentiated tumors, a second malignancy diagnosed within 5 years, cardiovascular disease/major event in the past 6 months, and uncontrolled hypertension (defined as blood pressure >150/100 mmHg).

The 615 patients were randomized in a 1:1 ratio on the basis of ECOG score and country of residence into either a group receiving sunitinib 50 mg daily or placebo daily for 4 weeks of a 6-week cycle. Patients were treated for a total of 1 year. The primary endpoint of the study was DFS. Secondary endpoints included OS and safety assessments. The median DFS in the sunitinib group was 6.8 years (95% CI,

5.8-not reached) compared to 5.6 years in the placebo group (95% CI, 3.8–6.6). The HR was 0.76 (95% CI, 0.59–0.98; $P = 0.03$). Of note, there was an improved DFS with sunitinib compared to placebo according to both the independent central review group (6.8 years vs 5.6 years) and the investigators' review (6.5 years vs 4.5 years), but this improvement was only statistically significant according to the independent central review group, not the investigators' review group (HR, 0.81 [95% CI, 0.64–1.02]; $P = 0.08$). OS data was not mature at the time of data cutoff, with an HR of 1.01 (95% CI, 0.72–1.44; $P = 0.94$).

AEs occurred among 99.7% of patients in the sunitinib group and 88.5% of patients in the placebo group. The most common grade 3 or higher AEs in the sunitinib group were hand-foot syndrome (16%), neutropenia (8.5%), hypertension (7.8%), thrombocytopenia (6.2%), fatigue (4.9%), mucositis (4.6%), and diarrhea (3.9%).

The findings of the S-TRAC trial showed a DFS benefit for adjuvant sunitinib following nephrectomy compared to placebo. This was not seen in the ASSURE trial, which compared adjuvant sunitinib, sorafenib, and placebo. The authors of the S-TRAC trial posited numerous reasons for this discrepancy in outcomes, including different trial methods and doses of sunitinib. In S-TRAC, all patients got sunitinib at a 50 mg dose, compared to ASSURE, in which patients in the sunitinib arm initially received the 50 mg dose and then TRAEs caused a dose reduction to an initial dose of 37.5 mg among patients who enrolled after the third year of the study. The ASSURE trial also enrolled patients who had non–clear cell histology (21% of patients), compared to the S-TRAC trial, which only enrolled patients with clear cell RCC.

Based on the results of the S-TRAC trial, the FDA approved sunitinib in the adjuvant setting in November 2017 [20]. It is important to note that, in clinical practice, sunitinib is not commonly used for multiple reasons, which are expanded upon later in the chapter "Impact of VEGFR TKIs as adjuvant therapy."

PROTECT Trial

The PROTECT (Pazopanib as Adjuvant Therapy in Localized/Locally Advanced RCC After Nephrectomy) trial, conducted after the ASSURE and S-TRAC trials, assessed the role of pazopanib in the adjuvant setting, given its efficacy as a first-line treatment [21]. The trial was conducted over a nearly 3-year span from December 2010 to September 2013. It enrolled 1538 patients at 263 centers across 26 countries. Patients were eligible if they had nonmetastatic clear cell or predominantly clear cell RCC histology that had been resected, along with Karnofsky PS > 80 and "adequate organ function."

Patients were originally randomized to 800 mg of pazopanib daily or placebo, but because of TRAEs and patients withdrawing from the study, the starting pazopanib dose was decreased to 600 mg daily. If patients tolerated the 600 mg dose, then it could be escalated to 800 mg after 8–12 weeks. A total of 403 patients were

enrolled and randomized when the starting dose was 800 mg (198 patients were randomized to pazopanib and 205 to placebo), and 1135 patients were enrolled and randomized on the subsequent lower starting dose of 600 mg (571 patients were randomized to pazopanib and 564 to placebo). Patients received treatment for a total of 1 year.

The primary endpoint of the study was DFS. Initially, the primary outcome was DFS for the cohort receiving 800 mg of pazopanib, but after the dose reduction, the primary outcome was changed to the DFS of the 600 mg cohort. The secondary endpoints were OS, DFS for the 800 mg cohort, DFS at yearly timepoints, safety, and patient-reported outcomes/quality of life. With respect to the primary endpoint (DFS for the 600 mg cohort), the HR was 0.86 (95% CI, 0.70–1.06; $P = 0.16$) but was not statistically significant. The secondary outcome of DFS for the 800 mg cohort showed a benefit from pazopanib (HR 0.69 [95% CI, 0.51–0.94]; $P = 0.02$). When both subgroups (600 mg and 800 mg) were combined, the HR was 0.80 (95% CI, 0.68–0.95; $P = 0.01$). There was not an OS benefit in any of the subgroups, as the OS in the 600 mg group had an HR of 0.79 (95% CI, 0.57–1.09; $P = 0.16$), the OS in the 800 mg group had an HR of 0.89 (95% CI, 0.54–1.46; $P = 0.65$), and the OS of the combined groups had an HR of 0.82 (95% CI, 0.62–1.07; $P = 0.15$).

A total of 98% of patients in the pazopanib group and 90% of patients in the placebo group experienced at least one AE. In terms of the different pazopanib dosage groups, 51% of patients in the 600 mg cohort and 60% of patients in the 800 mg cohort had dose reductions during treatment. Thirty-five percent of patients in the 600 mg cohort discontinued the drug because of TRAEs, whereas 39% of patients in the 800 mg cohort discontinued pazopanib because of TRAEs. Of note, 21% of patients in the 600 mg of pazopanib cohort had a dose escalation by week 12 because of tolerability. The most common AEs were diarrhea (64%), hypertension (52%), hair color changes (41%), nausea (40%), and fatigue (39%). The most common grade 3 and above AEs were hypertension (25%), increased alanine aminotransferase (16%), diarrhea (7%), and increased aspartate aminotransferase (6%).

The PROTECT trial showed that there was not an increased DFS in the cohort of patients who received 600 mg of pazopanib; however, its secondary endpoint of improved DFS for the 800 mg cohort was met. It is important to consider that the cohort that received the 800 mg dose was roughly one-third the size of the 600 mg cohort, largely because of toxicity and TRAEs.

ATLAS Trial

The most recent phase 3, randomized trial to explore the effect of adjuvant TKI therapy after nephrectomy was the ATLAS (Adjuvant Axitinib Therapy of Renal Cell Cancer in High Risk Patients) trial. This study evaluated the effect of axitinib among patients with locoregional RCC with high risk of recurrence after nephrectomy [22]. Like the previous trials (ASSURE, S-TRAC, and PROTECT),

investigators of this study questioned whether the benefit of VEGFR TKIs in the metastatic setting could be extended into the adjuvant setting following nephrectomy.

The trial enrolled 724 patients across 137 multinational centers in 8 countries. Patients were eligible if they had RCC following nephrectomy (with greater than a 50% clear cell component) without metastatic disease, had not received any prior systemic treatment, and had an ECOG PS of 0–1. Patients were randomized 1:1 to receive axitinib 5 mg twice daily or placebo and were treated for a minimum of 1 year and up to 3 years based on individualized decision-making by the patient and the site investigator. Thirty-one percent of patients on the axitinib arm were treated for less than 1 year, 27% of patients were treated for 1–2 years, 23% of patients were treated for 2–3 years, and 20% of patients completed 3 years of treatment.

The primary endpoint was DFS and secondary endpoints were OS and safety. The trial was stopped early because of futility at a preplanned interim analysis because 203 DFS events were reached. The HR was 0.87 (95% CI, 0.66–1.147; $P = 0.321$). Prespecified subgroup analyses of high-risk and low-risk recurrence showed a potential difference in HR depending on analyses from an independent review committee (IRC) vs investigators assessment. In the high-risk patient subgroup, IRC review showed an HR of 0.735 (95% CI, 0.525–1.028; $P = 0.07$) and investigator review showed an HR of 0.641 (95% CI, 0.468–0.879; $P = 0.005$). In the low-risk patient subgroup, there was no statistically significant difference between axitinib and placebo, as the IRC review showed an HR of 1.016 (95% CI, 0.62–1.666; $P = 0.948$) and the investigator review showed an HR of 1.048 (95% CI, 0.654–1.681; $P = 0.845$).

The most common side effects with axitinib were hypertension (64%), diarrhea (47%), dysphonia (42%), and hand-foot syndrome (32%). The most common TRAEs with axitinib were hypertension (60%) and dysphonia (38%). Due to AEs, the dose of axitinib was reduced for 56% of patients, interrupted for 51% of patients, and discontinued for 23% of patients. The most common AEs requiring discontinuation of axitinib were hypertension (4%), proteinuria (3%), and hand-foot syndrome (2%).

ATLAS was stopped early for futility at a preset interim analysis and did not meet its primary endpoint for improved DFS with axitinib. However, prespecified subset analyses showed potential improvement in DFS in the high-risk patient cohort, although this effect is questionable as it was statistically significant per the investigators' review but not according to IRC review.

Impact of VEGFR TKIs as Adjuvant Therapy

To date, 4 trials have explored the impact of VEGFR TKIs as adjuvant therapies following nephrectomy for localized/locoregional RCC. The ASSURE trial found no benefit for either adjuvant sunitinib or adjuvant sorafenib. The S-TRAC trial found a DFS benefit for adjuvant sunitinib following nephrectomy. The PROTECT

trial did not meet its primary endpoint of improved DFS for patients treated with 600 mg of adjuvant pazopanib but did show improvement in DFS at the 800 mg dose. Lastly, the ATLAS trial was stopped early because of futility and did not meet its primary endpoint of improved DFS with axitinib, but preplanned subset analyses showed a potential benefit in the high-risk patient cohort.

There are several limitations to comparing these trials. ASSURE, S-TRAC, PROTECT, and ATLAS varied greatly in their inclusion criteria as they all had slightly different definitions of *high-risk disease* and guidelines for what stages of RCC were included (local vs locoregional). Baseline histology also differed between studies—S-TRAC included pure clear cell RCC, PROTECT included predominantly clear cell RCC, ATLAS included RCCs with over 50% clear cell component, and ASSURE allowed all RCC histologies (21% of patients had non–clear cell histology). Patients enrolled in ASSURE and S-TRAC were required to start adjuvant therapy within 12 weeks of surgery, whereas the PROTECT and ATLAS trials did not mandate this. Length of treatment also differed between trials—1 year of sunitinib in S-TRAC, 1 year of pazopanib in PROTECT, 54 weeks of either sunitinib or sorafenib in ASSURE, and up to 3 years of axitinib in ATLAS.

Currently, only sunitinib is FDA approved as adjuvant therapy following nephrectomy for patients with stage III RCC [20]. However, its use is controversial, and it is a category 3 recommendation in the NCCN guidelines [5]. The use of sorafenib, axitinib, or pazopanib is not standard practice, despite some potential marginal benefit seen in certain subgroups. A meta-analysis by Sun and colleagues examining the ASSURE, S-TRAC, and PROTECT trials (but not ATLAS) found that their pooled analyses did not show statistically significant benefit in DFS (HR, 0.92 [95% CI, 0.82–1.03]; $P = 0.16$) or OS (HR 0.98 [95% CI, 0.84–1.15]; $P = 0.84$) from adjuvant VEGFR TKIs but did show higher risk of grade 3 and grade 4 AEs (OR, 5.89 [95% CI, 4.85–7.15]; $P < 0.001$). They found in exploratory analyses that patients who were initiated on full-dose VEGFR TKIs had improved DFS with adjuvant therapy (HR, 0.83 [95% CI, 0.73–0.95]; $P = 0.005$). Based on the results of the 4 trials, the questionable benefit of adjuvant therapy in the setting of numerous side effects means that many oncologists do not view this as a good treatment option and it is not routinely used in standard practice.

KEYNOTE-564

KEYNOTE-564 was a phase 3, multicenter, randomized, double-blind, placebo-controlled trial which explored the use of adjuvant pembrolizumab following nephrectomy [23]. It was the first published investigation exploring the use of adjuvant immunotherapy following nephrectomy (unlike the ASSURE, S-TRAC, PROTECT, and ATLAS trials, which all examined the effect of adjuvant tyrosine kinase inhibitors).

The trial enrolled 994 patients between June 2017 and September 2019, and at the prespecified interim analysis in December 2020, the median follow-up was

24.1 months (range 14.9–41.5 months). Patients were eligible if they had biopsy-proven clear cell RCC (did allow for a sarcomatoid component), intermediate/high risk disease (or M1 metastatic disease with no evidence of disease following nephrectomy and metastasectomy of soft tissue metastases), had not received prior systemic therapy, were less than 12 weeks from nephrectomy, and had ECOG PS 0–1.

Patients were randomized to pembrolizumab 200 mg intravenously every 3 weeks for up to 17 cycles or placebo. The primary outcome was DFS (assessed by the investigator), and secondary outcomes included OS, safety, and tolerability. DFS was not reached in either arm (HR 0.68, 95% CI 0.53–0.87), $p = 0.0010$, with an estimated 24-month DFS of 77.3% with pembrolizumab compared to 68.1% with placebo. The median OS was also not reached in either arm (HR 0.54, 95% CI 0.30–0.96), $p = 0.0164$, with an estimated 24-month OS of 96.6% with pembrolizumab vs 93.5% with placebo.

Treatment-related adverse effects were reported in 79.1% of the pembrolizumab population and 53.4% of those treated with placebo. Grade 3–5 AEs were seen in 18.9% of patients treated with pembrolizumab but only 1.2% of those treated with placebo. The most common TRAEs were fatigue (20.3% pembrolizumab, 14.3% placebo), pruritus (18.6% pembrolizumab, 11.5% placebo), diarrhea (15.8% pembrolizumab, 10.3% placebo), and rash (15.0% pembrolizumab, 7.3% placebo). The most common immune-mediated AEs were hypothyroidism (21.1% pembrolizumab, 3.6% placebo), hyperthyroidism (11.9% pembrolizumab, 0.2% placebo), and pneumonitis (2.3% pembrolizumab, 1.0% placebo).

The findings of KEYNOTE-564 are important as they represent a potential new therapeutic avenue in treating high-risk localized RCC following nephrectomy using immunotherapy, an area that is currently lacking beneficial options. While the data is still in the process of maturing, statistically significant benefits in DFS and OS are promising, and it will be of utmost importance to continue to follow future updates from this trial. To determine the effect of pembrolizumab on this patient population, this data, as well as data from other clinical trials exploring adjuvant immunotherapy following nephrectomy (see below), will be of utmost importance to determine if there is a potential role for immunotherapy in this setting, and potentially improve patient outcomes.

Trials Exploring Adjuvant Immunotherapy Following Nephrectomy

There are now a variety of ongoing clinical trials exploring the benefit of adjuvant IO after nephrectomy among patients with localized or locoregional RCC (Table 15.2 and Fig. 15.4). IMmotion010 is a phase 3, multicenter, randomized, placebo-controlled, double-blind study using atezolizumab. Patients randomized to the treatment arm receive 1200 mg of atezolizumab intravenously every 3 weeks for 16 cycles or 1 year, whichever occurs first. The primary outcome is DFS, which will

Table 15.2 Ongoing clinical trials exploring adjuvant immunotherapy

Trial	Phase	Intervention	N	Primary endpoint	Estimated completion date
NCT03024996 (IMmotion010)	Phase III	Atezolizumab vs placebo	778	Disease-free survival	February 2024
[a]NCT03142334 (KEYNOTE-564)	Phase III	Pembrolizumab vs placebo	950	Disease-free survival	December 2025
NCT03138512 (CheckMate914)	Phase III	Nivolumab + ipilimumab vs nivolumab vs placebo	1600	Disease-free survival	July 2024
NCT03288532 (RAMPART)	Phase III	Durvalumab + tremelimumab vs durvalumab vs placebo	1750	Disease-free survival and overall survival	December 2034

[a]Findings of KEYNOTE-564 presented at the American Society of Clinical Oncology meeting, June 2021, and are summarized further in the main text

be assessed by an IRC. Enrollment began in January 2017, and 778 patients have been accrued. It is no longer recruiting and is estimated to be completed in February 2024 (NCT03024996).

CheckMate914 is a phase 3, randomized, double-blind study comparing nivolumab monotherapy, nivolumab and ipilimumab combination therapy, and placebo among high-risk patients with localized RCC after nephrectomy. The primary outcome is DFS, which will be assessed by an independent central review. It began enrolling in July 2017 and has an estimated enrollment of 1600 patients. It is currently recruiting and is estimated to finish in July 2024 (NCT03138512).

RAMPART (Renal Adjuvant Multiple Arm Randomized Trial) is a phase 3, randomized, open-label trial examining adjuvant durvalumab, durvalumab and tremelimumab combination, and placebo among patients with RCC following primary resection. Durvalumab is given intravenously in 1500 mg doses every 4 weeks for a maximum of 13 cycles (1 year), and tremelimumab is given intravenously in 75 mg doses every 4 weeks for 2 total cycles. The primary outcomes are DFS and OS. It began enrolling in July 2018 and has an estimated enrollment of 1750 patients. It is currently recruiting and is estimated to finish in December 2034 (NCT03288532).

It is important to note the different inclusion criteria with respect to histological subtypes of RCC. The first two trials discussed, NCT03024996 examining atezolizumab, and NCT03138512 examining nivolumab and nivolumab with ipilimumab require patients to have clear cell RCC but allow for a sarcomatoid component. NCT03288532, on the other hand, which is examining durvalumab and durvalumab with tremelimumab, allows for variant histologies of all RCC cell types, with the exception of collecting duct, pure oncocytoma, medullary, and transitional cell cancer.

Conclusion

The current treatment paradigms for locally advanced RCC are nearly entirely based on surgical management followed by surveillance with serial imaging. More focus should be placed on identifying patients who are at the highest risk of disease recurrence and identifying molecular and radiomic markers of disease response to therapies. Numerous clinical trials have explored the role of neoadjuvant and adjuvant therapies among this patient population, but they have not been practice-changing or incorporated into the standard of care (with the possible exception of adjuvant pembrolizumab). Neoadjuvant trials exploring the roles of axitinib, pazopanib, sorafenib, and sunitinib showed that neoadjuvant therapy with these TKIs can lead to downstaging, allowing patients to undergo nephrectomy when they would not have been able to without neoadjuvant therapy or even allowing some patients to undergo partial nephrectomy rather than a total nephrectomy. These studies, however, have numerous limitations, including small sample sizes, different study endpoints, and a lack of data with single-agent IO in the neoadjuvant setting at the current time. Adjuvant trials, on the other hand, examining the same 4 TKIs—sunitinib, sorafenib, pazopanib, and axitinib—have shown mixed results, with none yet impacting the standard of care of postnephrectomy surveillance thus far. The S-TRAC trial showed a DFS benefit from adjuvant sunitinib, but this was not shown in the ASSURE trial. The PROTECT trial showed a potential benefit of adjuvant pazopanib as a secondary outcome at a higher dose. Lastly, the ATLAS trial showed a potential benefit of adjuvant axitinib in a high-risk patient cohort. Optimal selection of high-risk patients who would benefit from adjuvant therapy, including those who can tolerate higher doses, should be a future direction of examination.

Overall, though neither neoadjuvant therapy nor adjuvant therapy is currently the standard of care, there is evidence that neoadjuvant therapy can lead to preoperative downstaging and improved surgical outcomes, and adjuvant therapy for a select patient subset may also improve outcomes. It is important to note that all evidence to date has been with VEGFR TKIs (with the exception of KEYNOTE-564 exploring adjuvant pembrolizumab), and the multitude of open and currently accruing clinical trials are exploring the utility of neoadjuvant or adjuvant IOs. Pending the results of these ongoing clinical trials, the incorporation of IOs may prove to be useful for this patient population (as in the metastatic setting), and future clinical trials incorporating both IO and a VEGF TKI as a combination therapy may be a potential avenue to explore as well. It will be important to monitor the treatment landscape of these trials, as, if patients treated with combination therapies including IOs in the neoadjuvant setting have recurrence of disease, this will affect potential subsequent treatment options as well as monitoring for and exploring patterns of resistance.

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Chapter 16
The Search for the Optimal Immunotherapy Sequencing in the Perioperative Setting of RCC

Georgia Sofia Karachaliou, Deborah R. Kaye, Daniel J. George, and Tian Zhang

Introduction

Surgical excision with nephrectomy remains the definitive treatment of localized renal cell carcinoma [1–4]. However, about 30–40% of patients who have locally advanced RCC, involvement of regional lymph nodes, or high nuclear grade at the time of nephrectomy will experience disease recurrence or metastatic disease. While perioperative systemic treatment has been established as a common strategy in other solid tumors (i.e., breast, lung, colon, and bladder cancer) to eliminate micrometastatic disease and to improve cure rates [5], perioperative treatment in renal cell carcinoma remains an unproven path.

Tyrosine kinase inhibitors (TKIs) of the vascular endothelial growth factor (VEGF) pathway have improved clinical outcomes in patients with metastatic disease [6], and the role of sunitinib has been shown to improve disease-free survival but not overall survival in the adjuvant post-operative setting. Recently, multiple immunotherapy checkpoint inhibitor (ICI) combinations (i.e., ipilimumab/nivolumab or ICI/TKI combinations such as pembrolizumab/axitinib and avelumab/axitinib) have shown clinical benefit in metastatic RCC [7]. Thus far, however, no perioperative therapy has been proven to prolong overall survival [8]. Using

G. S. Karachaliou · D. J. George · T. Zhang (✉)
Division of Medical Oncology, Department of Medicine, Duke University, Durham, NC, USA

Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA
e-mail: tian.zhang2@duke.edu

D. R. Kaye
Duke Cancer Institute Center for Prostate and Urologic Cancers, Durham, NC, USA

Division of Urology, Department of Surgery, Duke University, Durham, NC, USA

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immunotherapies earlier in the localized RCC setting therefore holds promise for further clinical benefit and to potentially improve clinical outcomes.

In this chapter, we will highlight the search for optimal immunotherapy sequencing in the perioperative setting of localized as well as metastatic RCC, along with ongoing clinical trials.

Immunotherapy Sequencing in Perioperative Setting in Patients with Localized/Locally Advanced RCC

The administration of neoadjuvant therapy in patients with localized or locally advanced RCC remains controversial. The main controversy lies in the delay during neoadjuvant therapy that might incur to impact good clinical outcomes from surgical resection, hypothetically increasing time for the tumor to further progress prior to nephrectomy [9, 10]. Additionally, possible toxicities of neoadjuvant medical therapy, along with the increased surgical morbidities, also argue against using neoadjuvant treatments [11]. From case series and phase 2 studies, however, neoadjuvant therapy with VEGF TKIs has been shown to facilitate debulking and allow initially unresectable locally advanced RCC to become operable, by inhibiting angiogenesis [12–23]. The latter is of paramount importance since in situ tumors may release angiogenic or proinflammatory cytokines that physiologically enhance the therapeutic targeting of the neoadjuvant therapy. In the same setting, in situ tumors express nascent tumor antigens that enable immunologic targeting. As ICIs have improved outcomes with good immune-mediated responses and tolerable toxicities in metastatic RCC, there is indeed equipoise and rationale to embark on using ICIs in the perioperative setting.

Neoadjuvant Therapy in Locally Advanced RCC

The goals of neoadjuvant therapy for RCC include reducing the tumor burden prior to surgical resection, thereby allowing prior inoperable tumors to become operable, and selecting patients with appropriate disease response to achieve better clinical results with definitive nephrectomy [12, 13]. Early studies of sunitinib and pazopanib showed that in some instances, larger tumors could be cytoreduced with antiangiogenic treatment for patients to undergo nephrectomy or even nephron-sparing partial nephrectomy [13–23]. Patients with RCC undergoing surgical resection also had higher counts of circulating PD-1+ myelomonocytic cells, effector T cells, and natural killer (NK) cells in advanced disease stages, which decreased after resection

of the primary tumor [24]. Neoadjuvant immunotherapy promotes a proinflammatory response against the tumor, including proliferation of effector T cells, priming of T cells in peripheral lymph nodes, and decreases in the numbers and function of regulatory T cells. Together with surgical resection of the tumor, neoadjuvant immune checkpoint inhibition allows an enhanced immune response prior to consolidative surgery. Finally, if PD-1-targeted immune checkpoint inhibition and T-cell activation depend on the presence of tumor antigens and an intact microenvironment of tumor-infiltrating lymphocytes, then neoadjuvant immunotherapy may well improve overall clinical outcomes.

Two phase 1 studies of perioperative PD-1 blockade have investigated neoadjuvant nivolumab in patients with localized/locally advanced RCC (NCT02575222 and NCT02595918). Initial results from one showed preliminary feasibility and safety data with no additional surgical delays or other unexpected complications [25]. Other studies are currently investigating neoadjuvant ICIs with PD-1 inhibitors with or without CTLA-4 inhibitors (i.e., durvalumab with or without tremelimumab (NCT02762006) and nivolumab with or without ipilimumab (NCT02210117)).

While we await the results of these phase 1 studies, the largest ongoing perioperative trial to evaluate the utility of perioperative immunotherapy for patients with locally advanced RCC is PROSPER RCC study. PROSPER RCC is an unblinded, randomized phase 3 study that includes patients with RCC (any histology except oncocytoma) and clinical stage higher than T2 or any nodal involvement, planning for radical or partial nephrectomy. This trial randomizes patients to either nephrectomy or administration of nivolumab before and for 9 months after nephrectomy, with both cohorts followed by standard post-operative follow-up and monitoring [26]. PROSPER RCC aims to improve clinical outcomes by priming the immune system with neoadjuvant nivolumab prior to surgical resection of the tumor followed by continued immune system engagement with adjuvant nivolumab in patients with high-risk RCC. These patients' outcomes will be compared to standard of care surgery alone. Selected patients with oligometastatic disease ($\leq$3 metastases; no brain, bone, or liver) are permitted to be included on PROSPER RCC as long as all metastatic disease can be resected at the time of surgery. The primary endpoint is to improve recurrence-free survival, and the sample size of 766 randomized (up to 805 enrolled) patients will provide 84.2% power to detect a 14.4% absolute benefit in recurrence-free survival at 5 years (based on the historical recurrence-free survival rate of 56% from ASSURE (HR 0.70)). This study will also evaluate secondary endpoints in OS as well as critical perioperative safety, feasibility, and quality-of-life metrics. Furthermore, the PROSPER RCC study also embeds a wealth of translational studies to evaluate the contribution of the baseline immune milieu and neoadjuvant priming with anti-PD1 therapies on the tumor microenvironment. The trial anticipates completing accrual in 2021. If the study is positive and the nivolumab approval includes this setting, nivolumab will become the first neoadjuvant treatment approach for localized renal cancer.

Using Immunotherapy as Adjuvant Treatment

In the adjuvant setting, only sunitinib has an approved indication currently for treatment up to 1 year, based on the S-TRAC trial [27]. As immunotherapies have improved outcomes in metastatic clear cell RCC, there is further impetus to evaluate the clinical efficacy of immune checkpoint inhibitors in the adjuvant setting. Preclinical murine models have shown that effector T cells proliferate in the tumor microenvironment after PD-1 blockade and then migrate to distant sites where they can elicit cytotoxicity on micrometastatic disease [28]. These effector T cells can also transform into memory cells and offer continual suppression or elimination of metastatic disease, decreasing the possibility of recurrence.

There are multiple ongoing trials investigating the administration of immunotherapy in the adjuvant setting in RCC (Table 16.1). IMmotion010 is a phase 3, multicenter, randomized, placebo-controlled, double-blind study evaluating the efficacy and safety of the administration of atezolizumab versus placebo in patients with RCC who are at high risk of disease recurrence following nephrectomy (NCT03024996). The participants in the experimental cohort received atezolizumab 1200 mg IV infusion every 3 weeks for 16 cycles or 1 year (whichever occurs first), whereas the control cohort received placebo every 3 weeks for 16 cycles or 1 year (whichever occurs first). KEYNOTE-564 (NCT03142334) is a separate phase 3, randomized, placebo-controlled, double-blind study evaluating the safety and efficacy of pembrolizumab for patients with RCC who have undergone nephrectomy and have intermediate- to high-risk, high-risk, or oligometastatic disease after metastasectomy, with no evidence of disease on scans. Patients in the experimental cohort received pembrolizumab 200 mg IV infusion on day 1 of each 3-week cycle for up to 17 cycles, whereas the control cohort received placebo IV infusion on day 1 of each 3-week cycle for up to 17 cycles [29]. Both trials are powered on the primary endpoint of disease-free survival (DFS, IMmotion010 from independent review and KEYNOTE-564 from investigator assessment) and have completed target trial enrollment. KEYNOTE-564 reported improvement in DFS for patients treated with pembrolizumab compared to placebo (HR 0.68, 95%CI 0.53-0.87, p=0.002). IMmotion010 is still awaiting sufficient events to occur for full statistical analysis and final results.

The last phase 3, multicenter, placebo-controlled trial, CheckMate 914 (NCT03138512), will evaluate whether nivolumab alone or combination nivolumab and ipilimumab will be effective in the adjuvant setting for patients with RCC who have undergone prior nephrectomy. During part A, patients are randomized 1:1 to receive nivolumab plus ipilimumab or placebo infusions, and in part B, patients are randomized 1:1:2 to receive nivolumab plus ipilimumab, placebo infusions, or nivolumab with ipilimumab placebo. All treatments are given for 24 weeks or until evidence of disease recurrence, unacceptable toxicity, or withdrawal of consent [30]. CheckMate 914 is also powered around disease-free survival from blinded independent central review.

Table 16.1 Ongoing clinical trials investigating perioperative immune checkpoint inhibitors in patients with RCC

Localized/locally advanced RCC			
Phase (number of participants)	Treatment	Setting status (neoadjuvant/adjuvant)	Clinicaltrials.gov registration
1 ($n = 17$)	Nivolumab	Neoadjuvant	NCT02575222
1 ($n = 29$)	Nivolumab	Neoadjuvant ([a]non-metastatic/metastatic)	NCT02595918
1 ($n = 29$)	Durvalumab +/- Tremelimumab	Neoadjuvant	NCT02762006
1 ($n = 10$)	Pembrolizumab	Neoadjuvant	NCT02212730
1 ($n = 105$)	Nivolumab, Nivolumab + Bevacizumab, Nivolumab + ipilimumab	Neoadjuvant	NCT02210117
N/A ($n = 5$)	AGS-003 (vaccine)	Neoadjuvant	NCT02170389
2 ($n = 20$)	Sitravatinib + Nivolumab	Neoadjuvant	NCT03680521
3 ($n = 766$[a])	Nivolumab	Neoadjuvant/adjuvant	NCT03055013 (PROSPER RCC)
3 ($n = 778$[a])	Atezolizumab	Adjuvant	NCT03024996 (IMmotion010)
3 ($n = 950$[a])	Pembrolizumab	Adjuvant	NCT03142334 (KEYNOTE-564)
3 ($n = 1600$[a])	Nivolumab, Nivolumab + Ipilimumab	Adjuvant	NCT03138512 (CheckMate 914)
A. *Metastatic RCC*			
2 ($n = 19$)	Nivolumab	Neoadjuvant/adjuvant	NCT02446860 (ADAPTeR)
3 ($n = 364$[a])	Immunotherapy-based combination with cytoreductive nephrectomy vs no CN	Synchronous metastatic	NCT04510597 (PROBE)
3 ($n = 1046$[a])	Nivolumab + ipilimumab → nivolumab versus nivolumab + cabozantinib Allows consolidative nephrectomy	First-line metastatic IMDC intermediate-poor risk	NCT03793166 (PDIGREE)
3 ($n = 400$[a])	Nivolumab + ipilimumab → CN + nivolumab (maintenance) versus nivolumab (maintenance)	Synchronous metastatic	NCT03977571 (NORDIC-SUN)

[a]Estimated number of participants

With the completion and study maturation of the primary DFS endpoints from these three large adjuvant immunotherapy trials, sufficient clinical trial evidence will either support or refute the use of immunotherapies for prolonging disease-free survival. More time will be needed for the secondary overall survival endpoints to be reached. Certainly the patient characteristics on each of these studies will be important in assessing initial risk for disease recurrence and how these immune checkpoint inhibitors will impact these critical clinical endpoints.

Immunotherapy Sequencing with Cytoreductive Nephrectomy in Patients with Metastatic RCC

Many targeted agents have shown clinical efficacy in patients with metastatic RCC, and as a result, the landscape of systemic treatment regimens for metastatic RCC has expanded dramatically over the past 15 years [31]. Initially approved cytokine treatments of interferon-α (IFN-α) and high-dose interleukin-2 (IL-2) had limited clinical efficacy for metastatic RCC. During the cytokine era, two randomized trials of cytoreductive nephrectomy with IFN-α versus IFN-α showed improved overall survival outcomes when cytoreductive nephrectomy was paired with IFN-a [32, 33]. However, as systemic agents have improved, this management paradigm and the perceived benefit of cytoreductive nephrectomy have been challenged. Of note, in metastatic RCC, disease prognosis can be categorized according to favorable-, intermediate-, or poor-risk disease depending on the presence of well-characterized clinical and laboratory risk factors. A commonly used, validated prognostic model was initially developed from the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC criteria: <1 year from nephrectomy to systemic therapy, poor performance status, hypercalcemia, neutrophilia, anemia, and thrombocytosis) [34, 35]. These IMDC criteria have now been used in multiple trials as stratification criteria and can be used as a set of predictive biomarkers for immunotherapy treatment responses.

Two contemporary trials have shown that for de novo metastatic RCC, cytoreductive nephrectomy may be less beneficial than starting immediate systemic treatments for patients who present with synchronous metastases. The open-label, multicenter randomized phase 3 clinical trial SURTIME was designed to compare immediate versus deferred cytoreductive nephrectomy in patients receiving sunitinib for synchronous metastatic RCC. Between July 2010 and March 2016, patients were randomized to either immediate cytoreductive nephrectomy followed by sunitinib or who received three cycles of sunitinib followed by cytoreductive nephrectomy and maintenance sunitinib. A total of 99 patients were enrolled in SURTIME trial by 19 different institutions; the trial was stopped early due to poor accrual. At the time of the final analysis, median follow-up was 3.3 years (range, 0–6.2 years). All patients but one in the deferred cohort received preoperatively sunitinib, and 83% (40/48) successfully completed three cycles of sunitinib. Thirty-four patients

finally underwent deferred cytoreductive nephrectomy per protocol. Before nephrectomy, 23% (11/48) of patients had a radiographic partial response, and 29% (14/48) had progression of disease [36]. After immediate cytoreductive nephrectomy, the 28-week progression-free rate was 42% (90% CI, 30–55%) versus 43% (90% CI, 31–56%) in the deferred cytoreductive nephrectomy cohort. While PFS did not differ between the deferred and immediate cytoreductive nephrectomy cohorts (HR 0.88, 95% CI, 0.56–1.37; $p = 0.57$ [36]), OS did differ with median OS in the deferred cytoreductive nephrectomy cohort at 32.4 months compared to 15 months for the immediate cytoreductive nephrectomy cohort (HR 0.57, 95% CI 0.34–0.95; $p = 0.03$).

The open-label, multicenter, randomized phase 3 clinical trial CARMENA evaluated the importance of nephrectomy in patients with metastatic RCC also treated with sunitinib in a non-inferiority design. Patients were randomized in two cohorts, immediate nephrectomy followed by sunitinib versus sunitinib alone. Cytoreductive nephrectomy was planned within the first 28 days and then between 3 and 6 weeks sunitinib was started, whereas in the sunitinib-alone group, treatment began within 21 days after randomization. The dose of sunitinib was 50 mg/day in 6-week cycles of 4 weeks on, 2 weeks off. Between September 2009 and September 2017, 450 patients were enrolled in this study from 79 different institutions: 226 in the nephrectomy-sunitinib cohort and 224 in sunitinib-alone cohort. Of note, of the patients in the nephrectomy-sunitinib cohort, 56% had MSKCC intermediate-risk disease, and 44% had MSKCC poor-risk disease. In the sunitinib-alone cohort, 59% and 42% of the patients had MSKCC intermediate- and poor-risk disease, respectively. At a median follow-up of 50.9 months, median OS was 18.4 months (95% CI, 14.7–23.0) and 13.9 months (95% CI, 11.8–18.3) in patients treated with sunitinib alone and those treated with upfront nephrectomy, respectively [37]. In the nephrectomy cohort, 16 patients did not proceed with the intended surgical resection, and 40 patients did not receive sunitinib, whereas in sunitinib-alone cohort, 11 patients did not receive sunitinib, while 38 patients eventually underwent consolidative nephrectomy. The objective response rates were comparable in both groups, 27% in the nephrectomy-sunitinib cohort and 29% in the sunitinib-alone cohort. Regarding the adverse events, 33% of the patients in the nephrectomy-sunitinib cohort and 43% of the patients in the sunitinib cohort experienced grade 3 or 4 adverse events.

Based on SURTIME and CARMENA, upfront cytoreductive nephrectomy in the setting of synchronous metastatic RCC is now reserved for patients who have large primaries that are symptomatic and/or for patients who have one metastatic disease site and only one IMDC risk factor. Instead, for the majority of patients diagnosed with synchronous metastatic disease, effective systemic therapies are preferred first, followed by consolidative nephrectomy for those who have favorable responses. A recent phase 1 study investigated the safety of immune checkpoint inhibitors (specifically, nivolumab alone or in combination with either bevacizumab or ipilimumab) prior to surgery in patients with metastatic RCC [38]. Of the 104 total patients enrolled, 29 were treated with nivolumab, 45 with combination nivolumab and bevacizumab, and 30 with combination nivolumab and ipilimumab. At a median follow-up of 29 months, the median PFS was 14.5 months (95%CI: 5.5–not reached)

for the nivolumab group, 7.6 months (95%CI: 4.8–8.9) for the nivolumab plus bevacizumab group, and 7.5 months (95%CI: 2.0–12.4) for the nivolumab plus ipilimumab group. The 2-year overall survival rates were 72%, 60%, and 56% for the three groups, respectively [38]. Across cohorts, patients who underwent cytoreductive nephrectomy ($n = 44$) did well, with 2-year OS of about 84%, whereas patients who did not undergo cytoreductive nephrectomy ($n = 59$) had a median OS of 19.6 months (95% CI 14.2 mo–not reached) [38]. Patients had generally acceptable and manageable toxicities, with ≥ grade 3 treatment-related adverse events occurring in 28%, 38%, and 43% of patients treated with nivolumab, nivolumab with bevacizumab, and nivolumab with ipilimumab, respectively. The correlative studies from this study showed that interferon expression and tumor-infiltrating CD8+ T cells were associated with better responses to nivolumab or nivolumab with bevacizumab but not to nivolumab with ipilimumab. PD-L1 positivity, high tumor mutational burden, and predicted tumor neoantigens were not associated with responses. While patients who underwent cytoreductive nephrectomy in this neoadjuvant study had better outcomes in general, clinical efficacy was not the primary endpoint of this study, and further prospective studies are needed for preoperative immunotherapy in the setting of synchronous metastases.

The size of the primary tumor may also play a role in the decision for cytoreductive nephrectomy at any point. Another recently published study from the Memorial Sloan Kettering Cancer Center (MSKCC) nephrectomy database of 304 patients with metastatic RCC and underwent cytoreductive nephrectomy between 1989 and 2016 evaluated the impact of tumor size on survival [39]. Data from 778 similar patients from the International Metastatic Database Consortium (IMDC) were used as validation. The investigators reported a prolonged OS in patients who had small clear cell primary tumors 4 cm or less in both the MSKCC (HR 0.35, 95%CI: 0.17–0.72, $p = 0.004$) and IMDC (HR 0.54, 95%CI: 0.36–0.83, $p = 0.004$) cohorts. This finding suggests that a subgroup of patients with metastatic RCC but small primaries could be achieved more clinical benefit from cytoreductive nephrectomy at any point. The main limitation of this retrospective study is the inherent selection bias without including patients who were considered poor surgical candidates.

Ongoing Clinical Trials Investigating Immunotherapeutic Agents in the Perioperative Setting in Metastatic RCC

The ongoing search regarding optimal timing of immunotherapy in patients with metastatic RCC includes three therapeutic clinical trials in the US National Clinical Trials Network (Table 16.1). The PROBE trial was activated in November 2020 and will randomize patients with IMDC intermediate- or poor-risk metastatic RCC to either ipilimumab-nivolumab or ipilimumab-nivolumab for 12 to 18 weeks followed by consolidative nephrectomy and continuation of maintenance nivolumab

(NCT04510597). Another phase 3 trial, NORDIC-SUN, is investigating the role of cytoreductive nephrectomy in patients with metastatic RCC receiving ipilimumab and nivolumab. Patients with fewer than 3 IMDC risk features and a resectable tumor are randomized after four cycles of combination nivolumab and ipilimumab to maintenance nivolumab with or without cytoreductive nephrectomy (NCT03977571). The primary endpoint of NORDIC-SUN is overall survival, while secondary endpoints include progression-free survival and objective response rates. Finally, the randomized, multicenter phase 3 trial, PDIGREE [40], is investigating the efficacy of the combination of nivolumab plus ipilimumab followed by randomization to nivolumab with or without cabozantinib in patients with previously untreated intermediate- or poor-risk metastatic RCC (NCT03793166). While the primary endpoint is overall survival, 1-year complete response rate is a key secondary endpoint, and patients who achieve excellent partial responses in metastatic disease are allowed to stop study treatments and undergo consolidative nephrectomy (Fig. 16.1).

As cytoreductive nephrectomy has become less favored for upfront management for metastatic RCC, patient selection for who would benefit from consolidative nephrectomy will become more important to understand. Comparing the two phase 3 first-line CheckMate trials (9ER and 214), CheckMate 9ER enrolled a lower proportion of patients (70%) with prior nephrectomy (presumed more with synchronous metastatic disease) than those enrolled on the CheckMate 214 trial (81%). Understanding clinical outcomes for these patients who subsequently undergo

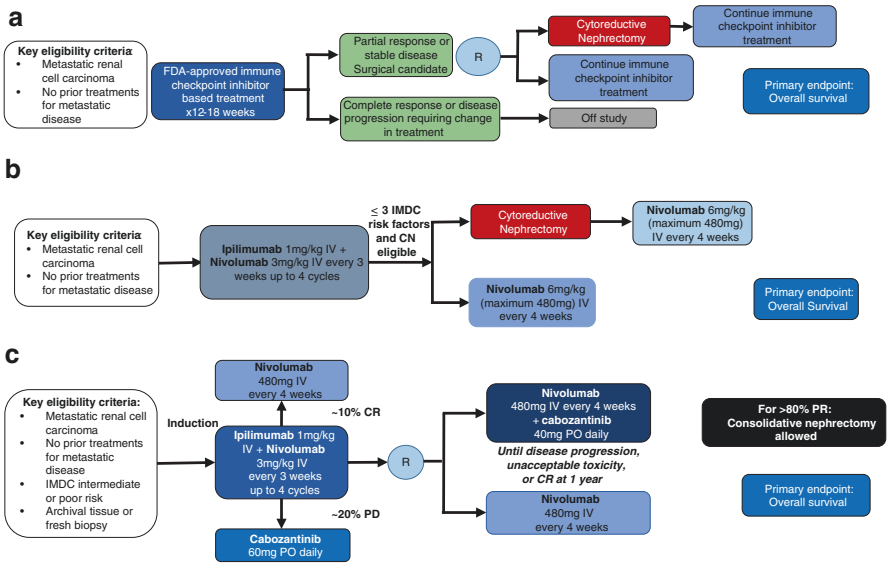

Fig. 16.1 Ongoing phase 3 clinical trials for patients with metastatic RCC and cytoreductive nephrectomy. (**a**) PROBE study. (**b**) NORDIC-SUN study. (**c**) **PDIGREE study**

consolidative nephrectomy on these and other ongoing phase 3 immunotherapy trials for metastatic RCC (COSMIC-313, CLEAR, PDIGREE, and PIVOT-09) will provide future evidence for patient characteristics that might benefit from consolidative nephrectomy. Ultimately, the randomized PROBE trial will provide definitive clinical trial evidence for the role of consolidative nephrectomy in the era of immune checkpoint inhibitors.

Future Perspectives for Perioperative Immunotherapy in Patients with RCC

Immunotherapy constitutes an established treatment option for other solid tumors in the adjuvant and neoadjuvant settings. Immune checkpoint inhibitors have transformed treatment for metastatic RCC, and many ongoing trials are evaluating the utility of immune checkpoint inhibitors for neoadjuvant and adjuvant treatment for localized RCC [41, 42]. In the management of localized or metastatic RCC, level 1 evidence is still lacking to determine the optimal timing of immunotherapy relative to nephrectomy. Some smaller studies indicate that neoadjuvant immunotherapy may downstage the tumor and facilitate the surgical management of initially unresectable bulky tumors. Completion of the randomized trials PROSPER RCC, KEYNOTE-564, IMmotion010, and CheckMate 914 will further define when and which immune checkpoint inhibitors will be used in the peri-nephrectomy setting for localized disease. In the near future, these randomized phase 3 trials should mature to give level 1 evidence to inform timing of immunotherapies before or after definitive nephrectomy for localized RCC.

On the other hand, not many systemic therapies have been investigated nor have dramatically improved clinical outcomes in patients with non-clear cell renal cell carcinoma (nccRCC) [43, 44]. Therefore, cytoreductive nephrectomy continues to be an optimal treatment option for patients with nccRCC who are eligible for surgical resection [45–47]. In the era of immune checkpoint inhibitors, retrospective case series and trials evaluating the efficacy of PD-1 or PD-L1 inhibitors for patients with nccRCC have shown objective response rates around 25–30% [48–51]. Ongoing clinical trials continue to assess of immune checkpoint inhibitors alone or in concurrent administration with other agents in patients with nccRCC [52–55]. This continues to be an area of clinical need, and as more effective systemic treatments are found for nccRCC, the question of role and timing of cytoreductive nephrectomy should be addressed.

In the metastatic setting for ccRCC, as effective systemic therapies are favored as first-line treatments, the optimal timing of consolidative nephrectomy is still a pertinent clinical question. While the PROBE study is specifically designed to study nephrectomy after immunotherapy versus immunotherapy alone, other trials like PDIGREE will allow consolidative nephrectomy after excellent tumor responses.

All of these trials will inform upon patient selection for future clinical decision-making on timing of consolidative nephrectomies.

Further efforts should be invested in future clinical trials to identify patient characteristics and biomarkers for those who may achieve a good clinical outcome through immunotherapies and nephrectomy. Ultimately, whether in the localized or metastatic settings, multimodality care will most likely be standard in future RCC management.

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Chapter 17
Predictive Biomarkers of Immunotherapy Efficacy in RCC and Their Role in Non-metastatic Stages

Jasnoor Malhotra, Luis Meza, Nicholas Salgia, and Sumanta Kumar Pal

Introduction

In 2020, an estimated 73,750 number of patients will be diagnosed with renal cell carcinoma (RCC), and of these, 14,830 may die of the disease [1]. Of those diagnosed, the vast majority (~90%) will present with localized disease. For patients with localized disease, the cornerstone of therapy is surgery – depending on a number of factors including size and extent, partial nephrectomy or radical nephrectomy may be attempted. For individuals who have significant comorbidity and for those with smaller lesions, local approaches such as cryoablation or radiofrequency ablation may be feasible. One challenge is that, despite the use of these definitive interventions, patients may still recur with metastatic disease. While improvements in systemic therapy have occurred, the unfortunate reality is that most patients with metastatic disease are incurable.

For this reason, aggressive efforts have been made to develop adjuvant therapies for RCC. The US FDA approved adjuvant therapy with sunitinib in 2017; this was based on the phase III S-TRAC clinical trial [2]. However, this study was quite controversial given the demonstration of only a modest benefit in disease-free survival (DFS) with sunitinib over placebo in patients with high-risk localized RCC. Furthermore, the study demonstrated no benefit in overall survival (OS).

J. Malhotra · L. Meza · S. K. Pal (✉)
Department of Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA, USA
e-mail: spal@coh.org

N. Salgia
University at Buffalo Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA

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Flanking this dataset were several other publications that cited no benefit with adjuvant targeted therapy. For example, the ASSURE trial compared sunitinib and sorafenib to placebo and showed no benefit in DFS or OS, and the PROTECT trial comparing pazopanib to placebo had similar results [3]. With this in mind, attention has turned to the development of adjuvant immunotherapy approaches for localized RCC. Several completed and ongoing studies compare checkpoint inhibitor (CPI) monotherapy or combination therapy for varying durations. These are the subject of other chapters in this textbook.

In the current chapter, we will focus our attention on the role of biomarkers in identifying benefit with immunotherapy for RCC. It is well recognized that not all patients benefit from immunotherapy, and in the adjuvant setting, where a high therapeutic index is essential, biomarkers may allow for optimized application of immunotherapy.

Predictive Markers of Immunotherapy

PD-L1 Expression

In current clinical practice, CPIs abrogate signaling through three primary targets: (1) cytotoxic T-lymphocyte-associated protein 4 (CTLA4), (2) programmed cell death protein 1 (PD-1), and (3) programmed cell death ligand 1 (PD-L1). PD-1 and CTLA4 are on the T-cell surface, while PD-L1 is expressed on the antigen-presenting cell (APC). Trials in adjuvant therapy for RCC utilize agents directed at each of these targets. The first adjuvant immunotherapy trial to be initiated was IMmotion010, a trial comparing atezolizumab (a PD-L1 inhibitor) to placebo. KEYNOTE-564 was initiated shortly thereafter, comparing pembrolizumab to placebo. Other perioperative trials have since emerged evaluating nivolumab, durvalumab with tremelimumab, and nivolumab with ipilimumab. Each of these agents has data in the context of metastatic RCC (mRCC), and in most, there is detailed information regarding the prognostic and predictive potential of PD-L1 status.

PD-L1 status is complex – there are a variety of antibodies used to characterize PD-L1, and each has a different sensitivity as well as specificity for tumor and/or immune cells. The phase III CheckMate 214 trial comparing nivolumab with ipilimumab to sunitinib is perhaps the best opportunity to evaluate the role of PD-L1, as the study juxtaposes a targeted therapy regimen against an immunotherapy regimen. A recent update at the 2020 American Society of Clinical Oncology (ASCO) Annual Meeting offered a detailed assessment of PD-L1 status in this trial, using a separate assessment of PD-L1 in tumor cells and a combined positive score (CPS) evaluating PD-L1 expression in both tumor cells and immune cells. For this assessment, the Dako PD-L1 IHC 28-8 antibody was used. In the tumor cell PD-L1 assessment, 754 patients were noted to have <1% expression, while 236 patients had 1% or greater

expression. Although the magnitude of survival benefit was greater in those individuals with 1% or greater expression, an OS advantage was seen in both subsets with nivolumab and ipilimumab as compared to sunitinib. Using the PD-L1 CPS yielded a similar observation. Notably, using this method, a total of 384 patients had a score of <1, while 596 had a score of 1 or greater – a more even distribution of patients.

The role of PD-L1 has also been assessed in the context of two other recent pivotal trials in the front-line setting of mRCC, namely, KEYNOTE-426 and JAVELIN Renal 101. The KEYNOTE-426 study compared axitinib with pembrolizumab to sunitinib, while the JAVELIN Renal 101 study compared axitinib with avelumab to sunitinib. Both studies met their primary endpoint of demonstrating a progression-free survival benefit with the combination of axitinib with CPI. Only KEYNOTE-426 has yielded a survival advantage to date, however [4]. Both studies included an assessment of PD-L1 status, with the benefit of combination therapy occurring in both PD-L1-positive and PD-L1-negative groups. Currently, there are no adjuvant trials evaluating a combined strategy of targeted therapy with immunotherapy, but these data may be informative in smaller neoadjuvant trials exploring this concept (e.g., an ongoing study of axitinib with avelumab).

Gene Signatures

One of the most informative biomarker studies to date in mRCC is the randomized, phase II IMmotion150 trial. This study assessed the combination of bevacizumab with atezolizumab compared to atezolizumab monotherapy, with sunitinib representing the control arm. With over 100 patients per arm and robust correlatives, this was a sizeable experience. RNA sequencing allowed for determination of three unique subtypes of patients – (1) an angiogenic profile; (2) T effector high, myeloid inflammation low profile; and (3) T effector high, myeloid inflammation high profile. As the profile name implies, patients with an angiogenic profile fared better with VEGF-directed therapy. Patients with a T effector high, myeloid inflammation low profile did similarly well with atezolizumab monotherapy and the combination with bevacizumab. Interestingly, patients with a T effector high, myeloid inflammation high profile "required" the combination of bevacizumab with atezolizumab [5].

This gene signature from IMmotion150 has been assessed in the context of several recent studies. In the JAVELIN Renal 101 study, several signatures were assessed. Notably, a 26-gene score was derived within the JAVELIN study that included a diverse array of immune response genes – a high score using this metric was able to discern superior outcome with the combination of axitinib with avelumab as compared to sunitinib. When the IMmotion150 score was applied in the JAVELIN Renal 101 study, it was interesting to note that the angiogenesis-high group fared better with sunitinib – no other significant differences were noted [6].

RNA sequencing data was also recently presented from the CheckMate 214 study. In this undertaking, 109 specimens were available from patients receiving nivolumab with ipilimumab, and 104 samples were available from patients receiving sunitinib. Within this limited subset, various gene signatures (including the IMmotion150 and JAVELIN Renal 101 signatures) were explored. Interestingly, the only notable differences were in the angiogenic score once again. Consistent with the previous experience, patients with a high angiogenic score appear to fare better with sunitinib as compared to nivolumab with ipilimumab with respect to progression-free survival. However, there is no significant difference in OS [7].

Genomic Predictors of Outcome

There is emerging evidence that, beyond genomic signatures, single genes may be effective predictors of clinical outcome. *PBRM1* is a chromatin remodeling gene that may be associated with immunotherapy response. In a study combining patients from a prospective assessment of nivolumab in mRCC with several institutional registries, response to immunotherapy appeared to be enriched in those patients with *PBRM1* mutation. This high-profile publication was followed by several efforts to validate *PBRM1* in recent phase III clinical trials. In the JAVELIN Renal 101 study, no association between *PBRM1* and outcome was reported [6]. Furthermore, in a recent assessment of patients in CheckMate 214, there was also no significant difference in PFS or OS based on *PBRM1* status [7].

As the quest for novel biomarkers continues, our group has recently reported an assessment of 91 patients with mRCC. In our study, we identified 58 patients who had received VEGF-TKI and/or immunotherapy [8]. *TERT* promoter mutations were associated with lack of clinical benefit with immunotherapy in this cohort. Given the limited sample size, prospective validation of these findings is warranted.

Microbiome

A fascinating but admittedly early foray in biomarker research in mRCC is evaluation of the microbiome. Our group was the first to characterize the microbiome in mRCC. In a small cohort of 20 patients with mRCC, we collected stool and performed 16S ribosomal RNA profiling at varying timepoints during VEGF-TKI therapy [9]. Our findings indicated that higher levels of *Bacteroides* spp. were found in patients with treatment-induced diarrhea; lower levels of *Prevotella* spp. were found in the same patients.

More sophisticated metagenomic sequencing has since evolved to characterize gut bacterial composition. This was applied in a large series of patients with both mRCC and metastatic non-small cell lung cancer treated at the Institut Gustave

Roussy. In 40 patients with mRCC, it was suggested that higher levels of *Akkermansia* spp. were associated with treatment response [10]. In separately published studies, the same group has also identified that antibiotic use may potentially influence clinical outcome, perhaps by reducing populations of "immunotherapy-sensitizing bacteria" [11].

Our group has recently performed a detailed study of 31 patients with mRCC initiating therapy with checkpoint inhibitors. Stool was collected at baseline and at varying timepoints during therapy. In contrast to the French group, our study pointed most strongly to *Prevotella copri* as a predictor of immunotherapy response. As we were able to temporally characterize changes in stool profile, our findings did suggest an increase in *Akkermansia* spp. in those patients who responded to immunotherapy. Finally, our results also suggested that stool bacterial diversity correlated with treatment response.

These cumulative results have led to the design of a randomized, phase I study exploring the probiotic CBM-588 in combination with immunotherapy. CBM-588 represents spores of *Clostridium butyricum.* Spores of *C. butyricum* theoretically generate and release butyrate in the lower intestinal tract. By doing so, they may enhance proliferation of *Bifidobacteria* and other species that have been associated with treatment response.

Conclusions and Future Directions

The biomarkers discussed herein are the most well established we have to predict outcome with immunotherapy in patients with RCC. Of course, these have all been validated in the metastatic setting. Definitive data for these biomarkers in the non-metastatic setting will only emerge once there are preliminary data available from the phase III trials evaluating adjuvant immunotherapy. Smaller studies of neoadjuvant therapy may provide some insight but will unlikely be practice changing.

It is of critical importance that this research progresses in the adjuvant setting. Immunotherapy, although well tolerated by many patients, does come with potentially severe consequences. Reports of rapid and fatal myocarditis have recently emerged in the literature, and truthfully, any immune-related toxicity can have dire consequences if not managed aggressively and in a timely fashion. Using biomarkers to identify those individuals who are most prone to benefit from immunotherapy will ultimately limit unnecessary exposure to these agents. Although a considerable investment in time, money, and effort, this line of research is bound to save lives (Fig. 17.1 and Table 17.1).

Disclosures SKP reports previous consulting roles for Genentech, Aveo, Eisai, Roche, Pfizer, Novartis, Exelixis, Ipsen, BMS, and Astellas.

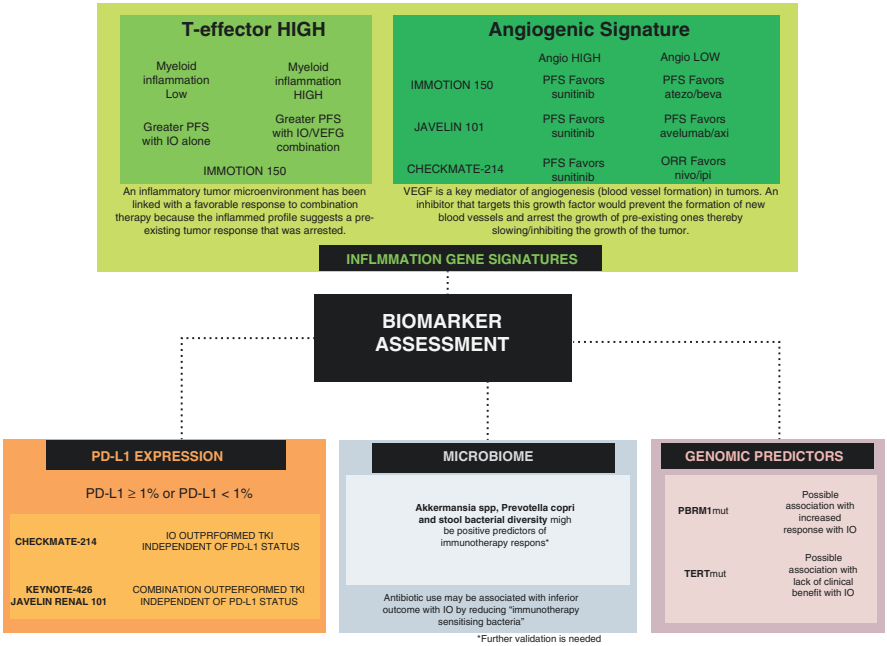

Fig. 17.1 Comprehensive overview of potential biomarkers in the setting of RCC as assessed in various clinical trials

Table 17.1 IMmotion150 PFS results based on Angio gene signature and validation in separate studies. Angio high ≥ median, Angio low < median

Author (reference)	Study	Result
McDermott (Nat Med 2018)	IMmotion150	**HR 0.31 (0.18–0.55)** in favor of Angio^{High} in patients treated with sunitinib. $p < 0.001$ No significant PFS difference based on Angio gene signature when treated with atezolizumab + bevacizumab or atezolizumab alone **HR 0.59 (0.35–0.98)** in favor of atezolizumab + bevacizumab vs sunitinib in Angio^{Low} patients
Choueiri (JCO 2019)	JAVELIN renal 101	**HR of 0.64 (0.48–0.85)** in favor of Angio^{High} in patients treated with sunitinib. $p = 0.0018$ No significant PFS difference based on Angio gene signature within the avelumab + axitinib arm In the Angio^{Low} subset, avelumab + axitinib improved PFS vs sunitinib
Motzer (JCO 2020)	CheckMate 214	**HR 0.58 (0.37–0.92)** in favor of Angio^{High} in patients treated with sunitinib. $p < 0.05$ No significant PFS difference based on Angio gene signature when treated with nivolumab + ipilimumab

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Part IV
Safety of Immune-Checkpoint Inhibitors in the Perioperative Setting of GU Malignancies

Chapter 18
Safety of Immune Checkpoint Inhibitors in the Peri-operative Setting

Mohamed E. Ahmed, Vidhu B. Joshi, and Philippe E. Spiess

Principles of Immune Checkpoint Blockade

In recent years, immune checkpoint inhibitors (ICIs) have become a mainstay of modern cancer immunotherapeutics. ICIs are capable of increasing the amplitude of the systemic antitumor immune response through the inhibition of immune checkpoint proteins such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4; alternatively called CD152), programmed cell death protein 1 (PD1; alternatively called CD279), and programmed death-ligand 1 (PDL1; alternatively called CD274 or B7-H1) [1]. In normal cells, these proteins function as inhibitory molecules that maintain immune tolerance through modulation of the immune system. Specifically, CTLA4 dampens T-cell activation, while PD1 and PDL1 function in concert to reduce both T-cell activation and T-cell effector function in activated T-cells [2]. Interestingly, cancer cells often avoid T-cell-mediated destruction in the tumor microenvironment (TME) by presenting PDL1 on the cell surface as part of both innate and adaptive immune resistance mechanisms [3–5]. Thus, the goal of immune checkpoint blockade (ICB) is to revive antitumor immunity mainly via anti-CTLA4, anti-PD1, and anti-PDL1 therapies. In clinical practice, ICIs have demonstrated high efficacy in a subset of patients, and several ICIs are approved for use in the treatment of various malignancies at varying stages, including genitourinary cancers [6].

Mohamed E. Ahmed and Vidhu B. Joshi contributed equally with all other contributors.

M. E. Ahmed · V. B. Joshi
Department of Urology, Mayo Clinic, Rochester, MN, USA

P. E. Spiess (✉)
Department of GU Oncology, Moffitt Cancer Center, Tampa, FL, USA
e-mail: Philippe.Spiess@moffitt.org

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Approved Indications for ICIs in Genitourinary Malignancies

In the treatment of urothelial carcinomas of the bladder (UC), atezolizumab (anti-PDL1) and pembrolizumab (anti-PD1) are approved for use by the United States Food and Drug Administration (FDA) as first-line agents in patients with advanced or metastatic disease who are not candidates for platinum-based chemotherapy [7]. In the second-line setting, nivolumab (anti-PD1) and durvalumab (anti-PDL1) are approved for use when patients have locally advanced or metastatic disease and have failed platinum-based chemotherapy [8, 9]. In patients with locally advanced or metastatic disease who have not progressed on platinum-based chemotherapy, avelumab (anti-PDL1) can be used as maintenance therapy [10]. Currently, pembrolizumab is the only approved drug for organ-confined disease, specifically, as a second-line option in patients who are Bacillus Calmette-Guerin (BCG)-unresponsive, are ineligible for cystectomy, and do not show evidence of muscle invasion. Thus, immune checkpoint blockade therapy is not approved for use in the peri-operative setting for patients with muscle-invasive or locally advanced disease (i.e., cT2-T4NXM0). Of note, no anti-CTLA4 therapies are approved to treat urothelial carcinoma, but pre-clinical and clinical investigations are ongoing [11].

In the treatment of renal cell carcinoma (RCC), nivolumab and ipilimumab (anti-CTLA4) are approved in combination for use in treatment-naïve patients with poor- to intermediate-risk advanced RCC [12]. Avelumab is approved for use in combination with axitinib – a small molecule tyrosine kinase inhibitor (TKI) – in the first-line setting for untreated, advanced RCC [13]. Pembrolizumab is also approved in combination with axitinib, but in patients with advanced RCC who have no prior exposure to systemic treatment [14]. While there are multiple immunotherapy options available to patients with advanced disease, there are no ICIs approved for use in patients with localized disease, including those who are ineligible for surgical resection of the tumor (partial or radical nephrectomy).

Finally, within the setting of prostate cancer, there is a paucity of ICIs approved for use. Specifically, pembrolizumab is approved for use only in the treatment of castration-resistant prostate cancer (CRPC) in patients who have exhausted all available treatment options (i.e., androgen deprivation therapy, chemotherapy, second-generation hormone therapy, radium-223, sipuleucel-T) and have progressed. These patients must also exhibit microsatellite instability in the tumor and/or possess mutations in DNA mismatch repair (MMR) genes [15].

ICI Immune-Related Toxicities

As described, immune checkpoint inhibitors are currently seldom used to treat patients with localized genitourinary malignancies in the peri-operative setting outside of a clinical trial. This is in part due to the potential albeit rare likelihood of the development of immune-related adverse events (irAEs) that could delay surgery or

lead to worse surgical outcomes. In a 2019 meta-analysis of treatment-related adverse events (AEs) in 20,128 patients across 125 clinical trials investigating anti-PD1 and anti-PDL1 therapies, including 22 trials on genitourinary cancers, nearly 2 in 3 patients experienced ≥1 AE, and 1 in 7 patients experienced ≥1 grade 3 or higher AE. Fatigue (18.3%; 95% CI: 16.5–20.1), pruritus (10.6%; 95% CI: 9.5–11.8), and diarrhea (9.5%; 95% CI: 8.4–10.6) were the most common all-grade AEs, while fatigue (0.9%; 95% CI: 0.7–1.1), anemia (0.8%; 95% CI: 0.6–1.02), and aspartate aminotransferase (AST) increase (0.8%; 95% CI: 0.6–0.9) were the most common grade 3 or higher AEs. Immune-related adverse events were divided into endocrine dysfunctions and all other irAEs (Table 18.1). Hypothyroidism (6.1%; 95% CI: 5.4–6.9) and hyperthyroidism (2.8%; 95% CI: 2.4–3.3) were the most common all-grade irAEs, while hyperglycemia (0.24%; 95% CI: 0.1–0.4), adrenal insufficiency (0.18%; 95% CI: 0.1–0.3), type 1 diabetes (0.18%; 95% CI: 0.1–0.3), hypophysitis (0.16%; 95% CI: 0.1–0.3), and hypothyroidism (0.08%; 95% CI: 0.04–0.1) were the most common grade 3 or higher irAEs. Among all other irAEs, diarrhea (9.5%; 95% CI: 8.4–10.6), AST increase (3.4%; 95% CI: 2.9–3.9), vitiligo (3.3%; 95% CI: 2.8–3.8), alanine aminotransferase (ALT) increase (3.1%; 95% CI; 2.7–3.6), pneumonitis (2.8%; 95% CI: 2.4–3.2), and colitis (1.2%; 95% CI: 0.9–1.5) were the most common all-grade irAEs. AST increase (0.75%; 95% CI: 0.6–0.9), ALT increase (0.70%; 95% CI: 0.5–0.9), pneumonitis (0.67%; 95% CI: 0.5–0.9), diarrhea (0.59%; 95% CI: 0.5–0.8), and colitis (0.47%; 95% CI: 0.3–0.7) were the most common grade 3 or higher irAEs. The overall mean incidence of AEs was 1.7% (95% CI: 1.4–2.0) in the genitourinary cancer studies examined, and this did not vary significantly compared to other cancer types evaluated in the meta-analysis. Overall, 82 treatment-related deaths were observed with respiratory causes resulting in 48% of treatment-related deaths (pneumonitis was the most common; 28%) [16]. As noted in the study, some of the lower-grade irAEs can be early indicators of more serious irAEs; thus, timely management of irAEs using available guidelines is necessary [17–19]. In general, initial management of many irAEs (e.g., dermatologic/mucosal, diarrhea/colitis, hepatotoxic, pneumonitis) may involve treatment with immunosuppressive medications, such as glucocorticoids and either

Table 18.1 Most common endocrine irAEs and non-endocrine irAEs in clinical trials investigating anti-PD1 and anti-PDL1 therapies

Endocrine irAEs	Non-endocrine irAEs
All-grade: Hypothyroidism (6.1%) Hyperthyroidism (2.8%) Hyperglycemia (1.2%)	All-grade: Diarrhea (9.5%) AST increase (3.4%) Vitiligo (3.3%) Alanine aminotransferase (ALT) increase (3.1%)
Grade ≥ 3: Hyperglycemia (0.24%) Adrenal insufficiency (0.18%) Type 1 diabetes (0.18%) Hypophysitis (0.16%) Hypothyroidism (0.08%)	Grade ≥ 3: AST increase (0.75%) ALT increase (0.70%) Pneumonitis (0.67%) Diarrhea (0.59%) Colitis (0.47%)

temporary or permanent discontinuation of the ICI. For more severe irAEs that do not improve with glucocorticoid use, other immunosuppressives such as infliximab may be provided [17–19].

Based on the current literature, patients who are treated with anti-CTLA4 therapy (e.g., ipilimumab) experience irAEs more frequently than those who are treated with anti-PD1/anti-PDL1 therapy (e.g., nivolumab). For example, 71% of patients experienced irAEs in a pooled analysis of four clinical trials involving nivolumab, while 85% of patients experienced irAEs while receiving ipilimumab. Interestingly, the use of immunosuppressive medications in the management of irAEs did not adversely impact objective response to immunotherapy in the nivolumab trial or overall survival (OS) in the ipilimumab trial [20, 21]. Other studies report similar findings regarding the greater incidence of irAEs in anti-CTLA4-treated patients versus anti-PD1/PDL1-treated patients across multiple classes of irAEs, including systemic irAEs (e.g., fatigue), dermatologic/mucosal irAEs, diarrhea/colitis, and pneumonitis [22–27].

Potential Benefits of ICI Use in the Peri-operative Setting

Given that utilization of ICIs is rare peri-operatively, it is difficult to accurately evaluate the potential risks of neoadjuvant and adjuvant ICI use due to the paucity of clinical data available. With respect to the potential benefits of neoadjuvant immunotherapy, pre-clinical and limited clinical studies have shown that ICI use prior to surgery may lead to a reduction in tumor burden (rendering potentially highly challenging or non-resectable tumors into those highly amenable to extirpation), eliminate/control micrometastatic disease, and help improve recurrence-free survival (RFS) and overall survival (OS) – all of which are potential benefits of a conventional neoadjuvant systemic chemotherapeutic approach as well [28–30]. However, neoadjuvant immunotherapy may offer additional benefits most notably in those with imperative contraindication to effective chemotherapy such as pre-treatment renal impairment, neurotoxicity, or hearing loss, e.g.

First, patients with contraindications to chemotherapy can be offered neoadjuvant ICIs as an alternative with fewer side effects. In fact, a recent meta-analysis of 22 clinical trials involving 12,727 patients compared the incidence of AEs in patients treated with ICIs (anti-CTLA4, anti-PDL1, and anti-PD1) versus standard-of-care (SOC) chemotherapy; the authors found that only 16.5% of patients treated with immunotherapy experienced a grade 3 or higher AE, versus 41.1% of patients who were treated with SOC chemotherapy. The patients treated with immunotherapy were also overall less likely to experience an AE, discontinue treatment, or experience death secondary to a treatment-related AE [31]. However, the subset of immune-related adverse events that occur uniquely as a result of immune checkpoint blockade must be noted [32]. Second, given that the primary tumor is the principal source of tumor antigen, neoadjuvant ICI use would result in enhanced activation and expansion of tumor-specific T-cells compared to the immune response

observed in immunotherapy delivered in the absence of primary tumor. This phenomenon has been demonstrated in both pre-clinical and clinical models of solid, resectable tumors [29, 33].

In the adjuvant setting, immune checkpoint blockade in the immediate post-operative period could be used to mitigate the negative effects of the surgical stress response, specifically, changes in angiogenic, inflammatory, endocrine, and immunosuppressive signaling pathways that can improve the survival of any residual cancer cells and potentially contribute to disease recurrence [34–37].

Currently, there are a number of recent trials investigating peri-operative use of immune checkpoint blockade for genitourinary malignancies. While a portion of the efficacy data has not been published, early safety data on both irAEs and surgery-related complications have been released. Thus, the purpose of this chapter is to provide a summary of the latest bladder, kidney, and prostate cancer clinical trials investigating peri-operative immunotherapy and review available pre- and peri-operative safety data from these clinical investigations.

Peri-operative Immunotherapy in Bladder Cancer

In the setting of urothelial carcinoma of the bladder, the American Urological Association (AUA) recommends definitive therapy (i.e., radical cystectomy; RC) for patients who have muscle-invasive disease (MIBC), do not have involvement beyond the common iliac lymph nodes, and have no evidence of distant metastases. Prior to surgery, neoadjuvant cisplatin-based chemotherapy (NAC) is recommended for eligible patients. If patients do not receive cisplatin-based NAC and demonstrate non-organ-confined disease at cystectomy (pT3/T4a and/or N+), they are recommended to receive adjuvant cisplatin-based NAC [38].

While cisplatin-based NAC is widely recommended for MIBC and has demonstrated an overall survival benefit, utilization remains relatively low [39–41]. Nearly half of patients are ineligible due to contraindications such as renal insufficiency, and those who are prescribed cisplatin-based NAC experience major toxicities [42, 43]. Given that recurrence rates are high after surgery alone, aggressive treatment upfront is critical in the management of MIBC [44]. Thus, peri-operative immune checkpoint blockade may expand the number of patients eligible for systemic neoadjuvant or adjuvant treatment and lead to improved MIBC outcomes. Given that the tolerability of neoadjuvant systemic therapy is of paramount importance, the purpose of this section is to review safety data from ongoing clinical investigations. Currently, there are five completed or ongoing clinical trials evaluating the use of neoadjuvant ICIs for MIBC that have safety data available. In the adjuvant setting, there is a paucity of data from prospective clinical trials evaluating either chemotherapy or ICIs; specifically, only one trial will be discussed. Finally, a single trial investing peri-operative immune checkpoint blockade therapy (i.e., neoadjuvant and adjuvant) will be reviewed. Table 18.2 summarizes the safety findings from these trials.

Table 18.2 Most commonly reported medical irAEs and surgical complications in MIBC ICI trials

Trial (NCT #)	Agent(s)	Most common toxicities
PURE-01 (NCT02736266)	Pembrolizumab (anti-PD1)	Medical: Thyroid dysfunction (all-grade), AST/ALT increase, pruritus, pyrexia Surgical: Sepsis, subocclusion
ABACUS (NCT02662309)	Atezolizumab (anti-PDL1)	Medical: Fatigue, anorexia, transaminitis, pruritus Surgical: UTI, paralytic ileus, anemia, wound dehiscence
NABUCCO (NCT03387761)	Ipilimumab (anti-CTLA4) and nivolumab (anti-PD1)	Medical: Increased lipase, ALT increase, diarrhea Surgery: NA
NCT02812420	Durvalumab (anti-PDL1) and tremelimumab (anti-CTLA4)	Medical: Hepatitis, amylase/lipase increase Surgery: NA
DUTRENEO (NCT03472274)	Durvalumab (anti-PDL1) and tremelimumab (anti-CTLA4)	Medical: 21.7% of patients experienced grade 3 or 4 irAEs Surgical: NA
IMvigor010 (NCT02450331)	Atezolizumab (anti-PDL1)	Medical: 16% of patients experienced grade 3 or 4 irAEs Surgical: NA
SAKK 06/17 (NCT03406650)	Durvalumab (anti-PDL1)	Medical: 24% of patients experienced grade 3 or 4 irAEs Surgical: Infection

Abbreviations: *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *UTI* urinary tract infection, *NA* not available

Single-Agent Neoadjuvant Immunotherapy Clinical Trials in MIBC

The PURE-01 trial is a phase 2, open-label, single-arm study of pembrolizumab (anti-PD1) (3 cycles, 200 mg every 3 weeks) as a neoadjuvant therapy for cT2-3bN0M0 predominant urothelial carcinoma histology MIBC [45]. In total, 50 patients were treated with pembrolizumab followed by radical cystectomy, with a median time to RC of 22 days (IQR 15–30). With respect to medical AEs, there were 28 grade 1–2 AEs and 3 grade 3 or higher AEs observed, with thyroid dysfunction representing the most common all-grade medical AE (18%). One patient experienced an increase in AST/ALT and discontinued pembrolizumab. Additionally, the grade 1–2 AEs of pruritus (6%), pyrexia (6%), and xerostomia (4%) all had a post-RC onset within 2 months of surgery. Notably, neoadjuvant pembrolizumab did not result in any delays in surgery. With respect to surgical AEs, 30% of patients experienced a >2 Clavien-Dindo complication, with sepsis (20%) and subocclusion (16%) being the most common. The authors reported that the post-surgery complications observed were in line with previous literature on robotic-assisted and open radical cystectomies [46].

The ABACUS trial is a phase 2, open-label, single-arm study of atezolizumab (anti-PDL1) (1–2 cycles, 1200 mg every week) as a neoadjuvant therapy for cT2-T4aN0M0 MIBC in patients who either refused or were ineligible for cisplatin-based NAC and have no evidence of nodal or metastatic disease [47]. In total, 95 patients were treated with atezolizumab ($n = 75$ received 2 cycles; $n = 20$ received 1 cycle), and 87 patients underwent radical cystectomy, with a median time to RC of 39 days (IQR 28–48). Of the eight patients who did not proceed with RC, three could not receive a RC due to atezolizumab-related AEs; specifically, these irAEs were pneumonia, myocardial infarction, and deterioration of performance status. With respect to medical AEs, 52% of patients experienced at least one all-grade irAE, 11% of patients experienced a grade 3 or 4 AE, and one patient died due to dyspnea. In total, there were $n = 99$ grade 1–2 irAEs, $n = 14$ grade 3–4 irAEs, and $n = 1$ grade 5 irAE. In general, fatigue (21%), anorexia (8%), transaminitis (7%), and pruritus (7%) were the most common irAEs. In regard to surgical AEs, 45% of patients who underwent RC experienced grade 1–2 Clavien-Dindo surgical complications. The most common grade 1–2 complications were urinary tract infection (UTI) (26%), paralytic ileus (7%), and anemia (6%). Only 17% of patients experienced a grade 3–4 Clavien-Dindo surgical complication, of which the most common was wound dehiscence. Finally, one patient died post-operatively due to cardiovascular complications.

Combination Neoadjuvant Immunotherapy Clinical Trials in MIBC

The remaining clinical trials investigating neoadjuvant immunotherapy reported here used a combination therapy approach.

The NABUCCO trial is a single-arm, open-label, feasibility study of ipilimumab (anti-CTLA4) and nivolumab (anti-PD1) (2 doses each, 3 mg kg^{-1}) as neoadjuvant combination therapy for cT3-4aN0N0 and cT1-4aN1-3 M0 urothelial carcinoma in patients who either refused or were ineligible for cisplatin-based NAC. Of note, the NABUCCO trial included patients with lymph node metastases (42%) and one patient with upper tract urothelial carcinoma (UTUC) (unlike the PURE-01 and ABACUS trials) [48]. Additionally, the primary endpoint of the NABUCCO trial was the feasibility to perform surgery within 12 weeks of beginning immune checkpoint blockade therapy. In total, 24 patients were treated with neoadjuvant ipilimumab and nivolumab, followed by radical cystectomy or nephro−/urethrectomy. 75% of patients were able to tolerate the three treatment cycles, while the remaining 25% did not receive the second nivolumab dose due to irAEs. Overall, 100 of patients experienced at least one all-grade irAE, while 41% of patients experienced grade 3–4 irAEs with increased lipase (25%), ALT increase (12%), and diarrhea (12%) being the most common. Additionally, the primary endpoint of resection within 12 weeks was achieved for 23/24 (96%) of patients, while 1 patient

experienced delayed resection due to an irAE of hemolysis. Interestingly, the authors did not share data on surgical AEs or post-operative complications.

The following two neoadjuvant immunotherapy trials that will be discussed here are both investigating the use of durvalumab (anti-PDL1) in combination with tremelimumab (anti-CTLA4). Both studies have not yet published their final results, but limited safety data is available. First, NCT02812420 is a single-arm, open-label pilot study in patients with cT2-T4a MIBC who were either ineligible for or refused cisplatin-based NAC [11]. Patients are scheduled to receive two doses of combined durvalumab and tremelimumab at weeks 1 and 5, followed by radical cystectomy between weeks 9 and 11. Per available data, the study has enrolled 28/35 patients, and 21/35 have undergone both neoadjuvant ICI therapy and surgery. The authors reported grade 3 or 4 irAEs in 17% (5/28) patients, noting hepatitis and amylase/lipase increases without indicating the frequency of these irAEs. Additionally, only 2/28 (7%) of patients were required to delay surgery for >4 weeks due to irAEs. The authors of NCT02812420 did not share data on surgical AEs or post-operative complications.

The second trial, called the DUTRENEO trial, is a phase 2, open-label, multi-arm study in patients with cT2-T4aN≤1 MIBC [49]. Unlike the other trials discussed, the DUTRENEO trial enrolled patients who were eligible for cisplatin-based NAC. Following enrollment, patients were further stratified by a pro-inflammatory interferon-gamma signature (tumor immune score, TIS). Patients who exhibited a "hot" tumor were randomized to receive either combined durvalumab (1500 mg) and tremelimumab (75 mg) (3 cycles, every 4 weeks) or standard-of-care cisplatin-based NAC, while patients who exhibited a "cold" tumor received SOC cisplatin-based NAC and were not randomized. In total, 16 patients were in the cisplatin-based NAC "cold" arm, 22 patients were in the cisplatin-based NAC "hot" arm, and 23 patients were in the "hot" ICI arm. With respect to medical AEs, 62.5% and 36.4% of patients experienced grade 3 or 4 AEs in the "cold" and "hot" cisplatin-based NAC arms, while only 21.7% of patients experienced grade 3 or 4 AEs in the "hot" ICI arm. The majority of patients in all three groups completed cystectomy; specifically, 93.8%, 90.9%, and 87.0% of patients completed surgery in the cisplatin-based NAC "cold" arm, cisplatin-based NAC "hot" arm, and ICI arm, respectively. The full results of the investigation are not yet published; thus, the authors did not share data on surgical AEs or post-operative complications.

Adjuvant Immunotherapy Clinical Trials in MIBC

Currently, there are limited data available on investigations of adjuvant immune checkpoint blockade. The IMvigor010 trial is a phase 3, open-label, randomized trial of adjuvant atezolizumab versus observation in patients with either (1) pT2-4a or pN+ if patients had cisplatin-based NAC or (2) pT3-4a or pN+ if they did not receive cisplatin-based NAC due to ineligibility or refusal [50]. Patients were eligible if they underwent a radical cystectomy/nephroureterectomy within 14 weeks

of study enrollment. Patients were randomized to either receive atezolizumab (16 cycles, 1200 mg every 3 weeks) or continue on observation following surgery. With respect to medical AEs, grade 3 or 4 irAEs were observed in 16% of patients treated with atezolizumab. While the authors do not indicate the number, patients who discontinued ICI treatment commonly did so due to skin and gastrointestinal irAEs.

Combination of Adjuvant-Neoadjuvant Immunotherapy Clinical Trials in MIBC

As mentioned, early results from a single trial evaluating combined neoadjuvant and adjuvant ICI therapy are available.

The SAKK 06/17 trial is a phase 2, open-label, single-arm trial of durvalumab in combination with cisplatin or gemcitabine in patients with cT2-T4a MIBC or UTUC [51]. Unlike previous trials discussed, this study evaluated immunotherapy in combination with chemotherapy. In total, 33 patients with MIBC and 1 patient with UTUC were enrolled, and all 34 patients received the combination therapy. Cisplatin/gemcitabine was administered over four cycles every 3 weeks, while durvalumab (1500 mg) was administered in combination with cisplatin/gemcitabine for three cycles pre-operatively and continued as a single agent for a total of ten cycles post-operatively. With respect to medical AEs, 24% of patients experienced grade 3 or 4 irAEs. Notably, surgery was performed without delays in 30 of 34 patients. Of the four patients who did not undergo surgery, three patients declined surgery, and one patient was ineligible for surgery due to a "frozen pelvis" upon assessment. Overall, 27% of patients experienced Clavien-Dindo complications that were grade 3 or higher; specifically, infections represented the most frequent complication (17%).

Peri-operative Immunotherapy Clinical Trials in Kidney Cancer

In the setting of a suspected, localized case of renal cell carcinoma (RCC), the AUA recommends definitive therapy in the form of a partial nephrectomy or radical nephrectomy, with a nephron-sparing approach preferred for clinically localized renal masses due to similar oncologic outcomes of both approaches for low-stage (T1-T2N0M0) disease [52, 53]. However, a subset of patients with an intermediate-high risk of recurrence have a paucity of systemic treatment options in the peri-operative setting, as previous trials using targeted agents have not demonstrated a survival benefit [54, 55]. However, a number of recent trials have emerged investigating immune checkpoint inhibitors in the adjuvant setting after resection of

localized disease; in this section, three ongoing trials will be mentioned. Of note, none of these trials have published any data.

The KEYNOTE-564 trial is a phase 3, randomized, double-blind, placebo-controlled study of adjuvant pembrolizumab for patients with intermediate- to high-risk RCC (pT2N0M0, grade 4 or sarcomatoid; pT3N0M0, any grade; pT4N0M0, any grade; pTanyN+M0, any grade; M1 NED). The patients will receive either placebo or pembrolizumab (17 cycles, 200 mg every 3 weeks). Treatment will be stopped due to drug-related toxicities or disease recurrence. The primary and secondary endpoints are disease-free survival (DFS) and OS, respectively [56]. As noted, results are forthcoming.

The IMmotion010 trial is a phase 3, randomized, double-blind, placebo-controlled study of adjuvant atezolizumab for patients with high-risk RCC (T2, grade 4; T3a, grades 3–4; T3b/c, any grade; T4, any grade; TxN+, any grade). The patients will receive either placebo or atezolizumab (16 cycles/1 year, 1200 mg every 3 weeks). The primary and secondary endpoints include DFS and OS, respectively [57]. As noted, results are forthcoming.

The RAMPART trial is a phase 3, randomized study of adjuvant durvalumab alone (every 4 weeks for 1 year), durvalumab (every 4 weeks for 1 year) in combination with tremelimumab (2 doses), or active surveillance for patients with intermediate-high risk of recurrence, based on the Leibovich score [3–11]. The primary endpoints are DFS and OS [58]. As noted, results are forthcoming.

Peri-operative Immunotherapy Clinical Trials in Prostate Cancer

Due to early detection of disease and the relatively low risk of prostate cancer-specific mortality for the majority of patients treated with definitive therapy, there is limited interest in neoadjuvant or adjuvant systemic therapies for patients with very-low- to intermediate-risk, clinically localized disease [59–66]. In the setting of high- to very-high-risk disease, management includes radical prostatectomy, radiotherapy, and/or androgen deprivation therapy and leads to favorable outcomes [67]. For example, 10-year prostate cancer-specific survival has been shown to range between 83 and 93% in patients treated with radical prostatectomy [68]. Thus, a single trial evaluating neoadjuvant immunotherapy will be briefly mentioned.

NCT03753243 is a phase 2, open-label, single-arm study of neoadjuvant pembrolizumab combined with enzalutamide for patients with high-risk, localized prostate cancer (cT3a, Gleason grades 8–10, PSA > 20 ng/mL) who are scheduled to undergo radical prostatectomy. Patients will receive pembrolizumab (200 mg every 3 weeks) and enzalutamide (160 mg/day) for a period of 14–16 weeks. The primary endpoint is pathologic complete response, and the secondary endpoints include safety and biochemical complete response [69]. As noted, results are forthcoming.

Conclusion

This chapter provided a brief summary of current investigations into the peri-operative use of immune checkpoint inhibitors in the management of genitourinary malignancies. Specifically, early safety data from several trials in MIBC, RCC, and prostate cancer were summarized, including both drug-related toxicities (irAEs) and medical complications. Based on the data presented, peri-operative immunotherapy does not result in toxicities that are divergent in type or frequency from previous literature characterizing anti-PD1, anti-PDL1, and anti-CTLA4 toxicity profiles. Additionally, the trials discussed here do not report delays in surgery for the vast majority of patients who were treated with neoadjuvant immunotherapy. These data, pending final reports and further investigations, provide promising early evidence to support the feasibility and tolerability of peri-operative immunotherapy.

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Part V
The Role of Imaging in Tumor Staging and Response Assessment: Envisioning an Application into the Next-Generation Neoadjuvant Trials

Chapter 19
The Gut and Urinary Microbiota: A Rising Biomarker in Genitourinary Malignancies

Filippo Pederzoli, Valentina Murdica, Andrea Salonia, and Massimo Alfano

Since the first observations of human-associated microorganisms by Antonie van Leeuwenhoek in the second half of the seventeenth century, the study of the human microbiome has raised increasing interest for its influence in health and diseases, including cancer. Perturbances of the homeostatic microbiome, known as *dysbiosis*, have been implicated directly and indirectly in the tumorigenesis of several malignancies, so much that specific tumor microbial signatures have been identified [1] and the microbiome/dysbiosis has been proposed as a novel "hallmark of cancer" [2]. The most famous and well-studied example of the direct contribution of a bacterial species to cancer is the case of *Helicobacter pylori* in the stomach, where a direct stimulation of the gastric cell lining by bacterial virulence factors and the establishment of a chronic, pro-inflammatory environment are linked to the development of gastric cancer [3]. In addition to the pathobiological role played by specific bacterial taxa or by the whole microbiome on cancer development and progression, there are several examples of a beneficial contribution of the microbiome or specific bacteria to cancer therapy and eradication. A clear example of the exploitation of a bacterium to treat cancer is the use of the Bacillus Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium*, in the management of high-grade, non-muscle invasive bladder cancer [4]. Moreover, the role of the gut and other

F. Pederzoli · A. Salonia
Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

Vita-Salute San Raffaele University, Milan, Italy

V. Murdica · M. Alfano (✉)
Division of Experimental Oncology/Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy
e-mail: alfano.massimo@hsr.it

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tissue-specific microbiome in the metabolism of several drugs has been established, highlighting their implications for drug inactivation, efficacy, and toxicity [5–8]. For instance, it has been shown that an oral administration of *Bifidobacterium* alone in a mouse model of melanoma improved tumor control similarly to treatment with checkpoint inhibitors, and the combination treatment nearly abolished tumor outgrowth [9]. The gut microbiome may also mediate side effects, as it was shown for dose-limiting severe diarrhea following irinotecan administration, caused by the reactivation of the drug within the intestinal lumen by bacterial β-glucuronidases [10].

The Role of the Microbiome in Cancer and Cancer Treatment

A number of studies have been published over the last years showing the association between the disruption of a tissue-specific microbial niche (i.e., dysbiosis) and pathologic conditions. The evidence can be demonstrated by the fact that restoring the gut microbiome by fecal microbiome transplantation represents a successful treatment of ulcerative colitis [11], and it has been shown to have effects also on distant organs. With regard to dysbiosis in cancer [12–14], the effect of the microbiome on cancer has been demonstrated at different stages, from carcinogenesis [15–18] to treatment response [19–22] and through local (e.g., intratumoral [19]) and systemic (e.g., gut microbiome-immune system-tumor axis [20]) mechanisms. The strict relationship between microbial communities and response to cancer therapy has aroused much interest in the possibility of exploiting the former to predict and boost the latter.

An increasing number of studies has made clear that the gut microbiome is central in the modulation of the response to immune checkpoint inhibition in several malignancies [9, 20, 23–25]. A common experimental design can be identified in these studies: the gut microbiota is analyzed in its potential predictive and prognostic value of the endpoint of choice (generally, pathological response, disease downstaging, progression-free survival) to identify a favorable microbial "signature." Then, the beneficial role of the identified bacterial taxa is demonstrated by microbiome transplantation in a germ-free or antibiotic-conditioned animal model of the study malignancy, showing that the "responder" microbiome is able to boost the antitumor activity in the animal models. Despite the growing amount of reports in the field, only a few bacteria have been found in multiple studies, highlighting the need for the inclusion of other potential influencing factors (e.g., diet and concurrent medications) in the study of the microbiome. The close crosstalk between immunotherapy and the microbiome may be explained by several mechanisms, for instance through direct modulation of the T-cell response by microbial metabolites and chemokines or through indirect effects by increasing the efficiency of antigen presentation and T-cell activation [26].

A role of the gut microbiome in the modulation of therapy response has been also demonstrated for chemotherapy. Using animal models, Viaud and colleagues [21] showed that the antineoplastic drug cyclophosphamide caused shortening of small intestinal villi, disruption of intestinal epithelial barrier, and dysbiosis of the

mucosa-associated microbiome, resulting in a significant translocation of several commensal Gram-positive bacteria into the mesenteric lymph nodes and the spleen. Within these secondary lymphoid structures, the translocated bacteria stimulated the generation of a specific subset of "pathogenic" T helper 17 (pT(H)17) cells and memory Th1 cells, which indeed contributed to the systemic antitumor effect of cyclophosphamide. In another preclinical study, Iida and colleagues [22] showed that disruption of the gut microbiome in mice by antibiotics decreased the antitumor efficacy of CpG-oligodeoxynucleotides-based immunotherapy and platinum-based chemotherapy (oxaliplatin). Moreover, they demonstrated that the therapy response was dependent on the expression of specific microbiome-induced inflammatory genes and the production of reactive oxygen species within the tumor microenvironment.

Another area in which the gut microbiome seems to play a major role is cancer therapy toxicity. For instance, a protective effect against immunotherapy-induced colitis has been linked to the presence of *Bacteroidetes* and *Bifidobacterium* [27, 28] in the gut. Interestingly, some studies showed that uncoupling favorable antitumoral effects and undesired toxicity of the microbiome is not always trivial and straightforward. For instance, chemotherapy-induced peripheral neuropathy is a quite common adverse event of oxaliplatin-based chemotherapy, affecting as much as 30% of treated patients. This neuropathy can last up to several years, and it can be so severe and debilitating to prevent the administration of adequate oxaliplatin dosages [29]. Shen and colleagues [30] demonstrated that the gut microbiome mediated oxaliplatin-induced mechanical hyperalgesia in mice through a direct impact on the inflammatory status of the dorsal root ganglia, showing that mechanical hyperalgesia was reduced in germ-free animals or in animals treated with antibiotics to deplete the gut flora. A potential mechanism behind these findings was found in a synergic-activating effect of the microbiome and oxaliplatin on macrophages, which sustained a proinflammatory environment in response to oxaliplatin and bacterial molecules like lipopolysaccharide (LPS). As mentioned previously, the gut microbiome is also the central mediator of oxaliplatin antitumoral activity [22], highlighting the potential double-edged sword role of the microbiome in therapy and the need for further studies to carefully distinguish the mechanisms behind efficacy vs. toxicity.

Another area of research is about the role of intratumoral bacteria in response to cancer treatments. Living bacteria can alter the effective concentration and activity of chemical compounds in the tumor microenvironment by means of their own metabolism. For instance, Geller and colleagues [19] found that, in a colon cancer model, the chemotherapeutic drug gemcitabine (2′,2′-difluorodeoxycytidine) was transformed into its inactive form 2′,2′-difluorodeoxyuridine by the activity of bacterial (especially *Gammaproteobacteria*) cytidine deaminase enzymes released in the tumor microenvironment, thus resulting in a lower antitumor activity. Moreover, the efficacy of gemcitabine was restored if the animals were concurrently treated with the antibiotic ciprofloxacin, reinforcing the causal link between intratumoral bacteria metabolism and antineoplastic activity. Moreover, Yu and colleagues [31] demonstrated that *Fusobacterium nucleatum* promotes resistance to chemotherapy in colorectal cancer by mounting a complex biological network that includes the toll-like receptor 4

(TLR4)/myeloid differentiation primary response 88 (MYD88) signaling pathway and specific microRNAs to ultimately activate autophagy and cancer cell death. As far as cancer immunotherapy is concerned, there have been reports pointing to either an immunosuppressive or immunostimulatory role of intratumoral bacteria. For instance, some hemagglutinating strains of *Fusobacterium nucleatum* can inhibit natural killer (NK) cells-mediated killing of colon cancer cells in the tumor microenvironment by the bacterial protein Fap2 [32]. Fap2 can interact with the human T cell immunoglobulin and ITIM domain (TIGIT) receptor, expressed on the surface of NK cells, leading to the inhibition of their cytotoxicity. Moreover, the Fap protein can also interact with the TIGIT receptor expressed on tumor-infiltrating lymphocytes, inhibiting them, thus contributing further to the tumor immune evasion. On the other hand, bacteria can also play an immunostimulatory role in the tumor microenvironment by favoring the recruitment and activation of immune cells, enhancing in this way the antitumor immune function. For instance, Zheng and colleagues [33] showed that colonization of the murine colon cancer microenvironment by engineered *Salmonella typhimurium* secreting the heterologous *Vibrio vulnificus* flagellin B (FlaB) protein resulted in a higher infiltration of monocytes/macrophages and neutrophils. Moreover, release of FlaB skewed the polarization of intratumoral macrophages toward the M1 phenotype, with a reciprocal decrease in M2 immunosuppressive phenotype. It is evident from the abovementioned evidence that any intervention aiming at modifying the intratumoral microbiome (e.g., antibiotics and pre-/pro-biotics) needs to be carefully designed and tested to avoid having the opposite effect.

The Urine Is Not Sterile! The Rise of the Urinary Microbiome

"*The fresh and healthy urine is perfectly free from bacteria or other minute organisms. The ordinary types of morbid urine, although they may contain blood, pus, or casts of tubes, are equally free from organisms.*" This was the opinion expressed by bacteriologist William Roberts in 1881 [34], recapitulating the dogma of the "sterility of urine" that held in place for over a century, with few dissenting opinions against the mainstream idea [35]. One of the main reasons why the idea of the sterility of urine held stable through the years may be found in the inadequate standard culture technique applied to urinary microbiology and the derived false equation "culture-negative = sterile". Indeed, this belief has been proven incorrect with the recent spread of culture-independent methods, such as 16S ribosomal RNA (rRNA) gene amplicon sequencing, applied to urine samples [36–38], and with the introduction of novel culture protocols based on a combination of different growing conditions [39]. For instance, Hilt and colleagues [39] hypothesized that the lack of growing bacteria in routine urine cultures could be attributed to the low number of inoculated bacteria and/or the need for special culture conditions (e.g., aerobic/anaerobic/microaerophilic conditions and prolonged incubation time). Applying this differential culture and incubation protocol, which they called "expanded quantitative urine culture" (EQUC), they were able to grow bacterial species in most (52/65, 80%) of the analyzed urine samples collected from women suffering from

overactive bladder and from healthy controls. Of note, most (48/52, 92%) of the EQUC-positive samples were reported negative using standard urine culture protocols. Moreover, they showed that the majority of the bacteria found by EQUC were also identified using 16S rRNA gene sequencing in the same specimens, providing evidence of the viability of the bacteria present in the urinary microenvironment. Although the field of urinary microbiota, and especially of bladder microbiome, is still at its beginning compared to the study of other human microbial ecosystems, it is arousing growing interest as a mediator of therapy efficacy in genitourinary (GU) malignancies and as a potential predictor of treatment response.

Microbiota and GU Malignancies – Kidney Cancer

In the field of renal malignancies (Table 19.1), to date the microbiome has been mostly studied as a potential biomarker of response to therapy, especially in the advanced/metastatic stages. For instance, Routy and colleagues [24] reported that

Table 19.1 Main studies investigating the role of microbiome in kidney cancer

Study	Main findings	Reference
Pal et al. (2015)	20 mRCC patients receiving vascular endothelial growth factor – TKIs Higher abundance of *Bacteroides spp.* and lower abundance of *Prevotella spp.* were found in patients with drug-induced diarrhea	[42]
Routy et al. (2018)	Evaluation of the effect of ABTs in patients receiving PD-1/PD-L1 monoclonal antibodies, including 67 patients with RCC PFS and OS were shorter in patients receiving ABTs, in the overall and RCC-only cohorts In Cox regression analyses, ATBs was confirmed as a predictor of resistance to PD-1 blockade in RCC *A. muciniphila* was identified as the gut commensal most significantly associated with favorable clinical outcome in RCC patients undergoing anti-PD-1/PD-L1 therapy	[24]
Derosa et al. (2020)	69 stage IV RCC patients who progressed during or after one or more prior regimens, and treated with nivolumab in the NIVOREN GETUG-AFU 26 trial ATBs administration (11 patients, 16%) resulted in a lower objective response rate, progression-free survival, and overall survival, and it affected the composition of the gut microbiota (e.g., expansion of *C. hathewayi*) Prior therapy with TKIs (e.g., axitinib) resulted in significant shifts in the gut microbiome of RCC patients	[40]
Salgia et al. (2020)	31 mRCC patients treated with nivolumab or nivolumab+ipilimumab Greater alpha diversity (Shannon index) was associated with clinical benefit, defined as complete response, partial response, or stable disease >4 months per RECIST v1.1 criteria *B. adolescentis*, *B. intestinihominis*, *O. splanchnicus*, and *B. eggerthii* were the most significantly enriched taxa in patients with clinical benefit	[41]

Abbreviations: *ABTs* antibiotics, *mRCC* metastatic renal cell carcinoma, *OS* overall survival, *PFS* progression-free survival, *RECIST* response evaluation criteria in solid tumors, *TKI* tyrosine kinase inhibitors

patients with advanced lung (non-small cell lung carcinoma, NSCLC), kidney (renal cell carcinoma, RCC), and bladder (urothelial carcinoma, UC) malignancies treated with antibiotics (beta-lactam+/−inhibitors, fluoroquinolones, or macrolides) in the 2 months before and 1 month after administration of anti-PD-1/PD-L1 immunotherapy had a shorter progression-free survival and overall survival compared to similar antibiotic-free patients. Moreover, antibiotic treatment emerged as an independent predictor of resistance to immunotherapy in lung and kidney malignancies. The mechanism linking antibiotics and the resistance to immunotherapy was found in an antibiotic-induced gut dysbiosis that caused a decrease in the relative abundance of *Akkermansia muciniphila*, which plays an immunostimulatory effect on cytotoxic T-cell, thus increasing tumor control and therapy response. Using a murine model, oral administration of *A. muciniphila* to mice previously treated by antibiotics was sufficient to restore efficacy of anti-PD-1/PD-L1 immunotherapy, further supporting the paramount importance of this bacterial species in influencing response to therapy. The RCC cohort originally examined in Routy et al. [24] was expanded in the work by Derosa and colleagues [40], who analyzed the gut microbiota of 69 patients with stage IV RCC treated with nivolumab. They confirmed the detrimental role of antibiotics on nivolumab efficacy, and they identified gut microbial fingerprints associated with response vs. lack of response. Furthermore, they also found that previous treatment with tyrosine-kinase inhibitors (e.g., axitinib) caused a major shift in the gut microbial communities, with a potential effect on following treatment strategies (i.e., nivolumab in this study). Another independent cohort [41] of 31 patients with metastatic RCC confirmed the presence of different bacterial taxa in the gut of patients responding or not to nivolumab or ipilimumab plus nivolumab. They found that patients with clinical benefit from checkpoint inhibitors had greater gut microbial diversity (Shannon index, $p = 0.001$) and they identified bacterial species differently enriched in responders (e.g., *Prevotella copri*, *Bifidobacterium adolescentis*, *Barnesiella intestinihominis*) or non-responders (e.g., *Bacteroides ovatus*). Of interest, among patients with clinical benefit to immunotherapy, there was a general increase in relative abundance of bacteria associated with response (e.g., *P. copri* and *A. muciniphila*). The gut microbiome also seems to play a role in the response to another class of compounds used in the treatment of metastatic RCC, which is the one of vascular endothelial growth factor tyrosine kinase inhibitors (TKI; e.g., axitinib, sunitinib, pazopanib). It was known that a higher amount of *Bacteroides spp.* was found in patients with TKI-induced diarrhea [42]. Hahn and colleagues [43] showed that patients treated with TKI and who received antibiotics effective against *Bacteroides* (beta-lactam/beta-lactamase inhibitor combinations, clindamycin, carbapenems, metronidazole, select cephalosporins, and tetracyclines) had an improved progression-free survival (PFS) compared to antibiotic-untreated patients. Although the PFS benefit in the antibiotic-treated cohort may be partially explained by a higher adherence to therapy due to less toxicity (i.e., diarrhea), the mechanisms behind this beneficial role of antibiotics seem to be more complex and probably due to a shift of the gut

microbiome toward a more TKI-beneficial composition. More studies are awaited to further elucidate the crosstalk between microbiome and response to therapy in kidney cancer.

Microbiota and GU Malignancies – Prostate Cancer

Prostatic bacterial inflammation has been traditionally regarded as a potential risk factor for the development of prostate cancer, although conflicting epidemiological evidence has emerged over the years [44, 45]. Chronic inflammation is a common finding in adult prostatic tissue, and bacteria have been thought to be the source of this inflammation. However, no single bacterial species, either the ones most commonly causing prostatitis or the bacterial species linked to sexually transmitted diseases, has emerged as a strong risk factor for prostate cancer carcinogenesis in clinical studies [46, 47]. Efforts to demonstrate whether an intraglandular commensal prostate microbiome exists in healthy status has led to controversial findings (Table 19.2). Hochreiter and colleagues [48] applied 16S rDNA PCR to detect the presence of bacterial DNA in prostate tissue samples from healthy deceased organ donors, patients with prostate cancer undergoing radical prostatectomy, and patients undergoing simple prostatectomy for benign prostatic hyperplasia (BPH). While they found bacterial rDNA PCR products in prostate cancer and BPH samples, no PCR amplification occurred in normal healthy prostate samples, suggesting the absence of a commensal bacterial flora in healthy men. Studies conducted on prostatectomy specimens with sampling of different areas of the prostate (e.g., neoplastic, para-neoplastic, and benign) found bacteria in all of them, even if the presence of bacteria was not homogeneous throughout the whole gland [49–51]. Therefore, it seems plausible that the prostate gland does not have a ubiquitous flora, but instead bacteria grow within limited foci, most likely in association with areas of acute and/or chronic inflammation. A recent work from the Sfanos's Lab seems to provide a strong mechanistic evidence of the link between inflammation, bacteria, and prostate carcinogenesis [52]. As it is known that inflammation-induced oxidative stress in the prostate tissue leads to DNA breaks in prostate cells resulting in TMPRSS2-ERG gene fusions [53, 54] – a hallmark genetic alteration of prostate cancer – Sfanos and colleagues [52] demonstrated that these fusions initiate in early inflammation-associated prostate cancer precursor lesions, such as proliferative inflammatory atrophy, and that the bacterial genotoxin colibactin was a potential cause of DNA instability and break both *in vivo* and *in vitro*.

In addition to the prostate tissue microbiome, the urinary microbiome has gained increasing research interest as a potential predictive and prognostic biomarker of prostatic diseases. In men with chronic prostatitis/chronic pelvic pain syndrome, microbiome analyses on midstream urine samples revealed a higher isolation of *Clostridia* and *Bacteroides* compared to controls, who have higher prevalence of

Table 19.2 Main studies investigating the role of microbiome in prostate cancer

Study	Main findings	Reference
Hochreiter et al. (2000)	Samples from normal prostate from organ donors (n = 28), radical prostatectomy from prostate cancer patients (n = 14) and from simple prostatectomy for BPH (n = 6) No PCR amplification occurred in normal healthy prostate samples compared to prostate cancer and BPH samples, suggesting a lack of a commensal microbiota in the prostate gland Presence of bacteria in the prostate is focal and associated with areas of inflammation	[48]
Cavarretta et al. (2017)	Paired tumor, peri-tumor, and non-tumor biopsies from 16 radical prostatectomy specimens *Propionibacterium* was the dominant bacterial genus found across all the different areas *Staphylococcus spp.* were more represented in the tumor/peri-tumor biopsies	[49]
Yow et al. (2017)	20 snap-frozen tissue biopsies from 10 "aggressive" (Gleason score ≥8 and tumor stage ranging from pT2c to pT3b) *Enterobacteriaceae spp.* identified in all samples, *P. acnes* in 95% of them	[50]
Sfanos et al. (2008)	170 samples from 30 prostate cancer patients analyzed by 16S rDNA gene sequencing The majority of the bioptic cores were negative for presence of bacterial DNA, suggesting the absence of a ubiquitous commensal flora in the prostate gland Compared to sequencing, culture of the bioptic specimens resulted in fewer species, suggesting the presence of either difficult-to-growth bacteria or the presence of non-viable bacteria	[51]
Shrestha et al. (2018)	135 urine samples collected from men undergoing biopsies for prostate cancer; 65 men were diagnosed with cancer No significant differences in α or β diversity between patients with/without cancer A group of pro-inflammatory bacteria (*S. anginosus, A. lactolyticus, A. obesiensis, A. schaalii, V. cambriense*, and *P. lymphophilum*) was identified to be enriched in a subgroup composed mostly of cancer patients	[56]
Sfanos et al. (2018)	Fecal samples from 30 men (healthy controls + prostate cancer patients with localized, biochemically recurrent, and metastatic disease) Different alpha diversity among men with vs. without prostate cancer Men taking oral androgen receptor axis-targeted therapies showed an increased abundance of pro-immunotherapy bacteria like *A. muciniphila* and *Ruminococcaceae* spp. Men treated with oral androgen receptor axis-targeted therapies showed a functional shift of the gut microbiota toward increased expression of pathways involved in steroid biosynthesis and steroid hormone biosynthesis	[60]

Abbreviations: *BPH* benign prostatic hyperplasia, *PCR* polymerase chain reaction

Bacilli [55]. In prostate cancer, Shrestha and colleague [56] analyzed the urinary microbiome of men with and without a biopsy-proven cancer. They found no differences in the bacterial load or diversity between patients and controls. However, prostate cancer patients had higher abundance of potentially pro-inflammatory bacteria, including *S. anginosus*, *A. lactolyticus*, and *P. lymphophilum*. Moreover, known pathogens of the urinary tract like *Ureaplasma* spp. were differentially abundant among cancer and benign samples, and they also differed in relation to cancer aggressiveness and degree of inflammation.

In the management and treatment of prostate cancer, it is important to recall that bacteria have also been involved in the metabolism of steroid hormones, contributing to define their systemic levels [57, 58]. Androgens play a fundamental role in the growth and survival of both benign and hormone-sensitive malignant prostate cancer cells. The importance of the androgen-derived signaling pathways in prostate cancer led to the use of androgen blockade as treatment for prostate cancer. In this setting, the potential role of specific gut and intraprostatic bacteria in modulating the concentrations of steroid hormones and androgens may have a great impact on the effectiveness of androgen deprivation therapy, potentially affecting disease progression and patient survival. It is worth mentioning that the interplay between androgens and gut microbiome is bidirectional. Harada and colleagues [59] showed that androgen deprivation following castration in C57BL/6J mice resulted in abdominal obesity in animals fed with high-fat diet, but not in animals fed with standard diet. Interestingly, disrupting the native gut microbiome by antibiotic treatment was protective against the increase in body weight and visceral fat. Performing taxonomic analysis of the fecal microbiome in the different mice cohorts, an increase in the *Firmicutes/Bacteroidetes* ratio and in the abundance of *Lactobacillus* species was implicated in increased obesity after castration. Exploratory analyses from Sfanos and colleagues [60] showed that the gut microbiome of men taking anti-androgen therapy was characterized by a greater abundance of *A. muciniphila* and *Ruminococcaceae*, bacterial species implicated in the efficacy of immune checkpoint inhibitors. The available evidence suggest a strict crosstalk between the microbiome and the steroid hormonal milieu, with important effects on the efficacy and the onset of side effects of androgen deprivation therapy.

Microbiota and GU Malignancies – Bladder Cancer

In bladder cancer, few studies investigated differential microbial populations in the urine of patients and controls, and the available literature is far from being unambiguous (Table 19.3). Chipollini and colleagues [61] reported that urine collected from patients with invasive bladder cancer showed a significant enrichment of *Bacteroides* and *Faecalibacterium*, while urine from patients with superficial cancer samples did not yield differently expressed biomarker taxa. Wu and colleagues [62]

Table 19.3 Main studies investigating the role of microbiome in bladder cancer

Study	Main findings	Reference
Pederzoli et al. (2020)	166 biological specimens from bladder cancer patients and healthy controls, stratified according to the biological sex, including midstream urine and tissue biopsies (neoplastic and non-neoplastic) from radical cystectomy patients The genus *Klebsiella* was more common in the urine of female patients vs. controls In tissues, the genus *Burkholderia* was more abundant in the neoplastic vs. the non-neoplastic tissue in both sexes	[38]
Chipollini et al. (2020)	Urine samples from 38 urothelial carcinoma patients and 10 controls Cancer samples were enriched in *Bacteroides* and *Faecalibacterium*	[61]
Wu et al. (2018)	Midstream urine samples from 31 men with bladder cancer and 18 controls Cancer cohort was characterized by increased bacterial richness, higher abundance of *Acinetobacter*, *Anaerococcus*, and *Sphingobacterium* and lower abundance of *Serratia*, *Proteus*, and *Roseomonas*	[62]
Popović et al. (2018)	Urine samples from 12 males with bladder cancer and from 11 age-matched controls The genus *Fusobacterium*, a possible protumorigenic bacterium, was enriched in bladder cancer patients	[63]
Mai et al. (2019)	24 urine samples from patients with bladder cancer *Acinetobacter* abundance reported to be higher in bladder cancer samples	[64]

showed that the *Sphingobacteriaceae* family was more abundant in male bladder cancer patients, whereas Popović and colleagues [63] reported a greater abundance of *Fusobacterium*, *Actinobaculum*, *Facklamia*, and *Campylobacter* genera in their male bladder cancer patient cohort. Moreover, Mai and colleagues [64] analyzed a mixed cohort of male and female bladder cancer patients, identifying *Enterococcus*, *Enterobacteriaceae*, and *Lactobacillaceae* as the most common taxa.

The heterogeneous results from the above-mentioned studies can be explained by the different composition of the study cohorts, in terms of patients' race, sex, and extension of the disease (i.e., muscle invasive vs. non–muscle invasive urothelial carcinoma). For example, the reported differences can be partially attributed to sex-specific microbiome composition [65, 66]. Indeed, Pederzoli and colleagues [38] conducted a sex-based analysis of the urinary and tissue-associated microbiome in bladder cancer using a homogeneous cohort of Caucasian patients and controls from Northern Italy. Differently enriched taxa in the urine samples of patients were identified according to the biological sex: in men, the order *Opitutales* and subordinate family *Opitutaceae*, together with the isolated class *Acidobacteria-6*; in females, the genus *Klebsiella,* belonging to the family Enterobacteriaceae. These findings are in agreement with a study that reported an increased richness of the urinary *Klebsiella* genus in a mixed cohort of bladder cancer patients [64], although the study by Pederzoli and colleagues found its enrichment only in urine samples

from female patients. It is worth mentioning that *Klebsiella* spp. produce the colibactin toxin, which can cause direct DNA-strand damage and, consequently, genomic instability, as reported above in the prostate tissue [52].

Another relevant research question regards the presence and localization of a tissue-bound microbiome within the urinary bladder, and whether differences in microbial ecology can be detected between tumoral and benign urothelial areas. In an exploratory analysis using paired biopsies of neoplastic and non-neoplastic areas from the same bladder, Pederzoli and colleagues [38] identified higher abundance of only the genus *Burkholderia* in the neoplastic tissue coming from both male and female patients. This unexpectedly minor difference in terms of the microbial communities in the neoplastic vs. non-neoplastic bladder may be due to the multifocal nature of urothelial carcinoma and, therefore, the nearby non-neoplastic regions may be influenced by a "cancer field effect" able to modify the microbiome of those non-neoplastic areas. Another explanation might be that the urine shifts bacteria within the bladder according to movements caused by the daily human activities, thus making the whole bladder surface almost homogeneous in microbial community. Of note, the genus *Burkholderia* has been recently implicated in mediating response to immunotherapy in an animal model of sarcoma. Vetizou and colleagues [23] showed that the efficacy of immunotherapy with CTLA-4 antibody is influenced by the microbiome composition, specifically by *B. fragilis* and/or *B. thetaiotaomicron* and *Burkholderiales.* Moreover, the transplantation of those bacteria in antibiotic-conditioned animals had also a protective effect on colitis induced by CTLA-4 blockade, suggesting a promising role of those taxa as "anticancer probiotics".

The use of bacteria to treat bladder cancer dates back to the previous century, when patients with high-grade non–muscle invasive bladder cancer started to be treated with the intravesical instillation of a live, attenuated form of BCG. Since the first report by Morales and colleagues in 1976 [67], BCG has now become a mainstay in the management of bladder cancer patients. Despite our understanding of the mechanisms behind BCG antitumoral activity has still some open questions [68], it is plausible to hypothesize that the urinary and bladder-tissue microbiome may influence the efficacy of BCG immunotherapy. For example, the BCG might compete with the commensal bacterial species present in the bladder microbial niche for attachment to the extracellular protein fibronectin, the first step needed to mount a BCG-induced antitumor response. Moreover, the presence of certain subtypes of urothelial carcinoma in the bladder may cause a shift in the microbial communities toward a microbiome that prevents the BCG to bind to fibronectin, thus decreasing its therapeutic efficacy.

In the muscle-invasive disease, neoadjuvant immunotherapy by single-agent immune-checkpoint inhibitor has been proven effective in eradicating bladder cancer cells, aspiring to become a game changer in the management of bladder cancer patients [69–71]. Several studies have shown that the efficacy of immunotherapy is strictly linked to the modulation of the enteric commensal bacterial microbiome; therefore, a potential role of the gut microbiome in modulating the efficacy of immune checkpoint inhibitors in urothelial carcinoma is plausible and deserves further investigations. At the same time, it is not known if the urinary and

bladder-bound microbiome may play a role in the same setting. Further studies are needed to answer these open questions.

The urinary and bladder-associated microbiome provides several but different (potentially integrating) information, leading to many future applications. However, due to several variables influencing the microbiome, such as race, sex, diet, or exposure to environmental carcinogens, the dysbiosis of the urinary microbiome might not represent a universal prognostic marker of bladder cancer; on the other hand, classification of the above-listed variables might provide niches of urinary bacterial communities to be applied in different locations. On the contrary, the presence of the tumor in the bladder might contribute to modification of the environment (i.e., hypoxia and acidosis), creating a condition modulating richness and diversity of the microbial community.

Conclusions

The future of the microbiome in GU malignancies is bright and promising, and more and more reports are awaited on this emerging field of precision medicine, that is, the "precision urobiome" [72]. Firstly, the dysbiosis in the gastrointestinal and genitourinary systems of patients with tumor might be exploited as novel predictive and prognostic biomarkers of therapy response and disease recurrence. Moreover, the microbiome might also represent an actionable environment to improve therapy efficacy, either by oral pre-/pro-biotics or by more targeted interventions (e.g., phage therapy). Moreover, the presence of a defined consortium of bacteria may be introduced as variable in models to predict therapy efficacy, disease-specific survival, or onset of immune-mediated side effects [73, 74].

We are beginning to appreciate just now the potential influence of the microbiome present at different sites along the genitourinary tract on the pathobiology of genitourinary malignancies. A translational collaboration between different specialists, from basic scientists working on microbiology and tumor immunology to physicians taking care of patients with genitourinary malignancies, is necessary for a fruitful bench-to-bedside research in the field of the GU microbiome.

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Part VI
The Role of Imaging in Tumor Staging and Response Assessment: Envisioning an Application into the Next-Generation Neoadjuvant Trials

Chapter 20
The Future of Artificial Intelligence Applied to Immunotherapy Trials

Zuhir Bodalal, Stefano Trebeschi, Ivar Wamelink, Kevin Groot Lipman, Teresa Bucho, Nick van Dijk, Thierry Boellaard, Selam Waktola, and Regina G. H. Beets-Tan

Introduction

Clinical trials are a cornerstone of medical research, especially in the context of oncology. Rigorous trials act as a barrier of entry for novel interventions, treatments or treatment combinations. Traditional clinical trial approaches have been mostly

Zuhir Bodalal and Stefano Trebeschi contributed equally with all other contributors.

Z. Bodalal · S. Trebeschi · T. Bucho · R. G. H. Beets-Tan
Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands
e-mail: r.beetstan@nki.nl

I. Wamelink
Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Technical Medicine, University of Twente, Enschede, The Netherlands

K. G. Lipman
Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

Technical Medicine, University of Twente, Enschede, The Netherlands

N. van Dijk (✉)
Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
e-mail: n.v.dijk@nki.nl

T. Boellaard · S. Waktola
Department of Radiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

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unchanged for decades; a patient is assigned to either an experimental or control group, and their clinical outcome is recorded. However, the emergence of novel data analysis techniques provides a unique opportunity to augment decisions being made during a trial (e.g. prediction of resistance or adverse events) or even change to the way clinicians conduct trials (e.g. patient enrolment using AI or dynamic reassignment of patients between various treatment arms). Artificial intelligence (AI), in particular, has become the focus of a significant amount of research where groups explore the possible integration of these computational methods into a potential clinical decision support system.

In order to bring novel AI methods into clinical trials, it would be necessary to break down the components of a clinical trial and see where AI can be of added value. In its simplest form, we can think of a clinical trial as a process where an intervention/medication is first administered to a patient and then subsequently monitored continuously by diagnostic disciplines.

The principal diagnostic disciplines in the context of a clinical trial would be imaging (radiology), pathology and laboratory medicine. Each of these disciplines would generate large volumes of data per patient from the beginning of the trial to its conclusion. AI, in general, and deep learning, in particular, are notorious for their demand for data. As such, the implementation of AI has been most prevalent in these fields, particularly radiology.

In each of the principal diagnostic disciplines, several use cases for artificial intelligence methods have emerged. In the following sections, we will highlight the major applications of AI in these fields and discuss what a future 'AI-powered clinical trial' could look like.

AI in Medical Imaging

Artificial intelligence can be applied to many fields within medicine; however, generally speaking, a prerequisite is that AI requires access to vast amounts of data [1, 2]. The development of three-dimensional magnetic resonance imaging (MRI), computed tomography (CT) and other multiparametric imaging modalities significantly increased the availability of anatomical, functional and molecular information within routinely generated clinical images. AI algorithms have emerged to extract meaningful imaging properties, or features, from these imaging modalities that can be linked back to clinical endpoints [3, 4]. Research into medical image analysis has since blossomed with increasing interest from both radiologists and other physicians in the clinical implementation of these algorithms [5].

The novel domain of imaging features can be chiefly divided into qualitative, or semantic, imaging features and quantitative imaging features (Fig. 20.1). Semantic features are obtained from experienced readers (e.g. radiologists) who assess medical images and score specific parameters (e.g. presence of necrosis, lesion size and shape), thereby generating a feature vector (or a collection of features) that can be used for statistics or AI model construction. The scoring of semantic features is

generally dependent on expertise. Quantitative imaging features, however, are extracted by applying mathematical algorithms on the images. Examples are attenuation, tumour diameter, anatomically relevant angles or radiomics, among others [6–9]. Both types of features can be used in artificial intelligence models to link imaging data to clinical endpoints.

Semantic Features

Semantic (qualitative) features reflect intuitive tumour properties such as lesion size, shape, number of lesions, location, intensity and others [7, 9–11]. Several of these features have been added to the routine clinical workflow as they provide the radiologist extra information during the diagnosis of diseases and treatment response monitoring [8].

A number of semantic imaging features were significantly associated with progression-free survival (PFS) and overall survival (OS) in glioma and glioblastoma multiforme patients [12, 13]. In non-small cell lung cancer (NSCLC), semantic features were able to distinguish tumours with different genetic mutational statuses. ALK-positive nodules tended to show larger volume multi-focal thoracic lymphadenopathies on CT imaging [14], while pleural retraction [15], smaller nodules [15, 16] or spiculation [16] was indicative for an EGFR mutation. Tumour characteristics such as round shapes [15], the presence of multiple small nodules [16] or nodules in non-tumour lobes were associated with KRAS mutation [15].

Despite the implementation of different semantic features in the daily clinical workflow of radiologists, semantic features suffer from specific shortcomings, most notably standardisation [17]. Because semantic features are subject to human bias, two radiologists can score tumour properties very differently. Differences in experience between readers could lead to different results in relation to diagnosis or treatment response [10]. Semantic features suffer from inter- and intra-observer variability [17–20], and a learning curve exists for readers to generate appropriate and accurate features [21].

Another disadvantage of semantic features is that they are bound to what is discernible to the human eye. This limitation could lead to the missing of high-dimensional and potentially important imaging traits which, as a result, will not be taken into account [4, 22, 23]. The last limitation can generally be overcome with quantitative analysis [24, 25].

Radiomics

In the field of radiomics, advanced mathematical algorithms are applied to medical images to convert them into quantitative minable data [11, 26–28]. The field of radiomics is built on the principle that medical images contain valuable information

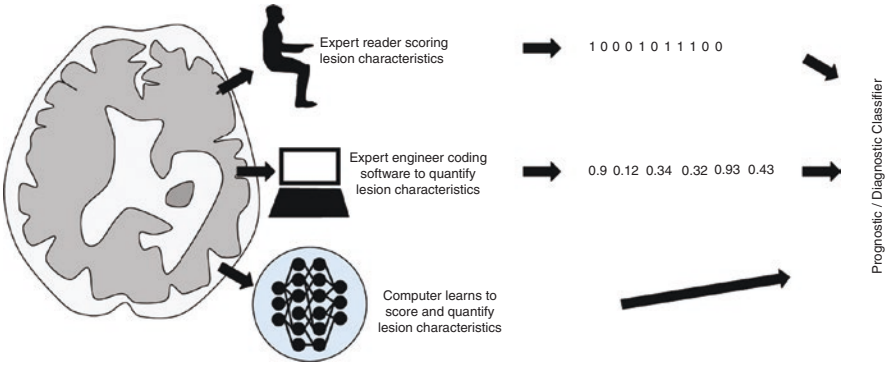

Fig. 20.1 Schematic representation of the methods of imaging feature generation and application. In the top path, an expert reader scores specific (usually binary) parameters in the image. In the second path, handcrafted radiomic features are extracted automatically using predefined algorithms/formulae. In the final path, a deep learning neural network acts as an end-to-end solution, where the image is input, features are automatically learned and a classification is made based on the clinical endpoint

beyond what is discernible to the human eye. Radiomics is capable of extracting predictive high-dimensional information from the images by means of quantitative image analysis [29, 30].

Radiomic data could improve our understanding of medical domains such as treatment-related adverse events, therapeutic and post-therapeutic changes and underlying biology, among others [4]. Depending on the way that radiomic features are extracted, the field of radiomics can be divided into two main approaches: handcrafted, or classical, radiomics and deep learning radiomics [7, 29].

In handcrafted/classical radiomics, visual aspects in the medical images are converted into features by means of predefined mathematical formulae [7, 29, 31]. These types of features are generally based on morphological, phenotypic characteristics, such as image intensities, shape or textural attributes [4, 32]. A prerequisite for handcrafted radiomic features, and generally considered a limitation, is the requirement of manual delineation of regions of interest. An experienced radiologist generally performs the region of interest delineation. The workflow of manual delineation followed by handcrafted feature extraction is characteristic of classical radiomics. Classical radiomics can then be analysed by conventional statistical methods or by machine learning artificial intelligence models.

The second and upcoming approach that makes use of radiomic features is deep learning. Deep learning, in itself, is a term that can be explained in several books. The concept of deep learning describes a number of neural networks that can be taught, a process commonly known as *training*, to generate features by itself. While creating these features, deep neural networks can perform classification without human involvement [33, 34]. Within the medical community, the convolutional neural network (CNN) is generally accepted as the go-to class of deep neural networks as it is free from human interference and is capable of extracting many more

features than classical radiomics or semantic features [7]. A major advantage of deep learning is that feature extraction, selection and classification all happen within the same network.

Radiomics, with either machine learning or deep learning, have been employed in a number of applications in medical imaging [35–37]; two prominent ones can be put forward in this book chapter:

Prediction of Response to Immunotherapy

AI has been used for different objectives within the field of medicine. One of the proposed applications is response prediction by distinguishing probable responders from non-responders, with the predictive performance being measured by the area under the receiver operating characteristic curve (AUC). Ultimately, such predictive AI algorithms may exclude patients from exposure to unnecessary treatment, mitigating both potential adverse effects for the patients [38] and loss of precious funds for the healthcare facility [39].

Publications about radiomics/deep learning for prediction of response to immunotherapy are relatively scarce but are increasingly being published. Trebeschi et al. used CT-based radiomic biomarkers to predict treatment response to immune checkpoint blockade in melanoma and non-small cell lung cancer (NSCLC) [40]. Similarly, non-invasive CT biomarkers were able to distinguish between high tumour mutational burden and low tumour mutational burden in patients with NSCLC (AUC = 0.81). These biomarkers were also able to predict clinical outcomes of NSCLC patients receiving anti-PD-1/PD-L1 treatment [41]. Radiomic features derived from PET/CT showed promising results in determining which NSCLC patients would likely benefit from anti-PD-1/PD-L1 immunotherapy [42, 43]. A deep learning model trained on FDG-PET images also appeared to be predictive of immunotherapy in patients with lung adenocarcinoma [44]. Another study used time to progression and pretreatment CT-based features to identify NSCLC patients unlikely to benefit from immunotherapy [45]. Similarly, delta radiomic features (resulting from the subtraction of pre- and post-treatment features) could recognise early immunotherapy response in NSCLC patients [46].

Sun et al. found that a combination of CT-based features and CD8 gene expression signature showed promising results when predicting clinical outcome in four independent cohorts with advanced solid tumours treated with immunotherapy [47].

Response prediction has also been studied for urothelial cell cancer patients treated with immunotherapy. In metastatic urothelial carcinoma, a radiomic model involving CT-based features showed promising results when predicting immunotherapy response and survival outcome (AUC = 0.88) [48]. The use of a deep learning network radiomic pipeline in bladder cancer achieved 86% accuracy when distinguishing between potential responders and non-responders to immunotherapy [49].

Radiogenomics for Prognostication and Precision Medicine

Radiogenomics is a novel research field that links imaging phenotypes to genetic characteristics (such as gene profiles, gene expression and gene mutation status) [50, 51]. The term has expanded its meaning beyond 'just genomics' and now also encompasses the linking of imaging markers with other biological parameters in the tumour microenvironment, such as proteomics and metabolomics [7].

Radiogenomics addresses a number of the shortcomings of conventional biopsy-based approaches. Biopsies suffer from sampling bias, fail to take interlesional and intralesional heterogeneity into account and have increased morbidity and invasiveness for patients. Additionally, biopsies are limited to sites of the body that are more accessible. Using AI radiomics, we can gain insight into the tumour biology of the full tumour burden (i.e. the primary lesion and metastases) across time. A key advantage of radiogenomics is the possibility of using serial and longitudinal imaging, whereas serial or multi-lesion biopsies are often unavailable.

By linking different biopsy results to radiomic features, radiogenomics could potentially map the genomic landscape of a patient's entire tumoural burden, completely non-invasively. In the context of a clinical trial, radiogenomics can be used to identify early biological markers of resistance or the emergence of a targetable mutation for precision medicine. The value of visualising the genomic profile of the full tumour burden longitudinally cannot be overstated.

We envision that, in the future, a generalisable radiogenomic model may ultimately be used as a non-invasive approach that mimics and expands biopsy function.

AI in Pathology

The application of artificial intelligence in medical imaging has started with considerable focus on 2D imaging, such as histopathological slides. In an effort to promote digitalisation and long-term preservation of the biological data collected, pathology departments have long started the digitalisation of image acquisition, processing and storage. This, in turn, allowed AI researchers to make use of such data for purposes of diagnosis and prognostication (Fig. 20.2).

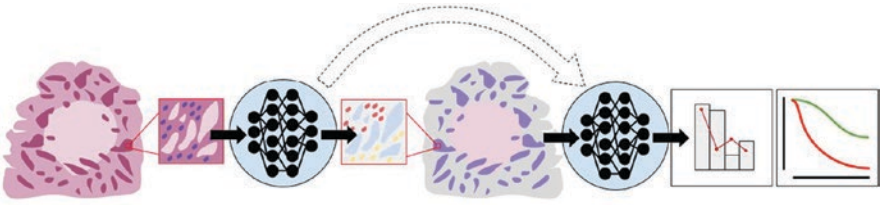

Fig. 20.2 An illustration of possible applications of AI in pathology. Using AI, we can apply computational stainings on pathological slides (as shown by the transition from an H&E image to an IHC image). Additionally, a digital image of a slide can be used for biomarker identification (or for classification purposes)

Several aspects are unique to pathology, including the multidimensional information of the staining encoded in the colours, the relatively high dimension of the image derived from the microscopic scale resolution and the level of heterogeneity resulting from different intra- and inter-patient variability, as well as biopsy parameters. These aspects, among others, have been reported to require specific, tailored-made solutions [52–55].

AI-Assisted Pathological Assessment

In the realm of AI-driven pathological diagnosis, one of the first applications is the automatic extraction of biological parameters of interest. In this sense, AI was developed to be used as a tool to convert complex, high-dimensional data to cellular and tissue phenotypes at a large scale, to be used for scientific research and prognostication. The simplest example is the identification and segmentation of single cells [56–58]. In this case, the algorithm would automatically discern pixels that belong to cells from background ones, separating different cells from each other. This enables high-throughput processing of information regarding cellularity and cellular distribution from massive datasets, which would have otherwise been impossible by manual labelling. Further research redefined and proposed more precise methods for the segmentation of finer structures, like the cell nuclei and cytoplasm [59–61], and classification of tumour and immune cells [52, 62] and tumour epithelium and stroma [63–65]. AI promises to unlock further information encoded in these pathological slides with the aim of supporting scientists and clinicians.

AI-Driven Pathological Biomarker Discovery

AI has the potential to unlock automatic quantification of biological parameters of interest through the identification and quantification of known structures and patterns. This raises the question of whether AI-based features can be used for biomarker discovery and the extent of their application. A study from Stein et al. revealed how common pathological scoring, featuring immune activation, cell death, tissue repair and regression grade, had the potential for pan-tumour scoring of response to anti-PD1 therapies [66]. If these findings hold, it would be a logical step to explore AI applications able to quantify these aspects from pathological slides and potential associations with pan-cancer therapy-specific response. Steps in this direction have been taken already with deep learning methods developed for predicting known biological immune biomarkers. These include PD-L1 status [67], TMB [68, 69] and microsatellite instability [70], among others. It is yet to be seen whether these AI algorithms can generalise beyond the tumour type in their training set. Most statistical methods used for these applications (of which deep learning networks are part of) are based on the creation and synthesis of domain-specific

knowledge. Whether the AI would manage to extrapolate its knowledge to cancer types it was not trained upon has yet to be seen.

Unique Aspects of AI Pathology Imaging and Immunotherapy

Most of the approaches reported so far encompass the application of AI for the quantification of specific biological quantities of interest. However, immunotherapy depends on more complex mechanisms which frequently rely on the relation and distribution of biological entities (and their respective properties) in the microenvironment. In this case, it would be beneficial to harness the full potential of these large computational models (often >1 M parameters) to track and quantify these complex patterns. In a study from Saltz et al. [52], researchers found an association between tumour-infiltrating lymphocyte (TIL) patterns (exposed by AI from haematoxylin and eosin (H&E) staining) and tumour and immune molecular features and ultimately treatment outcome. These were automatically extracted and analysed from a public cohort of 5202 H&E high-resolution pathological slices, a study otherwise unfeasible if it were to be completed by human manual labour. These aspects are particularly researched in the immune-oncological world, as they enable scientists to gain additional insights into the complex immunotherapy functioning mechanisms. The application of AI in pathology imaging of immunotherapy patients is becoming more relevant with the enlargement of immunotherapy to the neoadjuvant settings. As these patients often present tumour in situ, AI would allow to perform an initial assessment of the sample, classifying the microenvironment, quantifying immune infiltration and estimating the likelihood of micrometastases in the surrounding tissue.

Data Rediscovery

The last aspect for which AI is set to transform the field of pathology is through data rediscovery or, in other words, rediscovering older datasets for novel purposes. Currently, clinical pathology is moving away from standard H&E staining to more complex and customised stains for the purpose of personalised medicine. This is the case for immunohistochemistry, which is now becoming the de facto standard in immunotherapy clinical settings. While normally this would mean that retrospective observational studies would not be able to harness the data collected in the time when H&E was the only standard, AI allows us to do just that. This process is commonly termed *computational staining* and has been used to generate H&E imaging from unstained tissue samples [71], staining of TILs from H&E [52] and even commercial solutions for computational IHC staining from H&E. Once deployed, these technologies will enable us to harness the full potential of the

datasets collected by hospitals during the last decades and gain novel insights that are still hidden in old data.

AI in Laboratory Medicine

Laboratory medicine represents a vast source of healthcare data [72], paving the way for numerous potential AI applications, including laboratory operation optimisation, laboratory test analysis, early diagnosis and personalised patient care, among others [73]. In addition, laboratory data can be particularly appealing in machine learning because of its tabular and codified nature. Traditional machine learning algorithms depend on structured data and are typically organised in a tabular format on which to train. Despite this immense potential, the application of AI to traditional laboratory results appears to be relatively unexplored [74].

In clinical oncology, the treatment technology is rapidly improving with advanced techniques and new types of therapies, including immunotherapy, chemotherapy and radiotherapy. Developing precision medicine with the aid of AI techniques is becoming a major trend. AI research in laboratory medicine also has been growing, although the total number of publications remains relatively low, especially in immunotherapy. Recently, machine learning was used to analyse rhabdomyosarcoma patients treated with vincristine (IVA) chemotherapy to predict their blood cell count dynamics and reproduce the dynamic profiles of the haematologic toxicities. In the study, 24 patients with rhabdomyosarcoma treated with IVA chemotherapy courses were included, and during each cycle, routine multiple blood cell counts were performed [75]. Such kinds of AI-based studies could also be extended and applicable in immunotherapy treatments.

In immunotherapy trials, laboratory parameters have been investigated as predictive and prognostic biomarkers and as a monitoring tool for treatment response. Particularly, biomarkers exploring the immunologic aspects of the tumour and its microenvironment (e.g. PD-L1 expression, tumour-infiltrating lymphocytes, TMB) have been broadly studied [76–78].

However, these require a tissue biopsy that can be challenging to obtain considering the invasiveness of the procedure, high tumour heterogeneity, high turnaround time or tissue insufficiency [79, 80]. Thus, routinely collected laboratory values, which are easily obtained at baseline and follow-ups, have also gained interest as biomarkers. Serum-based markers such as the neutrophil-to-lymphocyte ratio [81–85] and lactate dehydrogenase [82, 85, 86], for instance, have been found to have prognostic or predictive power in NSCLC and melanoma patients receiving immunotherapy. Blood TMB was shown to be correlated with tissue TMB and associated with longer progression-free survival in NSCLC patients [79], which could, therefore, obviate the need for resorting to tumour tissue. Biomarkers that can be derived directly from routinely collected laboratory values do not require extra resources [87] and allow to quickly

evaluate dynamic changes during treatment [83], assisting, for example, in restaging decisions when imaging assessment is uncertain [88].

Integrated Artificial Intelligence

As seen in the previous sections, artificial intelligence paves its way into many branches of medicine, for a wide variety of use cases. Quite often, the key advantage offered by AI methods is that of automation and labour reduction. This is mainly due to the historical advantage that computational methods have when it comes to monotonous, repetitive tasks. AI models tend to perform better when there is less variability with a single class in the input. However, real-world data is heterogeneous, particularly in the medical setting. In part, due to this high variability, the predictive performance of AI models in each of the disciplines has often been below what is expected from trusted clinically implemented models (AUC > 0.9).

This limitation in the predictive performance of medical AI models has triggered the call for the development of an 'Integration AI System' (also known as integrated diagnostics) [89]. The fundamental hypothesis of this concept is that data from different medical disciplines would contain complementary information that could be harnessed by a neural network to boost its predictive performance (Fig. 20.3).

In modern healthcare facilities, it is very often the case that a patient generates data from the moment that they enter the front door. A clinician will take their medical history and perform a physical examination, radiological images and pathological slides are obtained and even genomic data/fluid biopsy data is acquired. Each of these disciplines has identified prognostic and predictive markers within their respective fields. However, it is likely that individual biomarkers alone might not have sufficient predictive power. We believe that future immunotherapy biomarkers

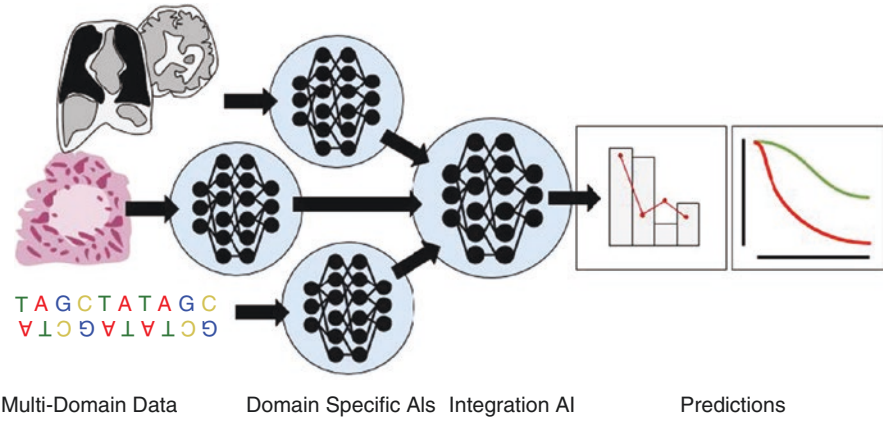

Fig. 20.3 Representation of an integrated AI system, where individual predictive models receive a separate input and then the output of those models is integrated into a new neural network

with sufficient discriminatory power to predict response/prognosis will involve a comprehensive multiparametric approach, including multidimensional biomarkers obtained from whole-body imaging, pathology, peripheral blood markers and omics-based biomarkers rather than single-analyte biomarkers alone. By simultaneously integrating composite biomarkers and their dynamic interactions, machine learning allows superior response prediction and prognostic performance when compared to manual biomarker selection.

The Clinical Trial of the Future

Newly discovered treatments for patients have to endure a clinical trial before they can enter the market. However, problems that arise with developing a clinical trial are numerous, driven by the ever-increasing complexity of these trials [90–94]. AI has the potential to counter these problems and can improve parts of the clinical trial workflow, such as trial design, patient selection and patient monitoring, which could have a massive impact on the speed of implementing cures for cancer [94, 95]. AI can explore massive datasets and find relations humans cannot comprehend. However, it holds new challenges for implementation [96]. The objective of specialised AI algorithms that we have at the moment is not to supplant clinical trials but rather augment them – with the ultimate aim of optimising clinical benefit.

In this section, we discuss the key challenges in clinical trials for immunotherapy and propose AI-based solutions, hypothesising the 'clinical trial of the future' (Fig. 20.4).

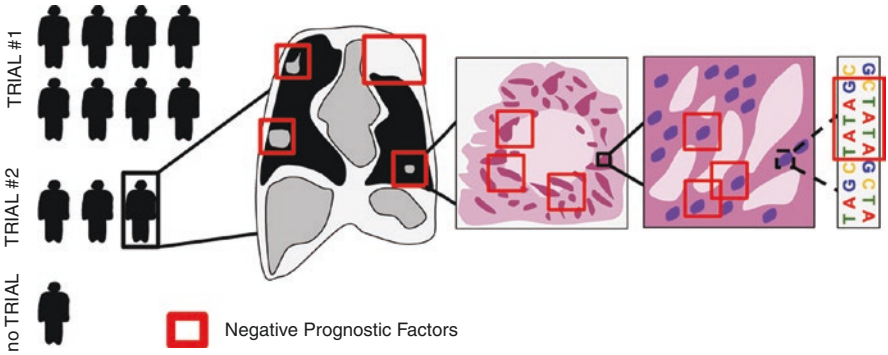

Fig. 20.4 Visual representation of how AI could be implemented in clinical trials. In this figure, patients are classified into different trials (or recommended against a trial altogether) based on the presence of predictive and prognostic factors in the input data (e.g. radiological image, pathological slides, genetic data)

Challenges Currently Faced by Clinical Trials

Testing a newly discovered immunotherapeutic agent in a clinical trial for FDA approval is laborious, costly and logistically challenging. Here, the design of the trial is of the utmost importance. The inclusion and exclusion criteria should generate a statistically similar population to the targeted population [92]. The inclusion of unsuitable patients in the cohort is detrimental, and the response criteria should be accurately defined since an offset in thresholds for inclusion criteria can doom the entire trial [90, 91]. The lack of a standardised method to determine tumour immune response that is universally accepted troubles this process further [90], although the irRC, irRECIST and iRECIST criteria offer guidelines [97–99]. However, these guidelines track tumour progression by measuring the diameters of the tumour, resulting in possible inadequate estimations of the tumour growth due to the inability to calculate tumour volume. Biomarkers reflect dynamics of deeper mechanisms on the molecular level. Generally, biomarkers are obtained upon assessment of biopsies from single-tumour lesions. Here, biomarker dynamics, tissue heterogeneity and spatiotemporal dynamics in biomarker expression limit the reliability and representability of potential biomarkers obtained from limited tumour tissue [100].

Furthermore, preventing underpowered clinical trials due to troublesome patient recruitment is another challenge [94]. A study found that 25% of cancer trials did not meet the required enrolment [101]. Other studies found that for a given cohort of patients, only 5% end up enrolled in a cancer trial [102], while only 18% of the cohort would be ineligible for a trial [103]. Moreover, clinical trials compete for patients to enrol, causing a higher risk of underpowered results for competing trials.

Solutions that Can Be Offered by AI

AI's ability to navigate through the massive maze of clinical trial criteria exceeds the capability of humans. It can learn features from thousands of trials and is able to return the best matches based on the results of previous trials. This can yield problems for normal computer programs as they are unable to extract contextual information. Natural language processing (NLP) is a specific AI method to retrieve and process the textual context. Currently, GPT-3 [104] is an incredibly powerful NLP model that can accurately extract context out of the text and generate text itself if the user describes the concept.

We can implement such a model for several vital improvements:

1. It can compare the proposed specifics of the study to the ongoing studies with a similarity measure when presenting a new study. To prevent competing trials, the authors can decide whether they want to cancel a trial when the proposed study is almost identical to an ongoing one. Moreover, one could think of an application database where studies are preliminarily compared to detect potential collaboration, increasing trial study capacity.

2. To improve patient enrolment and decrease dropout, the NLP model can analyse information about the patient to retrieve the best fit for a study, yielding higher power for statistical analysis.
3. To predict the probability of success, the AI can analyse previous studies, indicating the risks stakeholders are taking. As long as the studies are documented in an accessible database, such an NLP model can continuously learn. These applications have the potential to improve the pipeline of clinical trials but still rely on human designs for the trials. Here, AI can boost the design to enhance cohort composition and patient monitoring [105]. Based on the specifics, like cancer type and molecular structure of the proposed therapeutic agent, AI can predict optimal inclusion and exclusion criteria to involve. Furthermore, AI can learn relations in biomarker expression and discover complex characteristics beyond human ability. By clustering the AI's predictions based on the biomarkers, we can gain insight into the discovered relations.

AI can improve the tracking of tumour growth by automatically segmenting the tumour within seconds after acquiring the imaging scan. By tracking the segmented tumour volume over time, the AI model gives a more accurate indication of the response to the treatment than the current state of the art of measuring the diameters in three axes, which will improve the robustness of the clinical trials. Naturally, clinical trials, as we currently conduct them, will benefit greatly from the implementation of artificial intelligence in each of the diagnostic disciplines that form the backbone of the clinical trial.

An AI-Powered Clinical Trial

Traversing even further into the (far) future, the most exciting concept would be to transcend traditional clinical trials completely. Imagine a powerful AI model that enables feature matching of genomes to generate patient-tailored therapeutic agents that activate the right immune response. Here, AI has the potential to not only accurately predict the success rate of the discovered agent but also predict the side effects for the patient-agent combination. The AI model can find the optimal agent for each specific patient by processing the vast amount of data acquired over the years. This, in turn, could yield therapeutic agents' characteristics that are effective for particular cancerous cells without damaging healthy cells. The aim is not to test the therapeutic agent that targets specific cells in a clinical trial but rather the AI that generates these agents. Once the trained AI can generate reliable, safe therapies for each individual patient, it only needs to be validated once versus the current state-of-the-art in a massive trial. The CE and FDA can approve the method of generating the therapies, instead of approving each patient-tailored therapy after a separate trial. The moment such an immensely powerful method is available and validated, clinical immunotherapy trials will become redundant.

Regulations in clinical trials are of utmost importance to guarantee patient safety and standardised comparison methods. When AI is available to improve trials, regulations specifically for AI should already have been constructed. The SPIRIT-AI and CONSORT-AI guidelines are defined for developing and reporting clinical trials involving AI [106, 107]. Researchers should always be up-to-date with the current guidelines since the rapidly evolving nature of AI will constantly result in new challenges and concepts to regulate.

To conclude, clinical trials in immunotherapy suffer from challenges that prevent the treatment from reaching its full potential. AI can improve the pipeline of clinical trials and has the potential to resolve multiple challenges. When regulated correctly, it holds tremendous capabilities, and we hypothesise that AI has to be part of the clinical trial of the future to develop a patient-tailored immunotherapy treatment.

Conclusions

Artificial intelligence has only just begun to have an impact on the medical field. Research is ongoing in the diagnostic disciplines, particularly radiology, to test the limits of existing predictive algorithms. The future of radiomics is especially promising considering the trend towards increasing resolution of radiological images. This trend could unlock even more information encoded in the image and yield better imaging markers/phenotypes. Despite the impressive achievements of predictive models in research, many of these networks are only tasked with detecting small abnormalities, neglecting a number of biological/clinical characteristics of the tumour for the sake of simplicity [108]. This serves as a reminder that AI is not intended as a replacement for the healthcare team but rather as a support tool to help guide decisions.

One long-standing challenge for medical AI has been generalisability of the predictive model to real-world data. A classical technical solution would be to expand the training data, but in the medical setting, this is often infeasible due to limited patient cohorts. This challenge may yet be overcome with the integration of different data types within an integrated AI system.

Finally and possibly the most profound question that needs to be solved before AI can be implemented in the clinics is: ‘Who is responsible for the predictions an AI algorithm makes?’ [109]. This socio-philosophical/medicolegal enigma remains unsolved and may prove to be one of the largest hurdles for mainstream adoption of AI in clinical trials and daily practice. While many open questions have yet to be answered, the impact that artificial intelligence will have on the domain of healthcare is undeniable. Man and machine need to work together for the betterment of patient care.

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Chapter 21
The Role of Imaging in Tumor Staging and Response Assessment: Envisaging an Application for the Next-Generation Trials

Antonella Messina, Giuseppina Calareso, and Alessandra Alessi

Introduction

Bladder cancer (BC) represents 4.6% of total cancer diagnoses and is more frequent in males [1]. The great majority of BC are urothelial cell carcinomas (UCC), and based on histopathology, they are defined as muscle-invasive (MIBC) and non-muscle invasive BC (NMIBC) [2]. Identification of the disease is usually made by cystoscopy after the insurgence of hematuria and/or dysuria [3]. Diagnosis is surgical with trans-ureteral resection of bladder cancer (TURBT), which is generally used as a definitive treatment in the NMIBC and for diagnosis in the MIBC [4]. A correct staging of the BC, based on the evaluation of the primitive lesion and lymph nodes involvement, is essential for prognosis and therapy. For many years, chemotherapy has been the only choice of treatment, but in recent years with the introduction of immunotherapy, a new criteria of response assessments have been developed. Imaging plays an important role in assessing the extent of BC for which ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography/computed tomography (PET/CT) are used [5].

A. Messina (✉) · G. Calareso
Department of Radiology, Foundation IRCCS National Cancer Institute, Milan, Italy
e-mail: Antonella.messina@istitutotumori.mi.it

A. Alessi
Nuclear Medicine Unit, Department of Radiology, Foundation IRCCS National Cancer Institute, Milan, Italy

A. Necchi, P. E. Spiess (eds.), *Neoadjuvant Immunotherapy Treatment of Localized Genitourinary Cancers*,
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Ultrasonography

Ultrasonography (US) is a noninvasive, first-level diagnostic method used as a screening test in the presence of hematuria. It is performed with a curvilinear probe (2–5 MHz) in a patient with a well-distended bladder. By using a high-frequency probe, US can differentiate three out of four layers of the bladder wall (the muscolaris propria appears as an hypoechoic line between the superficial serous layer and the hyperechoic mucous and submucous layers) [6, 7]. Urothelial bladder carcinoma appears echographically as an irregular focal or diffuse thickening of the bladder wall or as a plaque of the wall [8]. It may appear hypoechoic, isoechoic, or hyperechoic depending on the presence of fibrosis, calcification, and hemorrhage. To stage the BC, it is necessary to assess the degree of infiltration of the bladder wall and invasion of the muscolaris layer is suggested by the disappearance of the hypoechoic intermediate layer. Although some studies report an 80% accuracy of US to detect BC [9], there is only limited experience to support the use of US for the staging of BC. In fact it plays only a limited role in the diagnosis of bladder tumor, in particular in the identification of small-size carcinomas. The use of contrast medium, however, improves the diagnostic accuracy of this technique; it is reported that accuracy of Contrast Enhanced Ultrasound (CEUS) to detect BC is 90.9%. [10, 11]Despite this evidence, US is operator dependent and the ability to identify the lesion is affected by the presence of adjacent organs and compliance of the patient.

Computed Tomography

CT scan is a second-level technique, which requires ionizing radiation. It is useful in the pre-operative staging of the tumor, in the evaluation of the response to neoadjuvant treatment, and in the follow-up after radical cystectomy. According to the latest guidelines of the National Comprehensive Cancer Network (NCCN), the presence of a solid tumor of high grade or potentially invasive solid tumor necessitates either a CT or an MR for the staging of the local lesion before TURBT [12]. CT plays an important role in evaluating macroscopical cancers invasive of the adipose tissue or the adjacent organs (T3b; 83.3% accuracy and 100% precision); however, the thickening of the adipose tissue may sometimes be related to the inflammatory reaction or post-bioptic desmoplastic reaction, thus causing false-positive results, such as loss of the clivage planes, which is not always a sign of infiltration of the adjacent organs [6, 13]. In such a case by the use of multiplanar reconstruction, it is possible to detect an involvement of the adjacent organs. Furthermore, CT does not permit the detection of infiltration of the muscolaris mucosa, even though it has been suggested that a retraction of the wall of the BC represents muscle involvement [6, 14]. However, many studies by multidetector CT show an accuracy degree between of 89% and 91% and specificity between 92% and 95%, [13, 15]. CT is useful, however, to detect distant metastases

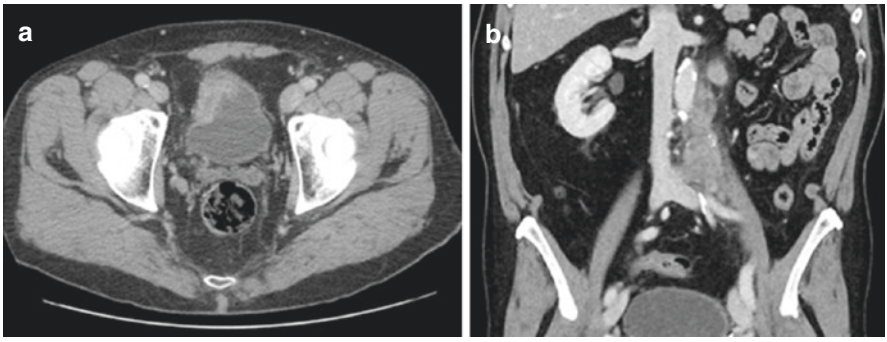

Fig. 21.1 Axial CT image of the bladder (**a**). Large lesion of the anterior right lateral wall of the bladder. Coronal CT image of the abdomen (**b**) shows paraaortic lymphadenectomy

(lymphogenous or hematogenous) and is recommended before cystectomy to exclude other different causes of hematuria (urinary stones, trauma, infection, and renal cancer). CT scan detect lymph node metastases in BCa with a sensitivity ranging between 31% and 50% and a specificity ranging between 68% and 100% [16]. CT has a limited role in the assessment of locoregional response after neoadjuvant therapies to differentiate residual tumor from inflammatory processes; to avoid this limit, some studies suggest the use of computer-aided diagnosis (CAD). Using radiogenic learning algorithms and characteristics can increase the accuracy of CT in identifying the complete response in the infiltrating muscle tumor [17].The usefulness and accuracy of CT in predicting lymph node response after adjuvant therapies are not fully shared. Recist criteria are used to evaluated the response to treatment in BC, as in other solid tumors also in BC are used the criteria RECIST 1.1 or irRECIST; among the limits of these criteria, the cutoff indicated for lymph node size must be considered. According to some studies, the reduction of the cutoff to 6 mm and assessments of morphological or contrast criteria increase the accuracy of CT in diagnosing lymph node involvement after neoadjuvant therapy [18] which, as it is known, affects the survival of BC patients after cystectomy and lymphadenectomy (Fig. 21.1).

Magnetic Resonance Imaging

The European Association of Urology (EAU) guidelines have not recommended any well-defined criteria for the diagnosis of bladder tumor, and MR is requested only when CT cannot be performed. MR, unlike CT, does not use ionizing radiation, offers superior soft tissue contrast, and provides more anatomical and functional information [19]. MR also differentiates MIBC from NMIBC and visualizes extramural invasion and T3b and T4 disease [20, 21].

Table 21.1 The 5 scores used in the VI-RADS classification system

Score	VI-RADS (Vesical Imaging-Reporting and Data System)	T2	DCE	DWI	ADC
1	Small (<1 cm) exophyitc tumor with or without peduncle with a thickend internal layer, but with an inatct muscolaris propria which appears as an uninterrupted line of low signal				
2	A larger tumor(> 1cm) exophytic with a larger base peduncle with thickening of the internal layer if present, and an uninterrpted line of the mouscolaris propria				
3	A lack of the findings of SC2 with a exophytic tumor without peduncle or sessile tumor, with wide base without thickening of the internal layer and without clear interruption of the muscolaris propria				
4	Interruption of the inferior line suggesting the invasion by the tumor of the muscolaris propria				
5	Extension of the tumor to the adipose tissue outside the bladder indicating invasion of the entire wall and other exterior bladder tissue				

Multiparametric MRI (mpMR) is the most accurate method for studying BC. The mpMR includes a morphologic study with multiplanar high definition T2 weighted sequences, a cellular density study with DWI sequences (Diffusion Weight Imaging) with b 0-800-1000 and ADC map and a Perfusional study with contrast medium intravenous (DCE) to evaluate the vascularization of the lesion. Correct bladder filling is required with or without cateheterization.

In 2018, Panebianco and colleagues introduced a new way to diagnose and stage primary BC with the development of an mpMR imaging protocol called "VI-RADS" (Vesical Imaging-Reporting and Data System), consisting of a 5-score tool as indicated in Table 21.1 (modified from Panebianco et al. [26]).Today, this system has been endorsed by the guidelines of the European Association of Urology (EAU). This protocol provides higher level of accuracy in the staging and diagnosis of BC, in particular in the differentiation of superficial from muscle-invasive tumor, thus requiring less-invasive methods for diagnosis and staging, particularly useful in patients with severe comorbidities. There are however some drawbacks in VI-RADS protocol regarding the staging of the primary lesion (30% are multifocal) and the fact that the upper urinary tract cannot be reliably assessed. According to the available data, VI-RADS criteria cannot be also applied to images from patients undergoing treatment, and it is not validated as a method to assess tumor response to treatment. Based on experiences reported by other authors, mpMR images could also represent a further aid to physicians in response assessment to neoadjuvant therapies. It is important to have baseline and post treatment MR exame to evaluate tumore response (complete response or partial response) (Figs. 21.1, 21.2, and 21.3) or disease progression is considered (Fig. 21.4). Treatment for BC may currently include chemotherapy (CT) and immunotherapy. Regardless of the

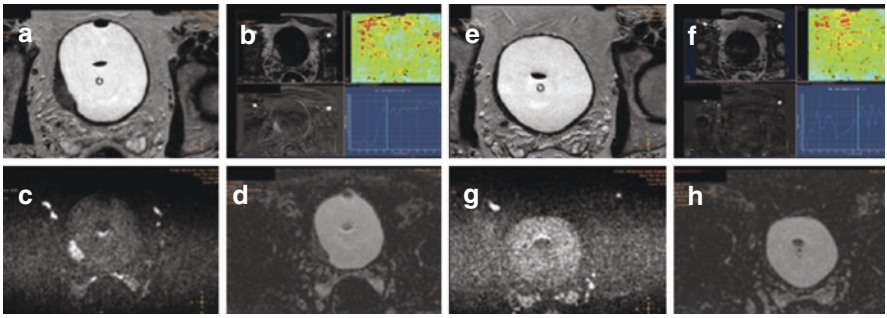

Fig. 21.2 Pre-treatment images (**a**–**d**) and post-treatment images (**e**–**h**) . In the pre-treatment images, a large muscle-infiltrating lesion of the right lateral wall of the bladder is observed: hypointense in T2 (**a**), with enhancement after contrast medium with a type 2 contrast intensity-time curve (**b**); hyperintense in native DWI (**c**), and hypointense in ADC maps (**d**). The post-treatment images show the disappearance of the lesion with bladder wall returning within normal limits. In this case, there was a complete response

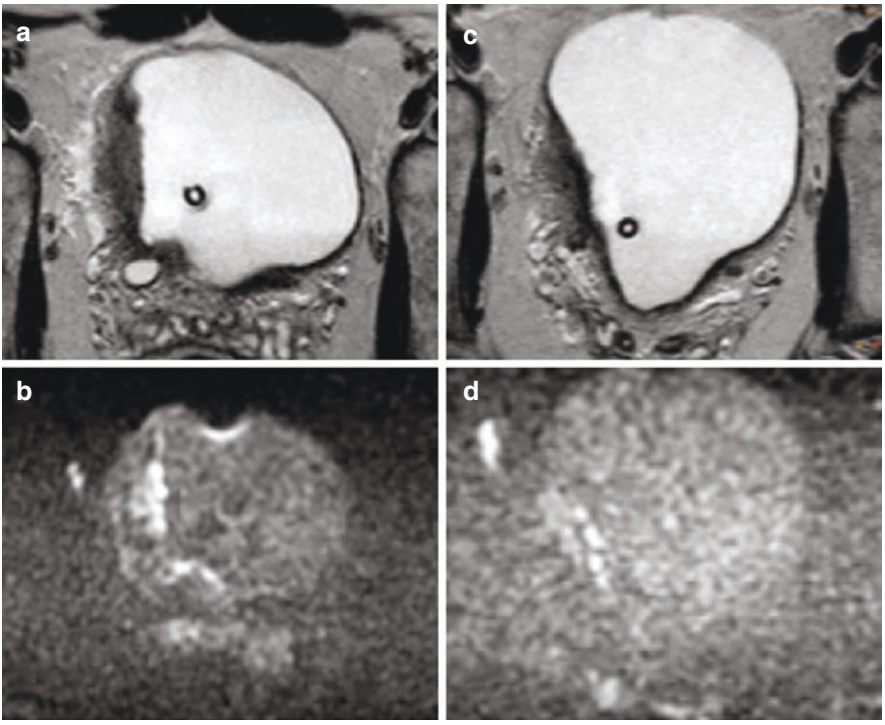

Fig. 21.3 In baseline MR images (**a**–**b**), there is a plurinodular lesion, hypointense in T2 (**a**) and hyperintense in DWI in sequences with a high b-value. After treatment (**c**, **d**), there is a moderate size reduction of the lesions. Most visible micronodularities persist in DWI (**d**) sequences with high b-value. Thsee features indicate a partial response to treatment

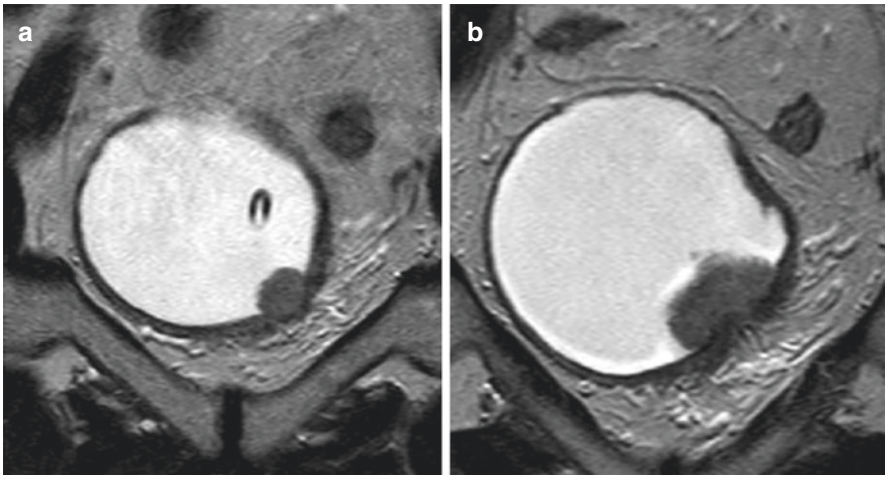

Fig. 21.4 T2- weighted coronal image pre treatment (**a**) and T2 weighted coronal image post tretment (**b**) These images show an increas in size of the lesion of the left lateral bladder wall (progression desease)

therapeutic option, patients are always subjected to cystoscopy and endoscopic resection of the existing lesions, including those with thickening of the bladder walls due to inflammation. Therefore, the baseline MRI images may be difficult to interpret due to the persistence of disease in the context of an inflamed thickening of the bladder wall. In this case, the T2 and DWI sequences are more reliable if they are jointly assessed. While the evaluation of response to standard chemotherapy may be easier, in patients treated with immunotherapy, the post-therapy stromal tissue is generally more complex than after chemotherapy, as it is usually characterized by a significant recruitment of T-cells that surround the residual disease resulting in a major inflammation in the bladder wall [14]. All these features can make hardly detect any micronodular disease (Fig. 21.2). DWI sequences seemed to be the most reliable sequences in association with T2 sequences. In the near future, a biparametric MR study of the bladder could be performed without using contrast medium. This feature might in part explain the discordance between the mean values of ADC after neoadjuvant chemotherapy and the hystopathological response which can result in unreliable quantitative post-immunotherapy evaluation. On the contrary, the changes in the mean ADC values after neoajuvant chemotherapy or chemoradiotherapy have been reported to be the first markers of response in bladder tumors [22, 23]. The finding that 20% of patients who are thought to have had a total tumor eradication, although in agreement with some published data, should be taken with extreme caution, because it might be inconsistent with the hystopathological final results. Such limitations are entirely consistent with previous studies, which show that there is no advantage in staging the tumor by MR (including mpMR) with a total accuracy ranging from 56% to 62% and with an overestimate

ranging from of 32% to 38% [24, 25]. The findings obtained with mpMRI are interesting and could be used in the future to evaluate the individual role of each parameter of the pathological response [26]. Nonetheless, it is evident that the morphological response of the tumor alone is insufficient to evaluate the total response and, in a substantial percentage of reports, difficult to interpret because they are either mixed or incomplete, or there have been tissue changes in the lesion after treatment. Such a method to evaluate the pathological response to neoadjuvant treatment in a noninvasive way may have important implications in clinical practice and in the design of future studies on neoadjuvant approaches [27]. In particular, the use of equipment which may lead to identification of patients who might obtain either a complete or major pathological response could be relevant to identify those patients who are suitable for bladder conservation strategies, thus avoiding cystectomy after an immunotherapy-induced response. In the future, particular attention will be likely attributed to radiomics which, when routinely available, might help radiologists in disease staging and in evaluating the response to treatment of the bladder wall.

Positron Emission Tomography/Computed Tomography

Over the last decade, positron emission tomography in combination with computed tomography (PET/CT) has become an important tool in the oncology field, covering a major role in staging, response assessment, early response monitoring, and the prognosis of many types of tumors. 18Fluorine-2-deoxy-2-fluorodeoxyglucose (^{18}F-FDG) is the most commonly used radiopharmaceutical in PET/CT imaging, which is excreted through the kidney. Therefore, differentiation of bladder pathology or pelvic lymph node involvement from physiological 18F-FDG activities is difficult [28]; to overcome these limitations, several strategies can be applied such as bladder catheterization and forced diuresis, but they are rarely used in clinical practice. Many authors also evaluated the use of alternative tracers such as 11C-choline, 11C-acetate, and 11C-methionine, which have a lower urinary excretion, but these radiopharmaceuticals are not always available [29]. PET/CT may be helpful in the detection of disease outside the bladder at nodal or more distant sites and in the assessment of recurrent disease. The European Urology Guidelines (EAU) do not recommend the routine use of PET/CT in the staging or in the follow-up of BC; therefore, CT and MR remain the first choice. The NCCN guidelines suggest the possible use of PET/CT with FDG for the staging of selected patients (>cT2 stage), to establish the presence of locoregional or distant lymph nodal involvement, and to evaluate suspicious relapses and/or metastasis [12]. According to some studies, FDG PET/CT has a sensibility of 56% and a specificity of 98% in revealing lymph nodal metastases of BC, thus demonstrating major diagnostic accuracy in staging with regard to the exclusive use of CT [30, 31]. Meta-analysis aimed at comparing imaging methods to assess pelvic lymph nodes involvement in patients with BC showed a slightly

reduced percentage (22%) in recognizing metastases by MRI (22%) compared to CT and PET/CT (both 29%). However, the values showed great variability. The accuracy of CT imaging ranges between 56% and 60%, MR between 67% and 95%, and PET/CT between 64% and 94%. An accurate clinical staging of pelvic lymph nodes is still an open challenge in the field of diagnostic imaging. The use of hybrid methods such as PET/MRI might increase accuracy and resolution in the pelvic disease. The diagnostic performance of MRI has been compared to that of PET/MRI in a study conducted in 22 patients with BC. PET/MRI showed greater accuracy in detecting the primary lesion (86% vs 77%), the pathological pelvic lymph nodes (95% vs 76%), and extranodal disease (100% vs 91%) [32]; its use is still controversial. For several decades chemotherapy has been the only therapeutic alternative in BC: either as neoadjuvant therapy for muscle-invasive tumors localized to the bladder, or as first-line treatment of locally-advanced or metastatic tumors, or for post-surgical adjuvant purposes [33]. The recent approval of anti-PD-1/PD-L1 treatment in urothelial cancer has expanded the therapeutic approach. The different mechanisms of therapeutic action led to unusual pictures of treatment response, with the recognition of phenomena of "flare" or response patterns that simulate a "pseudo-progression" mistakenly interpreted as progression of disease [34]. The consequence of this phenomenon led to the proposal of new criteria of response assessment by PET/CT. Among the major changes proposed by this new approach, we find the concept of "total burden of disease," according to which the tumor extension must be evaluated as a whole and not as appearance/remission of single lesions. Currently, preliminary clinical data, PURE-01 trial (NCT02736266), which proposed the use of pembrolizumab as neoadjuvant, before radical cystectomy in patients with MIBC, does not justify the use of FDG PET/CT in clinical practice [35]. In treatment with immune checkpoint inhibitors, PET/CT may be helpful in the early detection of immuno-related adverse events (irAE), whose long-term impact has yet to be defined. The opportunity to radiolabel monoclonal antibodies PD-1 and PD-L1 [36], in order to recognize and trace the distribution of the drug "cold," assess the extent of tumor collection, as well as its variations over time, and identify the subjects that would benefit from treatment, could give PET/CT an important role in this scenario.

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Chapter 22
The Future of Artificial Intelligence Applied to Perioperative Immunotherapy Trials: Prostate Cancer

Alberto Martini and Francesco Montorsi

Prostate cancer (PCa) represents the first non-cutaneous malignancy for incidence in men [1]. Its prognosis can be highly heterogeneous, spanning from a relatively indolent course to a more aggressive and rapidly progressive disease. While most men with PCa will likely succumb *with* it, almost one in eight men during their lifetime will be diagnosed with this tumor. This gives the reader the idea of how important is to properly address the fraction of patients with more aggressive disease and the magnitude of such potential benefit.

Starting from few years ago, the major urological guidelines have incorporated multiparametric prostate magnetic resonance imaging (mpMRI) in the diagnostic pathway of PCa. Patients with a clinical suspicious of PCa should now undergo mpMRI before any biopsy is performed; thus virtually all patients would have imaging before any active treatment is considered [2]. This implementation has led to the study of radiomic features to identify pathological and clinical behaviors of the area(s) suspicious for tumor. This evaluation by means of artificial intelligence (AI) has important clinical implications. It can help in avoiding unnecessary biopsies but also can help characterizing the tumor pre-biopsy and ultimately, in case a neoadjuvant therapy is administered, can help in evaluating the eventual response to neoadjuvant therapy.

Briefly, AI is currently used as an umbrella term to describe those processes that focus on creating an artificial and intelligent machine that can successfully perform human tasks. This is realized by nonlinear mathematical modelling systems that are

A. Martini (✉) · F. Montorsi
Unit of Urology, University Vita-Salute, San Raffaele Scientific Institute, Milan, Italy

Division of Experimental Oncology, URI, Urological Research Institute, IRCCS San Raffaele Scientific Institute, Milan, Italy
e-mail: martini.alberto@hsr.it

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composed of basic blocks which mimic the human neurons [3]. The ultimate goal of AI is to recognize ways in which humans think and/or perform tasks, thus translating human intelligence into machines. When the algorithms are developed from existing data and the "machine" performing the task is learning from this data, one can refer to the overall process as "machine learning." If this process is performed on images, it is called "computer vision." Yet, the ultimate goal of all the different artificial intelligence is to mimic and/or be more precise than human intelligence. The application of these processes can span from the diagnosis to the evaluation of treatment response in different cancers. Regarding PCa, many studies have evaluated the role of AI on biopsy specimens to help diagnosing and grading cancer on biopsy.

Arvaniti et al. reported on an AI score annotator and used the model's predictions to assign patients into low-, intermediate-, and high-risk groups, achieving stratification results similar to those of an experienced pathologist [4]. Donovan et al. proposed an innovative platform which is able to discriminate between low-, intermediate-, and high-risk PCa and also predict the likelihood of recurrence during follow-up [5]. These findings can be taken into account when designing novel prospective studies, especially if the same AI algorithm is used to evaluate pre- and posttreatment pathology changing, together with the same pathologist.

Regarding pre-biopsy and pretreatment imaging, few studies have evaluated the role of AI for the identification of suspicious tumor area(s) and the characterization of such area(s) [6]. In combination with the prostate imaging reporting and data system (PIRADS), accurate AI algorithms can increase the reliability of the exam and improve the diagnostic accuracy of mpMRI and ultimately its interpretation [7]. On this matter, Ishioka et al. developed AI algorithms aimed at estimating the area in which a targeted biopsy may detect the presence of cancer ultimately leading to a reduced number of unnecessary biopsies [8].

Interestingly, Beksac et al. found that PIRADS score is correlated with a genomic classifier based on 22 genes (Decipher®, GenomeDx). Ideally, changes in the PIRADS score post-neoadjuvant therapies could correspond to changes in the tumor genomics [9]. Similar findings by Hectors et al. support that radiomic features are correlated with the genomics of the prostate tumor [10]. If these findings are confirmed in future studies, the incorporation of PIRADS lesion changes, in terms of radiomic features, in response evaluation could have an important impact in practice, especially to evaluate the potential response to neoadjuvant immunotherapy and delay or avoid radical treatment.

In conclusion, there are encouraging findings in the field of AI in PCa. No study has yet evaluated the role of AI applied to imaging to evaluate the response to perioperative therapy. Yet, there are encouraging findings on the correlation of radiomic features and pathologic features and radiogenomic features, which are the correlation between radiomic and genomic tumor characteristics.

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Chapter 23
The Future of Artificial Intelligence Applied to Perioperative Immunotherapy Trials: Renal Cell Carcinoma

Alberto Martini and Alessandro Larcher

Renal cell carcinoma (RCC) encompasses a spectrum of different malignant disease that represents the sixth cancer in men and the tenth in women for incidence overall [1]. Over time, the incidence of RCC has been increasing, and at the same time, there has been a shift toward more localized disease at presentation; this phenomenon is particularly true in high-income settings [2]. Despite that, up to 40% of the patients still has metastatic or locally advanced disease at diagnosis [3].

While surgery still maintains its pivotal role in the treatment of RCC, the relatively poor prognosis of certain subgroup of patients has led to the introduction of therapies in the perioperative settings. Such studies are aimed to reduce the risk of local and distant disease recurrence, with the ultimate goal of improving patient's prognosis while maintaining an adequate quality of life [4–6].

Similarly to other tumors, medical therapies aimed at improving patients' prognosis have been initially studied in the setting of metastatic disease. Following positive results, the administration of these therapies has then been "shifted" toward earlier stages. The latter is the case for sunitinib, a medication that determined changed the field for the treatment of RCC [7].

More recently, newer therapies that trigger the individuals' immune response have been first investigated in patients with metastatic disease and are now tested in the adjuvant and neoadjuvant settings. This is the case of nivolumab, another medication that determined a paradigm shift for the treatment of RCC. This medication was first introduced in 2015 [8]. Immune checkpoint inhibitors have been thoroughly described in the prior chapters of the book.

Presently, there are nine studies aimed to evaluate the role of immunotherapy in the neoadjuvant setting and four studies in the adjuvant setting [4, 5]. It is obvious

A. Martini · A. Larcher (✉)
Unit of Urology, Division of Experimental Oncology, URI – Urological Research Institute, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

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that if one can ascertain the pathological complete response to neoadjuvant immunotherapy through imaging, a period of oncological surveillance could be offered, and extirpative surgery could potentially be delayed or, in the best-case scenario, avoided completely. Furthermore, to predict response to anticancer drug is key in oncology, and in this context, the use of artificial intelligence (AI) algorithms could provide detrimental information and help clinicians and patients to take a shared decision on, for example, oncologic surveillance in case of apparent downstaging, oncologic surveillance, and delayed surgery.

In the prior chapter, we have already introduced the concept of AI. We are now going to focus on the current evidence in RCC and its potential future developments.

A recent systematic review was performed to evaluate the role of the application of AI on imaging to identify benign and malignant masses. The authors found a lot of heterogeneity in the studies and in the methodologies, and many of the articles included did not compare the performance of AI to the one of radiologists. Artificial intelligence refers to the extensive diversity of methodology aimed at the development of models without a priori strict rule-based programming but with the ability to improve and correct themselves through experience. For instance, machine learning methods fall into such definition. However, a precise threshold distinguishing machine learning from traditional statistical modeling is still lacking, and hybrid modeling represents the most common scenario in the field of medical research.

The authors concluded saying that before bringing an AI-based characterization into practice, more studies are needed [9]. Certainly, the characterization of benign and malignant renal masses represents the first step for bringing AI into practice. This is the first issue that needs to be addressed and will have important implications also for patient selection for clinical trials and could potentially avoid renal mass biopsy [10].

Kocak et al. have evaluated a computed tomography (CT) texture analysis for differentiating histological subtypes based on texture features. They concluded that their machine learning algorithm is able to distinguish non-clear cell RCC from clear cell RCC with a Matthews correlation coefficient of 0.8 [11]. This information can be precious, especially in the era of targeted therapies and personalized medicine for the selection of the best perioperative treatment regimen. In a similar study, Lin et al. report that an AI algorithm is able to differentiate quite well low- from high-grade clear cell RCC [12]. This would have important implication for candidates to neoadjuvant therapy as well.

Only one study so far has studied the use of AI in the evaluation of response to nivolumab. Khene et al. evaluated the radiomic characteristics of patients with metastatic RCC pre- and post-nivolumab. They evaluated 279 radiomic features from the CT scans (pre- and posttreatment) of 48 patients (Fig. 23.1).

They report that their best AI model was able to predict response in more than 90% of the patients. One limitation of the study is the fact that all patients had received anti-angiogenic therapy before nivolumab and, as the authors appropriately pointed out, the radiomic features would best be explored in patients receiving first-line immunotherapy [13]. Nonetheless, this report clearly recapitulated the major advantages of machine-learning based radiomics, namely, that it gives

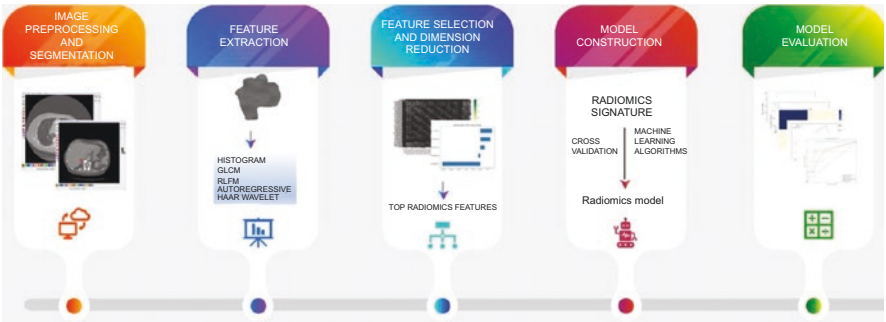

Fig. 23.1 Simplified flowchart showing the machine learning-based radiomic analysis pathway. (Adapted with the permission of the authors from Khene et al. Radiomics can predict tumor response in patients treated with nivolumab for a metastatic renal cell carcinoma: an artificial intelligence concept- WJU 2020)

immediate results, it is an objective method that does not need human interpretation, it could be easily integrated into routine radiological assessment, it is noninvasive, and finally it has the ability to take into account tumor heterogeneity in time and space.

In conclusion, there is definitely room for improvement and implementation of AI algorithm in the context of perioperative immunotherapy for RCC. Hopefully, the current ongoing clinical trials will provide more data and results on whether imaging features are reliable enough to potentially delay or avoid surgery.

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Chapter 24
Clinical Trial Corner

Shilpa Gupta and Guru P. Sonpavde

Emerging Evidence and Future Role of Neoadjuvant Immunotherapy in Muscle-Invasive Bladder Cancer (MIBC)

Checkpoint inhibitors have revolutionized the treatment paradigms of advanced and metastatic urothelial cancer and shown promising efficacy and safety in early phase trials when used as neoadjuvant treatment as single agent, anti-PD-1/PD-L1 agents, anti-PD-1/PD-L1/CTLA 4 doublets, or in combination with chemotherapy in early phase trials [1–8] (Table 24.1).

Although limited data is available for long-term survival from the early phase trials, these trials have demonstrated that immunotherapy can be used safely and effectively prior to definitive surgery in bladder cancer. Ongoing phase III trials are investigating the role of immunotherapy in perioperative setting, both in patients who are eligible to receive cisplatin-based neoadjuvant chemotherapy (Table 24.2) and those who are cisplatin-ineligible (Table 24.3). Notably, these trials are extending the use of immunotherapy post-surgery as well.

Results from these trials will help determine the role of immunotherapy in perioperative setting and provide an insight on whether combination trials of checkpoint inhibitors with antibody drug conjugates (ADCs) like enfortumab vedotin (EV) can help eliminate the use of cisplatin-based chemotherapy.

S. Gupta (✉)
Department of Hematology and Oncology, Taussig Cancer Institute, Cleveland Clinic Foundation, Cleveland, OH, USA
e-mail: Guptas5@ccf.org

G. P. Sonpavde
Department of Medicine, Dana Farber Cancer Institute, Boston, MA, USA
e-mail: GuruP_Sonpavde@dfci.harvard.edu

A. Necchi, P. E. Spiess (eds.), *Neoadjuvant Immunotherapy Treatment of Localized Genitourinary Cancers*,
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Table 24.1 Phase I/II clinical trials of neoadjuvant immunotherapy in MIBC

	GU14–188 Gemcitabine + pembrolizumab NCT02365766	NABUCCO Ipilimumab + nivolumab NCT03387761	ABACUS atezolizumab NCT02662309	PURE-01 pembrolizumab NCT02736266	BLASST-1 Nivolumab + GemCis NCT03294304	Durvalumab + tremelimumab NCT02812420
N	37	24	88	114	41	28
cT2	43%	0	73%	43%	90%	58%
cT3/T4	57%	58%	27%	57%	7%	42%
cN+	0	42%	0	0	3%	0
pT0N0 rate	45.2%	46%	31% (includes CIS)	37%	49% (includes CIS)	37.5%
pT ≤ 1N0 rate	51.6%	58%		55%	66%	58%
RFS	93.6% at 12 mo	92% at 15.6 mo	79% at 12 mo	91%	Not mature	82.8%
Gr 3–4 AEs	84%	54% (irAE)	11%	Initial report 5% (N = 43)	0	21%
RC delay/withheld due to TRAE	No	Yes 4%	Yes 3%	No	No	Yes 14%
Surgical complications	Not reported	Not reported	60% 1 death	34%	Not reported	Not reported

Table 24.2 Phase III perioperative immunotherapy trials in cisplatin-eligible patients with MIBC

Chemo/ADC-immunotherapy combinations					
Clinical trial	Estimated N	Treatment arms	Eligibility	Primary endpoint(s)	Status
KEYNOTE-866 (NCT03924856)	870	Pembrolizumab + Gemcitabine-cisplatin	T2-4aN0M0	pCR rate, EFS	Ongoing
		Gemcitabine-cisplatin			
KEYNOTE-B15/EV-304 (NCT04700124)	784	Pembrolizumab + EV	T2-T4aN0M0 or T1-T4aN1M0	pCR rate, EFS	Ongoing
		Gemcitabine-cisplatin			
NIAGARA (NCT03732677)	1050	Durvalumab + gemcitabine-cisplatin	T2-4aN0M0	pCR rate, EFS	Ongoing
		Gemcitabine-cisplatin			
ENERGIZE (NCT03661320)	1200	Placebo + nivolumab + gemcitabine-cisplatin	T2-4aN0M0	pCR rate, EFS	Ongoing
		Linrodostat + nivolumab + gemcitabine-cisplatin			
		Gemcitabine-cisplatin			

EV Enfortumab vedotin (ADC), linrodostat (IDO inhibitor), *pCR* pathologic complete response, *EFS* event-free survival

Table 24.3 Phase III perioperative immunotherapy trials in cisplatin-ineligible patients with MIBC

Clinical trial	Estimated N	Treatment arms	Eligibility	Primary endpoint(s)	Status
KEYNOTE-905/ EV-303 (NCT03924895)	836	Surgery alone	T2-4aN0M0	pCR rate, EFS	Ongoing
		Pembrolizumab			
		Pembrolizumab + EV			
NCT04209114	540	Surgery alone	T2-4aN0M0	pCR rate, EFS	Ongoing
		Nivolumab + bempegaldesleukin (NKTR-21)			
		Nivolumab			

EV Enfortumab vedotin (ADC), bempegaldesleukin (CD-122 agonist), *pCR* pathologic complete response, *EFS* event-free survival

Role of Adjuvant Immunotherapy in MIBC

Results from the IMvigor010, a phase III randomized trial of adjuvant atezolizumab versus observation in high-risk MIBC, were recently reported [9]. The study failed to meet its primary endpoint of disease-free survival (DFS), and atezolizumab did not improve DFS in the overall population or patients with PD-L1 high tumors. Atezolizumab did not improve overall survival (OS) either [9]. On the other hand, recent results from the phase III CheckMate 274 study showed that up to 1 year of adjuvant nivolumab improved DFS compared to placebo in a similar patient population [10]. A total of 353 patients were enrolled, and the primary endpoint of DFS was met in the overall population and in patients with PD-L1 high tumors. Median DFS was 21 months with nivolumab compared to 10.9 months with placebo in all comers, and in patients with high PD-L1 tumors, DFS was not reached with nivolumab, indicating a more pronounced benefit. Overall survival (OS) data was not reported as it is event driven [10]. CtDNA analysis from the IMvigor010 study provides some evidence that those patients with ctDNA had improved disease-free and overall survival with atezolizumab. The role of ctDNA for identifying patients who may best respond to immunotherapy is warranted in a prospective trial. Long-term follow-up from CheckMate 274 study and results from the ongoing AMBASSADOR study will help establish the role of adjuvant immunotherapy in urothelial cancer. Table 24.4 highlights the completed and ongoing phase 3 adjuvant immunotherapy trials in MIBC. Notably, the perioperative immunotherapy trials in MIBC as outlined in Tables 24.2 and 24.3 also harness adjuvant immunotherapy treatment after neoadjuvant use, and results from these trials will truly establish the most appropriate role of immunotherapy in MIBC. Another ongoing adjuvant trial, PROOF-302, is studying the role of FGFR inhibitor, infigratinib, in patients with invasive urothelial cancer with FGFR alterations [11].

Conclusions and Future Directions

The emerging evidence from early phase trials exploring immunotherapy alone or as combinatorial approaches appears to be promising and generally safe. Ongoing randomized perioperative phase III trials will further elucidate the efficacy of immunotherapy in tumor downstaging and long-term survival and functional outcomes. The definite role of adjuvant immunotherapy in MIBC will be further established in the future as well. The early phase trials have enhanced our understanding of some of the complex biomarkers that can predict response and resistance to immunotherapy in bladder cancer, and we should aim to develop more precision-based immunotherapy trials in the 2020s. Now, more than ever in the brave new world post-COVID-19, we need to rethink how we conduct biomarker-based trials in a cost- and time-effective manner to advance therapeutic options for bladder cancer patients around the world. Traditional large and costly phase III randomized control

Table 24.4 Phase III adjuvant immunotherapy trials in muscle-invasive urothelial cancer (including upper tract urothelial carcinoma)

Clinical trial	Patients accrued or estimated	Treatment arms	Eligibility	Primary endpoint (s)	Status
IMvigor010 (NCT02450331)	809	Atezolizumab Observation	≥ypT2 disease and/or N+ at surgery after neoadjuvant chemotherapy or ≥ypT3 disease and/or N+ at surgery if did not receive prior neoadjuvant chemotherapy	DFS	Completed; no improvement in DFS [9]
CheckMate 274 (NCT02632409)	700	Nivolumab Observation	≥ypT2 disease and/or N+ at surgery after neoadjuvant chemotherapy or ≥ypT3 disease and/or N+ at surgery if did not receive prior neoadjuvant chemotherapy	DFS	Completed; DFS improvement [10]
AMBASSADOR (NCT03244384)	739	Pembrolizumab Observation	≥pT2 disease and/or N+ at surgery after neoadjuvant chemotherapy or ≥pT3 disease and/or N+ at surgery if did not receive prior neoadjuvant chemotherapy	DFS, OS	Ongoing

DFS disease-free survival, *OS* overall survival

trials take years to read out and can become outdated in a rapidly evolving therapeutic landscape. Developing a framework of an adaptive phase 2 clinical trial design in the neoadjuvant setting for localized MIBC to evaluate immunotherapy and novel agents with the primary endpoint of pathological responses can target rapid and individualized clinical development of drugs based upon comprehensive biomarker selection to identify patients best suited for specific immunotherapy-based approaches.

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Index

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