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Resistance to Immunotherapy: A Challenge of Cancer Treatment

Lead Guest Editor: Xuelei Ma
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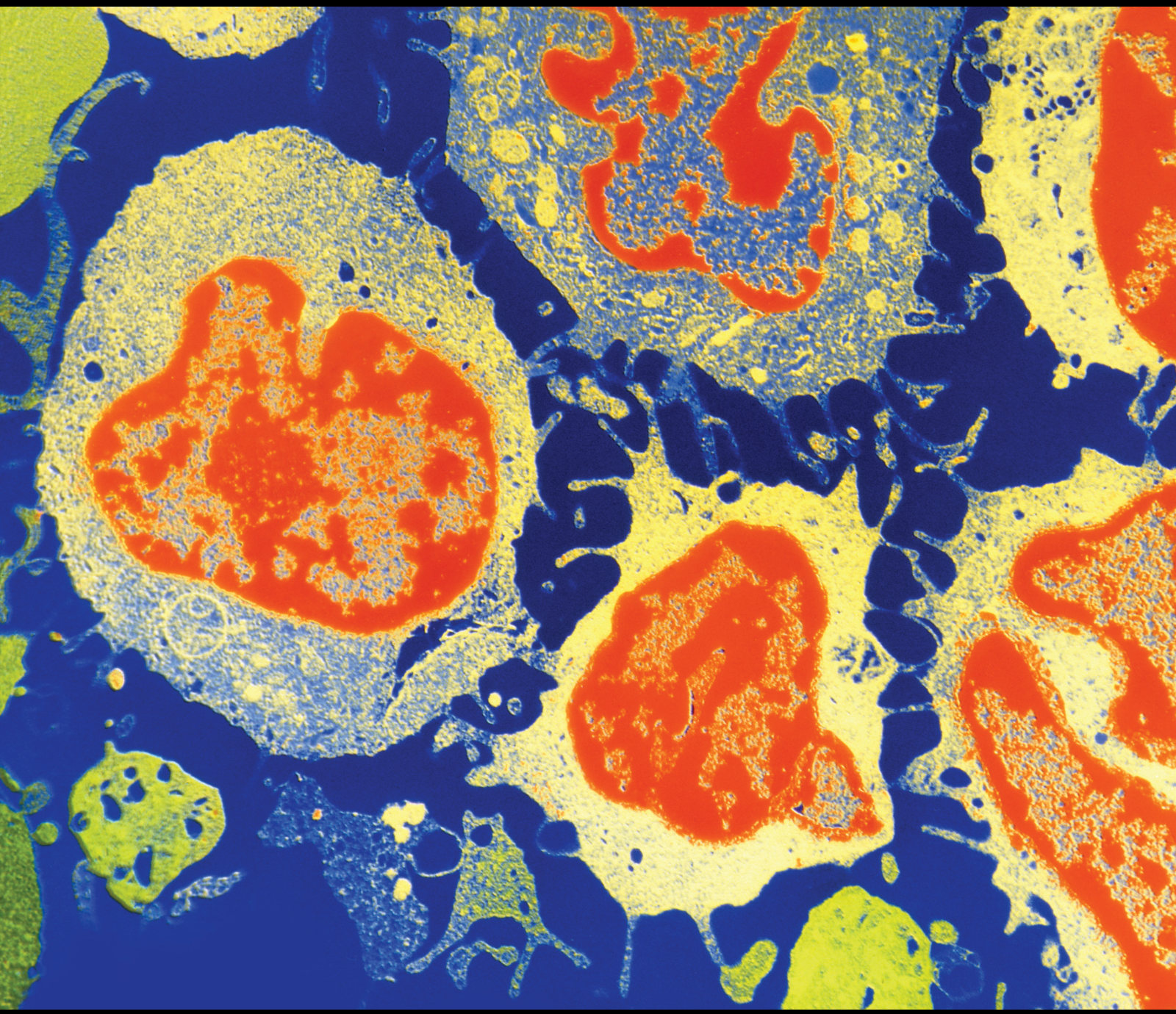

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Contents

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Review Article

Combination with Stereotactic Body Radiotherapy Offers a Promising Strategy to Overcome Resistance to Immunotherapy in Advanced Renal Cell Cancer

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Guest Editor: Shuang Zhou

Immunotherapy for renal cell cancer (RCC) has witnessed several developments for more than two decades. Checkpoint inhibitors, including anti-CTLA-4 and anti-PD-1/PD-L1 blockers, have changed the treatment landscape for patients with advanced RCC in the past 3 years. Despite these advances, more than 55% RCC patients become resistant to different immunotherapies without other treatment combination. Among various attempts at overcoming resistance to immunotherapy, stereotactic body radiotherapy (SBRT) has been found to potentiate the activity of immunotherapy agents through several potential mechanisms, including normalization of microvessels to alleviate tumor hypoxia, improvement in efficient delivery of drugs, abundant neoantigen exposure, and recruitment of antitumor immune cells to alter the immunosuppressive tumor microenvironment. Preclinical studies and clinical case reports have predicted that the combination of SBRT, an immunotherapy, may lead to remarkable results. This review aims to provide the biological basis for the feasibility of combining SBRT to overcome immunotherapy resistance and to review the currently available clinical evidence of this combination therapy in patients with advanced RCC.

1. Introduction

Renal cell cancer (RCC) is the third most common urological carcinoma, and over 90% cases of RCC in adults is clear cell in histology [1, 2]. The prognosis of RCC cases depends on the disease stage, tumor properties, the state of tumor metastasis, accurate diagnosis, proper treatment, and so on [2]. Advance and metastatic cases still carry a poor prognosis with a 5-year survival of about 9–12% [3]. Furthermore, nearly 30% of RCC cases with early-stage diagnosis will suffer from recurrence and progression after surgical procedures partly because of pre-existing micrometastatic loci before the surgery or some uncertain reasons [4].

Therapeutic options for advanced RCC patients should be based on histology (clear cell or not clear cell) and the most widely used prognostic factor model is from the Memorial Sloan Kettering Cancer Center (MSKCC) with stratification in three prognostic categories (favorable, intermediate, and poor risk) [5]. Prognostic factors for multivariable analysis included five variables—Karnofsky performance status (KPS) less than 80%, interval from diagnosis to treatment of less than 1 year, serum lactate

dehydrogenase (LDH) greater than 1.5 times the upper limit of normal (ULN), corrected serum calcium greater than the ULN, and serum hemoglobin less than the lower limit of normal (LLN). Patients with none of these risk factors are considered low risk or with good prognosis, those with one or two factors present are considered intermediate risk, and patients with three or more of the factors are considered poor risk. First-generation systemic therapy, comprising cytokine-based procedures including interferon-alpha (IFN-α) and interleukin-2(IL-2), is recommended for advanced RCC patients since there is documented evidence for its effectiveness against advanced RCC. Targeted therapies including tyrosine kinase (TKI) and mTOR inhibitors, and antibodies against vascular endothelial factor (VEGF) and platelet-derived growth factor (PDGF), have tremendously improved clinical outcomes compared with cytokine therapy alone.

Development and progression of advanced RCC have been slowed or even arrested through immune checkpoint inhibitor (ICI) combination therapy (ipilimumab plus nivolumab), which, in patients with intermediate or poor risk, showed a better overall survival (OS) than VEGF target therapy recently [6]. However, the objective response rate (ORR) is 42% in ICI combination therapy suggesting that most RCC patients are resistant to ICI combination therapy [6]. The lack of predictive biomarkers of high quality has resulted in missed treatment opportunities for RCC patients who could not benefit from ICI therapy. Therefore, it is crucial that RCC patients overcome resistance to treatment and to expand applicable people who could benefit from ICI therapies.

Though RCC was considered to be resistant to radiotherapy, this concept is being challenged, particularly in the past decade, due to the continuous advances and innovation in radiotherapy technology. Increased doses of radiotherapy to tumor lesions has been observed following significant improvement in the accuracy of radiotherapy, which achieved better control of the damage in surrounding normal tissue. Stereotactic body radiotherapy (SBRT), which comprises high doses of radiation delivered in fractions (usually ≤ 5), has evolved to become an important treatment strategy for both primary lesions and metastatic diseases in different organs for RCC patients. Several key biological pathways triggered by SBRT prime the system immune to eliminate tumor cells. Therefore, SBRT and immunotherapy display synergistic effects, which are reviewed in this study to determine the biological basis and current preclinical and clinical evidence for combination treatment of SBRT and immunotherapy.

2. Current Immunotherapy in Clinical Trials for Patients with Advanced RCC

Currently, five immunotherapy agents, IL2, IFN-α, ipilimumab, nivolumab, and pembrolizumab, have been approved for treating advanced RCC, either alone or in combination with other drugs. Current immunotherapies for patients with advanced clear cell or non-clear cell RCC are described in Table 1.

IL-2 and IFN-α are reported to achieve durable complete or partial response in only a small population of patients [9, 17]. For the majority, the benefit from cytokine-based therapy is limited and the trials to improve the effectiveness have met with efficacy uncertainties. High-dose IL-2 showed substantial toxicity in patients [18]. Thus, selection of patients treated with high-dose IL-2 mainly depends on safety and the tumor histology (clear cell approved), medical comorbidities, patient's performance status, risk scores, and the patient's attitude to treatment risk.

IFN-α plus VEGF-targeted therapies such as bevacizumab may improve the prognosis of RCC to a certain degree [10, 11], but whether toxicity was greater in the combination therapy arm remains controversial. However, IFN-α alone was inferior compared to the sorafenib (VEGF TKI) [12] or temsirolimus (mTOR inhibitor) monotherapy [13].

Ipilimumab is a selective antibody blocking the interaction between cytotoxic T-lymphocyte antigen 4 (CTLA-4) and its ligands CD80/CD86. Nivolumab selectively blocks the interaction between programmed death-1 (PD-1) and its ligands. The FDA approved nivolumab for previously treated advanced RCC patients. A multicenter phase III trial (CheckMate 214) compared ipilimumab plus nivolumab (ICI combination) followed by nivolumab monotherapy ($N = 425$) versus sunitinib monotherapy ($N = 422$) in patients with advanced RCC [6]. Both groups showed intermediate or poor risk. In comparison with sunitinib, patients receiving ICI had higher ORR (42% vs. 27%, $p < 0.001$), and ICI group showed a significant improvement in complete response (CR) rate (9% vs. 1%, $p < 0.001$) in intermediate- or poor-risk patients. The 18-month OS rate in the ICI group was 75% (95% confidence interval (CI): 70–78%), while it was 60% in the sunitinib group [6].

There is controversy over ICI combination therapy in previously untreated favorable-risk patients. Also, the study population in CheckMate 214 included favorable-risk patients treated with ICI combination ($N = 125$) or sunitinib ($N = 124$) [6]. Exploratory analyses of 18-month OS rate found that the favorable-risk patients benefited more from sunitinib (88% vs. 93%). The ORR (29% and 52%; $p < 0.001$) and median progression-free survival (PFS) (14.3 months vs 25.1 months; HR: 2.18; $p < 0.001$) were lower in favorable-risk patients taking ICI combination than sunitinib in this trial. However, the CR rates were 11% and 6% for the ICI combination and sunitinib groups, respectively. Conversely, a phase I trial (CheckMate 016) supported the use of ICI combination in patients at any risk with confirmed advanced clear cell RCC, including those who received prior therapy [14]. The study included patients with poor ($N = 6$), intermediate ($N = 47$), or favorable ($N = 47$) risks. Patients with favorable risk comprised 44.7% of those taking ICI combination. The data for the favorable-risk patients alone were not published, but the 2-year OS for the entire cohort was 67.3%. The confirmed ORR for the cohort was similar in both arms (40.4%) [14]. Because of these conflicting results, the FDA approval for nivolumab plus ipilimumab only included patients with intermediate- or poor-risk RCC for first-line therapy.

In another randomized phase III clinical trial (CheckMate 025), patients ($N = 821$) with previously treated (excluding mTOR inhibitors) advanced clear cell RCC were assigned to receive nivolumab or everolimus (a mTOR

Table 1: Main clinical trials of immunotherapy for advanced RCC.

Type of RCC	Drug	Phase	No. of pts	Line of therapy	ORR	mPFS (month)	mOS (month)	Reference
Undifferentiated	High-dose IL2	2	71	ND	ORR = 17% CR = 5.6%	NA	15.5	Atkins et al., [7]
Undifferentiated	High-dose IL2	3	96	ND	ORR = 23.3% CR = 8.4%	14	17.1	McDermott et al., [8]
Undifferentiated	IL2 plus IFNα-2a	3	140	ND	ORR = 13.6% CR = 3.5%	NA	17	Negrier et al. [9]
Clear cell	Arm 1: bevacizumab plus IFNα-2a; Arm 2: IFNα-2a	3	325 289	ND	Arm 1: ORR = 31% CR = 1%; Arm 2: ORR = 13% CR = 2%	10.2 5.4	18.3 17.4	Escudier et al. [10]; Rini et al. [11]
Clear cell	IFNα-2a	2	189	First line	ORR = 39% CR = 2%	5.6	NA	Escudier et al. [12]
Both clear cell and non-clear cell enrolled	Arm 1: temsirolimus; Arm 2: IFNα-2a; Arm 3: both	3	209 207 210	First line	Arm 1: ORR = 8.6%; Arm 2: ORR = 4.8%; Arm 3: ORR = 8.1%	Arm 1: 3.8; Arm 2: 1.9; Arm 3: 3.7.	10.9 7.3 8.4	Hudes et al. [13]
Clear cell	Nivolumab (N) plus ipilimumab (I)	1	N3I1 = 47; N1I3 = 47	First line	Both ORR = 40.4% in the N3I1 and N1I3 arms; CR = 10.6% in the N3I1 arm and none in the N1I3 arm.	N3I1 = 7.7; N1I3 = 9.4	Not reached in the N3I1 arm and 32.6 months in the N1I3 arm	Hammers et al. [14]
Clear cell	Arm 1: nivolumab plus ipilimumab Arm 2: sunitinib	3	550 546	First line	Arm 1: ORR = 42%; CR = 9%; Arm 2: ORR = 27%; CR = 1%;	Arm 1: 11.6; Arm 2: 8.4.	Not reached in arm 1 and 26 months in arm 2	Motzer et al. [6]
Clear cell	Arm 1: nivolumab Arm 2: everolimus	3	821	Second line or third line	Arm 1: ORR = 25%; CR = 1%; Arm 2: ORR = 5%; CR < 1%;	Arm 1: 4.6; Arm 2: 4.4.	Arm 1: 25; Arm 2: 19.6	Motzer et al. [15]
Clear cell	Arm 1: pembrolizumab plus axitinib Arm 2: sunitinib	3	432 429	First line	Arm 1: ORR = 59.3%, CR = 5.8%; Arm 2: ORR = 35.7%, CR = 1.9%;	Arm 1: 15.1; Arm 2: 11.1.	Not reached in both arms	Rini et al. [16]

RCC: renal cell cancer; pts: patients; ND: not demanded; ORR: objective response rate; mPFS: median progression-free survival; mOS: median overall survival; IL2: interleukin-2; CR: complete response.

inhibitor). The median OS of the nivolumab group and everolimus group were 25.0 months and 19.6 months, respectively. The ORR was also 5 times greater with nivolumab (25% vs. 5%; $p < 0.001$) [15].

Recently, an open-label, randomized phase III clinical trial (KEYNOTE-426) compared the efficacy of pembrolizumab (Keytruda, a PD-1 blocker) plus axitinib (a multitargeted tyrosine kinase inhibitor for VEGFR, c-kit, and PDGFR, $N = 432$) with sunitinib (a multitargeted tyrosine kinase inhibitor for PDGFR, VEGFR, and c-kit, $N = 429$) in previously untreated advanced RCC patients [16]. As a result, 89.9% patients in the pembrolizumab-axitinib group and 78.3% patients in the sunitinib group survived at 12 months in 12.8 months median follow-up. Median PFS durations were 15.1 months and 11.1 months in the pembrolizumab plus axitinib group and in the sunitinib group, respectively (HR 0.69; 95% CI, 0.57–0.84; $p < 0.001$); ORRs were 59.3% and 35.7% in the pembrolizumab-axitinib group (95%CI, 54.5–63.9%) and in the sunitinib group (95% CI, 31.1–40.4%). Regardless of PDL-1 expression, pembrolizumab combined with axitinib benefited patients in all risk groups (favorable, intermediate, and poor risk) [16]. Due to the conspicuous advantage of pembrolizumab plus axitinib over sunitinib on ORR and PFS, the FDA approved pembrolizumab plus axitinib as first-line therapy of all risk groups in advanced RCC on April 19, 2019.

A retrospective analysis of 35 patients with metastatic, non-clear cell RCC who received at least one dose of nivolumab showed that 20% of patients had partial response and 29% of patients had stable disease in 8.5 months median follow-up and 3.5 months median PFS [19]. McKay et al. found that of 43 patients with metastatic, non-clear cell RCC, 8 (19%) patients had modest responses to PD-1/PD-L1 and 4 (13%) patients who received PD-1/PD-L1 monotherapy showed an objective response [20].

In general, the next generation of immunotherapies (ICI: ipilimumab, nivolumab, and pembrolizumab) raised hopes for patients with advanced RCC. From the results reported

so far, clear cell RCC and intermediate-risk/poor-risk populations could benefit more than others. ICI therapies showed the potential of improving the ORR and PFS with or without anti-VEGF therapy, which also resulted with lower severe toxicities than high-dose IL2. However, the OS benefit of pembrolizumab plus axitinib over sunitinib remains unknown. Considerable efforts are nevertheless needed to reduce the resistance rate to immunotherapy and improve its efficiency.

3. Potential Mechanisms of Adding SBRT to Overcome the Resistance to Immunotherapy

There are several underlying mechanisms explaining how SBRT enhances immunotherapy efficacy in the tumor microvasculature as depicted in Figure 1.

3.1. Tumor Microvasculature Response to SBRT. Folkman hypothesized that the most common pathway for new microvessel development in malignant tumor is angiogenesis [21]. In physiological conditions, pro- and anti-angiogenic factors maintain a dynamic balance for the normal development of blood vessels. However, in malignant tumors, this balance is perturbed by hypoxia. Excessive proangiogenic factors promote abnormal growth of microvessel, which become disorganized and form tortuous, dilated, hyperpermeable, and dysfunctional microvessels, resulting in intensifying hypoxia and poor transportation efficiency within the tumor microenvironment. These abnormal microvessels impede immune cell migration, function, and transportation of therapeutics. The response of microvessels to SBRT, their normalization structure, and endothelial cell (EC) apoptosis determine the radiosensitivity of certain malignant tumors, including RCC. EC apoptosis might be particularly crucial for RCC because of its extensive microvasculature.

In 2003, Garcia-Barros and colleagues discovered that high-dose SBRT (more than 8–11 Gy) facilitates apoptosis of EC in a dose-dependent manner and normalizes tumor microvasculature [22]. More than 8–11 Gy radiation induced EC apoptosis, and single dose of 15–20 Gy radiation resulted in rapid EC apoptosis. With a single dose of 15 Gy, EC apoptosis, involving acid sphingomyelinase (ASMase), is initiated in one hour, reaches its peak in four hours, and ceases in six hours. ASMase hydrolyses sphingomyelin, a proapoptotic messenger that coordinates transmembrane signaling of FAS-FASL-mediated and tumor necrosis factor- (TNF-) receptor-mediated apoptosis and DR5-TRAIL-mediated apoptosis through death-inducing signaling complexes within seconds after irradiation and without DNA damage. Clustering of receptor-bearing rafts facilitates the stimulation of receptor-mediated apoptosis. Exclusion of survival-regulating proteins and growth factors from these clustered rafts might cause EC apoptosis. A previous study showed that $ASMase^{-/-}$ mice had double the growth rate of MCA129 fibrosarcoma and half the rate of EC apoptosis than $ASMase^{+/+}$ mice [22], suggesting that EC apoptosis plays an important role in tumor cell death. Sathishkumar et al. observed that patients having a complete or partial response after SBRT (15 Gy/1f) had substantially augmented or higher levels of a secretory form of ASMase (S-ASMase) activity before radiotherapy was given (high basal activity), while little-to-no increase in low basal activity was observed in nonresponders [23]. Furthermore, 60% of patients with clear-cell renal cancer are highly vascularized owing to transcriptional silencing (hypermethylation) or mutation of *von Hippel–Lindau (VHL)*. Degradation of hypoxia-inducible factor-1 (HIF-1) requires pVHL, and deficiency in pVHL results in HIF-1 accumulation and angiogenesis.

Given that renal cancer is assumed to be sensitive to SBRT [24], it was found that EC damage appears to be induced by both SBRT and conventional fractionated radiation (CFRT). These contrasting results may be due to the fact that EC apoptosis contributes significantly to tumor cell elimination in SBRT, and EC apoptosis was merely due to low-dose irradiation of CFRT which may not induce tumor cell death effectively, as death signaling in EC is repressed by activation of HIF-1 in tumor cells [25].

Apart from EC apoptosis, SBRT enhances involvement of pericytes in tumor microvessels, and the pericyte-covered microvessels were functional with an increase in perfusion, which could alleviate hypoxia and improve transportation efficacy [26]. Thus, there is a normalization of blood microvessels, offering a "window of opportunity" for immune-cell migration and transportation of therapeutics.

3.2. The Systemic Antitumor Effect of SBRT. Basic biological and clinical research in tumor radiotherapy have revealed that local radiotherapy, especially SBRT, can induce systemic antitumor effect in tumor lesions beyond the radiated field, termed the abscopal effect, which has been reported in various malignancies including melanoma, lymphoma, neuroblastoma, and RCC and particularly in pulmonary metastases. A valid hypothesis explaining the mechanism behind abscopal effect is that high-dose radiation can cause tumor cells to die within a short period and expose new tumor antigens, so that radiated tumor cells function as natural tumor vaccines after radiation exposure [27–29]. Concurrently, during the process of tumor cell death, damage-associated molecular patterns, such as HMGB1, ATP, and heat shock proteins, are also released in large quantities. These molecules can effectively induce dendritic cells (DCs) to recognize tumor-specific antigens resulting in their capture and migration of DCs to draining lymph nodes, where tumor antigens are presented to T cells [30], which in turn get activated and undergo massive proliferation. Activated effector T cells enter the circulatory system, recognize tumor cells far from the radiated field, and exert antitumor effects [31, 32].

To explore whether SBRT can enhance the expression of tumor-associated antigens in patients with advanced RCC, Singh et al. studied the response to SBRT in patients with advanced RCC. This study evaluated patients receiving neoadjuvant SBRT following surgery and found SBRT patients had higher expression of tumor-associated antigens (MUC-1, CA-9, 5T4, and NY-ESO-1) and costimulatory molecules ICAM-1 and CD80 compared with patients

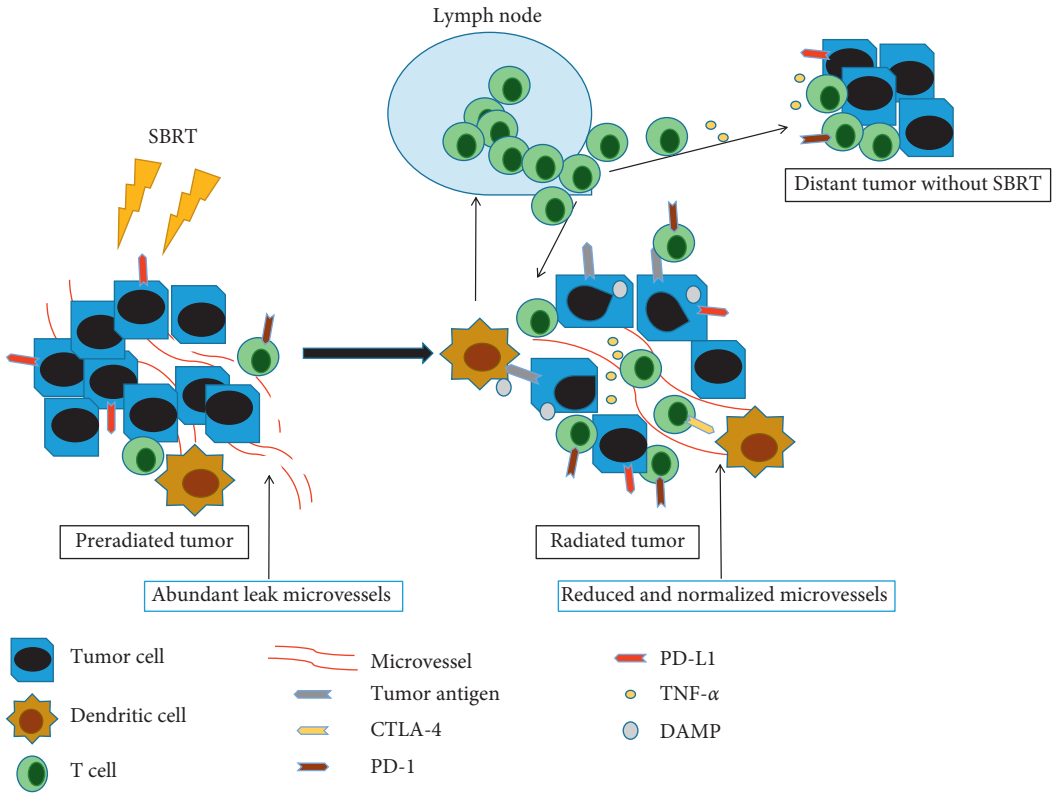

Figure 1: Potential mechanisms of SBRT enhance the efficacy of immunotherapy. SBRT (single dose >8 Gy) reduces and renormalizes the microvessels in tumor. On the other hand, SBRT increases infiltration of antitumor immune cells such as dendritic cells and T cells in the radiated tumor. Theoretically, these antitumor T cells could migrate to the unradiated tumor sites, which is called the abscopal effect. DAMP: damage-associated molecular patterns.

without SBRT [33]. Moreover, the apoptosis inducers TNF-α (24–72 h after SBRT), IL-1α, IL-1β, IL-6 FASL, and TGF-β were released during radiotherapy; higher levels of TNF-α agreed with the abscopal effect and complete tumor response [23, 34].

4. Efficacy of SBRT in Patients with Advanced RCC

Results from several studies support that SBRT differs from CFRT for RCC patients, and SBRT is effective at controlling both primary and metastatic lesions of RCC, as summarized in Table 2.

4.1. SBRT Differs from CFRT in Treating Patients with RCC. In recent years, SBRT has been delivered to patients with advanced RCC, with results showing a slow but persistent shrinkage of the renal tumor after SBRT [50]. Compared with CFRT, in RCC with bone metastasis, the median time to symptom relief between SBRT and CFRT was similar, but the symptom control rates of SBRT were much higher than those of CFRT [35]. Furthermore, the authors of the study also showed that the biologically effective dose (BED) ≥80 Gy was significant for better clinical response and was predictive of local control [35]. Similar results were reported by Altoos et al. showing SBRT-mediated control of thoracic, abdominal, and soft tissue lesions in RCC, with predictive factors for better local control being BED ≥100 Gy and dose per fraction ≥9 Gy [36]. An analysis of radiographic and symptomatic RT responses in 27 consecutive RCC patients with 37 lung lesions found that rates of radiographic local control with SBRT were much higher than CFRT [37]. To explore the difference between SBRT and CFRT on spine metastases from RCC, a total of 110 patients (34 CFRT; 76 SBRT) were retrospectively analyzed [51]. The researchers found that both CFRT (20 Gy/5f) and SBRT (15 Gy/1f) provided effective relief of symptomatic spine metastases from RCC, whereas CFRT relieved pain faster, and pain relief with SBRT was more durable [51].

4.2. SBRT Is Effective in Controlling Primary Renal Lesions. Results from several studies indicate that SBRT is effective in controlling primary renal lesions. For example, renal tumors treated with SBRT show significant reductions in growth rate and tumor size after radiation [52]. Furthermore, a prospective phase I trial suggested that SBRT might be an alternative to cytoreductive nephrectomy for inoperable patients with advanced RCC [39]. The median tumor size was increased 17.3% at 5.3 months, and the median OS was increased at 6.7 months [39]. Inadequate single doses (≤7 Gy) in this prospective study could be the reason for these moderate results. For asynchronous bilateral RCC

Table 2: SBRT is effective in primary lesions and metastases of advance RCC.

Study type	No. of patients/ lesions	SBRT target	SBRT regimen	Local control	OS	AE (≥Grade 3)	Ref
Retrospective study	50 lesions	Bone metastasis	Most common is 27 Gy/3f	Rates at 12 and 24 months were both 74.9%	NA	Grade 3 AE: 1 patient, dermatitis	Amini et al. [35]
Retrospective study	36 lesions	Thoracic, abdominal, and soft-tissue lesions	Most common is 50 Gy/5f	Rates at 12, 24, and 36 months were 100%, 93.41%, and 93.41%, respectively	Median OS about 32 months	Grade 3 AE: 1 patient, mucositis	Altoos et al. [36]
Retrospective study	27 pts/ 37 lesions	Lung metastasis	Median SBRT dose and fraction were 50 Gy (range 25–60) and 3 (range 1–6)	92.3% for median follow-up 16 months	NA	0	Altoos et al. [37]
Retrospective study	57 pts/ 88 lesions	Spinal metastases	Single fraction, median 15 Gy	Median 26 months	8.3 months (1.5–38)	0	Balagamwala et al. [38]
Prospective phase I trial	12 pts	Primary renal lesions	25 Gy, 30 Gy, or 35 Gy in 5 fractions	NA	6.7 months (1.5–16.4)	Grade 3 AE: 3 patients, fatigue (2) and bone pain (1)	Correa et al. [39]
Retrospective study	9 pts	Bilateral primary renal lesions	60–85 Gy was delivered at 5–7 Gy/fraction	Rates at 1, 3, and 5 years were 64.8, 43.2, and 43.2%, respectively	Rates at 1, 3, and 5 years were 66.7, 53.3, and 35.6%, respectively	0	Wang et al. [40]
Prospective phase I trial	15 pts	Primary renal lesions	24–48 Gy/4f	100% for median follow-up 13.67 months	Estimated 3-year OS post-treatment was 72%, 95% CI (0.44–0.87)	Grade 4 AE: 1 patient (5.3%) with duodenal ulcer possibly treatment-related	Ponsky et al. [41]
Prospective study	37 pts	Primary renal lesions	26 Gy/1f for tumors <5 cm and 42 Gy/3f for tumors ≥5 cm	Rates at 2 years was 100%	Rates at 2 years were 92%	Grade 3 AE: 1 patient (3%).	Siva et al. [42]
Retrospective study	21 pts	Primary renal lesions	48 Gy/3f	Rates at 1 year and 2 years were 92 and 84%, respectively	Rates at 1 year and 2 years were both 95%	0	Kaplan et al. [43]
Retrospective study	32 pts/ 52 lesions	Brain metastasis	22.0 Gy (range, 12.8–24.0 Gy)	NA	6.3 months (0.4–100.4 months)	NA	Shah et al. [44]
Retrospective study	16 pts/ 99 lesions	Brain metastasis (≥5)	SRS	91% of targets	50% after 6 months and 31% after 1 year	NA	Mohammadi et al. [45]
Retrospective study	81 pts/ 117 lesions	Brain metastasis (from melanoma or renal cancer)	18 Gy (range 15–20 Gy)	Rate at 1 year was 79.4% for renal cancer	Rates at 6 months and 1 year were 55.4% and 30.2%, respectively	NA	Feng and Lemons et al.
Retrospective study	15 pts	Brain metastasis	SRS	NA	8.4 months	NA	Feng et al. [46]
Retrospective study	18 pts/ 39 lesions	Oligometastatic renal cancer (extracranial)	8–14 Gy ∗ 3 fractions or 4–5 Gy ∗ 10 fractions	Rate at 2 years was 91.4%	2 years was 85%	NA	Ranck et al. [47]

Table 2: Continued.

Study type	No. of patients/ lesions	SBRT target	SBRT regimen	Local control	OS	AE (≥Grade 3)	Ref
Retrospective study	84 pts/ 175 lesions	Extracranial metastasis	(40–60 Gy/5f or 30–54 Gy/3f or 20-40 Gy/1f	1-year LC rate was 91.2%	NA	Grade 3 events: 8 patients (4.6%).	Wang et al. [48]
Retrospective study	48 patients/ 70 lesions	Spinal metastases	NA	Rate at 21 months was 72%	66 months (CI95% 54–79)	NA	Serrand et al. [49]

NA: not available; pts: patients; OS: overall survival; AE: adverse effect; SRS: stereotactic radiosurgery.

($N = 9$), SBRT resulted in an ORR of 55.6%, and the 1-, 3-, and 5-year OS rates were 66.7%, 53.3%, and 35.6%, respectively [40]. Among patients with localized RCC who were not suitable for surgery, a phase I study using SBRT (24–48 Gy/4f) showed three partial responses and 12 patients with stable disease among those with an evaluable response ($N = 15$) [41]. Siva et al. applied SBRT (26 Gy/1f for tumors <5 cm or 42 Gy/3f for tumors ≥5 cm) on inoperable primary kidney cancers and found freedom from local (100%) and distant (89%) progression, with an overall 2-year survival rate of 92% [42]. However, SBRT led to dose-dependent renal dysfunction at 1- and 2-years [42]. Therefore, sparing functional kidney from high-dose irradiation regions might help reduce the risk of renal dysfunction. In this context, SBRT (48 Gy/3f) was found to be effective for primary small renal tumors and results in a satisfactory local control rate [43].

4.3. SBRT Controls Intracranial and Extracranial Metastases in RCC. At present, several early studies have demonstrated that SBRT has an inhibitory effect on RCC metastases, including intracranial and extracranial metastases.

4.3.1. Intracranial Metastases Controlled by Stereotactic Radiosurgery (SRS). Brain metastasis (BM) usually indicates poor prognosis in patients with RCC. Whole brain radiation therapy (WBRT) is considered a standard treatment in patients with multiple (>5) BMs. However, WBRT (usually 2–3 Gy per fraction) has limited efficacy in patients with BM from radio-resistant tumors such as RCC and melanoma whose median survival is 2–4 months. Stereotactic radiosurgery (SRS) for BM from RCC has been regarded as an alternative to surgery and delivers high-dose radiation in no more than 3 fractions (usually only one fraction), but avoids the toxic effects of WBRT. Studies in this regard have shown local control in 24 of 32 renal patients with 52 metastases while 4 patients had local progression using SRS for brain metastases in patients, in which the median dose was 22.0 Gy (range, 12.8–24.0 Gy), and the median OS was 6.3 months (range, 0.4–100.4 months) [44]. To evaluate outcomes of SRS in 16 RCC patients with multiple (≥5) simultaneous BMs (99 lesions in total) treated with SRS showed OS after 6 months and 1 year to be 50% and 31%, respectively. The median OS was 7.1 months (range 1–21), and 91% patients were free from local failure [45]. Besides, it has been found that SRS dose >18 Gy was associated with improved survival in patients with RCC [53]. Using this dose (range 15–20 Gy), a study involving 81 patients treated with SRS for BM from melanoma or RCC showed actuarial OS rates at 6 months and 1 year of 55.4% and 30.2%, respectively, and one-year local control (LC) rate of 79.4% for RCC [46]. Another similar, but smaller, study involved BM from melanoma ($N = 26$) or RCC ($N = 15$) patients, which found the lack of statistical significant differences in OS between patients with RCC and melanoma (8.4 mo vs 5.0 mo, $p = 0.11$) [54].

The results of these studies indicated that the OS of patients with BM from RCC treated with SRS is about 6.3–8.4 months, which is much longer than patients who underwent WBRT. The lack of high-grade evidence in current retrospective studies warrants the need for prospective studies in order to guide clinical practice, with the inclusion of more numbers of BMs to make valid conclusions.

4.3.2. Extracranial Metastases Controlled by Stereotactic Radiosurgery (SRS). The ability of SBRT to control extracranial metastases in RCC was demonstrated in recent studies on 84 patients with 175 metastatic extracranial lesions who received SBRT (40–60 Gy/5f or 30–54 Gy/3f or 20–40 Gy/1f); the 1-year local control (LC) rate after SBRT was 91.2%, and one factor of local failure was BED <115 Gy [48]. Another retrospective study of 48 patients treated for 70 spine metastases showed that the spine recurrence rates of 60% were mainly associated with salvage SBRT, which was only 20% for upfront SBRT. The study suggested that an early SBRT with higher doses could be more effective than salvage SBRT [49]. As mentioned above, SBRT effectively relieves symptomatic spine metastases in RCC. Compared with CFRT, SBRT trends to produce more durable pain relief [51], as demonstrated in 57 RCC patients (88 treatment) with spine metastasis, wherein Balagamwala et al. found that a single fraction SBRT achieved a median survival of 8.3 months and relieved pain rapidly with a median duration of 5.4 months of pain relief [38].

The currently available evidence reviewed in this study suggests that SBRT alone is effective for RCC, including primary lesions treatment and intracranial and extracranial metastases control; especially, patients with multiple intracranial metastases face poor prognosis. Single dose <7 Gy

might be ineffective to achieve satisfactory treatment results in RCC patients, but higher dose radiation in SBRT monotherapy exerted robust disease control with acceptable clinical risk.

5. Preclinical and Clinical Evidence for the Inclusion of SBRT to Overcome Resistance to Immunotherapy

5.1. Preclinical Evidence. The introduction of ICIs, initially with anti-CTLA-4 antibodies, initiated a revolution in oncology. The inclusion of radiotherapy to ICI, animal models, or clinical studies focusing on the integrating radiation and related drugs followed in an attempt to find effect of radiotherapy on immune activation in several solid tumors [55]. Under this strategy, combining radiation with immune-checkpoint blockade increased locoregional control of tumors [31, 56]. Furthermore, combination of local radiation with anti-CTLA-4 and anti-PD-1/PD-L1 inhibitors increased systemic disease control mediated by the abscopal effect [57]. An increase in complete regression of the irradiated primary tumor and reduced size of nonirradiated tumors outside the radiation field were observed when SBRT was combined with PD-1 blockade in melanoma and RCC models [58]. This effect was not attributed to tumor histology or host genetic background, but as it was tumor-specific, the effect was potentiated by PD-1 blockade, an abscopal tumor-specific immune response induced by radiotherapy in nonirradiated tumors [58]. The abscopal effect was exerted only in a small proportion of patients who received anti-CTLA-4 combined with radiotherapy, leading to PD-1/PDL1-mediated resistance to ipilimumab [57]. Another study showed blockade of adaptive immune resistance mediated by anti-PD-1/PDL1 antibodies upon localized radiation with anti-CTLA-4 therapy. Furthermore, nonredundant immune mechanisms mediated the superior activity of radiation and dual immune checkpoint blockade [59].

5.2. Clinical Evidence. Clinical evidence reporting combination of SBRT with immunotherapy in advanced RCC is scant. A phase-2 trial combining high-dose IL2 and SBRT in patients with metastatic RCC [60] showed that 1–3 lesion sites were treated with SBRT with a dose of 21–27 Gy for single fraction or 25–33 Gy for 3 fractions. The primary endpoint of the study—response rate—was 40%, with 1 patient presenting CR and 3 patients showing PR. The median duration of overall response (including CR and PR) was 5 months, and median stable disease (SD) duration was 6 months. Addition of SBRT to IL-2 increased the response rate in metastatic RCC patients by about 2-folds compared with IL-2 alone. Two cases have reported the induction of abscopal effect when SBRT was combined with ICI therapy in advanced RCC patients. One case reported by Xie et al. showed a systemic complete response to SBRT (32 Gy/4f) and pembrolizumab (anti-PD-1 antibody) in a patient with metastatic RCC [61]. The metastatic lymph nodes in the left mediastinum were irradiated with a total of 32 Gy administered in four fractions on four consecutive days [61]. The second case was that of a 24-year-old male with advanced clear-cell RCC and bone, lung, and nodal metastases who received SBRT (27 Gy/3f) to the sacrum metastatic mass and subsequent ipilimumab and nivolumab therapy [62]. The sacrum mass was obviously shrunk with the therapy and no radiological evidence for lung and nodal metastases was found more than 12 months after SBRT [62].

To determine the effect of combining SBRT with immunotherapy, we searched ClinicalTrials.gov for studies and identified 13 ongoing clinical trials (Table 3). The vast majority of these trials were phase-2 studies and combined ICIs and (or) high-dose IL2.

6. Discussion

Recently, a single-arm phase-2 trial, which combined SBRT and a PD-1 blocker (pembrolizumab), suggested PFS improvement without serious safety signals in patients with oligometastatic NSCLC [63]. Immunotherapy (especially ICI) offers hope for patients with advanced RCC, particularly when SBRT is offered in combination. High dose of radiation effectively results in abundant ECs apoptosis which aids in reducing and renormalizing microvessels in the tumor for better transportation of therapeutics and migration of immune cells. Furthermore, SBRT has the potential to prime the immune system by exposing a mass of tumor antigens after irradiation. We acknowledge that there is limited evidence regarding this hypothesis and additional clinical studies are needed. However, in our humble opinion, SBRT offers a promising strategy for overcoming the resistance to immunotherapy in advanced RCC. Nevertheless, limitation of the combined therapy exists as follows:

First, there exists the possibility of severe treatment-related adverse events. High-dose IL2 itself has shown to induce substantial toxicity. Furthermore, ICI therapy-induced acute kidney injuries such as acute tubulointerstitial nephritis, acute interstitial nephritis, and increased blood creatinine or acute renal failure have been reported [64–66]. As mentioned previously, application of SBRT to renal primary lesions could lead to dose-dependent renal dysfunction. Therefore, the combination of SBRT with PD-1/PD-L1 inhibitors will probably increase therapy-associated severe adverse events. Moreover, the incidence of other common treatment-related adverse events such as hypothyroidism and hyperthyroidism, which were the most frequent endocrine immune-related adverse events for PD-1/PD-L1 inhibitor alone, must be considered [67].

Second, the dose, fractions, and targets of SBRT plan are crucial, whereas a single dose <8 Gy might be insufficient and a higher dose presents a higher risk, particularly when combined with immunotherapy. Therefore, a dose-escalation study is warranted to maximize clinical efficacy with acceptable toxicities for prospective clinical trials. Encouraging results from preclinical and clinical studies support the synergistic effect of SBRT and ICI therapy against brain metastases from melanoma [68, 69]. Marrow-derived suppressor cells (MDSC) and immunosuppressive B cells could impede the antitumor activity induced by SBRT and immune

Table 3: Ongoing clinical trials which combined SBRT and immunotherapy in advanced RCC.

Identifier	Phase/no. of patients	Status	Cancer	Immunological agents	Schedule of SBRT	Line of therapy
NCT03469713	NA/68	Recruiting	Metastatic RCC	Nivolumab	30 Gy in 3 consecutive fractions	II-III
NCT01884961	II/35	Recruiting	Metastatic melanoma or RCC	High-dose IL2	Three daily doses of SBRT at 6–12 Gy to at least 1 and up to a maximum of 5	NA
NCT02855203	NA/30	Recruiting	Oligometastatic renal tumors	Pembrolizumab	18–20 Gy in 1 fraction	≤III
NCT01896271	II/26	Active, not recruiting	Metastatic RCC	High-dose IL2	8Gy–20 Gy in 1–3 fractions	NA
NCT02306954	II/84	Recruiting	Metastatic RCC	High-dose IL2	40 in 2 fractions	NA
NCT03065179	II/25	Recruiting	Metastatic RCC with a clear-cell component	Nivolumab and ipilimumab	NA	Not limited
NCT02781506	II/35	Recruiting	Metastatic RCC	Nivolumab	Dose variable in 1–3 fractions	≥II
NCT03050060	II/120	Recruiting	Metastatic/recurrent RCC/ recurrent melanoma/ recurrent NSCLC	Nelfinavir mesylate, pembrolizumab, nivolumab, and atezolizumab	Image-guided hypofractionated radiotherapy	Not limited
NCT03115801	II/112	Recruiting	Metastatic RCC	Nivolumab	30 Gy in 3 fractions	Not limited
NCT03474497	I-II/45	Not yet recruiting	Metastatic NSCLC/ metastatic melanoma/ RCC/head and neck squamous cell carcinoma	IL-2, pembrolizumab	24 Gy in 3 fractions delivered on consecutive or every other day	NA
NCT03693014	II/60	Recruiting	Metastatic cancer: melanoma/lung cancer/ bladder cancer/RCC/head and neck cancers	Nivolumab for RCC	Image-guided, 27 Gy in 3 fractions	NA
NCT03511391	II/97	Recruiting	Urothelial carcinoma/ melanoma/RCC/NSCLC/ head and neck cancer	Pembrolizumab or nivolumab	24 Gy in 3 fractions	II for RCC
NCT02978404	II/60	Recruiting	Brain metastases of metastatic clear-cell RCC or metastatic NSCLC	Nivolumab	15–20 Gy in 1 fraction	≤IV

NA: not available; NSCLC: non-small-cell lung carcinoma; RCC: renal cell cancer.

therapy [70, 71]. These immunosuppressive cells and heterogeneity in tumor might be the reasons for low incidence of abscopal effect in the clinic. Brooks and Chang suggested that we should abandon single-site radiation and that radiotherapy could be delivered to all targetable disease sites to broaden the T-cell repertoire and maximize the activation of the immune response [72].

Third, an appropriate sequence of SBRT and immunotherapy should be planned with detailed consideration. Harris et al. reported that the highest antitumor immune response in the mouse model of prostate cancer was obtained by adding immunotherapy after 3–5 weeks of radiotherapy; however, there was no obvious antitumor immune response after the end of radiotherapy [73]. It has also been suggested that CTLA-4 antibodies should be used to deplete regulatory T cells prior to radiotherapy to obtain maximum immune effects [74].

Fourth, pembrolizumab plus axitinib have yielded outstanding results, suggesting the benefit of concurrent or sequential treatment with anti-VEGF therapy combined with SBRT and immunotherapy, especially for patients with multiple lesions, some of which may be unsuitable for SBRT. However, the potential toxicities of anti-VEGF therapy with SBRT and immunotherapy need more attention [75].

In conclusion, combination of SBRT with immunotherapy may unlock antitumor immune responses that have the potential of overcoming resistance to immunotherapy in patients with advanced RCC.

Conflicts of Interest

All the authors declare that there are no relevant conflicts of interest to disclose.

Authors' Contributions

Xiaowen SUN collected, reviewed the main literature, and wrote the manuscript. Lu GAN supplemented the literature and revised the manuscript. Jiaming LIU pointed out writing design and revised the manuscript. Runa A, Lingling GE, and Baoqing Chen put up with helpful comments on the manuscript. All authors read and approved the final manuscript.

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References

[1] L. Lipworth, R. E. Tarone, and J. K. McLaughlin, "The epidemiology of renal cell carcinoma," *Journal of Urology*, vol. 176, no. 6, pp. 2353–2358, 2006.

[2] J. Bedke, T. Gauler, V. Grünwald et al., "Systemic therapy in metastatic renal cell carcinoma," *World Journal of Urology*, vol. 35, no. 2, pp. 179–188, 2017.

[3] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2019," *CA: A Cancer Journal for Clinicians*, vol. 69, no. 1, pp. 7–34, 2019.

[4] A. C. Reese, J. M. Whitson, and M. V. Meng, "Natural history of untreated renal cell carcinoma with venous tumor thrombus," *Urologic Oncology: Seminars and Original Investigations*, vol. 31, no. 7, pp. 1305–1309, 2013.

[5] R. J. Motzer, J. Bacik, B. A. Murphy, P. Russo, and M. Mazumdar, "Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma," *Journal of Clinical Oncology*, vol. 20, no. 1, pp. 289–296, 2002.

[6] R. J. Motzer, N. M. Tannir, D. F. McDermott et al., "Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 378, no. 14, pp. 1277–1290, 2018.

[7] M. B. Atkins, J. Sparano, R. I. Fisher et al., "Randomized phase II trial of high-dose interleukin-2 either alone or in combination with interferon alfa-2b in advanced renal cell carcinoma," *Journal of Clinical Oncology*, vol. 11, no. 4, pp. 661–670, 1993.

[8] D. F. McDermott, M. M. Regan, J. I. Clark et al., "Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma," *Journal of Clinical Oncology*, vol. 23, no. 1, pp. 133–141, 2005.

[9] S. Negrier, B. Escudier, C. Lasset et al., "Recombinant human interleukin-2, recombinant human interferon alfa-2a, or both in metastatic renal-cell carcinoma," *New England Journal of Medicine*, vol. 338, no. 18, pp. 1272–1278, 1998.

[10] B. Escudier, A. Pluzanska, P. Koralewski et al., "Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial," *The Lancet*, vol. 370, no. 9605, pp. 2103–2111, 2007.

[11] B. I. Rini, S. Halabi, J. E. Rosenberg et al., "Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206," *Journal of Clinical Oncology*, vol. 28, no. 13, pp. 2137–2143, 2010.

[12] B. Escudier, C. Szczylik, T. E. Hutson et al., "Randomized phase II trial of first-line treatment with sorafenib versus interferon alfa-2a in patients with metastatic renal cell carcinoma," *Journal of Clinical Oncology*, vol. 27, no. 8, pp. 1280–1289, 2009.

[13] G. Hudes, M. Carducci, P. Tomczak et al., "Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 356, no. 22, pp. 2271–2281, 2007.

[14] H. J. Hammers, E. R. Plimack, J. R. Infante et al., "Safety and efficacy of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma: the CheckMate 016 study," *Journal of Clinical Oncology*, vol. 35, no. 34, pp. 3851–3858, 2017.

[15] R. J. Motzer, B. Escudier, D. F. McDermott et al., "Nivolumab versus everolimus in advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 373, no. 19, pp. 1803–1813, 2015.

[16] B. I. Rini, E. R. Plimack, V. Stus et al., "Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 380, no. 12, pp. 1116–1127, 2019.

[17] N. Javadpour and M. Lalehzarian, "A phase I-II study of high-dose recombinant human interleukin-2 in disseminated renal-cell carcinoma," *Seminars in Surgical Oncology*, vol. 4, no. 3, pp. 207–209, 1988.

[18] J. A. Sosman, P. C. Kohler, J. A. Hank et al., "Repetitive weekly cycles of interleukin-2. II. Clinical and immunologic effects of dose, schedule, and addition of indomethacin," *JNCI Journal of the National Cancer Institute*, vol. 80, no. 18, pp. 1451–1461, 1988.

[19] V. S. Koshkin, P. C. Barata, T. Zhang et al., "Clinical activity of nivolumab in patients with non-clear cell renal cell carcinoma," *Journal for ImmunoTherapy of Cancer*, vol. 6, no. 1, p. 9, 2018.

[20] R. R. McKay, D. Bossé, W. Xie et al., "The clinical activity of PD-1/PD-L1 inhibitors in metastatic non-clear cell renal cell carcinoma," *Cancer Immunology Research*, vol. 6, no. 7, pp. 758–765, 2018.

[21] L. M. Sherwood, E. E. Parris, and J. Folkman, "Tumor angiogenesis: therapeutic implications," *New England Journal of Medicine*, vol. 285, no. 21, pp. 1182–1186, 1971.

[22] M. Garcia-Barros, "Tumor response to radiotherapy regulated by endothelial cell apoptosis," *Science*, vol. 300, no. 5622, pp. 1155–1159, 2003.

[23] S. Sathishkumar, B. Boyanovsky, A. Karakashian et al., "Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis," *Cancer Biology & Therapy*, vol. 4, no. 9, pp. 979–986, 2005.

[24] E. Jonasch, P. A. Futreal, I. J. Davis et al., "State of the science: an update on renal cell carcinoma," *Molecular Cancer Research*, vol. 10, no. 7, pp. 859–880, 2012.

[25] Z. Fuks and R. Kolesnick, "Engaging the vascular component of the tumor response," *Cancer Cell*, vol. 8, no. 2, pp. 89–91, 2005.

[26] J. Lan, X.-L. Wan, L. Deng et al., "Ablative hypofractionated radiotherapy normalizes tumor vasculature in lewis lung carcinoma mice model," *Radiation Research*, vol. 179, no. 4, pp. 458–464, 2013.

[27] M. M. Ahmed, J. W. Hodge, C. Guha, E. J. Bernhard, B. Vikram, and C. N. Coleman, "Harnessing the potential of radiation-induced immune modulation for cancer therapy," *Cancer Immunology Research*, vol. 1, no. 5, pp. 280–284, 2013.

[28] S. C. Formenti and S. Demaria, "Radiation therapy to convert the tumor into an in situ vaccine," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 84, no. 4, pp. 879-880, 2012.

[29] S. Demaria, E. B. Golden, and S. C. Formenti, "Role of local radiation therapy in cancer immunotherapy," *JAMA Oncology*, vol. 1, no. 9, pp. 1325–1332, 2015.

[30] S. C. Formenti and S. Demaria, "Systemic effects of local radiotherapy," *The Lancet Oncology*, vol. 10, no. 7, pp. 718–726, 2009.

[31] A. B. Sharabi, C. J. Nirschl, C. M. Kochel et al., "Stereotactic radiation therapy augments antigen-specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen," *Cancer Immunology Research*, vol. 3, no. 4, pp. 345–355, 2015.

[32] A. B. Sharabi, M. Lim, T. L. DeWeese, and C. G. Drake, "Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy," *The Lancet Oncology*, vol. 16, no. 13, pp. e498–e509, 2015.

[33] A. K. Singh, T. B. Winslow, M. H. Kermany et al., "A pilot study of stereotactic body radiation therapy combined with cytoreductive nephrectomy for metastatic renal cell carcinoma," *Clinical Cancer Research*, vol. 23, no. 17, pp. 5055–5065, 2017.

[34] J. M. Kaminski, E. Shinohara, J. B. Summers, K. J. Niermann, A. Morimoto, and J. Brousal, "The controversial abscopal effect," *Cancer Treatment Reviews*, vol. 31, no. 3, pp. 159–172, 2005.

[35] A. Amini, B. Altoos, M. T. Bourlon et al., "Local control rates of metastatic renal cell carcinoma (RCC) to the bone using stereotactic body radiation therapy: is RCC truly radioresistant?," *Practical Radiation Oncology*, vol. 5, no. 6, pp. e589–e596, 2015.

[36] B. Altoos, A. Amini, M. Yacoub et al., "Local control rates of metastatic renal cell carcinoma (RCC) to thoracic, abdominal, and soft tissue lesions using stereotactic body radiotherapy (SBRT)," *Radiation Oncology*, vol. 10, no. 1, 2015.

[37] B. M. Altoos, A. Amini, and M. T. Bourlon, "Is RCC truly radioresistant? Local control rates of metastatic renal cell carcinoma (RCC) to the lung using stereotactic radiotherapy (SBRT)," *Journal of Clinical Oncology*, vol. 33, no. 7, p. 445, 2015.

[38] E. H. Balagamwala, L. Angelov, S. A. Koyfman et al., "Single-fraction stereotactic body radiotherapy for spinal metastases from renal cell carcinoma," *Journal of Neurosurgery: Spine*, vol. 17, no. 6, pp. 556–564, 2012.

[39] R. J. M. Correa, B. Ahmad, A. Warner et al., "A prospective phase I dose-escalation trial of stereotactic ablative radiotherapy (SABR) as an alternative to cytoreductive nephrectomy for inoperable patients with metastatic renal cell carcinoma," *Radiation Oncology*, vol. 13, no. 1, 2018.

[40] Y.-J. Wang, T.-T. Han, J.-X. Xue et al., "Stereotactic gamma-ray body radiation therapy for asynchronous bilateral renal cell carcinoma," *La Radiologia Medica*, vol. 119, no. 11, pp. 878–883, 2014.

[41] L. Ponsky, S. S. Lo, Y. Zhang et al., "Phase I dose-escalation study of stereotactic body radiotherapy (SBRT) for poor surgical candidates with localized renal cell carcinoma," *Radiotherapy and Oncology*, vol. 117, no. 1, pp. 183–187, 2015.

[42] S. Siva, D. Pham, T. Kron et al., "Stereotactic ablative body radiotherapy for inoperable primary kidney cancer: a prospective clinical trial," *BJU International*, vol. 120, no. 5, pp. 623–630, 2017.

[43] I. D. Kaplan, A. Wagner, A. Mahadevan, and R. N. Dagoglu, "Sterotactic body radiosurgery for primary small renal tumors: a retrospective analysis," *Journal of Clinical Oncology*, vol. 32, no. 4, p. 475, 2014.

[44] K. Shah, M. Surucu, E. Melian et al., "Intracranial control of renal cell carcinoma brain metastasis using Linac based stereotactic radiosurgery," *Neuro-Oncology*, vol. 14, no. 6, 2012.

[45] A. M. Mohammadi, S. T. Chao, B. I. Rini, J. A. Garcia, J. H. Suh, and L. Angelov, "The role of stereotactic radiosurgery in the treatment of renal cell carcinoma patients with five or more brain metastases," *Journal of Clinical Oncology*, vol. 30, no. 5, p. 410, 2012.

[46] C. Feng, J. Lemons, D. R. Raleigh et al., "Reconsidering radioresistance: Linacbased stereotactic radiosurgery for intracranial metastases from melanoma and renal cell carcinoma," *Neuro-Oncology*, vol. 18, no. 6, p. 181, 2016.

[47] M. C. Ranck, D. W. Golden, K. S. Corbin et al., "Stereotactic body radiotherapy for the treatment of oligometastatic renal cell carcinoma," *American Journal of Clinical Oncology*, vol. 36, no. 6, pp. 589–595, 2013.

[48] C. J. Wang, A. Christie, M.-H. Lin et al., "Safety and efficacy of stereotactic ablative radiation therapy for renal cell carcinoma extracranial metastases," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 98, no. 1, pp. 91–100, 2017.

[49] J. Serrand, M.-P. Sunyach, S. Negrier, C. Carrie, and R. Tanguy, "Stereotactic body radiation therapy (SBRT) on spine metastases from renal cell carcinomas (RCC): clinical results and impact of upfront vs salvage SBRT," *Journal of Clinical Oncology*, vol. 34, no. 15, p. e16073, 2016.

[50] S. Funayama, H. Onishi, K. Kuriyama et al., "Renal cancer is not radioresistant: slowly but continuing shrinkage of the tumor after stereotactic body radiation therapy," *Technology in Cancer Research & Treatment*, vol. 18, Article ID 1180781192, 2019.

[51] G. K. Hunter, E. H. Balagamwala, S. A. Koyfman et al., "The efficacy of external beam radiotherapy and stereotactic body radiotherapy (SBRT) for painful spinal metastases from renal cell carcinoma," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 81, no. 2, pp. S649–S650, 2011.

[52] M. R. M. Sun, A. Brook, M. F. Powell et al., "Effect of stereotactic body radiotherapy on the growth kinetics and enhancement pattern of primary renal tumors," *American Journal of Roentgenology*, vol. 206, no. 3, pp. 544–553, 2016.

[53] B. Hirshman, B. Wilson, M. A. Ali et al., "Optimal radiosurgery dose for treatment of melanoma and renal cell carcinoma metastases," *Neuro-Oncology*, vol. 18, no. 6, p. 34, 2016.

[54] J. Bates, P. Youn, C. R. Peterson, K. Y. Usuki, and M. T. Milano, "Efficacy of stereotactic radiosurgery (SRS) in the treatment of brain metastases from melanoma and renal cell carcinoma (RCC)," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 90, no. 1, p. S307, 2014.

[55] S. C. Formenti and S. Demaria, "Combining radiotherapy and cancer immunotherapy: a paradigm shift," *JNCI: Journal of the National Cancer Institute*, vol. 105, no. 4, pp. 256–265, 2013.

[56] A. B. Sharabi, M. Lim, T. L. DeWeese, and C. G. Drake, "Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy," *The Lancet Oncology*, vol. 16, no. 13, pp. e498–e509, 2015.

[57] C. T.-S. Victor, A. J. Rech, A. Maity et al., "Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer," *Nature*, vol. 520, no. 7547, pp. 373–377, 2015.

[58] S. S. Park, H. Dong, X. Liu et al., "PD-1 restrains radiotherapy-induced abscopal effect," *Cancer Immunology Research*, vol. 3, no. 6, pp. 610–619, 2015.

[59] S. F. Ngiow, G. A. McArthur, and M. J. Smyth, "Radiotherapy complements immune checkpoint blockade," *Cancer Cell*, vol. 27, no. 4, pp. 437-438, 2015.

[60] R. Hannan, D. Ishihara, K. Louder et al., "Phase II trial of high-dose interleukin-2 (IL-2) and stereotactic radiation therapy (SABR) for metastatic clear cell renal cell carcinoma (ccRCC): interim analysis," *Journal of Clinical Oncology*, vol. 34, no. 2, p. 532, 2016.

[61] G. Xie, D. Gu, L. Zhang, S. Chen, and D. Wu, "A rapid and systemic complete response to stereotactic body radiation therapy and pembrolizumab in a patient with metastatic renal cell carcinoma," *Cancer Biology & Therapy*, vol. 18, no. 8, pp. 547–551, 2017.

[62] Q. LaPlant, C. Deselm, N. A. Lockney, J. Hsieh, and Y. Yamada, "Potential abscopal response to dual checkpoint blockade in RCC after reirradiation using dose-painting SBRT," *Practical Radiation Oncology*, vol. 7, no. 6, pp. 396–399, 2017.

[63] J. M. Bauml, R. Mick, C. Ciunci et al., "Pembrolizumab after completion of locally ablative therapy for oligometastatic non-small cell lung cancer: a phase 2 trial," *JAMA Oncology*, vol. 5, no. 9, p. 1283, 2019.

[64] S. Manohar, P. Kompotiatis, C. Thongprayoon, W. Cheungpasitporn, J. Herrmann, and S. M. Herrmann, "Programmed cell death protein 1 inhibitor treatment is associated with acute kidney injury and hypocalcemia: meta-analysis," *Nephrology Dialysis Transplantation*, vol. 34, no. 1, pp. 108–117, 2019.

[65] A. C. Shirali, M. A. Perazella, and S. Gettinger, "Association of acute interstitial nephritis with programmed cell death 1 inhibitor therapy in lung cancer patients," *American Journal of Kidney Diseases*, vol. 68, no. 2, pp. 287–291, 2016.

[66] L. Bottlaender, A.-L. Breton, L. de Laforcade, F. Dijoud, L. Thomas, and S. Dalle, "Acute interstitial nephritis after sequential ipilumumab-nivolumab therapy of metastatic melanoma," *Journal for ImmunoTherapy of Cancer*, vol. 5, no. 1, p. 57, 2017.

[67] Y. Wang, S. Zhou, F. Yang et al., "Treatment-related adverse events of PD-1 and PD-L1 inhibitors in clinical trials," *JAMA Oncology*, vol. 5, no. 7, p. 1008, 2019.

[68] L. W. Pfannenstiel, C. McNeilly, C. Xiang et al., "Combination PD-1 blockade and irradiation of brain metastasis induces an effective abscopal effect in melanoma," *OncoImmunology*, vol. 8, no. 1, Article ID e1507669, 2019.

[69] B. Murphy, J. Walker, S. Bassale et al., "Concurrent radio-surgery and immune checkpoint inhibition: improving regional intracranial control for patients with metastatic melanoma," *American Journal of Clinical Oncology*, vol. 42, no. 3, pp. 253–257, 2019.

[70] J. Lan, R. Li, L.-M. Yin et al., "Targeting myeloid-derived suppressor cells and programmed death ligand 1 confers therapeutic advantage of ablative hypofractionated radiation therapy compared with conventional fractionated radiation therapy," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 101, no. 1, pp. 74–87, 2018.

[71] X. Sun, T. Zhang, M. Li, L. Yin, and J. Xue, "Immunosuppressive B cells expressing PD-1/PD-L1 in solid tumors: a mini review," *QJM: An International Journal of Medicine*, pp. 1–6, 2019.

[72] E. D. Brooks and J. Y. Chang, "Time to abandon single-site irradiation for inducing abscopal effects," *Nature Reviews Clinical Oncology*, vol. 16, no. 2, pp. 123–135, 2019.

[73] T. J. Harris, E. L. Hipkiss, S. Borzillary et al., "Radiotherapy augments the immune response to prostate cancer in a time-dependent manner," *The Prostate*, vol. 68, no. 12, pp. 1319–1329, 2008.

[74] E. B. Golden and S. C. Formenti, "Radiation therapy and immunotherapy: growing pains," *International Journal of Radiation Oncology∗Biology∗Physics*, vol. 91, no. 2, pp. 252–254, 2015.

[75] X. Sun, L. Deng, and Y. Lu, "Challenges and opportunities of using stereotactic body radiotherapy with anti-angiogenesis agents in tumor therapy," *Chinese Journal of Cancer Research*, vol. 30, no. 1, pp. 147–156, 2018.

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Review Article

Targeting L-Lactate Metabolism to Overcome Resistance to Immune Therapy of Melanoma and Other Tumor Entities

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Although immunotherapy plays a significant role in tumor therapy, its efficacy is impaired by an immunosuppressive tumor microenvironment. A molecule that contributes to the protumor microenvironment is the metabolic product lactate. Lactate is produced in large amounts by cancer cells in response to either hypoxia or pseudohypoxia, and its presence in excess alters the normal functioning of immune cells. A key enzyme involved in lactate metabolism is lactate dehydrogenase (LDH). Elevated baseline LDH serum levels are associated with poor outcomes of current anticancer (immune) therapies, especially in patients with melanoma. Therefore, targeting LDH and other molecules involved in lactate metabolism might improve the efficacy of immune therapies. This review summarizes current knowledge about lactate metabolism and its role in the tumor microenvironment. Based on that information, we develop a rationale for deploying drugs that target lactate metabolism in combination with immune checkpoint inhibitors to overcome lactate-mediated immune escape of tumor cells.

1. Introduction

Long regarded as merely a metabolic waste product, there is now growing evidence that L-lactate produced in excess by cancer cells favors tumor growth and metastasis. L-Lactate exerts this tumorigenic effect, at least in part, by disrupting the normal antitumor function of certain immune cells to create an immunosuppressive tumor microenvironment. This has important therapeutic implications because the localized immunosuppression blunts the efficacy of anticancer immunotherapies. Thus, in principle, targeting lactate metabolism could be a strategy to bolster the effectiveness of cancer therapies and improve patient outcomes. Before delving into these therapeutic possibilities, we begin with an overview of lactate metabolism, especially as it relates to energy production in cancer cells.

2. L-Lactate Biochemistry, Sources, and Transport

Lactate (2-hydroxypropanoate) is a hydroxycarboxylic acid. Two stereoisomers exist, L-lactate and D-lactate. L-Lactate is the predominant enantiomer in the human body [1]. L-Lactate is either produced or removed by a reversible oxidoreduction reaction catalyzed by the enzyme L-lactate dehydrogenase (LDH). Pyruvate is reduced to L-lactate, while reduced nicotinamide adenine dinucleotide (NADH) is oxidized to NAD^+ [2]. High levels of the LDHA isoform are found in muscles and tumors [3]. The two main sources of L-lactate in humans are pyruvate and alanine [4]. L-Lactate is the end-product of glycolysis and the pentose phosphate pathway [5]. Oxidation of L-lactate into pyruvate by LDH in the cytosol is the first step in L-lactate clearance. Lactate metabolism is a highly dynamic and tissue-specific process [6]. L-Lactate transport is mainly executed by monocarboxylate transporters (MCT1, MCT2, and MCT4) (Figure 1). MCT4 is responsible for excretion, whereas MCT1 and MCT2 work in both directions [7, 8]. In addition, two sodium-coupled monocarboxylate transporters, SMCT1 (SLC5A8) and SMCT2 (SLC5A12), mediate the cellular uptake of L-lactate [9–12]. While certain cell types excrete L-lactate, other cell types preferentially take it up, e.g., neurons and glial cells, respectively [6]. The same is true of

tumor cells, tumor stem cells, tumor-associated fibroblasts, and immune cells, which provides the basis for the formation of lactate-rich tumor niches and microenvironments that are highly inimical to therapy. Moreover, it has also been proposed that lactate facilitates metastasis via creation of a microenvironment toxic to normal cells by stimulating tissue lysis [13, 14].

3. The Warburg Effect

The Warburg effect describes the phenomenon, wherein cancer cells generate energy predominantly via glycolysis even if sufficient oxygen for respiration is present (Figure 1). But why would tumors use inefficient glycolysis instead of oxidative phosphorylation (OXPHOS) for energy production? There are several reasons which may explain this reprogramming of ATP generation.

In normal cells, one molecule of glucose produces 38 molecules of ATP during complete oxidation in mitochondria. In cancer cells, pyruvate oxidation is downregulated and replaced by lactate production, catalyzed by LDH, without ATP generation. Thus, in tumor cells, one molecule of glucose produces only two molecules of ATP [15–17]. However, aerobic glycolysis might not be as inefficient as often reported. The production of L-lactate from glucose occurs 10–100 times faster than the complete oxidation in mitochondria and the amount of ATP production is similar per unit of time [18]. The Warburg effect has been proposed to be an adaptive mechanism to support the biosynthetic requirements of uncontrolled proliferation. Glucose serves as a carbon source for anabolic processes. The excess carbon is diverted into branching pathways emanating from glycolysis and is used for the generation of building blocks such as nucleotides, lipids, and proteins [7, 16, 19, 20]. Another theory proposes that tumors shut down OXPHOS to reduce the damage caused by reactive oxygen species (ROS) while maintaining a level necessary for signaling, e.g., especially important for chromatin metabolism [20].

4. Other Models

In addition to the classic Warburg hypothesis, other models have been proposed. The two primary ones are the reverse Warburg effect and the lactate shuttle hypothesis (several additional models are more or less variations of these two hypotheses). An important feature of these two models is that they take into consideration cell-cell interactions, tumor microenvironment, and compartmentalization.

In 2009, a novel "two-compartment metabolic coupling" model, also named "the reverse Warburg effect," was proposed [21, 22]. In this model, epithelial cancer cells induce the Warburg effect (aerobic glycolysis) in neighboring stromal fibroblasts. Cancer-associated fibroblasts (CAFs) then undergo myofibroblastic differentiation and secrete lactate and pyruvate. Epithelial tumor cells are able to take up these energy-rich metabolites and use them in the mitochondrial tricarboxylic acid (TCA) cycle, thereby promoting efficient energy production (i.e., ATP generation via OXPHOS) [22].

The *intra*cellular lactate shuttle hypothesis posits that lactate formed during glycolysis can be continuously used as an energy source within mitochondria of the same cell [23]. The *inter*cellular or cell-cell lactate shuttle hypothesis proposes that lactate generated and exported from one cell can be taken up and utilized by another cell. The latter mechanism was described for neurons and astrocytes [24]. Several articles report that lactate can reach mitochondria via diffusion. LDH in the mitochondrial intermembrane space (IMS) generates NADH used by malate dehydrogenase, which converts oxaloacetate to malate. The malate-α-ketoglutarate (α-KG) antiporter (SLC25A11) transports malate into the mitochondrial matrix in exchange for α-KG that is transported to the IMS, where it is metabolized to glutamate by the enzyme aspartate aminotransferase (AAT). In addition, oxaloacetate is generated from aspartate. The aspartate in the IMS comes from the glutamate aspartate antiporter (SLC25A12 and SLC25A13). The glutamate in the matrix is metabolized to aspartate and the oxaloacetate to α-KG by AAT [23, 24].

5. Role of Hypoxia

A major player in the glycolytic response to hypoxia is the transcription factor hypoxia-inducible factor-1 α (HIF-1α) [25]. Following hypoxia-induced stabilization, HIF-1α mediates a pleiotropic reaction to hypoxia by inducing a plethora of genes, including glucose transporters, angiogenic growth factors (e.g., vascular endothelial growth factor (VEGF)), hexokinase II [26], and hematopoietic factors (e.g., transferrin and erythropoietin) [27]. Radioresistance, immune escape, and secretion of VEGF were reported to be linked to L-lactate accumulation [28–30]. Not surprisingly, MCTs are regulated by hypoxia and/or HIF-1α [31, 32]. Carbonic anhydrase IX (CAIX) is overexpressed in VHL-mutated clear renal cell carcinomas and hypoxic solid tumors [33, 34]. This enzyme catalyzes the reversible hydration of carbon monoxide and is thus involved in regulation of intracellular pH. CAIX is induced by HIF-1α [34]. Importantly, CAIX is considered to be a very reliable marker of hypoxic areas in tissue, whereas HIF-1α is not [35]. Hypoxia might not be important for melanomas. Although numerous articles describe changes of melanoma metabolism and behavior under hypoxic conditions, hypoxia in melanoma might not be present *in vivo*. CAIX is not expressed in the vast majority of melanocytic tumors although when it is expressed it is associated with worse overall survival (OS) [36–38]. Xu and colleagues likewise concluded that melanomas are not under hypoxic stress [39]. Although HIF-1α is induced by low oxygen, many other pathways can regulate HIF-1α in an oxygen-independent manner. The high HIF-1α expression observed in melanomas might be linked to increased lactate production. In other words, lactate may stimulate HIF expression independently of hypoxia [40–42]. In addition, the majority of the melanomas studied showed high OXPHOS enzyme expression, which suggests that they are OXPHOS competent. This is consistent with previous studies reporting that melanomas utilize OXPHOS in addition to glycolysis [39]. Therefore, functioning mitochondria in

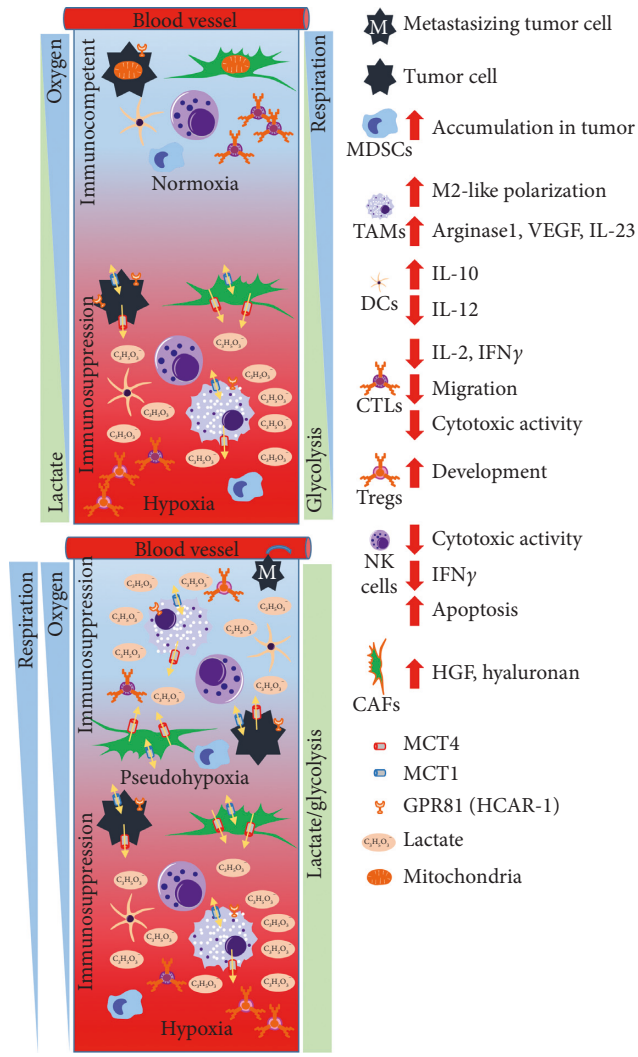

Figure 1: Different oxygen conditions determine the direction of the immune response in the tumor microenvironment. With increasing distance of tumor cells from blood vessels, the oxygen concentration drops. The tumor is not able to respire but instead uses primarily glycolysis for energy production with concomitant production of lactate, which in turn generates an immunosuppressive microenvironment that promotes tumor growth and metastasis (upper panel). Genetic alterations and high levels of lactate causing HIF-1α stabilization are responsible for the glycolytic switch. Tumors use glycolysis even if sufficient oxygen for respiration is present and express hypoxia-related genes and proteins, a state referred to as pseudohypoxia (lower panel). Mitochondria are not shown under hypoxic conditions. This represents a deficiency of OXPHOS, which can be caused by several mechanisms and not just loss of mitochondria. Cellular lactate transport is mainly executed by MCT1 (influx/efflux) and MCT4 (efflux). GPR81 is a G-protein-coupled receptor which senses extracellular levels of lactate. Increased extracellular lactate levels promote escape from immune surveillance of cancer cells, mostly through decreased cytotoxic activity of CTLs and NK cells. Furthermore, lactate induces the accumulation of MDSCs and promotes M2-like polarization and the development of tolerogenic DCs and Tregs. Secreted lactate also not only drives CAFs to produce hepatocyte growth factor, which can attenuate the activity of DCs and CTLs and promote the induction of Tregs, but also increases hyaluronan, which has been associated with cancer progression. Arrows pointing upwards indicate an increase and arrows pointing downwards a decrease. MDSCs: myeloid-derived suppressor cells; TAMs: tumor-associated macrophages; DCs: dendritic cells; CTLs: cytotoxic T lymphocytes; Tregs: regulatory T cells; NK cells: natural killer cells: CAFs: cancer-associated fibroblasts; MCT4: monocarboxylate transporter 4; MCT1: monocarboxylate transporter 1; GPR81: G-protein-coupled receptor 81; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor.

melanomas might be needed for oxidation of lactate produced by glycolysis.

A functioning OXPHOS system only makes sense if oxygen is present. Therefore, the majority of melanomas may be regarded as tumors that do not follow the classic Warburg rules. Several oxygen-independent pathways that regulate HIF-1α were identified in melanomas. Under normoxic conditions, HIF-1α can be stabilized by various growth factors, cytokines and oncogenes, as shown for BRAFV600E in melanoma [43]. HIF-1α was also identified as a microphthalmia-associated transcription factor (MITF) target [43–45]. Many factors important for neoangiogenesis are hypoxia-independent in melanomas [43]. A significant increase of LDHA expression was present in all melanomas. In addition, MCT4 was increased in single cells and areas of the melanomas, suggesting that shuttling of lactate does

indeed occur [36]. However, the lactate shuttle hypothesis is still a matter of debate since the presence of LDH and MCT1 in mitochondria is questioned [46, 47]. Increased expression of SLC25A11 was reported for melanomas in a proteomics study that analyzed 61 primary melanomas [48].

6. L-Lactate as a Biomarker in Melanoma and Other Neoplasms

As early as 1954, increased levels of LDH were detected in serum of melanoma patients [49]. Baseline serum LDH has been established as an independent prognostic factor for survival and since 2009 has been included in the American Joint Committee on Cancer (AJCC) staging system [50, 51]. Elevated serum LDH is also a strong negative predictor of survival in patients with other hematologic and solid neoplasms [52]. Pretreatment LDH levels represent a clinically significant factor associated with response, progression-free survival (PFS), and OS in targeted therapy and immune checkpoint therapy with anti-CTLA-4- and/or anti-PD1-antibodies in melanoma patients [52–57]. High pretreatment LDH levels are also significantly associated with shorter PFS and OS in patients with advanced non-small cell lung cancer treated with immune checkpoint inhibitors [58].

7. Lactate and the Tumor Microenvironment

Lactate has begun to be recognized as an active molecule capable of modulating the immune response. Tumor-derived lactate modulates the functionality of immune cells, contributing to the establishment of an immunosuppressive microenvironment which favors the development of tumors [59–61] (Figure 1). Inflammatory sites are characterized by an accumulation of lactate, which is partly responsible for the establishment of an acidic environment [62]. However, a recent review questions the presence of relevant lactate levels and its impact on immune cells in the tumor microenvironment [63].

7.1. Myeloid-Derived Suppressor Cells. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells and play a crucial role in mediating immunosuppressive effects in the tumor microenvironment [64]. MDSCs suppress both innate and adaptive immunity by preventing the maturation of dendritic cells (DCs), suppressing natural killer (NK) cell cytotoxicity, inhibiting T cell activation, and favoring the differentiation of regulatory T cells [59, 60]. Tumor-derived lactate promotes the development of MDSCs [65]. One possible mechanism of suppression of NK cell function is through the induction of natural killer group-2 member D (NKG2D) ligands in tumor-infiltrating myeloid cells and circulating monocytes via tumor-derived LDH, which downregulates the activating NKG2D receptor on NK cells [28].

7.2. Tumor-Associated Macrophages. Tumor-associated macrophages (TAMs) are one of the most abundant cells in the tumor stroma and contribute to tumor progression at different levels [66]. Tumor-derived lactate drives macrophage polarization toward a tumor-promoting phenotype in mice [67], where HIF-1α-dependent lactate-induced expression of arginase 1 and VEGF might also contribute to immunosuppression and tumor evasion [67–69]. Similarly, lactate from human cervical cancer cell lines caused polarization of macrophages to an immunosuppressive phenotype [70]. Lactic acid secreted from tumor cells enhances IL-23 production in murine and human macrophages [71], which contributes to the development of protumor immunity [72]. Moreover, pretreatment of bone marrow-derived murine macrophages with lactic acid inhibited proliferation of $CD8^+$ T cells [73]. Macrophages can sense lactate secreted from tumor cells via the G-protein-coupled receptors GPR132 (also known as G2A) and GPR81 (also known as hydroxycarboxylic acid receptor 1 (HCAR-1)) and respond with immunosuppressive activity [74, 75]. Both lactate and LDH in the tumor microenvironment can facilitate the protumor activity of TAMs [76].

7.3. Dendritic Cells and Monocytes. Some subsets of functionally distinct DC populations in the tumor microenvironment display a tolerogenic and immune suppressive phenotype [77]. High lactic acid concentrations in the tumor microenvironment possibly skew the differentiation of DCs to an immunosuppressive phenotype with increased production of IL-10 and loss of IL-12 [78, 79]. Furthermore, lactate inhibited the differentiation and lipopolysaccharide (LPS)-induced maturation of human monocyte-derived DCs [80]. Lactate also delayed the expression or suppressed the production of proinflammatory cytokines like TNF-alpha and IL-6 in LPS-stimulated human monocytes [81, 82]. The presence of lactic acid rendered tumor-associated DCs tolerogenic and led to concentration-dependent inhibition of T cell proliferation [78]. Lactate also promoted the synthesis of prostaglandin E2 and upregulation of COX2 in monocytes, both of which are involved in tumor progression and the development of therapeutic resistance [83, 84].

7.4. T Cells. Several studies demonstrate that lactate negatively affects tumor immunosurveillance by T cells. Lactate suppressed the proliferation and function of murine and human cytotoxic T lymphocytes (CTLs) *in vitro* [85–87]. The presence of lactate in an acidic environment has been shown to selectively target p38 and c-Jun N-terminal kinase activation, resulting in inhibition of IFN-γ production in CTLs [88]. Impairment of IL-2- and IFN-γ-production by CTLs *in vitro* was observed following incubation with either externally added or tumor-derived lactic acid [86, 89]. Lactic acid also impairs the recruitment of CTLs to the tumor microenvironment by blocking their motility [90]. Notably, a significant decrease in intratumoral CTLs was associated with high circulating LDH levels in patients with diffuse-large B cell lymphoma [91]. Lactic acid also diminishes the cytotoxic activity of CTLs by lowering the intracellular amounts of perforin and granzyme B and reducing lytic granule exocytosis [86, 88].

Murine tumors with reduced lactic acid production caused by *Ldha* knockdown showed significantly slower growth rates and greater infiltration by functionally active CTLs compared to control tumors in immunocompetent mice [85]. Importantly, a lactate-rich tumor microenvironment not only impairs effector T cells via LDH but also fosters the development of regulatory T cells to promote immune evasion by tumor cells [92].

7.5. Natural Killer Cells and Natural Killer T Cells. NK cells are part of the innate tumor immune surveillance system, but their contribution is diminished by the presence of lactic acid in an acidic tumor microenvironment [92]. Similar to its effect on T cells, lactic acid prevented the upregulation of the nuclear factor of activated T cells (NFAT) in NK cells, resulting in decreased IFN-γ production [92] and reduced cytotoxic activity [65]. Blocking the lactate flux by inhibition of MCT4 enhanced the cytotoxicity of NK cells in a murine model of breast cancer [93]. Conversely, lactate-mediated acidification of the tumor microenvironment induced apoptosis of NK cells, resulting in their depletion from human colorectal liver metastases [94]. A high-lactate microenvironment is also detrimental to the proliferation, survival, and effector function of NKT cells [95], which are important mediators of overcoming immune exhaustion in the tumor microenvironment [96].

7.6. Other Cell Types. Cancer-associated fibroblasts (CAFs) are a dynamic component of the tumor microenvironment. These cells modulate the interaction between tumor cells and the host stromal response, and CAF-associated metabolic reprogramming can facilitate tumor progression [97]. Secreted lactate drives CAFs to produce hepatocyte growth factor [98], which can attenuate the activity of DCs and CTLs and promote the induction of regulatory T cells [99, 100]. Lactate also increases hyaluronan production in fibroblasts [101], and elevated hyaluronan levels in the tumor microenvironment have been linked to cancer progression and unfavorable outcomes [102, 103].

Endothelial cells are another cell type involved in the crosstalk with tumor cells in the tumor microenvironment [104]. Human umbilical vein endothelial cells (HUVECs) have been shown to respond to lactate with enhanced production of VEGF and upregulation of several receptor tyrosine kinases, including VEGF receptor 2, thereby promoting angiogenesis [105–107]. The phosphoinositide 3-kinase/Akt and NF-$\varkappa$B/IL-8 signaling pathways have been reported to be involved in mediating the proangiogenic activity of HUVECs [107, 108].

8. Possible Targets of Lactate Metabolism and Their Potential to Improve Immunotherapy Outcomes

Due to the multitude of effects of lactate in promoting immune evasion of tumors and stimulating tumor angiogenesis, targeting lactate metabolism in combination with immunotherapy is a promising approach to enhance the efficacy of immune therapies. This was recently demonstrated in a murine melanoma model, where blockage of LDHA not only increased the number of NK cells and CTLs but also augmented their cytolytic activity, resulting in reduced melanoma growth in combination with anti-programmed cell death protein-1 (PD-1) therapy in comparison with PD-1 therapy alone [109]. In addition to LDH, there are other attractive molecules to target to interfere with lactate metabolism; these are described in detail below.

8.1. LDH. Although genetic disruption or silencing of LDHA was shown to inhibit tumor growth *in vitro* and *in vivo* in several studies [2, 110–112], it has been suggested that only disruption of LDHA and LDHB together can abolish the growth of tumor cell lines *in vitro* [113, 114].

Several LDH inhibitors have been tested preclinically for anticancer activity, but the majority of them have low potency and off-target effects and therefore are not suitable for clinical use [3].

Oxamate, a known LDH inhibitor for more than 60 years [115], is the most widely used substance for LDH inhibition in preclinical studies. However, due to its activity in the millimolar range, it has never been used in clinical trials [113, 116].

Quinoline-3-sulfonamides have been shown to have antitumor activity, but their clinical use is hampered by their poor bioavailability [112, 117].

A 2-amino-5-aryl pyrazine and a 2-thio-6-oxo-1,6-dihydropyrimidine were identified as potent inhibitors of human LDH, but they showed only minimal cellular activity in cancer cells [118, 119]. Modification of small molecule LDH inhibitors led to the development of the potent LDH inhibitor GNE-140, which inhibited murine B16 melanoma as well as human adenocarcinoma and pancreatic carcinoma cells *in vitro* dependent on their metabolic activity [114, 120].

Other drugs which target LDH by different mechanisms and exhibit preclinical antiproliferative activity against cancer cell lines, such as galloflavin [121, 122], FX11 [2], and *N*-hydroxyindole-2-carboxylate- [123, 124], and pyrazole-based inhibitors of LDH [125], have never been used clinically.

Recently, molecules with 1,4-triazole moieties have been reported as potent inhibitors of LDH, but they have not been tested for anticancer activity [126].

Several natural products, including the saffron derivative crocetin, have been identified as LDH inhibitors with antiproliferative activity against cancer cell lines [127].

Gossypol (also known as AT-101), derived from cotton plant seeds, is a nonselective inhibitor of LDH whose antitumor activity has been attributed to its additional capability to inhibit the antiapoptotic Bcl-2 protein family [128]. Gossypol has been tested in several phase I and phase II clinical trials in various tumor types either as a monotherapy or in combination with chemotherapy but produced negligible response rates in the majority of studies. Despite the multiple biological properties of gossypol, oral doses up to 40 mg per day were tolerated [129–134].

Oroxylin A, a bioactive flavonoid isolated from a Chinese medicinal plant, inhibited LDH and the production of lactate in human hepatocellular carcinoma cells [135]. However, the broadly reported anticancer activity of oroxylin A, including its inhibitory action on the generation of regulatory T cells in the tumor microenvironment of non-small cell lung cancer, appears to involve multiple targets and pathways [136, 137].

A recent high-throughput screen of 1280 drugs identified vitamin C as an LDH-lowering agent, which reduced lactate production and inhibited tumor growth of breast cancer cells in a chronic stress model [138].

There are several drugs currently approved for clinical use which could potentially be repurposed as LDH inhibitors such as the antiepileptic drug stiripentol [139] or the nonsteroidal anti-inflammatory drugs (NSAIDs) diclofenac and lumiracoxib [140].

8.2. MCTs. As knockdown of the lactate transporters MCT1 and MCT4 resulted in suppression of breast cancer and colorectal cancer *in vitro* and *in vivo* [141, 142], targeting MCTs has also been included in therapeutic strategies. Accordingly, analogs of α-cyano-4-hydroxycinnamic acid [143] as well as derivatives of 7-aminocarboxycoumarins [144] have been reported as MCT1 inhibitors with remarkable antitumor activity *in vitro* and *in vivo*. While some MCT1-inhibiting small molecules have been described as immunosuppressive compounds [145], a small molecule inhibitor of MCT1, AZD3965, has shown preclinical antitumor properties in several hematological tumors [146] and small cell lung cancer [147]. The compound has also entered a phase I trial (NCT01791595) in patients with advanced solid tumors or lymphoma, but no results of this trial have been published to date.

For MCT4, diclofenac [148] and bindarit (2-[(1-benzyl-1H-indazol-3-yl)methoxy]-2-methylpropanoic acid) [149] have been reported as selective inhibitors. Because the efficacy of the MCT4 inhibitor AZ93 to block the growth of various cancer cell lines was dependent of MCT1 inhibition [8], it is likely that only concurrent inhibition of MCT1 and MCT4 can impair tumor growth, especially under hypoxic conditions. Syrosingopine was recently identified as a dual inhibitor of MCT1 and MCT4 with potential antitumor benefits *in vivo* [150]. There is evidence that lonidamine, a well-tolerated anticancer drug which is particularly effective at selectively sensitizing tumors to other therapies, might also be capable of concurrently inhibiting MCT1 and MCT4 [151, 152].

8.3. GPR81. GPR81 (HCAR-1) is a lactate-sensing receptor found on monocytes and other immune cells [75, 153] and also on certain cancer cells. In the latter, GPR81 activation promotes proliferation, invasion [154], chemoresistance [155], and upregulation of programmed cell death protein 1-ligand (PD-L1) [156]. Knockdown of GPR81 in mice diminished the production of IL-10 and suppressed the generation of regulatory T cells [75]. Furthermore, silencing of GPR81 in tumor cells led to reduced PD-L1 expression [156] and attenuation of growth and metastatic potential [157]. These interesting findings elevate GPR81 as another target in lactate metabolism to be included in tumor therapy approaches.

9. Conclusion

The Warburg effect and altered tumor metabolism have been recognized as a hallmark of cancer for nearly a century. Lactate is one of the key "oncometabolites" regulating the interaction of cancer cells with the tumor microenvironment. Since elevated serum LDH is negatively associated with clinical efficacy of anticancer (immune) therapies, targeting this enzyme or other molecules involved in lactate metabolism clearly has potential to improve patient outcomes. Although several LDH inhibitors lack selectivity and clinical efficacy in monotherapy, there may be strong potential in combining them with immunotherapy, especially in patients with high LDH levels. Possible off-target effects (either beneficial or toxic) would need to be assessed. Repurposing of approved drugs which can inhibit LDH and have been well tolerated in clinical trials could circumvent toxicity concerns. Besides inhibition of LDH, there are other key molecules involved in lactate metabolism which could be targeted to overcome resistance to immune therapy.

Abbreviations

AAT:	Aspartate aminotransferase
AJCC:	American Joint Committee on Cancer
Akt:	Protein kinase B
α-KG:	α-Ketoglutarate
ATP:	Adenosine triphosphate
Bcl-2:	B cell lymphoma 2
BRAF:	v-Raf murine sarcoma viral oncogene homolog B
CAF:	Cancer-associated fibroblast
CAIX:	Carbonic anhydrase IX
COX2:	Cyclooxygenase 2
CTL:	Cytotoxic T lymphocyte
CTLA-4:	Cytotoxic T-lymphocyte-associated protein 4
DC:	Dendritic cell
GPR81:	G-protein coupled receptor 81
GPR132 (also known as G2A):	G-protein coupled receptor 132
HCAR-1:	Hydroxycarboxylic acid receptor 1
HIF-1α:	Hypoxia-inducible factor-1α
HUVEC:	Human umbilical vein endothelial cell
IFN-γ:	Interferon-γ
IMS:	Intermembrane space
LDH:	Lactate dehydrogenase
LPS:	Lipopolysaccharide
MCT1:	Monocarboxylate transporter 1
MCT2:	Monocarboxylate transporter 2
MCT4:	Monocarboxylate transporter 4
MDSC:	Myeloid-derived suppressor cell
MITF:	Microphthalmia-associated transcription factor
NADH:	Nicotinamide adenine dinucleotide

NFAT: Nuclear factor of activated T cells
NF-$\varkappa$B: Nuclear factor "kappa-light-chain-enhancer" of activated B cells
NK cell: Natural killer cell
NKG2D: Natural killer group 2 member D
OS: Overall survival
OXPHOS: Oxidative phosphorylation
PD1: Programmed cell death protein 1
PFS: Progression-free survival
ROS: Reactive oxygen species
SLC25A11: Solute carrier family 25 member 11 (malate-α-ketoglutarate antiporter)
SLC25A12: Solute carrier family 25 member 12 (glutamate aspartate antiporter)
SLC25A13: Solute carrier family 25 member 13 (glutamate aspartate antiporter)
SMCT1 (SLC5A8): Sodium-coupled monocarboxylate transporter 1
SMCT2 (SLC5A12): Sodium-coupled monocarboxylate transporter 2
TAM: Tumor-associated macrophage
VEGF: Vascular endothelial growth factor
VHL: von Hippel Lindau.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] H. Connor, H. F. Woods, and J. G. G. Ledingham, "Comparison of the kinetics and utilisation of *D*(−)- and *L*(+)-sodium lactate in normal man," *Annals of Nutrition and Metabolism*, vol. 27, no. 6, pp. 481–487, 1983.

[2] A. Le, C. R. Cooper, A. M. Gouw et al., "Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression," *Proceedings of the National Academy of Sciences*, vol. 107, no. 5, pp. 2037–2042, 2010.

[3] J. R. Doherty and J. L. Cleveland, "Targeting lactate metabolism for cancer therapeutics," *Journal of Clinical Investigation*, vol. 123, no. 9, pp. 3685–3692, 2013.

[4] J. Seheult, G. Fitzpatrick, and G. Boran, "Lactic acidosis: an update," *Clinical Chemistry and Laboratory Medicine*, vol. 55, no. 3, pp. 322–333, 2017.

[5] J. Lemire, R. J. Mailloux, and V. D. Appanna, "Mitochondrial lactate dehydrogenase is involved in oxidative-energy metabolism in human astrocytoma cells (CCF-STTG1)," *PLoS One*, vol. 3, no. 2, Article ID e1550, 2008.

[6] M. Adeva-Andany, M. Lopez-Ojen, R. Funcasta-Calderon et al., "Comprehensive review on lactate metabolism in human health," *Mitochondrion*, vol. 17, pp. 76–100, 2014.

[7] A. J. Levine and A. M. Puzio-Kuter, "The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes," *Science*, vol. 330, no. 6009, pp. 1340–1344, 2010.

[8] I. Marchiq and J. Pouyssegur, "Hypoxia, cancer metabolism and the therapeutic benefit of targeting lactate/H(+) symporters," *Journal of Molecular Medicine*, vol. 94, no. 2, pp. 155–171, 2016.

[9] E. Gopal, Y. J. Fei, M. Sugawara et al., "Expression of slc5a8 in kidney and its role in Na(+)-coupled transport of lactate," *Journal of Biological Chemistry*, vol. 279, no. 43, pp. 44522–44532, 2004.

[10] E. Gopal, S. Miyauchi, P. M. Martin et al., "Transport of nicotinate and structurally related compounds by human SMCT1 (SLC5A8) and its relevance to drug transport in the mammalian intestinal tract," *Pharmaceutical Research*, vol. 24, no. 3, pp. 575–584, 2007.

[11] H. Li, L. Myeroff, D. Smiraglia et al., "SLC5A8, a sodium transporter, is a tumor suppressor gene silenced by methylation in human colon aberrant crypt foci and cancers," *Proceedings of the National Academy of Sciences*, vol. 100, no. 14, pp. 8412–8417, 2003.

[12] P. M. Martin, E. Gopal, S. Ananth et al., "Identity of SMCT1 (SLC5A8) as a neuron-specific Na+-coupled transporter for active uptake of L-lactate and ketone bodies in the brain," *Journal of Neurochemistry*, vol. 98, no. 1, pp. 279–288, 2006.

[13] V. Estrella, T. Chen, M. Lloyd et al., "Acidity generated by the tumor microenvironment drives local invasion," *Cancer Research*, vol. 73, no. 5, pp. 1524–1535, 2013.

[14] R. A. Gatenby and E. T. Gawlinski, "A reaction-diffusion model of cancer invasion," *Cancer Research*, vol. 56, pp. 5745–5753, 1996.

[15] X. B. Li, J. D. Gu, and Q. H. Zhou, "Review of aerobic glycolysis and its key enzymes-new targets for lung cancer therapy," *Thoracic Cancer*, vol. 6, no. 1, pp. 17–24, 2015.

[16] M. G. Vander Heiden, L. C. Cantley, and C. B. Thompson, "Understanding the Warburg effect: the metabolic requirements of cell proliferation," *Science*, vol. 324, no. 5930, pp. 1029–1033, 2009.

[17] J. W. Locasale and L. C. Cantley, "Metabolic flux and the regulation of mammalian cell growth," *Cell Metabolism*, vol. 14, no. 4, pp. 443–451, 2011.

[18] A. A. Shestov, X. Liu, Z. Ser et al., "Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step," *eLife*, vol. 3, 2014.

[19] L. K. Boroughs and R. J. DeBerardinis, "Metabolic pathways promoting cancer cell survival and growth," *Nature Cell Biology*, vol. 17, no. 4, pp. 351–359, 2015.

[20] M. V. Liberti and J. W. Locasale, "The Warburg effect: how does it benefit cancer cells?," *Trends in Biochemical Sciences*, vol. 41, no. 3, pp. 211–218, 2016.

[21] Y. Fu, S. Liu, S. Yin et al., "The reverse Warburg effect is likely to be an Achilles' heel of cancer that can be exploited for cancer therapy," *Oncotarget*, vol. 8, no. 34, pp. 57813–57825, 2017.

[22] S. Pavlides, D. Whitaker-Menezes, R. Castello-Cros et al., "The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma," *Cell Cycle*, vol. 8, no. 23, pp. 3984–4001, 2009.

[23] G. A. Brooks, "Mammalian fuel utilization during sustained exercise," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 120, no. 1, pp. 89–107, 1998.

[24] S. Genc, I. A. Kurnaz, and M. Ozilgen, "Astrocyte-neuron lactate shuttle may boost more ATP supply to the neuron under hypoxic conditions--in silico study supported by in vitro expression data," *BMC Systems Biology*, vol. 5, no. 1, p. 162, 2011.

[25] G. L. Semenza, "Hypoxia-inducible factor 1: master regulator of O_2 homeostasis," *Current Opinion in Genetics & Development*, vol. 8, no. 5, pp. 588–594, 1998.

[26] S. Yasuda, S. Arii, A. Mori et al., "Hexokinase II and VEGF expression in liver tumors: correlation with hypoxia-inducible factor 1 alpha and its significance," *Journal of Hepatology*, vol. 40, no. 1, pp. 117–123, 2004.

[27] P. Carmeliet, Y. Dor, J. M. Herbert et al., "Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis," *Nature*, vol. 394, no. 6692, pp. 485–490, 1998.

[28] C. A. Crane, K. Austgen, K. Haberthur et al., "Immune evasion mediated by tumor-derived lactate dehydrogenase induction of NKG2D ligands on myeloid cells in glioblastoma patients," *Proceedings of the National Academy of Sciences*, vol. 111, no. 35, pp. 12823–12828, 2014.

[29] M. I. Koukourakis, A. Giatromanolaki, S. Winter, R. Leek, E. Sivridis, and A. L. Harris, "Lactate dehydrogenase 5 expression in squamous cell head and neck cancer relates to prognosis following radical or postoperative radiotherapy," *Oncology*, vol. 77, no. 5, pp. 285–292, 2009.

[30] S. Langhammer, M. Najjar, H. Hess-Stumpp, and K. H. Thierauch, "LDH-A influences hypoxia-inducible factor 1alpha (HIF1 alpha) and is critical for growth of HT29 colon carcinoma cells in vivo," *Target Oncology*, vol. 6, no. 3, pp. 155–162, 2011.

[31] F. Perez de Heredia, I. S. Wood, and P. Trayhurn, "Hypoxia stimulates lactate release and modulates monocarboxylate transporter (MCT1, MCT2, and MCT4) expression in human adipocytes," *Pflügers Archiv-European Journal of Physiology*, vol. 459, no. 3, pp. 509–518, 2010.

[32] M. S. Ullah, A. J. Davies, and A. P. Halestrap, "The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism," *Journal of Biological Chemistry*, vol. 281, no. 14, pp. 9030–9037, 2006.

[33] H. A. Al-Ahmadie, D. Alden, L. X. Qin et al., "Carbonic anhydrase IX expression in clear cell renal cell carcinoma: an immunohistochemical study comparing 2 antibodies," *The American Journal of Surgical Pathology*, vol. 32, no. 3, pp. 377–382, 2008.

[34] M. Benej, S. Pastorekova, and J. Pastorek, "Carbonic anhydrase IX: regulation and role in cancer," *Subcellular Biochemistry*, vol. 75, pp. 199–219, 2014.

[35] M. R. Ambrosio, C. Di Serio, G. Danza et al., "Carbonic anhydrase IX is a marker of hypoxia and correlates with higher Gleason scores and ISUP grading in prostate cancer," *Diagnostic Pathology*, vol. 11, no. 1, p. 45, 2016.

[36] R. G. Feichtinger, R. Lang, R. Geilberger et al., "Melanoma tumors exhibit a variable but distinct metabolic signature," *Experimental Dermatology*, vol. 27, no. 2, pp. 204–207, 2018.

[37] L. Syrjanen, T. Luukkaala, M. Leppilampi et al., "Expression of cancer-related carbonic anhydrases IX and XII in normal skin and skin neoplasms," *APMIS*, vol. 122, no. 9, pp. 880–889, 2014.

[38] S. C. Chafe, P. C. McDonald, S. Saberi et al., "Targeting hypoxia-induced carbonic anhydrase IX enhances immune-checkpoint blockade locally and systemically," *Cancer Immunology Research*, vol. 7, no. 7, pp. 1064–1078, 2019.

[39] K. Xu, X. Mao, M. Mehta, J. Cui, C. Zhang, and Y. Xu, "A comparative study of gene-expression data of basal cell carcinoma and melanoma reveals new insights about the two cancers," *PLoS One*, vol. 7, no. 1, Article ID e30750, 2012.

[40] D. Fukumura, L. Xu, Y. Chen, T. Gohongi, B. Seed, and R. K. Jain, "Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo," *Cancer Research*, vol. 61, pp. 6020–6024, 2001.

[41] H. Lu, R. A. Forbes, and A. Verma, "Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis," *Journal of Biological Chemistry*, vol. 277, no. 26, pp. 23111–23115, 2002.

[42] Q. Shi, X. Le, B. Wang et al., "Regulation of vascular endothelial growth factor expression by acidosis in human cancer cells," *Oncogene*, vol. 20, no. 28, pp. 3751–3756, 2001.

[43] S. Meierjohann, "Hypoxia-independent drivers of melanoma angiogenesis," *Frontiers in Oncology*, vol. 5, p. 102, 2015.

[44] R. Busca, E. Berra, C. Gaggioli et al., "Hypoxia-inducible factor 1{alpha} is a new target of microphthalmia-associated transcription factor (MITF) in melanoma cells," *The Journal of Cell Biology*, vol. 170, no. 1, pp. 49–59, 2005.

[45] M. L. Hartman and M. Czyz, "Pro-survival role of MITF in melanoma," *Journal of Investigative Dermatology*, vol. 135, no. 2, pp. 352–358, 2015.

[46] R. S. Cruz, R. A. de Aguiar, T. Turnes, R. Penteado Dos Santos, M. F. de Oliveira, and F. Caputo, "Intracellular shuttle: the lactate aerobic metabolism," *The Scientific World Journal*, vol. 2012, Article ID 420984, 8 pages, 2012.

[47] T. Hashimoto, S. Masuda, S. Taguchi, and G. A. Brooks, "Immunohistochemical analysis of MCT1, MCT2 and MCT4 expression in rat plantaris muscle," *The Journal of Physiology*, vol. 567, no. 1, pp. 121–129, 2005.

[48] S. D. Byrum, S. K. Larson, N. L. Avaritt et al., "Quantitative proteomics identifies activation of hallmark pathways of cancer in patient melanoma," *Journal of Proteomics & Bioinformatics*, vol. 6, no. 3, pp. 43–50, 2013.

[49] B. R. Hill and C. Levi, "Elevation of a serum component in neoplastic disease," *Cancer Research*, vol. 14, pp. 513–515, 1954.

[50] C. M. Balch, J. E. Gershenwald, S. J. Soong et al., "Final version of 2009 AJCC melanoma staging and classification," *Journal of Clinical Oncology*, vol. 27, no. 36, pp. 6199–6206, 2009.

[51] J. E. Gershenwald, R. A. Scolyer, K. R. Hess et al., "Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual," *CA: A Cancer Journal for Clinicians*, vol. 67, no. 6, pp. 472–492, 2017.

[52] J. Ding, J. E. Karp, and A. Emadi, "Elevated lactate dehydrogenase (LDH) can be a marker of immune suppression in cancer: interplay between hematologic and solid neoplastic clones and their microenvironments," *Cancer Biomarkers*, vol. 19, no. 4, pp. 353–363, 2017.

[53] J. Dick, N. Lang, A. Slynko et al., "Use of LDH and autoimmune side effects to predict response to ipilimumab treatment," *Immunotherapy*, vol. 8, no. 9, pp. 1033–1044, 2016.

[54] S. Kelderman, B. Heemskerk, H. van Tinteren et al., "Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma," *Cancer Immunology, Immunotherapy*, vol. 63, pp. 449–458, 2014.

[55] F. Petrelli, R. Ardito, B. Merelli et al., "Prognostic and predictive role of elevated lactate dehydrogenase in patients with melanoma treated with immunotherapy and BRAF inhibitors: a systematic review and meta-analysis," *Melanoma Research*, vol. 29, no. 1, pp. 1–12, 2019.

[56] N. B. Wagner, A. Forschner, U. Leiter, C. Garbe, and T. K. Eigentler, "S100B and LDH as early prognostic markers for response and overall survival in melanoma patients treated with anti-PD-1 or combined anti-PD-1 plus anti-CTLA-4 antibodies," *British Journal of Cancer*, vol. 119, no. 3, pp. 339–346, 2018.

[57] B. Weide, A. Martens, J. C. Hassel et al., "Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab," *Clinical Cancer Research*, vol. 22, no. 22, pp. 5487–5496, 2016.

[58] Z. Zhang, Y. Li, X. Yan et al., "Pretreatment lactate dehydrogenase may predict outcome of advanced non small-cell lung cancer patients treated with immune checkpoint inhibitors: a meta-analysis," *Cancer Medicine*, vol. 8, no. 4, pp. 1467–1473, 2019.

[59] D. I. Gabrilovich, S. Ostrand-Rosenberg, and V. Bronte, "Coordinated regulation of myeloid cells by tumours," *Nature Reviews Immunology*, vol. 12, no. 3, pp. 253–268, 2012.

[60] A. Morrot, L. M. da Fonseca, E. J. Salustiano et al., "Metabolic symbiosis and immunomodulation: how tumor cell-derived lactate may disturb innate and adaptive immune responses," *Frontiers in Oncology*, vol. 8, p. 81, 2018.

[61] I. Serganova, I. J. Cohen, K. Vemuri et al., "LDH-A regulates the tumor microenvironment via HIF-signaling and modulates the immune response," *PLoS One*, vol. 13, no. 9, Article ID e0203965, 2018.

[62] V. Pucino, M. Bombardieri, C. Pitzalis, and C. Mauro, "Lactate at the crossroads of metabolism, inflammation, and autoimmunity," *European Journal of Immunology*, vol. 47, no. 1, pp. 14–21, 2017.

[63] J. C. Garcia-Canaveras, L. Chen, and J. D. Rabinowitz, "The tumor metabolic microenvironment: lessons from lactate," *Cancer Research*, vol. 79, no. 13, pp. 3155–3162, 2019.

[64] S. Ostrand-Rosenberg, "Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity," *Cancer Immunology, Immunotherapy*, vol. 59, no. 10, pp. 1593–1600, 2010.

[65] Z. Husain, Y. Huang, P. Seth, and V. P. Sukhatme, "Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells," *The Journal of Immunology*, vol. 191, no. 3, pp. 1486–1495, 2013.

[66] A. Mantovani, F. Marchesi, A. Malesci, L. Laghi, and P. Allavena, "Tumour-associated macrophages as treatment targets in oncology," *Nature Reviews Clinical Oncology*, vol. 14, no. 7, pp. 399–416, 2017.

[67] O. R. Colegio, N. Q. Chu, A. L. Szabo et al., "Functional polarization of tumour-associated macrophages by tumour-derived lactic acid," *Nature*, vol. 513, no. 7519, pp. 559–563, 2014.

[68] H. Lemos, L. Huang, G. C. Prendergast, and A. L. Mellor, "Immune control by amino acid catabolism during tumorigenesis and therapy," *Nature Reviews Cancer*, vol. 19, no. 3, pp. 162–175, 2019.

[69] J. Yang, J. Yan, and B. Liu, "Targeting VEGF/VEGFR to modulate antitumor immunity," *Frontiers in Immunology*, vol. 9, p. 978, 2018.

[70] S. C. Stone, R. A. M. Rossetti, K. L. F. Alvarez et al., "Lactate secreted by cervical cancer cells modulates macrophage phenotype," *Journal of Leukocyte Biology*, vol. 105, no. 5, pp. 1041–1054, 2019.

[71] H. Shime, M. Yabu, T. Akazawa et al., "Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway," *The Journal of Immunology*, vol. 180, no. 11, pp. 7175–7183, 2008.

[72] J. Yan, M. J. Smyth, and M. W. L. Teng, "Interleukin (IL)-12 and IL-23 and their conflicting roles in cancer," *Cold Spring Harbor Perspectives in Biology*, vol. 10, no. 7, Article ID a028530, 2018.

[73] T. Ohashi, T. Akazawa, M. Aoki et al., "Dichloroacetate improves immune dysfunction caused by tumor-secreted lactic acid and increases antitumor immunoreactivity," *International Journal of Cancer*, vol. 133, no. 5, pp. 1107–1118, 2013.

[74] P. Chen, H. Zuo, H. Xiong et al., "Gpr132 sensing of lactate mediates tumor-macrophage interplay to promote breast cancer metastasis," *Proceedings of the National Academy of Sciences*, vol. 114, no. 3, pp. 580–585, 2017.

[75] P. Ranganathan, A. Shanmugam, D. Swafford et al., "GPR81, a cell-surface receptor for lactate, regulates intestinal homeostasis and protects mice from experimental colitis," *The Journal of Immunology*, vol. 200, pp. 1781–1789, 2018.

[76] P. Seth, E. Csizmadia, A. Hedblom et al., "Deletion of lactate dehydrogenase-A in myeloid cells triggers antitumor immunity," *Cancer Research*, vol. 77, no. 13, pp. 3632–3643, 2017.

[77] D. Laoui, J. Keirsse, Y. Morias et al., "The tumour microenvironment harbours ontogenically distinct dendritic cell populations with opposing effects on tumour immunity," *Nature Communications*, vol. 7, no. 1, p. 13720, 2016.

[78] E. Gottfried, L. A. Kunz-Schughart, S. Ebner et al., "Tumor-derived lactic acid modulates dendritic cell activation and antigen expression," *Blood*, vol. 107, no. 5, pp. 2013–2021, 2006.

[79] A. Nasi, T. Fekete, A. Krishnamurthy et al., "Dendritic cell reprogramming by endogenously produced lactic acid," *The Journal of Immunology*, vol. 191, no. 6, pp. 3090–3099, 2013.

[80] A. Puig-Kroger, O. M. Pello, O. Muniz-Pello et al., "Peritoneal dialysis solutions inhibit the differentiation and maturation of human monocyte-derived dendritic cells: effect of lactate and glucose-degradation products," *Journal of Leukocyte Biology*, vol. 73, no. 4, pp. 482–492, 2003.

[81] K. Goetze, S. Walenta, M. Ksiazkiewicz, L. A. Kunz-Schughart, and W. Mueller-Klieser, "Lactate enhances motility of tumor cells and inhibits monocyte migration and cytokine release," *International Journal of Oncology*, vol. 39, pp. 453–463, 2011.

[82] K. Peter, M. Rehli, K. Singer, K. Renner-Sattler, and M. Kreutz, "Lactic acid delays the inflammatory response of human monocytes," *Biochemical and Biophysical Research Communications*, vol. 457, no. 3, pp. 412–418, 2015.

[83] D. Tong, Q. Liu, L. A. Wang et al., "The roles of the COX_2/PGE_2/EP axis in therapeutic resistance," *Cancer and Metastasis Reviews*, vol. 37, no. 2-3, pp. 355–368, 2018.

[84] L. Wei, Y. Zhou, J. Yao et al., "Lactate promotes PGE2 synthesis and gluconeogenesis in monocytes to benefit the growth of inflammation-associated colorectal tumor," *Oncotarget*, vol. 6, no. 18, pp. 16198–16214, 2015.

[85] A. Brand, K. Singer, G. E. Koehl et al., "LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells," *Cell Metabolism*, vol. 24, no. 5, pp. 657–671, 2016.

[86] K. Fischer, P. Hoffmann, S. Voelkl et al., "Inhibitory effect of tumor cell-derived lactic acid on human T cells," *Blood*, vol. 109, no. 9, pp. 3812–3819, 2007.

[87] S. Roth, H. Gmunder, and W. Droge, "Regulation of intracellular glutathione levels and lymphocyte functions by lactate," *Cell Immunology*, vol. 136, no. 1, pp. 95–104, 1991.

[88] A. N. Mendler, B. Hu, P. U. Prinz, M. Kreutz, E. Gottfried, and E. Noessner, "Tumor lactic acidosis suppresses CTL function by inhibition of p38 and JNK/c-Jun activation," *International Journal of Cancer*, vol. 131, no. 3, pp. 633–640, 2012.

[89] C. Feder-Mengus, S. Ghosh, W. P. Weber et al., "Multiple mechanisms underlie defective recognition of melanoma cells cultured in three-dimensional architectures by antigen-specific cytotoxic T lymphocytes," *British Journal of Cancer*, vol. 96, no. 7, pp. 1072–1082, 2007.

[90] R. Haas, J. Smith, V. Rocher-Ros et al., "Lactate regulates metabolic and pro-inflammatory circuits in control of T cell

migration and effector functions," *PLoS Biology*, vol. 13, no. 7, Article ID e1002202, 2015.

[91] C. Keane, J. Tobin, D. Talaulikar et al., "A high LDH to absolute lymphocyte count ratio in patients with DLBCL predicts for a poor intratumoral immune response and inferior survival," *Oncotarget*, vol. 9, no. 34, pp. 23620–23627, 2018.

[92] A. Angelin, L. Gil-de-Gomez, S. Dahiya et al., "Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments," *Cell Metabolism*, vol. 25, no. 6, pp. 1282–1293, 2017.

[93] Y. Long, Z. Gao, X. Hu et al., "Downregulation of MCT4 for lactate exchange promotes the cytotoxicity of NK cells in breast carcinoma," *Cancer Medicine*, vol. 7, no. 9, pp. 4690–4700, 2018.

[94] C. Harmon, M. W. Robinson, F. Hand et al., "Lactate-mediated acidification of tumor microenvironment induces apoptosis of liver-resident NK cells in colorectal liver metastasis," *Cancer Immunology Research*, vol. 7, no. 2, pp. 335–346, 2019.

[95] A. Kumar, K. Pyaram, E. L. Yarosz et al., "Enhanced oxidative phosphorylation in NKT cells is essential for their survival and function," *Proceedings of the National Academy of Sciences*, vol. 116, no. 15, pp. 7439–7448, 2019.

[96] E. A. Bae, H. Seo, I. K. Kim, I. Jeon, and C. Y. Kang, "Roles of NKT cells in cancer immunotherapy," *Archives of Pharmacal Research*, vol. 42, no. 7, pp. 543–548, 2019.

[97] D. Wu, L. Zhuo, and X. Wang, "Metabolic reprogramming of carcinoma-associated fibroblasts and its impact on metabolic heterogeneity of tumors," *Seminars in Cell & Developmental Biology*, vol. 64, pp. 125–131, 2017.

[98] M. Apicella, E. Giannoni, S. Fiore et al., "Increased lactate secretion by cancer cells sustains non-cell-autonomous adaptive resistance to MET and EGFR targeted therapies," *Cell Metabolism*, vol. 28, no. 6, pp. 848–865, 2018.

[99] M. Benkhoucha, N. Molnarfi, G. Schneiter, P. R. Walker, and P. H. Lalive, "The neurotrophic hepatocyte growth factor attenuates CD8+ cytotoxic T-lymphocyte activity," *Journal of Neuroinflammation*, vol. 10, no. 1, p. 154, 2013.

[100] M. Benkhoucha, M. L. Santiago-Raber, G. Schneiter et al., "Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and CD25+Foxp3+ regulatory T cells," *Proceedings of the National Academy of Sciences*, vol. 107, no. 14, pp. 6424–6429, 2010.

[101] R. Stern, S. Shuster, B. A. Neudecker, and B. Formby, "Lactate stimulates fibroblast expression of hyaluronan and CD44: the Warburg effect revisited," *Experimental Cell Research*, vol. 276, no. 1, pp. 24–31, 2002.

[102] A. Josefsson, H. Adamo, P. Hammarsten et al., "Prostate cancer increases hyaluronan in surrounding nonmalignant stroma, and this response is associated with tumor growth and an unfavorable outcome," *The American Journal of Pathology*, vol. 179, no. 4, pp. 1961–1968, 2011.

[103] K. L. Schwertfeger, M. K. Cowman, P. G. Telmer, E. A. Turley, and J. B. McCarthy, "Hyaluronan, inflammation, and breast cancer progression," *Frontiers in Immunology*, vol. 6, p. 236, 2015.

[104] H. Choi and A. Moon, "Crosstalk between cancer cells and endothelial cells: implications for tumor progression and intervention," *Archives of Pharmacal Research*, vol. 41, no. 7, pp. 711–724, 2018.

[105] S. Beckert, F. Farrahi, R. S. Aslam et al., "Lactate stimulates endothelial cell migration," *Wound Repair and Regeneration*, vol. 14, no. 3, pp. 321–324, 2006.

[106] V. B. Kumar, R. I. Viji, M. S. Kiran, and P. R. Sudhakaran, "Endothelial cell response to lactate: implication of PAR modification of VEGF," *Journal of Cellular Physiology*, vol. 211, no. 2, pp. 477–485, 2007.

[107] G. X. Ruan and A. Kazlauskas, "Lactate engages receptor tyrosine kinases Axl, Tie2, and vascular endothelial growth factor receptor 2 to activate phosphoinositide 3-kinase/Akt and promote angiogenesis," *Journal of Biological Chemistry*, vol. 288, no. 29, pp. 21161–21172, 2013.

[108] F. Vegran, R. Boidot, C. Michiels, P. Sonveaux, and O. Feron, "Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kappaB/IL-8 pathway that drives tumor angiogenesis," *Cancer Res*, vol. 71, no. 7, pp. 2550–2560, 2011.

[109] S. Daneshmandi, B. Wegiel, and P. Seth, "Blockade of lactate dehydrogenase-A (LDH-A) improves efficacy of anti-programmed cell death-1 (PD-1) therapy in melanoma," *Cancers*, vol. 11, no. 4, p. 450, 2019.

[110] R. Bok, J. Lee, R. Sriram et al., "The role of lactate metabolism in prostate cancer progression and metastases revealed by dual-agent hyperpolarized (13)C MRSI," *Cancers*, vol. 11, no. 2, p. 257, 2019.

[111] L. Jin, J. Chun, C. Pan et al., "Phosphorylation-mediated activation of LDHA promotes cancer cell invasion and tumour metastasis," *Oncogene*, vol. 36, no. 27, pp. 3797–3806, 2017.

[112] H. Xie, J. Hanai, J. G. Ren et al., "Targeting lactate dehydrogenase--a inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor-initiating cells," *Cell Metabolism*, vol. 19, no. 5, pp. 795–809, 2014.

[113] C. J. Valvona and H. L. Fillmore, "Oxamate, but not selective targeting of LDH-A, inhibits medulloblastoma cell glycolysis, growth and motility," *Brain Sciences*, vol. 8, no. 4, p. 56, 2018.

[114] M. Zdralevic, A. Brand, L. Di Ianni et al., "Double genetic disruption of lactate dehydrogenases A and B is required to ablate the "Warburg effect" restricting tumor growth to oxidative metabolism," *Journal of Biological Chemistry*, vol. 293, pp. 15947–15961, 2018.

[115] W. B. Novoa, A. D. Winer, A. J. Glaid, and G. W. Schwert, "Lactic dehydrogenase. V. Inhibition by oxamate and by oxalate," *Journal of Biological Chemistry*, vol. 234, pp. 1143–1148, 1959.

[116] M. Manerba, L. Di Ianni, L. Fiume, M. Roberti, M. Recanatini, and G. Di Stefano, "Lactate dehydrogenase inhibitors sensitize lymphoma cells to cisplatin without enhancing the drug effects on immortalized normal lymphocytes," *European Journal of Pharmaceutical Sciences*, vol. 74, pp. 95–102, 2015.

[117] J. Billiard, J. B. Dennison, J. Briand et al., "Quinoline 3-sulfonamides inhibit lactate dehydrogenase A and reverse aerobic glycolysis in cancer cells," *Cancer & Metabolism*, vol. 1, no. 1, p. 19, 2013.

[118] P. S. Dragovich, B. P. Fauber, L. B. Corson et al., "Identification of substituted 2-thio-6-oxo-1,6-dihydropyrimidines as inhibitors of human lactate dehydrogenase," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 11, pp. 3186–3194, 2013.

[119] B. P. Fauber, P. S. Dragovich, J. Chen et al., "Identification of 2-amino-5-aryl-pyrazines as inhibitors of human lactate dehydrogenase," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 20, pp. 5533–5539, 2013.

[120] A. Boudreau, H. E. Purkey, A. Hitz et al., "Metabolic plasticity underpins innate and acquired resistance to LDHA

inhibition," *Nature Chemical Biology*, vol. 12, no. 10, pp. 779–786, 2016.

[121] F. Farabegoli, M. Vettraino, M. Manerba, L. Fiume, M. Roberti, and G. Di Stefano, "Galloflavin, a new lactate dehydrogenase inhibitor, induces the death of human breast cancer cells with different glycolytic attitude by affecting distinct signaling pathways," *European Journal of Pharmaceutical Sciences*, vol. 47, no. 4, pp. 729–738, 2012.

[122] M. Manerba, M. Vettraino, L. Fiume et al., "Galloflavin (CAS 568-80-9): a novel inhibitor of lactate dehydrogenase," *ChemMedChem*, vol. 7, no. 2, pp. 311–317, 2012.

[123] C. Granchi, S. Roy, A. De Simone et al., "N-Hydroxyindole-based inhibitors of lactate dehydrogenase against cancer cell proliferation," *European Journal of Medicinal Chemistry*, vol. 46, no. 11, pp. 5398–5407, 2011.

[124] M. A. Lea, Y. Guzman, and C. Desbordes, "Inhibition of growth by combined treatment with inhibitors of lactate dehydrogenase and either phenformin or inhibitors of 6-Phosphofructo-2-kinase/Fructose-2,6-bisphosphatase 3," *Anticancer Research*, vol. 36, pp. 1479–1488, 2016.

[125] G. Rai, K. R. Brimacombe, B. T. Mott et al., "Discovery and optimization of potent, cell-active pyrazole-based inhibitors of lactate dehydrogenase (LDH)," *Journal of Medicinal Chemistry*, vol. 60, no. 22, pp. 9184–9204, 2017.

[126] A. S. Altamimi, A. M. Alafeefy, A. Balode et al., "Symmetric molecules with 1,4-triazole moieties as potent inhibitors of tumour-associated lactate dehydrogenase-A," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 33, no. 1, pp. 147–150, 2018.

[127] C. Granchi, S. Fortunato, S. Meini et al., "Characterization of the saffron derivative crocetin as an inhibitor of human lactate dehydrogenase 5 in the antiglycolytic approach against cancer," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 28, pp. 5639–5649, 2017.

[128] M. Zhang, H. Liu, R. Guo et al., "Molecular mechanism of gossypol-induced cell growth inhibition and cell death of HT-29 human colon carcinoma cells," *Biochemical Pharmacology*, vol. 66, no. 1, pp. 93–103, 2003.

[129] M. Q. Baggstrom, Y. Qi, M. Koczywas et al., "A phase II study of AT-101 (Gossypol) in chemotherapy-sensitive recurrent extensive-stage small cell lung cancer," *Journal of Thoracic Oncology*, vol. 6, no. 10, pp. 1757–1760, 2011.

[130] P. Bushunow, M. M. Reidenberg, J. Wasenko et al., "Gossypol treatment of recurrent adult malignant gliomas," *Journal of Neuro-Oncology*, vol. 43, no. 1, pp. 79–86, 1999.

[131] M. R. Flack, R. G. Pyle, N. M. Mullen et al., "Oral gossypol in the treatment of metastatic adrenal cancer," *The Journal of Clinical Endocrinology & Metabolism*, vol. 76, no. 4, pp. 1019–1024, 1993.

[132] R. S. Heist, J. Fain, B. Chinnasami et al., "Phase I/II study of AT-101 with topotecan in relapsed and refractory small cell lung cancer," *Journal of Thoracic Oncology*, vol. 5, no. 10, pp. 1637–1643, 2010.

[133] G. Liu, W. K. Kelly, G. Wilding, L. Leopold, K. Brill, and B. Somer, "An open-label, multicenter, phase I/II study of single-agent AT-101 in men with castrate-resistant prostate cancer," *Clinical Cancer Research*, vol. 15, no. 9, pp. 3172–3176, 2009.

[134] C. Van Poznak, A. D. Seidman, M. M. Reidenberg et al., "Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase I/II clinical trial," *Breast Cancer Research and Treatment*, vol. 66, no. 3, pp. 239–248, 2001.

[135] Q. Dai, Q. Yin, L. Wei et al., "Oroxylin A regulates glucose metabolism in response to hypoxic stress with the involvement of Hypoxia-inducible factor-1 in human hepatoma HepG2 cells," *Molecular Carcinogenesis*, vol. 55, no. 8, pp. 1275–1289, 2016.

[136] L. Lu, Q. Guo, and L. Zhao, "Overview of oroxylin a: a promising flavonoid compound," *Phytotherapy Research*, vol. 30, no. 11, pp. 1765–1774, 2016.

[137] L. Shen, L. L. Zhang, H. Li et al., "Oroxylin A inhibits the generation of Tregs in non-small cell lung cancer," *Oncotarget*, vol. 8, no. 30, pp. 49395–49408, 2017.

[138] B. Cui, Y. Luo, P. Tian et al., "Stress-induced epinephrine enhances lactate dehydrogenase A and promotes breast cancer stem-like cells," *Journal of Clinical Investigation*, vol. 129, no. 3, pp. 1030–1046, 2019.

[139] N. Sada, S. Lee, T. Katsu, T. Otsuki, and T. Inoue, "Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy," *Science*, vol. 347, no. 6228, pp. 1362–1367, 2015.

[140] C. Brummer, S. Faerber, C. Bruss et al., "Metabolic targeting synergizes with MAPK inhibition and delays drug resistance in melanoma," *Cancer Letters*, vol. 442, pp. 453–463, 2019.

[141] H. K. Kim, I. Lee, H. Bang et al., "MCT4 expression is a potential therapeutic target in colorectal cancer with peritoneal carcinomatosis," *Molecular Cancer Therapeutics*, vol. 17, no. 4, pp. 838–848, 2018.

[142] F. Morais-Santos, S. Granja, V. Miranda-Goncalves et al., "Targeting lactate transport suppresses in vivo breast tumour growth," *Oncotarget*, vol. 6, no. 22, pp. 19177–19189, 2015.

[143] S. Gurrapu, S. K. Jonnalagadda, M. A. Alam et al., "Monocarboxylate transporter 1 inhibitors as potential anticancer agents," *ACS Medicinal Chemistry Letters*, vol. 6, no. 5, pp. 558–561, 2015.

[144] N. Draoui, O. Schicke, E. Seront et al., "Antitumor activity of 7-aminocarboxycoumarin derivatives, a new class of potent inhibitors of lactate influx but not efflux," *Molecular Cancer Therapeutics*, vol. 13, no. 6, pp. 1410–1418, 2014.

[145] C. Pahlman, Z. Qi, C. M. Murray et al., "Immunosuppressive properties of a series of novel inhibitors of the monocarboxylate transporter MCT-1," *Transplant International*, vol. 26, no. 1, pp. 22–29, 2013.

[146] N. J. Curtis, L. Mooney, L. Hopcroft et al., "Pre-clinical pharmacology of AZD3965, a selective inhibitor of MCT1: DLBCL, NHL and Burkitt's lymphoma anti-tumor activity," *Oncotarget*, vol. 8, no. 41, pp. 69219–69236, 2017.

[147] R. Polanski, C. L. Hodgkinson, A. Fusi et al., "Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer," *Clinical Cancer Research*, vol. 20, no. 4, pp. 926–937, 2014.

[148] S. Sasaki, Y. Futagi, M. Ideno et al., "Effect of diclofenac on SLC16A3/MCT4 by the Caco-2 cell line," *Drug Metabolism and Pharmacokinetics*, vol. 31, no. 3, pp. 218–223, 2016.

[149] Y. Futagi, M. Kobayashi, K. Narumi, A. Furugen, and K. Iseki, "Identification of a selective inhibitor of human monocarboxylate transporter 4," *Biochemical and Biophysical Research Communications*, vol. 495, no. 1, pp. 427–432, 2018.

[150] D. Benjamin, D. Robay, S. K. Hindupur et al., "Dual inhibition of the lactate transporters MCT1 and MCT4 is synthetic lethal with metformin due to NAD+ depletion in cancer cells," *Cell Reports*, vol. 25, no. 11, pp. 3047–3058, 2018.

[151] B. Nancolas, L. Guo, R. Zhou et al., "The anti-tumour agent lonidamine is a potent inhibitor of the mitochondrial pyruvate carrier and plasma membrane monocarboxylate transporters," *Biochemical Journal*, vol. 473, no. 7, pp. 929–936, 2016.

[152] G. Robustelli della Cuna and P. Pedrazzoli, "Toxicity and clinical tolerance of lonidamine," *Seminars in Oncology*, vol. 18, pp. 18–22, 1991.

[153] R. Hoque, A. Farooq, A. Ghani, F. Gorelick, and W. Z. Mehal, "Lactate reduces liver and pancreatic injury in Toll-like receptor- and inflammasome-mediated inflammation via GPR81-mediated suppression of innate immunity," *Gastroenterology*, vol. 146, no. 7, pp. 1763–1774, 2014.

[154] Y. J. Lee, K. J. Shin, S. A. Park et al., "G-protein-coupled receptor 81 promotes a malignant phenotype in breast cancer through angiogenic factor secretion," *Oncotarget*, vol. 7, no. 43, pp. 70898–70911, 2016.

[155] W. Wagner, K. D. Kania, A. Blauz, and W. M. Ciszewski, "The lactate receptor (HCAR1/GPR81) contributes to doxorubicin chemoresistance via ABCB1 transporter upregulation in human cervical cancer HeLa cells," *Journal of Physiology and Pharmacology*, vol. 68, pp. 555–564, 2017.

[156] J. Feng, H. Yang, Y. Zhang et al., "Tumor cell-derived lactate induces TAZ-dependent upregulation of PD-L1 through GPR81 in human lung cancer cells," *Oncogene*, vol. 36, no. 42, pp. 5829–5839, 2017.

[157] C. L. Roland, T. Arumugam, D. Deng et al., "Cell surface lactate receptor GPR81 is crucial for cancer cell survival," *Cancer Research*, vol. 74, no. 18, pp. 5301–5310, 2014.

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Review Article

Monoclonal Antibody Therapies in Multiple Myeloma: A Challenge to Develop Novel Targets

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The treatment options in multiple myeloma (MM) has changed dramatically over the past decade with the development of novel agents such as proteasome inhibitors (PIs); bortezomib and immunomodulatory drugs (IMiDs); thalidomide, and lenalidomide which revealed high efficacy and improvement of overall survival (OS) in MM patients. However, despite these progresses, most patients relapse and become eventually refractory to these therapies. Thus, the development of novel, targeted immunotherapies has been pursued aggressively. Recently, next-generation PIs; carfilzomib and ixazomib, IMiD; pomalidomide, histone deacetylase inhibitor (HDADi); panobinostat and monoclonal antibodies (MoAbs); and elotuzumab and daratumumab have emerged, and especially, combination of mAbs plus novel agents has led to dramatic improvements in the outcome of MM patients. The field of immune therapies has been accelerating in the treatment of hematological malignancies and has also taken center stage in MM. This review focuses on an overview of current status of novel MoAb therapy including bispecific T-cell engager (BiTE) antibody (BsAb), antibody-drug conjugate (ADC), and chimeric antigen receptor (CAR) T cells, in relapsed or refractory MM (RRMM). Lastly, investigational novel MoAb-based therapy to overcome immunotherapy resistance in MM is shown.

1. Introduction

The treatment options in MM has changed dramatically over the past decade with the emergence of novel agents including proteasome inhibitors (PIs, bortezomib) and immunomodulatory drugs (IMiDs, thalidomide and lenalidomide) and exerts a remarkable impact on the outcome of MM patients [1–3]. However, most patients who achieve a prolonged response following initial therapy may ultimately relapse or become refractory. Thus, the development of novel, targeted immunotherapies has been pursued aggressively. Recently, next-generation PIs (carfilzomib and ixazomib) [4–9], IMiDs (pomalidomide) [10–12], histone deacetylase inhibitor (HDACi, panobinostat) [13–15], and the monoclonal antibodies (MoAbs, elotuzumab and daratumumab) have emerged and further improved the clinical outcome in MM patients who are refractory to prior treatments [12, 16–36]. Importantly, MM remains a chronic disease, so in order to overcome the disease relapse, ongoing challenges to pursue novel therapeutic strategies as well as predictive biomarkers for response or resistance to immunotherapies are required. Furthermore, these novel therapies are expected to be potentially useful in the treatment options for patients who are ineligible for autologous stem cell transplantation (SCT) followed by high-dose chemotherapy [37].

Monoclonal antibody (MoAb) therapies have been accelerating and shown to be able to improve the outcome of cancers [38]. In hematological malignancies, rituximab, a chimeric murine/human anti-CD20 monoclonal $IgG_{1\kappa}$ antibody or of atumumab, a humanized anti-CD20 monoclonal $IgG_{1\kappa}$ antibody, targeting CD20 on B cells, is currently indicated for the treatment of B-cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). It exerts significant activity in combination with cytotoxic anticancer drugs [38, 39].

Although these progresses in immune therapies and their application for the treatment of MM have not succeeded until recently, these therapeutic strategies have finally attained a breakthrough with the development of the MoAb therapies targeting surface molecules, expressed in MM cells, such as elotuzumab, a humanized anti-CS1/SLAMF7 monoclonal antibody, and daratumumab, a humanized anti-CD38 monoclonal antibody, both of which have been approved in the treatment of relapsed or refractory MM (RRMM) patients who received at least three prior therapies including PIs and iMiDs [40–43]. Herein, we review an overview of the current status of MoAb therapies in RRMM. In addition, we introduce investigational novel MoAb therapies in RRMM and show future direction toward immunotherapy resistance in MM.

2. Monoclonal Antibodies (MoAbs) in MM

Potential MoAbs target various kinds of antigens including growth factors, signaling molecules, cell surface proteins, and molecule of adhesion. Ideally, these MoAb-therapeutic targets should be predominantly expressed on a majority of MM cells, but not on normal hematopoietic cells or non-hematopoietic tissues. MoAb therapies involve several mechanisms including direct cytotoxic effects, antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cellular cytotoxicity (CDC), and interference with cell-to-cell interactions [40–43]. Other mechanisms include the use of intracellular toxins or radioactive isotopes conjugated to MoAbs after its internalization into tumor cells, which reveal cytotoxicity against tumor cells beyond those bearing MoAb target antigens [40–43].

2.1. CD20 and Rituximab. CD20 is a transmembrane phosphoprotein expressed on committed B lymphoid cells through the all stages of their development, but its expression is reduced in plasma cells. Rituximab, a chimeric murine/human anti-CD20 monoclonal $IgG_{1\kappa}$ antibody targeting CD20 on B cells, is currently indicated for the treatment of B-cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) [39]. It exerts significant activity in combination with cytotoxic anticancer drugs. However, CD20 is present only in a few plasma cells and is absent in most of plasma cells in MM. Therefore, few selected MM patients achieved only minimal responses (MD) [44–46]. Moreover, MM cells express increased levels of complement-inhibitory proteins which result in the reduction of CDC via rituximab against tumor cells.

2.2. CS1/SLAMF7 and Elotuzumab. Elotuzumab is a humanized IgG_1 monoclonal antibody which targets SLAMF7, known as CS1, a glycoprotein, intensely expressed on MM cells and normal plasma cells as well as natural killer (NK) cells. It induces cytotoxicity against MM cells via NK cell-associated ADCC, NK cell activation, and inhibition of the interaction between MM cells and bone marrow stromal cells (BMSCs). Elotuzumab revealed intensive anti-MM efficacy and safety profiles when combined with IMiDs or PIs in previously treated RRMM [12, 16–21] (Table 1). The phase II results demonstrated that elotuzumab in combination with lenalidomide plus dexamethasone (Rd) in patients with RRMM showed safety and efficacy which was better than previously noted with Rd [17, 18]. Moreover, results of the phase III trial ELOQUENT-2 clearly proved the benefit of adding elotuzumab to Rd for the treatment of RRMM [18]. The overall response rates (ORRs) were 79% for the elotuzumab group and 66% for the control group; the PFS rate was 68 vs. 57% for the elotuzumab and control groups at 1 year and 41 vs. 27% at 2 years; the median PFS was 19.4 vs. 14.9 months for the elotuzumab and control groups [19]. Based on the results of these trials, elotuzumab attained food and drug administration (FDA) approval in 2015 in combination with Rd for the treatment of RRMM patients, who previously received two or three prior therapies. A phase III randomized study of Rd with or without elotuzumab in previously treated MM patients is currently ongoing. Phase II trials of elotuzumab plus pomalidomide and dexamethasone (EPd) vs Pd in 117 patients who received >2 prior therapies revealed that after a follow-up period of 9 months, EPd had a longer median PFS (10.3 vs 4.7 month) and a better ORR (53 vs 26%) [12]. Phase II trials of elotuzumab plus bortezomib and dexamethasone (EBd) vs Bd in 77 patients who had received one to three prior therapies showed that EBd had a longer median PFS (9.7 vs 6.9 months). However, there was no deference in ORR between EBd group and Bd group (66% vs 63%) [20, 21].

2.3. CD38 and Daratumumab. Daratumumab is a humanized IgG_1-kappa monoclonal antibody targeting CD38, which is 46-kDa type II transmembrane glycoprotein, broadly expressed on plasma cells as well as lymphoid cells, myeloid cells, and nonhematopoietic tissues. It is also expressed in OCs. CD38 retains multiple functions including ectoenzymatic activity, signal transduction, and receptor-mediated regulation of cell adhesion [22, 23]. In preclinical studies, daratumumab revealed anti-MM cytotoxicity through multiple mechanisms including ADCC, ADCP, CDC, and direct apoptosis via FcR-mediated cross linking of daratumumab *in vitro* [24–26] (Table 2). Of note, no difference was revealed in daratumumab-associated ADCC or CDC between newly diagnosed and RRMM patients. The level of CD38 expression in MM cells was reported to be related to daratumumab-associated ADCC and CDC [24–26]. Moreover, daratumumab has several effects on the immune system. It increases CD8+/CD4+ and CD8+ Treg ratios as well as memory T cells, while decreasing naïve T cells, which enhance the overall immune response to MM cells [27].

Daratumumab revealed anti-MM efficacy as monotherapy as well as in combination with novel agents in heavily pretreated RRMM patients, which resulted in FDA approval in 2015. The GEN501 and SIRIUS trials demonstrated that daratumumab is active as monotherapy in RRMM patients [28, 29]. It showed improved ORRs regardless of refractoriness to prior therapies including PIs and IMiDs (31%). [30]. Phase III Castor trials revealed that

Table 1: Summary of clinical trials in anti-CS1/SLAMF7 antibody in relapsed/refractory MM.

References		Phase	Regimen	ORR (%)	PFS (mo)	OS
Richardson et al. [17]		2	Elo + Rd	84.00%	NA	NA
Lonial et al. [18]	ELOAUENT2	3	Rd ± Elo	79% vs 66%	19.4 mo vs 14.9 mo	NA
Dimopoulos et al. [12]		2	Pd ± Elo	53% vs 26%	10.3 mo vs 4.7 mo	NA
Jakubowiak et al. [20]	Elo-Bd	2	Bd ± Elo	66% vs 63%	9.7 mo vs 6.9 mo	1 yr 85% vs 74%
Zonder et al. [16]	Phase1 Elo	1	Elo Dose Escalation	MTD not identified	NA	NA
Jakubowiak, et al. [21]	Elo-Bd	1	Elo + Bd	48.00%	9.5 mo	NA
Lonial, et al. [19]	Elo-Rd	1	Elo + Rd	82.00%	NA	NA

MM, multiple myeloma; Elo, elotuzumab; Rd, lenalidomide plus dexamethasone; Pd, pomalidomide plus dexamethasone; Bd, bortezomib plus dexamethasone, NA, not available; MTD, maximum tolerated dose.

Table 2: Summary of clinical trials in anti-CD38 antibody in relapse/refractory MM.

References		Phase	Regimen	ORR (%)	PFS (mo)	OS
Lokhorst et al. [28]	GEN501	1/2	Dara monotherapy	36%	5.6 mo	1 yr 77%
Lonial et al. [29]	SIRIUS	2	Dara monotherapy	17%	3.7 mo	1 yr 65%
Spencer et al. [32]	CASTOR	3	Bd ± Dara	83% vs 63%	1.5 yr 48% vs 8%	NA
Palumbo et al. [31]	CASTOR	3	Bd ± Dara	83% vs 63%	1 yr 61% vs 27%	NA
Dimopoulos et al. [33]	POLLUX	3	Rd ± Dara	93% vs 76%	1 yr 83% vs 60%	NA
Dimopoulos et al. [34]	POLLUX		Rd ± Dara	93% vs76%	2 yr 68% vs 41%	NA
Chari et al. [35]	EQULLEUS	1b	Pd ± Dara	60%	1 yr 42%	1 yr 89%

MM, multiple myeloma; Dara; daratumumab, Rd, lenalidomide plus dexamethasone; Bd, bortezomib plus dexamethasone; Pd, pomalidomide plus dexamethasone; NA, not available; MTD, maximum tolerated dose.

daratumumab significantly improved ORR, PFS, and time to progression (TTP) in combination with Bd, ORR (83% vs 63%), the 12-month rate of PFS (61% vs 27%), and TTP at 12 months (65% vs 29%) [31]. Another phase III Castor study also revealed a significant benefit of D-Bd over Bd regardless of treatment history or cytogenetic risk [32]. Phase III POLLUX trials demonstrated remarkable efficacy of daratumumab in combination with lenalidomide plus dexamethasone (DRd) in patients with RRMM [33, 34]. The ORR was 92.9% in DRd group versus 72.9% in Rd group. DRd improved PFS compared with Rd with 12-month PFS rates of 83.2% in DRd group versus 60.1% in Rd group and 24-month PFS rate of 68.0% versus 40.9%, restrictively [33, 34]. The EQUULEUS study led to the FDA approval of daratumumab in combination with Pd in 2017 for RRMM patients who have received 2 or more prior line of therapy including lenalidomide and a PI. The median PFS was 8.8 months, the 12-month PFS rate was 42%, the median OS was 17.5 months, and the median 12-month survival rate was 66% [35].

3. Novel Target Antigens in MoAb Therapies in MM

3.1. CD38 and Isatuximab. Isatuximab is a chimeric IgG_1-kappa anti-CD38 monoclonal antibody which selectively binds to a unique epitope on human CD38 receptor and elicits anti-MM activity by direct apoptosis, ADCC, and ADCP [47]. CDC was triggered in less than half of MM patients with high levels of CD38 in MM cells. A phase 1b open-label, dose escalation study showed that 57 patients who had received at least one prior line of therapy attained ORR of 52% by isatuximab plus Rd in 42 evaluable lenalidomide-refractory patients, and overall median PFS was 8.5 months [48]. Another phase 1b study of isatuximab plus Pd in patients with RRMM who had received more than 2 prior therapies also revealed that ORR was 62%; median duration of response was 18.7 months; and PFS was 17.6 months [49].

3.2. Interleukin-6 (IL6) and Siltuximab. Interleukin-6 is an important cytokine for the growth and survival of MM cells. It is chiefly produced by BMSCs and increased by several cytokines. A chimeric anti-IL-6 antibody, siltuximab, revealed cytotoxicity in MM patients who was refractory to dexamethasone [50]. In addition, it increased cytotoxicity with Bd in combination, whereas in a phase 2 randomized study of siltuximab plus bortezomib, the addition of siltuximab to bortezomib did not appear to improve PFS or OS in refractory MM patients [51]. The other study showed that there were no responses to siltuximab but combination therapy with dexamethasone yielded a partial or minimal response rate of 23%, in dexamethasone-refractory MM [51].

3.3. PD-1/PD-L1 Inhibitors. Programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway is a negative regulator of immune activation [52]. Recently, there are discrepancies concerning programmed death PD-L1 expression on plasma cells in MM. Several data demonstrated that PD-L1 is overexpressed on MM plasma cells but not on normal plasma cells [53–56]. It was reported that PD-L1 expression on plasma cells was associated with increased risk of progression from smoldering MM (SMM) into MM [57], whereas other reports showed that no difference was detected in PD-L1 expression on plasma cells between MM, SMM, monoclonal gammopathy of undetermined significance (MGUS), and healthy individuals

[58, 59]. Similarly, discordant results were reported regarding PD-1 expression on immune cells, including T cells and NK cells in MM. Paiva et al. showed that PD-1 was overexpressed on CD4+ and CD8+ T cells in MM patients [58]. Benson et al. demonstrated that PD-1 expression was increased on NK cells from MM patients, compared with normal NK cells, whereas Paiva et al. demonstrated there was no difference between these cells [58, 60].

Among hematological malignancies, antibody blockade of the PD-1/PD-L1 pathway is a highly effective therapeutic approach for patients with classical Hodgkin lymphoma, 97% of which typically exhibits an overexpression of PD-L1 due to the alteration in chromosome 9p24.1 (54). Therefore, the PD-1/PD-L1 axis is a good target for MoAbs, leading immune cells to kill tumor cells. The use of nivolumab, a human IgG4 MoAb which blocks the interaction with PD-L1 and PD-L2 by binding to the PD-1 receptor on activated immune cells, was approved by FDA in 2016 for the treatment of relapsed or progressed Hodgkin lymphoma [52]. However, the outcome of checkpoint blockade by monotherapy with PD-1/PD-L1 inhibitors is unsatisfactory in MM, compared with solid tumors due to the reduced immune dysfunction in MM [58, 59]. In contrast, lenalidomide enhances the effect of PD-1/PD-L1 blockade on both T cell- and NK cell-mediated cytotoxicity. The combination therapy of lenalidomide plus PD-1/PD-L1 inhibitors increased interferon γ by BM-derived effector cells in MM and was associated with increased apoptosis of MM cells, suggesting synergistic cytotoxic effects [56, 61, 62]. There are only limited data from clinical trials of PD1/PDL1 MoAbs in MM patients. The phase Ib trial of nivolumab monotherapy in 27 RRMM patients showed the stabilization of disease status in 17 patients, lasting a median of 11.4 weeks [63]. A phase I study of pembrolizumab with Rd in RRMM patients revealed a partial response rate of 50% [61, 62, 64, 65]. A phase 3 study of the combination of Rd with or without pembrolizumab was performed in transplant ineligible newly diagnosed MM patients (KEYNOTE-185 trial) [61, 62, 64]. A Phase 3 study of the combination of Pd with or without pembrolizumab was conducted in the KEYNOTE-183 trial, and it led FDA to discontinue the trial, due to increased risk of death of patients [61, 62, 65].

3.4. Bispecific T-Cell Engager (BiTE) Antibodies (BsAb). Bispecific T-cell engager (BiTE) antibodies (BsAbs) are constructs, composed of 2 linked MoAbs which target 2 epitopes. One arm of antibody, scFvs, binds to CD3 on tumor-specific T cells, while the other arm binds to tumor-specific antigen on tumor cells [66, 67]. Cross linkage of T cells to the tumor cells causes T cells to release cytotoxic molecules such as perforin, which creates transmembrane pores in tumor cells, and granzyme B, which initiates apoptosis toward tumor cells. In addition, cytokine production from T cells activates its proliferation to kill tumor cells. BsAbs are characterized by small size (5 kDa), which induces high efficacy toward tumor cells, but its serum half-life is short [66, 67]. B-cell maturation antigen (BCMA) belongs to tumor necrosis factor superfamily member 17, also named "TNFRSF17 or CD269," which is uniformly expressed in malignant plasma cells but not in normal essential non-hematopoietic tissues, and only restricted expression is detected in normal hematopoietic cells including normal plasma cells and mature B lymphocytes. Thus, it is a highly plasma cell specific antigen and has a central role in regulating B-cell maturation and differentiation into plasma cells by engaging a proliferation-inducing ligand (APRIL) cells. This expression pattern leads to the development of BCMA-specific mAbs, BsAbs, antibody-drug conjugates (ADCs), and chimeric T cell receptor (CAR) T cells [68–70]. BsAb, BI-836909 (AMG420), the first bispecific scFv, simultaneously binds to CD3+ T cells and BCMA + MM cells which make a cross linking between both cells to induce cytolytic synapse, activate T cells, and lyse BCMA + MM cells. In phase I study in RRMM patients, it exhibited potent and high efficacy by depleting BCMA + MM cells [68–70]. CD3xCD38 BsAb, engineered to direct T cells to CD38 on tumor cells, was also developed. The phase 1 multicenter study of GBR1342 is underway [71].

3.5. Antibody-Drug Conjugates (ADCs). Antibody-drug conjugate is composed of recombinant MoAbs, bound to cytotoxic chemical agents through synthetic chemical linkers. MoAbs bind to the cell surface antigen on tumor cells and are internalized with the chemicals. Thus, the cytotoxic chemicals are released and transported from lysosome into cytosol to kill tumor cells [72]. GSK2857916 is a humanized and IgG_1 MoAb with high affinity to BCMA with afucosylated Fc linked to auristatin F noncleavable linker, maleimidocaproyl. In preclinical study, it binds to BCMA + MM cells and induces G2/M arrest and apoptosis by the activation of caspase 3/7 and 8. The naked form of ADC augmented effector-mediated cytotoxicity including ADCC and ADCP against patient MM cells [72]. In MM xenograft models, GSK2857916 depletes MM cells but surrounding BCMA-BM accessory cells remain unharmed. Its cytotoxicity is further increased by GSK2857916 plus lenalidomide in combination. In phase 1 study of GSK2857916 in RRMM patients, GAK2857916 monotherapy revealed a 60% response rate and median PFS of 7.9 months [73, 74]. Anti-BCMA approaches, alone or in combination with iMIDs or immune checkpoint inhibitors, will be evaluated in clinical trials in MM [70].

3.6. Chimeric Antigen Receptor (CAR) T Cells. CARs are fusion proteins incorporating an antigen-recognition domain and T-cell signaling domain. T cells are genetically modified to express CARs, which specifically recognize target antigens on tumor cells [75–77]. CAR T-cell therapy has already approved by FDA and European Medicine Agency (EMA) for the treatment of relapsed of refractory B-acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma (DLBCL) [75–77]. CAR-expressing T cells targeting CD19 revealed efficacy in patients with acute lymphoblastic leukemia (ALL) or B-cell NHL. This success of CAR-T cells against leukemia or lymphoma has encouraged the development of CAR-T therapies for MM. In the first

Table 3: Investigational monoclonal antibodies in MM.

Target molecule	mAb	Type	Clinical trials
CD138	Indatuximab ravtansine	ADC	Inda ± Rena ORR 78% vs 4%
CD56	Lorvotuzumab	ADC	Lorv+/Rd ORR 56% vs 7%
CD40	Dacetuzumab, lucatumumab	Humanized	Luc; 4% attained prolonged PR
CD74	Milatuzumab	Humanized	No objective responses
BAFF	Tabalumab	Humanized	Bd + Taba; ORR 44%
BCMA	GSK2857916	ADC	MTD not determined
GRP78	PAT-SM6	Humanized	MTD not determined
IGF-1R	AVE1642	Humanized	No objective responses
ICAM-1	BI-505	Humanized	No objective responses
CD26	YS110 (huCD26mAb)	Humanized	Best responses 50%

ADC, antibody-drug conjugate; Lena, lenalidomide; Inda, indatuximab ravatansine, Rd, lenalidomide plus dexamethasone; Lorv, lorvotuzumab; Luc, lucatumumab; PR, partial response; Bd, bortezomib + dexamethasone; Taba, tabalumab; MTD, maximum tolerated doses.

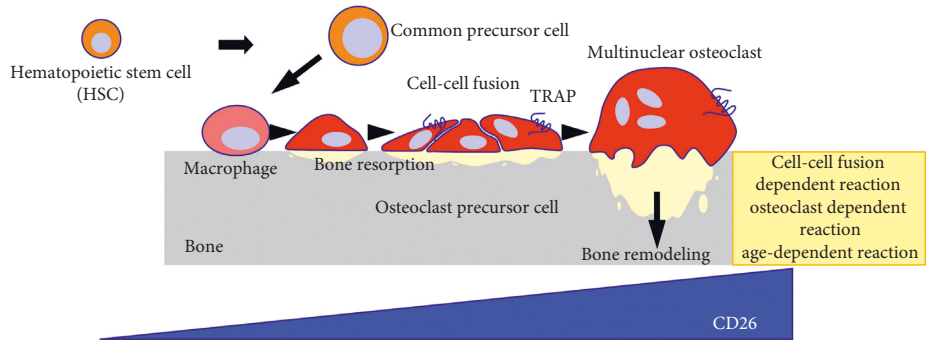

Figure 1: CD26 in human osteoclast development CD26 expression is increased during human osteoclast (OC) development.

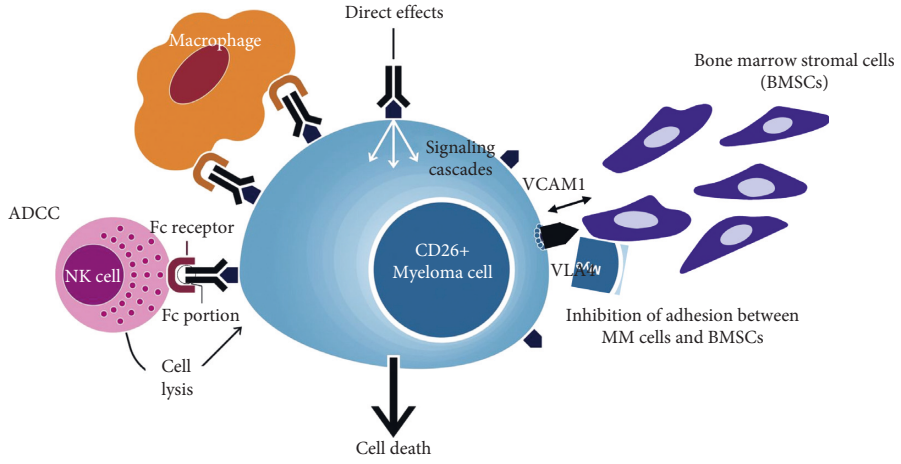

Figure 2: Humanized anti-CD26 monoclonal antibody (huCD26mAb): mechanisms of action huCD26mAb inhibits CD26 + MM cell growth chiefly via ADCC.

human clinical trials, Carpenter et al. designed the first novel CAR targeting BCMA in MM and demonstrated CAR-BCMA T cells had powerful activity against MM that was resistant to standard therapies [78, 79]. Moreover, bb2121 was produced by transducing autologous T cells with a lentiviral vector encoding a second-generation CAR incorporating an anti-BCMA single-chain variable fragment, CD137 costimulatory motif, and a CD3-zeta signaling domain [80]. A phase 1 clinical study of bb2121 in heavily pretreated RRMM patients revealed that 85% of the patients had a clinical response lasting a median of 10.9 months without any ongoing MM therapies [80]. Currently, CAR-T cell therapy for MM remains experimental. CAR-T cell therapy is a potentially life-threatening therapeutic approach, which needs to be administrated in experience hospitals. Now, phase 3 trials are just starting for RRMM in

2019. In addition, novel CARs targeting alternative plasma cell antigens including CD38, CD44v6, and SLAMF7(CS) are being developed [81, 82].

4. Experimental Research in Novel MoAb Therapy in RRMM

4.1. Investigational MoAbs. Target antigens for MoAb are either cell surface membrane proteins or soluble factors including cytokines or chemokines expressed or secreted in MM cells. Their functions include MM cell growth, cellular adhesion, angiogenesis, apoptosis, and cell-to-cell contact between MM cells microenvironmental cells. Investigational mAbs targeting CD138, CD56, CD40, CD74, BAFF, BCMA, GRP78, IGF-1R, and ICAM-1 are preclinically developed, and several of them are in clinical trials [83–92] (Table 3).

4.2. Humanized Anti-CD26 Monoclonal Antibody (huCD26mAb). CD26 is a 110 kDa transmembrane glycoprotein with dipeptidyl peptidase (DPPIV) activity, which is widely expressed in various normal cells such as T lymphocytes, natural killer (NK) cells, basophils, eosinophils, endothelial cells, and epithelial cells [93–96]. In addition, CD26 is expressed in several tumor cells including malignant lymphoma, mesothelioma, renal cell carcinoma, and hepatocellular carcinoma and is involved in T-cell activation and tumorigenesis [97, 98]. We have recently characterized CD26 as a potential therapeutic target for the treatment of MM [99]. We identified CD26 expression in human osteoclasts (OCs) in healthy individuals (Figure 1). Its expression is further increased in osteoclasts in osteolytic bone tumors including MM, adenocarcinoma, lung cancer, and osteosarcoma. huCD26mAb, a humanized IgG_1 monoclonal antibody that directly targets CD26, inhibits human OC differentiation *in vitro* and *in vivo* analysis [99]. In the bone marrow tissue of MM patients, we found that CD26 was present in plasma cells around OCs or endothelial cells. *In vitro* immunostaining or flow cytometry studies revealed that although CD26 expression was low or absent on MM cell lines cultured alone, it was intensely and uniformly expressed on MM cell lines cocultured with OCs [100]. The augmented CD26 expression in MM cells was exploited to enhance cytotoxicity of huCD26mAb chiefly via a substantial increase in antibody-dependent cytotoxicity (ADCC) against MM cells, direct effects or inhibition of the adhesion between MM cells and BM stromal cells (BMSCs) (Figure 2). Moreover, huCD26mAb in combination with the existing standards of care including bortezomib and lenalidomide synergistically enhanced huCD26mAb-induced ADCC activity against CD26 + MM cells compared with each agent alone [100]. Lastly, therapeutic effect of huCD26mAb against MM cell growth and its related osteolytic lesion was also validated *in vivo*, using a xenograft model: an intrabone tumor model of MM. Our preclinical results demonstrated that huCD26mAb elicited significant anti-MM efficacy by impairing both CD26 + MM cells and OCs *in vivo*, suggesting that CD26 could be an ideal therapeutic target of antibody-based therapy in RRMM [100].

5. Conclusion

During the last decades, therapeutic strategies in MM have dramatically changed. MoAbs act synergistically with backbone regimens including iMIDs, PIs, or HDACi and have benefits to overcome resistance to prior therapies. The future treatment options of MM to overcome resistance are promising by combination with MoAbs plus these novel agents, check point inhibitors or CAR T-cell therapy.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Supplementary Materials

CD26 in human osteoclast development humanized anti-CD26 monoclonal antibody (huCD26mAb): mechanisms of action summary of clinical trials in anti-CS1/SLAMF7 antibody in relapsed/refractory MM. Summary of clinical trials in anti-CD38 antibody in relapsed/refractory MM. Investigational monoclonal antibodies in MM. (*Supplementary Materials*)

References

[1] A. Palumbo and K. Anderson, "Multiple myeloma," *New England Journal of Medicine*, vol. 364, no. 11, pp. 1046–1060, 2011.

[2] P. Moreau and E. de Wit, "Recent progress in relapsed multiple myeloma therapy: implications for treatment decisions," *British Journal of Haematology*, vol. 179, no. 2, pp. 198–218, 2017.

[3] C. Varga, J. P. Laubach, K. C. Anderson, and P. G. Richardson, "Investigational agents in immunotherapy: a new horizon for the treatment of multiple myeloma," *British Journal of Haematology*, vol. 181, no. 4, pp. 433–446, 2018.

[4] H. Avet-Loiseau, R. Fonseca, D. Siegel et al., "Carfilzomib significantly improves the progression-free survival of high-risk patients in multiple myeloma," *Blood*, vol. 128, no. 9, pp. 1174–1180, 2016.

[5] A. K. Stewart, S. V. Rajkumar, M. A. Dimopoulos et al., "Carfilzomib, lenalidomide and dexamethasone for relapsed multiple myeloma," *New England Journal of Medicine*, vol. 372, no. 2, pp. 142–152, 2015.

[6] P. Moreau, M.-V. Mateos, J. R. Berenson et al., "Once weekly versus twice weekly carfilzomib dosing in patients with relapsed and refractory multiple myeloma (A.R.R.O.W.): interim analysis results of a randomised, phase 3 study," *The Lancet Oncology*, vol. 19, no. 7, pp. 953–964, 2018.

[7] P. Moreau, T. Masszi, N. Grzasko et al., "Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 374, no. 17, pp. 1621–1634, 2016.

[8] M. A. Dimopoulos, F. Gay, F. Schjesvold et al., "Oral ixazomib maintenance following autologous stem cell transplantation (TOURMALINE-MM3): a double-blind, randomised, placebo-controlled phase 3 trial," *Lancet*, vol. 393, no. 10168, pp. 253–264, 2019.

[9] C. Touzeau and P. Moreau, "Ixazomib in the management of relapsed multiple myeloma," *Future Oncology*, vol. 14, no. 20, pp. 2013–2020, 2018.

[10] J. S. Miguel, K. Weisel, P. Moreau et al., "Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial," *The Lancet Oncology*, vol. 14, no. 11, pp. 1055–1066, 2013.

[11] S. Ailawadhi, J. R. Mikhael, B. R. LaPlant et al., "Pomalidomide-dexamethasone in refractory multiple myeloma: long-term follow-up of a multi-cohort phase II clinical trial," *Leukemia*, vol. 32, no. 3, pp. 719–728, 2018.

[12] M. A. Dimopoulos, D. Dytfeld, S. Grosicki et al., "Elotuzumab plus pomalidomide and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 379, no. 19, pp. 1811–1822, 2018.

[13] J. F. San-Miguel, V. T. M. Hungria, S.-S. Yoon et al., "Overall survival of patients with relapsed multiple myeloma treated with panobinostat or placebo plus bortezomib and dexamethasone (the PANORAMA 1 trial): a randomised, placebo-controlled, phase 3 trial," *The Lancet Haematology*, vol. 3, no. 11, pp. e506–e515, 2016.

[14] J. F. San-Miguel, V. T. M. Hungria, S.-S. Yoon et al., "Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial," *The Lancet Oncology*, vol. 15, no. 11, pp. 1195–1206, 2014.

[15] D. Sivaraj, M. M. Green, and C. Gasparetto, "Panobinostat for the management of multiple myeloma," *Future Oncology*, vol. 13, no. 6, pp. 477–488, 2017.

[16] J. A. Zonder, A. F. Mohrbacher, S. Singhal et al., "A phase 1, multicenter, open-label, dose escalation study of elotuzumab in patients with advanced multiple myeloma," *Blood*, vol. 120, no. 3, pp. 552–559, 2012.

[17] P. G. Richardson, S. Jagannath, P. Moreau et al., "Elotuzumab in combination with lenalidomide and dexamethasone in patients with relapsed multiple myeloma: final phase 2 results from the randomised, open-label, phase 1b-2 dose-escalation study," *The Lancet Haematology*, vol. 2, no. 12, pp. e516–e527, 2015.

[18] S. Lonial, M. Dimopoulos, A. Palumbo et al., "Elotuzumab therapy for relapsed or refractory multiple myeloma," *New England Journal of Medicine*, vol. 373, no. 7, pp. 621–631, 2015.

[19] S. Lonial, R. Vij, J.-L. Harousseau et al., "Elotuzumab in combination with lenalidomide and low-dose dexamethasone in relapsed or refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1953–1959, 2012.

[20] A. Jakubowiak, M. Offidani, B. Pégourie et al., "Randomized phase 2 study: elotuzumab plus bortezomib/dexamethasone vs bortezomib/dexamethasone for relapsed/refractory MM," *Blood*, vol. 127, no. 23, pp. 2833–2840, 2016.

[21] A. J. Jakubowiak, D. M. Benson, W. Bensinger et al., "Phase I trial of anti-CS1 monoclonal antibody elotuzumab in combination with bortezomib in the treatment of relapsed/refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 30, no. 16, pp. 1960–1965, 2012.

[22] J. P. Laubach and P. G. Richardson, "CD38-targeted immunochemotherapy in refractory multiple myeloma: a new horizon," *Clinical Cancer Research*, vol. 21, no. 12, pp. 2660–2662, 2015.

[23] A. K. Nooka, J. L. Kaufman, C. C. Hofmeister et al., "Daratumumab in multiple myeloma," *Cancer*, vol. 125, no. 14, pp. 2364–2381, 2019.

[24] M. De Weers, Y.-T. Tai, M. S. van der Veer et al., "Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors," *The Journal of Immunology*, vol. 186, no. 3, pp. 1840–1848, 2011.

[25] M. B. Overdijk, S. Verploegen, M. Bögels et al., "Antibody-mediated phagocytosis contributes to the anti-tumor activity of the therapeutic antibody daratumumab in lymphoma and multiple myeloma," *mAbs*, vol. 7, no. 2, pp. 311–320, 2015.

[26] M. B. Overdijk, J. H. M. Jansen, M. Nederend et al., "The therapeutic CD38 monoclonal antibody daratumumab induces programmed cell death via fcγ receptor-mediated cross-linking," *The Journal of Immunology*, vol. 197, no. 3, pp. 807–813, 2016.

[27] J. Krejcik, T. Casneuf, I. S. Nijhof et al., "Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma," *Blood*, vol. 128, no. 3, pp. 384–394, 2016.

[28] H. M. Lokhorst, T. Plesner, J. P. Laubach et al., "Targeting CD38 with daratumumab monotherapy in multiple myeloma," *New England Journal of Medicine*, vol. 373, no. 13, pp. 1207–1219, 2015.

[29] S. Lonial, B. M. Weiss, S. Z. Usmani et al., "Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial," *The Lancet*, vol. 387, no. 10027, pp. 1551–1560, 2016.

[30] S. Z. Usmani, B. M. Weiss, T. Plesner et al., "Clinical efficacy of daratumumab monotherapy in patients with heavily pretreated relapsed or refractory multiple myeloma," *Blood*, vol. 128, no. 1, pp. 37–44, 2016.

[31] A. Palumbo, A. Chanan-Khan, K. Weisel et al., "Daratumumab, bortezomib, and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 375, no. 8, pp. 754–766, 2016.

[32] A. Spencer, S. Lentzsch, K. Weisel et al., "Daratumumab plus bortezomib and dexamethasone *versus* bortezomib and dexamethasone in relapsed or refractory multiple myeloma: updated analysis of CASTOR," *Haematologica*, vol. 103, no. 12, pp. 2079–2087, 2018.

[33] M. A. Dimopoulos, A. Oriol, H. Nahi et al., "Daratumumab, lenalidomide, and dexamethasone for multiple myeloma," *New England Journal of Medicine*, vol. 375, no. 14, pp. 1319–1331, 2016.

[34] M. A. Dimopoulos, J. San-Miguel, A. Belch et al., "Daratumumab plus lenalidomide and dexamethasone *versus* lenalidomide and dexamethasone in relapsed or refractory multiple myeloma: updated analysis of POLLUX," *Haematologica*, vol. 103, no. 12, pp. 2088–2096, 2018.

[35] A. Chari, A. Suvannasakha, J. W. Fay et al., "Daratumumab plus pomalidomide and dexamethasone in relapsed and/or refractory myeloma," *Blood*, vol. 130, no. 8, pp. 974–981, 2017.

[36] N. W. C. J. Van de Donk, P. Moreau, T. Plesner et al., "Clinical efficacy and management of monoclonal antibodies targeting CD38 and SLAMF7 in multiple myeloma," *Blood*, vol. 127, no. 6, pp. 681–695, 2016.

[37] H. M. Blommestein, C. H. Y. van Beurden-Tan, M. G. Franken, C. A. Uyl-de Groot, P. Sonneveld, and S. Zweegman, "Efficacy of first-line treatments for multiple myeloma patients not eligible for stem cell transplantation: a

network meta-analysis," *Haematologica*, vol. 104, no. 5, pp. 1026–1035, 2019.

[38] A. Im and S. Z. Pavletic, "Immunotherapy in hematologic malignancies: past, present, and future," *Journal of Hematology & Oncology*, vol. 10, no. 1, pp. 1–10, 2017.

[39] L. Falchi, A. Ferrajoli, I. Jacobs, and P. Nava-Parada, "An evidence-based review of anti-CD20 antibody-containing regimens for the treatment of patients with relapsed or refractory chronic lymphocytic leukemia, diffuse large B-cell lymphoma, or follicular lymphoma," *Clinical Lymphoma Myeloma and Leukemia*, vol. 18, no. 8, pp. 508–518, 2018.

[40] H. N. Abramson, "Monoclonal antibodies for the treatment of multiple myeloma: an update," *International Journal of Molecular Sciences*, vol. 19, no. 12, p. 3924, 2018.

[41] T. Ishida, "Therapeutic antibodies for multiple myeloma," *Japanese Journal of Clinical Oncology*, vol. 48, no. 11, pp. 957–963, 2018.

[42] P. G. Richardosn, S. Lonial, A. J. Jakubowiak, J. L. Harousseau, and K. C. Anderson, "Monoclonal antibodies in the treatment of multiple myeloma," *British Journal of Haematology*, vol. 154, no. 6, pp. 745–754, 2011.

[43] S. K. Kumar and K. C. Anderson, "Immune therapies in multiple myeloma," *Clinical Cancer Research*, vol. 22, no. 22, pp. 5453–5460, 2016.

[44] P. Kappor, P. T. Greipp, W. G. Morice, S. V. Rajkumar, T. E. Witzig, and P. R. Greipp, "Anti-CD20 monoclonal antibody therapy in multiple myeloma," *British Journal of Haematology*, vol. 141, no. 2, pp. 135–148, 2008.

[45] B. Huang, J. Li, J. Liu, and J. Gu, "CD20-positive multiple myeloma: can conventional chemotherapy still be used to achieve ideal outcome for these patients?," *Leukemia & Lymphoma*, vol. 57, no. 2, pp. 335–340, 2016.

[46] G. Grigoriadis, M. Gilbertson, N. Came et al., "Is CD20 positive plasma cell myeloma a unique clinicopathological entity? A study of 40 cases and review of the literature," *Pathology*, vol. 44, no. 6, pp. 552–556, 2012.

[47] L. Moreno, C. Perez, A. Zabaleta, I. Manrique et al., "The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma," *Clinical Cancer Research*, vol. 25, no. 10, pp. 3176–3187, 2019.

[48] T. Martin, R. Baz, D. M. Benson et al., "A phase 1b study of isatuximab plus lenalidomide and dexamethasone for relapsed/refractory multiple myeloma," *Blood*, vol. 129, no. 25, pp. 3294–3303, 2017.

[49] J. Mikhael, P. Richardson, S. Z. Usmani et al., "A phase 1b study of isatuximab plus pomalidomide/dexamethasone in relapsed/refractory multiple myeloma," *Blood*, vol. 134, no. 2, pp. 123–133, 2019.

[50] R. Z. Orlowski, L. Gercheva, C. Williams et al., "A phase 2, randomized, double-blind, placebo-controlled study of siltuximab (anti-IL-6 mAb) and bortezomib versus bortezomib alone in patients with relapsed or refractory multiple myeloma," *American Journal of Hematology*, vol. 90, no. 1, pp. 42–49, 2015.

[51] P. M. Voorhees, R. F. Manges, P. Sonneveld et al., "A phase 2 multicentre study of siltuximab, an anti-interleukin-6 monoclonal antibody, in patients with relapsed or refractory multiple myeloma," *British Journal of Haematology*, vol. 161, no. 3, pp. 357–366, 2013.

[52] T. Jelinek, J. Mihalyova, M. Kascak, J. Duras, and R. Hajek, "PD-1/PD-L1 inhibitors in haematological malignancies: update 2017," *Immunology*, vol. 152, no. 3, pp. 357–371, 2017.

[53] J. Liu, A. Hamrouni, D. Wolowiec et al., "Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway," *Blood*, vol. 110, no. 1, pp. 296–304, 2007.

[54] A. Ray, D. S. Das, Y. Song et al., "Targeting PD1–PDL1 immune checkpoint in plasmacytoid dendritic cell interactions with T cells, natural killer cells and multiple myeloma cells," *Leukemia*, vol. 29, no. 6, pp. 1441–1444, 2015.

[55] H. Tamura, M. Ishibashi, T. Yamashita et al., "Marrow stromal cells induce B7-H1 expression on myeloma cells, generating aggressive characteristics in multiple myeloma," *Leukemia*, vol. 27, no. 2, pp. 464–472, 2013.

[56] G. Görgün, M. K. Samur, K. B. Cowens et al., "Lenalidomide Enhances immune checkpoint blockade-induced immune response in multiple myeloma," *Clinical Cancer Research*, vol. 21, no. 20, pp. 4607–4618, 2015.

[57] M. V. Dhodapkar, R. Sexton, R. Das et al., "Prospective analysis of antigen-specific immunity, stem-cell antigens, and immune checkpoints in monoclonal gammopathy," *Blood*, vol. 126, no. 22, pp. 2475–2478, 2015.

[58] B. Paiva, A. Azpilikueta, N. Puig et al., "PD-L1/PD-1 presence in the tumor microenvironment and activity of PD-1 blockade in multiple myeloma," *Leukemia*, vol. 29, no. 10, pp. 2110–2113, 2015.

[59] K. R. Kelly, C. M. Espitia, W. Zhao et al., "Oncolytic reovirus sensitizes multiple myeloma cells to anti-PD-L1 therapy," *Leukemia*, vol. 32, no. 1, pp. 230–233, 2018.

[60] D. M. Benson, C. E. Bakan, A. Mishra et al., "The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody," *Blood*, vol. 116, no. 13, pp. 2286–2294, 2010.

[61] S. Oliva, R. Troia, M. D'Agostino, M. Boccadoro, and F. Gay, "Promise and Pitfalls in the Use of PD-1/PD-L1 inhibitors in multiple myeloma," *Frontiers in Immunology*, vol. 9, no. 2749, pp. 1–8, 2018.

[62] T. Jelinek, B. Pavia, and R. Hajek, "Update on PD1/PD-L1 inhibitors in multiple myeloma," *Frontiers in Immunology*, vol. 9, no. 2431, pp. 1–13, 2018.

[63] A. M. Lesokhin, S. M. Ansell, P. Armand et al., "Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study," *Journal of Clinical Oncology*, vol. 34, no. 23, pp. 2698–2704, 2016.

[64] S. Z. Usami, F. Schjesvold, A. Oriol et al., "Pembrolizumab plus lenalidomide and dexamethasone for patients with treatment-naïve multiple myeloma (KEYNOTE-185) randomized, open-label, phase 3 trial," *The Lancet Haematology*, vol. 6, no. 9, pp. e448–e458, 2019.

[65] M. V. Maetos, H. Blacklock, F. Schjesvold et al., "Pembrolizumab plus pomalidomide and dexamethasone for patients with relapsed or refractory multiple myeloma (KEYNOTE-183): a romdomised, open-label, phase 3 trial," *The Lancet Haematology*, vol. 6, no. 9, pp. e459–e469, 2019.

[66] P. A. Baeuerle and C. Reinhardt, "Bispecific T-cell engaging antibodies for cancer therapy," *Cancer Research*, vol. 69, no. 12, pp. 4941–4944, 2009.

[67] C. Caruso, "BiTE therapy active in multiple myeloma," *Cancer Discovery*, vol. 9, no. 2, pp. 157-158, 2019.

[68] S. Hipp, Y.-T. Tai, D. Blanset et al., "A novel BCMA/CD3 bispecific T-cell engager for the treatment of multiple myeloma induces selective lysis in vitro and in vivo," *Leukemia*, vol. 31, no. 8, pp. 1743–1751, 2017.

[69] A. Goyos, C. M. li, P. Deegen et al., "Generation of half-life extended anti-BCMA Bite antibody construct compatible with once-weekly dosing anti-BCMA Bite antibody

construct compatible with once-weekly dosing for the treatment of multiple myeloma (MM)," *Blood*, vol. 130, p. 5389, 2017.

[70] S. F. Cho, K. C. Anderson, and Y. T. Tai, "Targeting B cell maturation antigen (BCMA) in multiple myeloma: potential uses of BCMA-based immunotherapy," *Frontiers in Immunology*, vol. 9, no. 1821, pp. 1–15, 2018.

[71] J. R. Richter, C. O. Landgren, J. S. kauch et al., "Phase 1, multicenter open-label study of single-agent bispecific antibody t-cell engager GBR1342 in relapsed/refractory multiple myeloma," *Journal of Clinical Oncology*, vol. 36, no. 15_ suppl, Article ID TPS3132, 2018.

[72] Y.-T. Tai, P. A. Mayes, C. Acharya et al., "Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma," *Blood*, vol. 123, no. 20, pp. 3128–3138, 2014.

[73] A. D. Cohen, R. Popat, S. Trude et al., "First in human study with GSK2857916, an antibody drug conjugated to microtubule-disrupting agent directed against B-cell maturation antigen (BCMA) in patients with relapsed/refractory multiple myeloma," *Blood*, vol. 128, no. 22, p. 1148, 2016.

[74] S. Trudel, N. Lendvai, R. Popat, P. M. Voorhees, B. Reeves, and E. N. Libby, "Deep and durable responses in patients (Pts) with relapsed/refractory multiple myeloma (MM) treated with monotherapy GSK2857916, an antibody drug conjugate against B-cell maturation antigen (BCMA): preliminary results from Part 2 of Study BMA117159," *Blood*, vol. 130, no. Suppl 1, p. 741, 2017.

[75] K. Hasegawa and N. Hosen, "Chimeric antigen receptor T cell therapy for multiple myeloma," *Inflammation and Regeneration*, vol. 39, no. 1, Article ID e16771, 2019.

[76] L. Mikkilineni and J. N. Kochenderfer, "Chimeric antigen receptor T-cell therapies for multiple myeloma," *Blood*, vol. 130, no. 24, pp. 2594–2602, 2018.

[77] P. Moreau, P. Sonneveld, M. Boccadoro et al., "CAR-T cell therapy for multiple myeloma: a consensus statement from the European myeloma network," *Haematologica*, 2019.

[78] R. O. Carpenter, M. O. Evbuomwan, S. Pittaluga et al., "B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma," *Clinical Cancer Research*, vol. 19, no. 8, pp. 2048–2060, 2013.

[79] S. A. Ali, V. Shi, I. Maric et al., "T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma," *Blood*, vol. 128, no. 13, pp. 1688–1700, 2016.

[80] N. Raje, J. Berdeja, Y. Lin et al., "Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma," *New England Journal of Medicine*, vol. 380, no. 18, pp. 1726–1737, 2019.

[81] E. Drent, R. W. J. Groen, W. A. Noort et al., "Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma," *Haematologica*, vol. 101, no. 5, pp. 616–625, 2016.

[82] M. Casucci, B. Nicolis di Robilant, L. Falcone et al., "CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma," *Blood*, vol. 122, no. 20, pp. 3461–3472, 2013.

[83] K. R. Kelly, A. Chanan-khan, G. Aomlo et al., "Indatuximab ravtansine (BT062) in combination with lenalidomide and low-dose dexamethasone in patients with relapsed and/or refractory multiple myeloma: clinical activity in Len/Dex refractory patients," *Blood*, vol. 122, p. 758, 2013.

[84] J. Berdeja, "Lorvotuzumab mertansine: antibody-drug-conjugate for $CD56^+$ multiple myeloma," *Frontiers in Bioscience*, vol. 19, no. 1, pp. 163–170, 2014.

[85] M. Hussein, J. R. Berenson, R. Niesvizky et al., "A phase I multidose study of dacetuzumab (SGN-40; humanized anti-CD40 monoclonal antibody) in patients with multiple myeloma," *Haematologica*, vol. 95, no. 5, pp. 845–848, 2010.

[86] W. Bensinger, R. T. Maziarz, S. Jagannath et al., "A phase 1 study of lucatumumab, a fully human anti-CD40 antagonist monoclonal antibody administered intravenously to patients with relapsed or refractory multiple myeloma," *British Journal of Haematology*, vol. 159, no. 1, pp. 58–66, 2012.

[87] J. L. Kaufman, R. Niesvizky, E. A. Stadtmauer et al., "Phase I, multicentre, dose-escalation trial of monotherapy with milatuzumab (humanized anti-CD74 monoclonal antibody) in relapsed or refractory multiple myeloma," *British Journal of Haematology*, vol. 163, no. 4, pp. 478–486, 2013.

[88] N. S. Raje, P. Moreau, E. Terpos et al., "Phase 2 study of tabalumab, a human anti-B-cell activating factor antibody, with bortezomib and dexamethasone in patients with previously treated multiple myeloma," *British Journal of Haematology*, vol. 176, no. 5, pp. 783–795, 2017.

[89] P. J. Hengeveld and M. J. Kersten, "B-cell activating factor in the pathophysiology of multiple myeloma: a target for therapy?," *Blood Cancer Journal*, vol. 5, no. 2, p. e282, 2015.

[90] L. Rasche, J. Duell, I. C. Castro et al., "GRP78-directed immunotherapy in relapsed or refractory multiple myeloma—results from a phase 1 trial with the monoclonal immunoglobulin M antibody PAT-SM6," *Haematologica*, vol. 100, no. 3, pp. 377–384, 2015.

[91] P. Moreau, F. Cavallo, X. Leleu et al., "Phase I study of the anti-insulin-like growth factor 1 receptor (IGF-1R) monoclonal antibody, AVE1642, as single agent and in combination with bortezomib in patients with relapsed multiple myeloma," *Leukemia*, vol. 25, no. 5, pp. 872–874, 2011.

[92] S. Aichert, G. Juliusson, A. Johansson et al., "A single-arm, open-label, phase 2 clincal trial evaluating disease response following treatment with BI-505, a human anti-intracellular adhesion molecule-1 monoclonal antibody, in patients with smoldering multiple myeloma," *Plos One*, vol. 12, no. 2, Article ID e0171205, 2017.

[93] B. Fleischer, "CD26: a surface protease involved in T-cell activation," *Immunology Today*, vol. 15, no. 4, pp. 180–184, 1994.

[94] T. Tanaka, D. Camerini, B. Seed et al., "Cloning and functional expression of the T cell activation antigen CD26," *The Journal of Immunology*, vol. 149, no. 2, pp. 881–886, 1992.

[95] R. Dong, K. Tachibana, M. Hegen et al., "Correlation of the epitopes defined by anti-CD26 mAbs and CD26 function," *Molecular Immunology*, vol. 35, no. 1, pp. 13–21, 1998.

[96] Y. Torimoto, N. H. Dang, T. Tanaka, C. Prado, S. F. Schlossman, and C. Morimoto, "Biochemical characterization of CD26 (dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies," *Molecular Immunology*, vol. 29, no. 2, pp. 183–192, 1992.

[97] K. Ohnuma, N. Takahashi, T. Tamochu, O. Hosono, N. H. Dang, and C. Morimoto, "Role of CD26/dipeptidyl peptidase IV in human T cell activation and function," *Frontiers in Bioscience*, vol. 13, no. 13, pp. 2299–2310, 2008.

[98] P. A. Havre, M. Abe, Y. Urasaki, K. Ohnuma, C. Morimoto, and N. H. Dang, "The role of CD26/dipeptidyl peptidase IV in cancer," *Frontiers in Bioscience*, vol. 13, no. 13, pp. 1634–1645, 2008.

[99] H. Nishida, M. Hayashi, C. Morimoto, M. Sakamoto, and T. Yamada, "CD26 is a potential therapeutic target by humanized anti-body for the treatment of multiple myeloma," *Blood Cancer Journal*, vol. 8, no. 11, pp. 1–17, 2018.

[100] H. Nishida, H. Suzuki, H. Madokoro et al., "Blockade of CD26 signaling inhibits human osteoclast development," *Journal of Bone and Mineral Research*, vol. 29, no. 11, pp. 2439–2455, 2014.

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Review Article

Inflammatory Biomarkers as Predictors of Response to Immunotherapy in Urological Tumors

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Immunotherapy represents the new era of cancer treatment because of its promising results in various cancer types. In urological tumors, the use of the immune-checkpoint inhibitors (ICIs) is increasingly spreading. Although not all patients and not all diseases respond equally well to immunotherapy, there is an increasing need to find predictive markers of response to ICIs. Patient- and tumor-related factors may be involved in primary and secondary resistance to immunotherapy: tumor-derived protein and cytokines, tumor mutational burden, and patient performance status and comorbidities can condition tumor response to ICIs. Recently, some of these factors have been evaluated as potential biomarkers of response, with conflicting results. To date, the expression of programmed death-ligand 1 (PD-L1) and the presence of deficient mismatch repair (dMMR) in tumor tissue are the only biomarkers capable of guiding the clinician's decision in urothelial cancer and prostate cancer, respectively. In this review, we performed a comprehensive search of the main publications on biomarkers that are predictive of response to ICIs in urological cancers. Our aim was to understand whether existing data have the potential to drive clinical decision-making in the near future.

1. Introduction

Immunotherapy is fast becoming the new frontier of oncology, accompanied by the dream of being able to defeat cancer definitively. Although a substantial improvement in survival has been seen since immunotherapy was first used in melanoma, response remains low. The use of different types of immune-checkpoint inhibitors (ICIs), in particular the programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) axis, has led to significantly better results in terms of response and manageability. In recent years, advances have been made in the treatment of urological tumors, especially renal cell cancer (RCC) and urothelial cancer (UC). However, the issue of the identification of nonresponding patients persists. According to the tumor immunity in the microenvironment (TIME) classification [1], tumors can be divided into 4 subgroups based on the presence of inflammatory infiltrate (TIL) and PD-L1 expression: T1 (PD-L1$^-$, TIL$^-$), T2 (PD-L1$^+$, TIL$^+$), T3 (PD-L1$^-$, TIL$^+$), and T4 (PD-L1$^+$, TIL$^-$) (Figure 1). Although the TIME classification has significant predictive implications, there is an increasing need to find predictive markers of response to ICIs.

2. Factors Involved in Primary and Secondary Resistance to ICIs in Solid Tumors

Several factors can directly or indirectly influence the immune response and therefore contribute to triggering

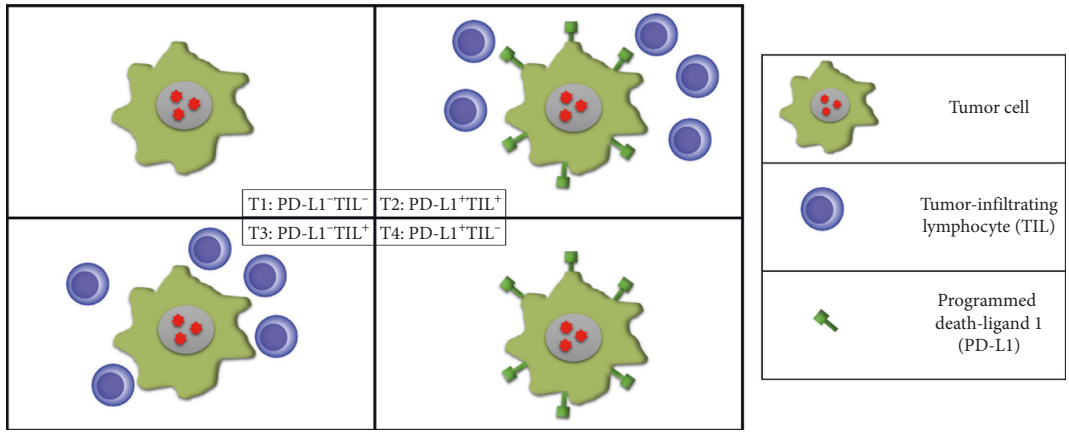

Figure 1: Four tumor subtypes according to the TIME classification based on the expression of PD-L1 in tumor cells and on the presence of TILs.

resistance mechanisms. As shown in Figure 2, these factors can be divided into two categories:

(1) Patient-related factors: it is acknowledged that patients in poor clinical conditions have a lower immune response. However, the underlying mechanism for this is still not understood. In fact, Pan et al. reported that an Eastern Cooperative Oncology Group performance score (ECOG PS) of 2 in melanoma patients was associated with worse prognosis when ICIs were used [2]. Conversely, a study carried out on patients with UC treated with atezolizumab showed that response rates (RRs) did not differ among patients with different PS [3]. Recently, several trials conducted on UC demonstrated a shorter overall survival (OS) in patients with ECOG PS > 2 compared with ECOG PS 0 [3–6]. Several comorbidities can also affect the immune response: autoimmune diseases [7, 8], diabetes [9], transplantations [10–12] (including bone marrow transplants), and infections [13]. Another important host-related factor is gut microbiota: several studies have shown that restoration of some bacterial families (Ruminococcaceae [14], *Akkermansia muciniphila* [15], and *Bacteroides fragilis* [16]) is correlated with a longer response in melanoma mice treated with anti-PD1 drugs. Thus, the use of antibiotics or steroids during ICI therapy may affect the outcome of treatment. In particular, 2 recent studies [17, 18] showed that the use of beta-lactams, quinolones, and macrolides during ICIs therapy also led to shorter progression-free survival (PFS) and poorer RR in RCC patients.

(2) Tumor-related factors: this category can be divided into 2 subcategories: intratumoral and microenvironmental factors.

2.1. Intratumoral Factors. Among tumor-related factors, different histologies and the presence of chromosomal alterations influence the immune response. For example, strongly aneuploid tumors have shown an intrinsic resistance to ICIs [19]. This is due to the poor expression of markers capable of activating the immune response. Conversely, a high expression of mutations, i.e., tumor mutational burden (TMB), especially if mismatch repair genes are involved, correlates with a high RR to ICIs, regardless of histology [20–23]. In UC, a recent study showed a higher RR in patients with alterations in the following genes: ATM, BRCA2, ERCC2, FANCA, MSH6, and POLE [24]. However, unlike solid tumors, elevated TMB has been associated with poor prognosis in hematological cancers, for example, multiple myeloma [25]. The growing interest in TMB has led to the development of studies aimed at testing the efficacy of neoantigens, structured within new molecules, such as chimeric antigen T-cell receptor therapy (CAR-T). Several studies are also underway for patients with RCC [26–28] and prostate tumors (PCa) [29].

PD-L1 expression in tumor tissue is one of the best known mechanisms for neutralizing immune system activity. A higher PD-L1 expression results in a poorer prognosis without the use of ICIs [13]. However, PD-L1 is not always capable of predicting response to ICIs [30, 31]. In fact, although response rates in UC differ significantly on the basis of PD-L1 status, this is not the case for RCC patients [32, 33].

To date, CTLA-4 and PD1/PD-L1 axis are not the only molecules involved in the modulation of the immune response. Other molecules are currently under investigation as potential immune checkpoint for new ICIs, e.g., lymphocyte-activation gene-3 (LAG-3), T-cell immunoglobulin mucin-3 (TIM-3), and B7-H3 and B7-H4/B7x/B7S1.

LAG-3 molecule is located on the cell surface of several immune cells; its ligand is Class II MHC and binds with higher affinity than CD4 [34]. LAG-3 downregulates the immune response of $CD4^+$- and $CD8^+$-activated cells. In fact, its negative activity has been observed in $CD8^+$ tumor-infiltrating lymphocytes (TILs) and in $CD4^+$ TRegs [35].

TIM-3 is a regulatory molecule expressed on the surface of innate immune cells; $CD8^+$ TILs usually coexpress PD-L1 and TIM-3, causing a strong inhibition of cytokine secretion [36]. To date, TIM-3/PD-L1 coexpression has also been studied in $CD8^+$ cells in melanoma patients. In one study, blocking both PD-L1 and TIM-3 led to a restoration of cytokine secretion [37].

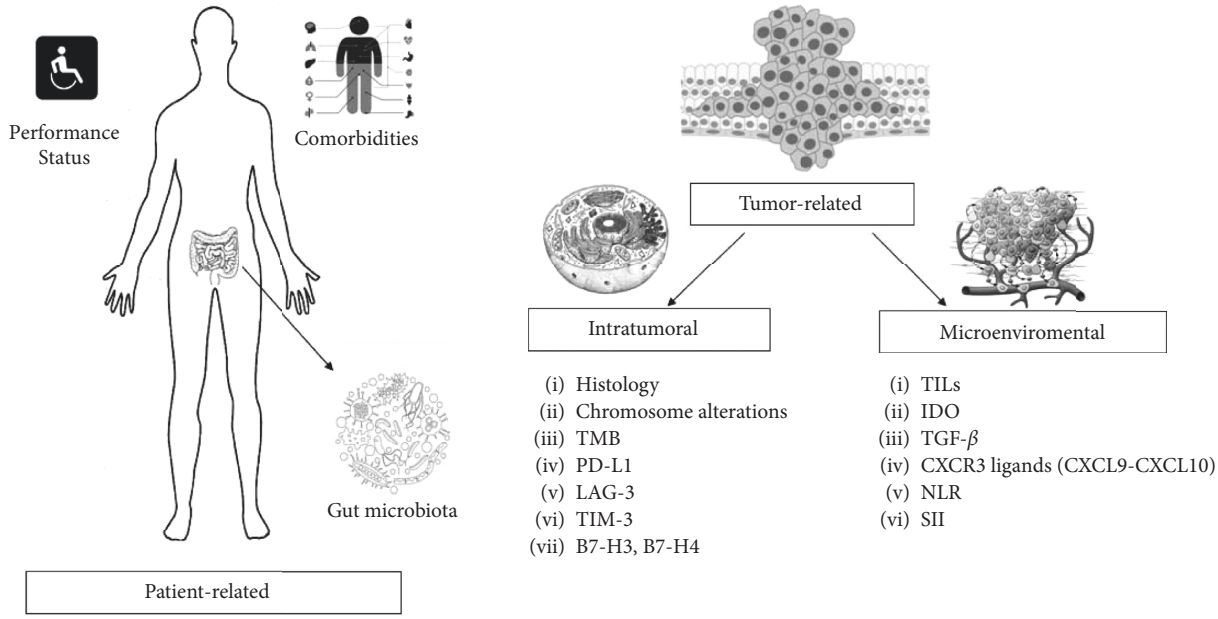

Figure 2: Factors influencing immune response and possibly related to resistance to immunotherapy. TMB: tumor mutational burden; PD-L1: programmed death-ligand 1; LAG-3: lymphocyte-activation gene-3; TIM-3: T-cell immunoglobulin and mucin domain 3; TILs: tumor-infiltrating lymphocytes; IDO: indoleamine-2,3-dioxygenase; TGF-β: transforming growth factor-β; CXCR: CXC chemokine receptors; CXCL: CXC chemokine receptors ligands; NLR: neutrophil-to-lymphocyte ratio; SII: systemic immune-inflammation index.

B7-H3 and B7-H4 (also known as B7x/B7S1) are 2 members of the B7 super-family expressed not only by immune cells but also by nonlymphoid tissues, including prostate and testis cells [38]. Although B7-H3 was initially characterized as a costimulatory molecule, recent studies have indicated its dual activity. In some cases, it acts as an upregulator of the immune responses and in others, a downregulator [39].

2.2. Microenvironmental Factors. Tumor microenvironment plays an important role in silencing the immune response. Usually, the presence of TILs is related to higher PD-L1 expression [40, 41] and to better response to ICI treatment [23]. The KEYNOTE 028 study tested the efficacy of pembrolizumab in 20 different tumors. Results showed that treatment with ICIs was more effective in patients with TILs, independently of tumor histology [42].

On the other hand, the aforementioned TIME classification [1] has emphasized the link between TILs and PD-L1 in determining the response to ICIs. However, its correlation with response in UTs is still under evaluation [43]. The T2 subgroup, for example, is characterized by the presence of TILs and higher PD-L1 expression, stimulated by the TIL-mediated production of interferon-gamma (IFN-γ). This subgroup is associated with high RRs when treated with ICIs. Unlike T2, the T3 subgroup expresses TILs but not PD-L1 (probably due to a nonexpression of inducing factors, such as IFN-γ). In this context, the use of OX-40 or 4-1BB agonists may convert tumors classified as T3 into T2 [44, 45]. T1 and T4 subgroups differ because of their lack of TILs. Many tumors have this characteristic, which is usually associated with a nonresponse to treatment with ICIs. There are different ways to stimulate the immune response, for example, by using anti-CTLA4 antibodies or CAR-T-cell therapy. However, some negative PD-L1 tumors may respond to an anti-PD-L1 drug. Positivity or negativity of the histological examination may not reflect a common characteristic of the overall tumor. Thus, tumor heterogeneity may be responsible for ICI response in patients with PD-L1-negative biopsy [1]. It is also a unstable characteristic over time; in fact, treatment may select altered tumor cells capable of activating the process of immune escape, blocking the immune system activation, and even transforming positive TIL into negative TIL tumors. This condition has been described in different tumor types, such as lung and breast cancer and RCC [46–48]. In particular, discordance in PD-L1 status between primary and metastatic sites has been observed in 20% of RCC patients [49]. The immune-silencing process is ascribed to several mechanisms: activation of the Wnt–β-catenin pathway [50]; loss of PTEN associated with AKT activation [51]; and loss of immunogenicity [52] through several mechanisms (including downregulation of MHC class I molecules and reduced production of immunogenic antigens).

The study of the tumor microenvironment has led to the discovery of other molecules involved in immune-silencing mechanisms. For example, indoleamine-2,3-dioxygenase (IDO) is a molecule produced in TILs capable of stimulating the immune infiltrate, reducing the concentration of tryptophan which is necessary for the activation of cytotoxic T cells, and permitting their transformation into regulatory T cells (TRegs). This promotes an immunosuppressive microenvironment near the tumor. Consequently, IDO is a promising biomarker, and high concentrations are associated with worse prognosis. However, IDO as a target for new drug development has been disappointing, and the use of IDO inhibitors has not shown any advantages over ICI

treatment [53]. In addition to IDO, there is a high expression of other molecules in tumor microenvironment, including TGF-β secreted by fibroblasts [54], and various other cytokines involved in immune-silencing mechanisms. Among these molecules, CXCL9 and CXCL10, two CXCR3 ligands, have shown to be correlated with the TIL-positive TIME subgroups, whereas TIL-negative subgroups lack these chemokines [55, 56].

Furthermore, several studies have evaluated the prognostic/predictive role of some parameters, such as the neutrophil-to-lymphocyte ratio (NLR) and the systemic immune-inflammation index (SII). NLR is the most widely tested prognostic index and correlates with prognosis in different tumor types [57]. Similarly, SII, combining neutrophils, lymphocytes, and platelet count in a single parameter, demonstrates a significant correlation with prognosis in different cancers [58–60]. Among UTs, SII and NLR have shown a prognostic and predictive role of response to conventional treatment in several retrospective trials [61–63]. In particular, Lalani et al. recently demonstrated that an early reduction in NLR (at 6 weeks) was associated with a significantly improved outcome in mRCC patients after ICI treatment [64]. Moreover, Raccioppi et al. found that preoperatory NLR value was a predictor of response to BCG therapy in non-muscle-invasive bladder cancer [65].

3. Potential Prognostic and Predictive Biomarkers in UCs Treated with ICIs

3.1. PD-L1 and TILs. PD-L1 is the most widely studied (potential) biomarker in immunotherapy, and several studies have investigated its predictive value in UCs. Table 1 lists the clinical trials that evaluated PD-L1 expression by immunohistochemistry (IHC) or the IHC-based combined positive score (CPS) to develop a reproducible PD-L1 scoring method that can be used to identify patients most likely to respond to therapy. CPS is obtained as follows: CPS = 100 × PD-L1 stained cells (tumor cells, lymphocytes, macrophages)/total viable tumor cells. In RCC, PD-L1 is not a useful predictor of response to ICI treatment. Both PD-L1-negative and PD-L1-positive tumors respond to immunotherapy, despite higher rates of RR and PFS in patients with PD-L1 expression. In fact, in the metastatic RCC population of the CheckMate 214 trial, the combination of nivolumab plus ipilimumab obtained an objective RR of 37% in patients with PD-L1 expression <1%, compared to 58% of those with PD-L1 expression >1% [31]. In the IMmotion 151 trial, patients with PD-L1 ≥ 1% showed longer PFS when treated with bevacizumab plus atezolizumab [66]. Conversely, the combination of axitinib with pembrolizumab (KEYNOTE 423 trial) or axitinib with avelumab (Javelin Renal 101) did not produce different efficacy results on the basis of different PD-L1 statuses [67, 68]. Similarly, Motzer et al. observed that the use of nivolumab after treatment with anti-VEGFR inhibitors improved OS independently of PD-L1 status [69]. Unlike RCC, PD-L1 has been recognized as a predictive biomarker in UCs. In metastatic/locally advanced UC, atezolizumab and pembrolizumab demonstrated antitumor activity and acceptable tolerability in the first-line treatment of cisplatin-ineligible patients [3, 5]. Based on these results, the Food and Drug Administration (FDA) approved atezolizumab and pembrolizumab in this subgroup. However, the FDA updated the prescribing information for first-line pembrolizumab and atezolizumab in cisplatin-ineligible patients, making it compulsory to use an approved PD-L1 diagnostic test (Dako PDL-1 ICH $22C_3$ PharmDx Assay® and Ventana PDL-1 Assay®) to select patients. Therefore, FDA indications were modified as follows: cisplatin-unfit patients are eligible for pembrolizumab and atezolizumab if the tumor expresses PD-L1 (CPS ≥ 10 for pembrolizumab and PD-L1 ≥ 5% for atezolizumab) [70]. In patients not eligible for any platinum, pembrolizumab and atezolizumab can be administered in first-line regardless of tumor PD-L1 expression. In postplatinum UC patients, several trials have demonstrated ICI efficacy [71–75], with ICI-treated PD-L1-positive TIL-positive UCs showing higher RRs. In the IMvigor 210 trial, the use of atezolizumab obtained an overall response rate (ORR) of 16%, which was higher (28%) in patients with ≥5% PD-L1 expression [71]. In CheckMate 275, patients with tumor cluster III proved most likely to obtain a better response to nivolumab (30%) [73]. Similar results were obtained in 2 other studies. In the JAVELIN trial, avelumab demonstrated an ORR of 17% in all patients and 50% in those showing PD-L1 expression [75]. In a phase 1/2 trial, durvalumab obtained an ORR of 31% in the overall population, 46% in patients with PD-L1 expression, and 0% in those without PD-L1 expression [76]. Based on these results, the FDA approved pembrolizumab as the preferred drug, with atezolizumab, nivolumab, and durvalumab as alternative preferred agents, regardless of PD-L1 expression. The European Medicines Agency (EMA) recently approved pembrolizumab for the treatment of metastatic/unresectable UCs in relapsed patients after first-line platinum-based therapy and also in nonpretreated cisplatin-unfit patients with CPS>10. The EMA has also approved atezolizumab for the first- and second-line treatment of UC and nivolumab for use in a second-line setting. Although the cancer vaccine, sipuleucel-T, has shown activity in prolonging OS in PCa, none of the new ICIs have been approved. This is due to limited antitumor immune infiltrates and poor PD-L1 expression in this tumor type [77, 78]. In germ-cell tumors, PD-L1 expression has been observed in 73% and 64% of patients with seminoma and nonseminoma types, respectively [79] and correlates with outcome. Low levels of PD-L1 are associated with better PFS [80]. Despite the prognostic value of PD-L1 expression, pembrolizumab has not shown activity as a single agent in the treatment of refractory germ-cell tumors [81]. Therefore, PD-L1 is the only recognized biomarker in patients with UC, but its prognostic and predictive role is still open to debate in nonurothelial urological tumors. A recent study of 160 UC patients showed that although PD-L1 positivity ≥5% in tumor cells was not predictive of OS, it was predictive if expressed in TIL cells [82]. Mariathasan et al., after evaluating data from the IMvigor 210 phase 2 trials, reported that differences in PD-L1 also existed between tumor cells and inflammatory cells in TILs [54]. Hence, the debate about the

Table 1: Potential predictive biomarkers in urological tumors treated with ICIs.

Histology	Biomarker	Trial/author	Drugs	Setting	Study results
Urothelial	PD-L1 (CPS)	KEYNOTE 052 (phase 2)	Pembrolizumab	1-line CDDP ineligible	24% ORR, highest ORR in patients with CPS ≥ 10%
	PD-L1 (CPS)	KEYNOTE 045 (phase 3)	Pembrolizumab vs CHT	Second line after platinum-based CHT	Higher ORR in pembrolizumab group than CHT, regardless of tumor PD-L1 expression
	PD-L1 (IHC)	NCT02108652 (phase 2)	Atezolizumab	≥2-line after platinum-based CHT (cohort 2)	ORR: 26% (PD-L1 ≥ 5%) vs 15% (all patients) OS: 11.4 (PD-L1 ≥ 5%) vs 7.9 (all patients) months
	PD-L1 (IHC)	NCT02108652 (phase 2)	Atezolizumab	First-line CDDP ineligible	No significant enrichment of response and OS by PD-L1 expression
	PD-L1 (IHC)	NCT01772004 (phase 1b)	Avelumab	≥2-line treatment after platinum-based CHT	Patients with higher PD-L1 ≥ 5% showed higher response rates and longer PFS and OS
	PD-L1 (IHC)	CheckMate 275 (phase 2)	Nivolumab	≥2-line treatment after platinum-based CHT	ORR: 28.4% (PD-L1 ≥ 5%) vs 23.8% (PD-L1 ≥ 1%) vs 16.1 (PD-L1 < 1%); OS: 11.3 (PD-L1 ≥ 1%) vs 5.9 (PD-L1 < 1%) months
	CXCL9, CXCL10 cytokines	CheckMate 275 (phase 2)	Nivolumab	≥2-line treatment after platinum-based CHT	Positive predictors of response to nivolumab
	CXCL9, CXCL10 cytokines PD-L1 rabbit SP142 (Ventana)	IMvigor 210 (phase 2)	Atezolizumab	≥2-line after platinum-based CHT (cohort 2)	Positive predictors of response to atezolizumab; PD-L1 expression on IC (>5% of cells) was significantly associated with response. In contrast, PD-L1 expression in tumor cells was not associated with response
	PD-L1 (IHC)	NCT01693562 (phase 2)	Durvalumab	≥2-line treatment after platinum-based CHT	No differences in PFS and ORR between high and low/negative PD-L1 patients
	dMMR or MSI-H	G. Iyer et al., J Clin Oncol 2017	ICIs	Metastatic setting	dMMR caused a high mutation load and was associated to durable responses to ICIs
Kidney	PD-L1 rabbit 28-8 (Dako)	CheckMate 214 (phase 3)	Nivolumab ipilimumab vs sunitinib	First line	Greater benefit in ORR, PFS, and OS for patients with PD-L1 ≥ 1% treated with nivolumab and ipilimumab
	PD-L1 (IHC)	Javelin renal 101	Avelumab plus axitinib vs sunitinib	First line	Greater benefit in ORR and PFS in patients with treated with avelumab plus axitinib, independently from PD-L1
	PD-L1 (IHC)	KEYNOTE 423 (phase 3)	Pembrolizumab plus axitinib vs sunitinib	First line	Greater benefit in ORR, OS, and PFS in patients with treated with pembrolizumab plus axitinib, independently of PD-L1

Table 1: Continued.

Histology	Biomarker	Trial/author	Drugs	Setting	Study results
	PD-L1 (IHC) rabbit SP142 (Ventana)	IMmotion 151 (phase 3)	Bevacizumab/ atezolizumab vs sunitinib	1-line	PFS in PD-L1 ≥ 1% patients: 11.2 mo (with atezolizumab plus bevacizumab) vs 7.7 mo (with sutent), HR 0.74, $P = 0.0217$
	PD-L1 (IHC) rabbit 28-8 (Dako)	CheckMate 025 (phase 3)	Nivolumab vs everolimus	≥2-line treatment after anti-VEGFR therapy	No differences in OS on the basis of PD-L1 status
	SII rabbit 28-8 (Dako)	De Giorgi et al., Clin Cancer Research 2019	Retrospective analysis of EAP of nivolumab	≥2-line treatment after anti-VEGFR therapy	Normal body mass index combined with higher SII tripled the risk of death
Prostate	dMMR	Le DT et al., Science 2017	Pembrolizumab	Advanced dMMR cancers	ORR: 53% of patients and complete responses were achieved in 21% of patients

PD-L1 = programmed death-ligand 1; CPS = combined positive score; ICIs = immune-checkpoint inhibitors; ICH = immunohistochemistry; SII = systemic inflammation index; dMMR = mismatch repair genes deficiency; MSI-H = higher microsatellite instability; CHT = chemotherapy; EAP = expanded access program; ORR = overall response rate; PFS = progression-free survival; OS = overall survival.

different value of PD-L1 expression in tumor and nontumor cells (TILs) is still open.

3.2. Prognostic and Predictive Role of TIM-3, B7-H3, and B7-H4. Tumor-associated macrophages induce a more immunosuppressive phenotype, leading to an enhanced expression of TIM-3 and PD-1 on $CD4^+$ and $CD8^+$ T cells. The concentration of TIM-3 and PD-1-positive $CD4^+$ and $CD8^+$ T cells is higher in TILs than in peripheral blood in RCC patients [83]. Recently, Granier et al. demonstrated that $PD\text{-}1^+Tim\text{-}3^+CD8^+$ T cells could not be enhanced *in vitro* by a strong stimulus, suggesting that these cells cannot be reactivated after PD-1-PD-L1 blockade [84]. In PCa patients, malignant cells show higher TIM-3 expression than benign cells, expression correlating with TNM staging system, grading, and PFS [85]. Piao et al. demonstrated that Tim-3 expression in both CD4+ and CD8+ T cells closely correlated with advanced disease and poor prognosis in PCa patients [86]. Other studies have evaluated the prognostic role B7-H3 and B7-H4 in UTs. In both RCC and PCa, the overexpression of B7-H3 and B7-H4 was correlated with poor prognosis and a higher risk of recurrent and metastatic disease [87, 88]. Moreover, in RCC, B7-H3 and B7-H4 were expressed by both immune and endothelial cells: among 743 RCC patients, B7-H3-positive TILs were observed in 17% of tumor samples and in 95% of tumor vasculature [89]. Another study reported a B7-H4 positive expression in tumor vasculature of 211 RCC patients [90, 91]. In UCs, B7-H3 is overexpressed in all tumor stages and its expression can be stimulated by Bacillus Calmette–Guérin-based therapy [92].

3.3. Prognostic Role of NLR and SII. In the last few years, the prognostic role of NLR and SII has been evaluated in urological and nonurological cancers. Although several studies have demonstrated a correlation between NLR and prognosis and NLR and treatment response, its prognostic role remains uncertain [93, 94]. In UC and RCC, NLR is significantly associated with prognosis [95–97]. As seen in breast cancer [98], lymphopenia is also associated with poor prognosis in patients with RCC [99]. In a study on an elderly mRCC population treated with first-line sunitinib, lymphopenia proved to be a negative prognostic factor [100]. Thrombocytosis has also been identified as a negative prognostic factor in RCC patients [101]. A recently published study evaluated the role of SII in RCC patients treated with the PD-1 inhibitor nivolumab and enrolled in an Italian Expanded Access Program. The authors demonstrated that normal body mass index combined with higher SII tripled the risk of death, suggesting that SII is a critical prognostic factor for OS in pretreated RCC patients during treatment with nivolumab [102]. A recent article confirmed the prognostic role of SII (and its variations during therapy) in mRCC patients treated with sunitinib [103]. Recently, a study evaluated the combination of SII and the monocyte/lymphocyte ratio (MLR) as new prognostic factor in upper-tract UC. The authors demonstrated that SII was significantly associated with PFS and OS, whereas MLR significantly correlated with OS but not with PFS. Both SII and MLR correlate with an enhanced risk of disseminated disease [104]. In PCa, Fan et al. reported that SII has a negative independent prognostic role in terms of OS in patients treated with both abiraterone and docetaxel, independently of the treatment sequence [105].

3.4. Predictive Role of IFN-γ and Other Cytokines. A 25-gene IFN-γ signature was evaluated in patients with metastatic UC enrolled in the phase II trial CheckMate 275, a trial focusing nivolumab used as a single agent. The analysis demonstrated that a higher IFN-γ signature was expressed in the basal-1 subgroup, corresponding to cluster III of the TCGA classification. The patients in this group were more likely to respond to ICIs [72, 73]. Recently, IFN-γ-induced

cytokines (CXCL9 and CXCL10) were also shown to be positive predictors of response to atezolizumab in the IMvigor trial [71].

3.5. Prognostic and Predictive Role of TMB and Genetic Instability. In PCa, 2 large phase III trials on unselected patients reported the failure of anti-CTLA4 (ipilimumab) [106, 107]. Initial clinical data had shown that 5%–12% of patients with metastatic PCa may benefit from ICIs [108, 109], probably due to the low mutational loads of PCa, which is correlated with low neoantigen burden [110]. The mismatch repair (MMR) gene is a DNA single-strand repair mechanism. Mismatch repair-deficient (dMMR) cancers are characterized by microsatellite instability and hypermutator phenotype, both associated with chemotherapy resistance but immunotherapy sensitivity [111]. In a study by Iyer et al., dMMR or high MSI (MSI-H) were found in 3% of 424 UC patients [112], both subgroups showing a higher response to ICIs [112]. A recently published phase II trial including patients with cholangiocarcinoma, colorectal, endometrial, gastric, and small bowel cancer demonstrated that dMMR predicted clinical benefit from pembrolizumab [20]. In PCa, the prevalence of dMMR varies between 12% and 22% in different studies, probably because of the different assays used to detect the genomic aberrations [113, 114]. Recent evidence that dMMR cancers may benefit from pembrolizumab [20] has led to FDA approval of pembrolizumab for the treatment of metastatic/unresectable solid tumors with dMMR or MSI-H in patients who progress on prior treatment. Initially, this indication included several cancer types but not PCa. After the results from the KEYNOTE-028-phase 1b trial were published [109], the FDA expanded the previous indication to include patients with pretreated metastatic PCa with MSI-H or dMMR deficiency [115]. However, dMMR cancers do not always respond to immunotherapy, and not all cancers responding to ICIs are dMMR [20, 21, 116]. In fact, a recent study showed that dMMR tumors constitute a subtype with decreased survival time but that only a proportion has a high mutation load and show PD-L1 IHC staining. Thus, dMMR tumors represent a heterogeneous group and may require further subclassification to understand their clinical behaviour and response to ICIs [117]. However, NCCN guidelines still recommend DNA-repair gene mutation testing for all patients with high-risk regional or metastatic PCa [115].

4. Conclusions

In UCs, several ICIs have been approved in metastatic disease and several studies are ongoing in a nonmetastatic setting. To date, 2 biomarkers have been recognized in clinical practice: PD-L1 and dMMR. The FDA and EMA permit the use of pembrolizumab and atezolizumab in UC cisplatin-ineligible patients expressing PD-L1 and undergoing first-line treatment for metastatic disease. The presence of dMMR or MSI-H also represents a predictive factor of response to ICIs in PCa and has led to FDA approval of pembrolizumab in this subgroup. Notwithstanding, several unanswered questions remain: Why do some tumors express TILs and some do not? Why do some tumors not express PD-L1? What regulates immune escape mechanisms? The role of PD-1 and PD-L1 expression as a predictive biomarker is still unclear, the use of different methods and cutoff points in trials complicating its validation. As suggested by Mariathasan et al., another difference may derive from different PD-L1 expressions in both tumor cells and immune cells [54]. Moreover, patients with low or negative PD-L1 expression respond to ICIs. Consequently, more suitable biomarkers must be sought. In the near future, it is hoped that the biological characterization of tumors will be able to drive clinical decision-making, leading to more personalized treatment. In UCs, new classification systems such as TCGA will add further valuable information, allowing for better patient selection. Furthermore, classification of biomarker expression into the three immunological phenotypes "immune inflamed," "immune excluded," and "immune desert" could improve our knowledge of distinct immunological pathways, enabling a more effective use of ICIs such as mono- or combination therapies [118].

In the past, nanoparticle-based drugs have been hypothesized for the treatment of cancer. These drug nanocarriers can improve the therapeutic efficacy of a drug by penetrating deep into tissue and overcoming the physical barriers linked to drug release [119]. In this scenario, the identification of new cancer-specific biomarkers could lead to the development of new nanocarrier drugs directed against cancer-specific driver biomarkers. In the near future, the identification of new biomarkers capable of predicting outcome and of acting as molecular targets for cancer treatment will be possible, thanks to a greater understanding of the intrinsic mechanisms that regulate immune system activity. Meanwhile, the search for new and reliable predictive biomarkers will proceed in 3 main directions: humoral (cytokines), immunohistochemical (new or unexplored checkpoints), and genomic (mutations, genetic instability).

Conflicts of Interest

Ugo De Giorgi has received personal fees for advisory board/consultancy from Astellas, Bayer, BMS, Ipsen, Janssen, Merck, Novartis, Pfizer, and Sanofi. Other authors declare no conflicts of interest.

References

[1] Y. Zhang and L. Chen, "Classification of advanced human cancers based on tumor immunity in the MicroEnvironment (TIME) for cancer immunotherapy," *JAMA Oncology*, vol. 2, no. 11, pp. 1403-1404, 2016.

[2] M. Pan, M. Alavi, and L. J. Herrinton, "Association of inflammatory markers with disease progression in patients with metastatic melanoma treated with immune checkpoint inhibitors," *The Permanente Journal*, vol. 22, pp. 17–149, 2018.

[3] A. V. Balar, M. D. Galsky, J. E. Rosenberg et al., "Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial," *Lancet*, vol. 389, pp. 67–76, 2017.

[4] C. N. Sternberg, Y. Loriot, N. James et al., "Primary results from SAUL, a multinational single-arm safety study of atezolizumab therapy for locally advanced or metastatic urothelial or nonurothelial carcinoma of the urinary tract," *European Urology*, vol. 76, no. 1, pp. 73–81, 2019.

[5] A. V. Balar, D. Castellano, P. H. O'Donnell et al., "First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study," *The Lancet Oncology*, vol. 18, no. 11, pp. 1483–1492, 2017.

[6] A. Necchi, G. Sonpavde, S. Lo Vullo et al., "Nomogram-based prediction of overall survival in patients with metastatic urothelial carcinoma receiving first-line platinum-based chemotherapy: retrospective international study of invasive/advanced cancer of the urothelium (RISC)," *European Urology*, vol. 71, no. 2, pp. 281–289, 2017.

[7] A. M. Menzies, D. B. Johnson, S. Ramanujam et al., "Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab," *Annals of Oncology*, vol. 28, pp. 368–376, 2017.

[8] D. B. Johnson, R. J. Sullivan, P. A. Ott et al., "Ipilimumab therapy in patients with advanced melanoma and preexisting autoimmune disorders," *JAMA Oncology*, vol. 2, no. 2, pp. 234–240, 2016.

[9] E. S. Scott, G. V. Long, A. Guminski, R. J. Clifton-Bligh, A. M. Menzies, and V. H. Tsang, "The spectrum, incidence, kinetics and management of endocrinopathies with immune checkpoint inhibitors for metastatic melanoma," *European Journal of Endocrinology*, vol. 178, no. 2, pp. 173–180, 2018.

[10] S. Herz, T. Höfer, M. Papapanagiotou et al., "Checkpoint inhibitors in chronic kidney failure and an organ transplant recipient," *European Journal of Cancer*, vol. 67, pp. 66–72, 2016.

[11] T. Alhamad, K. Venkatachalam, G. P. Linette, and D. C. Brennan, "Checkpoint inhibitors in kidney transplant recipients and the potential risk of rejection," *American Journal of Transplantation*, vol. 16, no. 4, pp. 1332-1333, 2016.

[12] L. Spain, R. Higgins, K. Gopalakrishnan, S. Turajlic, M. Gore, and J. Larkin, "Acute renal allograft rejection after immune checkpoint inhibitor therapy for metastatic melanoma," *Annals of Oncology*, vol. 27, no. 6, pp. 1135–1137, 2016.

[13] T. Seto, D. Sam, and M. Pan, "Mechanisms of primary and secondary resistance to immune checkpoint inhibitors in cancer," *Medical Sciences*, vol. 7, no. 2, p. 14, 2019.

[14] V. Gopalakrishnan, C. N. Spencer, L. Nezi et al., "Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients," *Science*, vol. 359, no. 6371, pp. 97–103, 2018.

[15] B. Routy, E. Le Chatelier, L. Derosa et al., "Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors," *Science*, vol. 359, no. 6371, pp. 91–97, 2018.

[16] M. Vetizou, J. M. Pitt, R. Daillere et al., "Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota," *Science*, vol. 350, no. 6264, pp. 1079–1084, 2015.

[17] L. Derosa, M. D. Hellmann, M. Spaziano et al., "Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer," *Annals of Oncology*, vol. 29, no. 6, pp. 1437–1444, 2018.

[18] N. Tinsley, C. Zhou, S. Villa et al., "Cumulative antibiotic use and efficacy of immune checkpoint inhibitors in patients with advanced cancer," *Journal of Clinical Oncology*, vol. 36, no. 15, p. 3010, 2018.

[19] T. Davoli, H. Uno, E. C. Wooten, and S. J. Elledge, "Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy," *Science*, vol. 355, no. 6322, Article ID eaaf8399, 2017.

[20] D. T. Le, J. N. Uram, H. Wang et al., "PD-1 blockade in tumors with mismatch repair deficiency," *Journal of Clinical Oncology*, vol. 33, Article ID LBA100, 2015.

[21] D. T. Le, J. N. Durham, K. N. Smith et al., "Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade," *Science*, vol. 357, pp. 409–413, 2017.

[22] M. J. Overman, R. McDermott, J. L. Leach et al., "Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study," *The Lancet Oncology*, vol. 18, no. 9, pp. 1182–1191, 2017.

[23] N. McGranahan, A. J. S. Furness, R. Rosenthal et al., "Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade," *Science*, vol. 351, no. 6280, pp. 1463–1469, 2016.

[24] M. Y. Teo, K. Seier, I. Ostrovnaya et al., "Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers," *Journal of Clinical Oncology*, vol. 36, no. 17, pp. 1685–1694, 2018.

[25] A. Miller, The MMRF CoMMpass Network, Y. Asmann et al., "High somatic mutation and neoantigen burden are correlated with decreased progression-free survival in multiple myeloma," *Blood Cancer Journal*, vol. 7, no. 9, p. e612, 2017.

[26] Y. Klaver, S. C. L. van Steenbergen, S. Sleijfer, R. Debets, and C. H. J. Lamers, "Plasma IFN-γ and IL-6 levels correlate with peripheral T-cell numbers but not toxicity in RCC patients treated with CAR T-cells," *Clinical Immunology*, vol. 169, pp. 107–113, 2016.

[27] E. R. Suarez, D.-K. Chang, J. Sun et al., "Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model," *Oncotarget*, vol. 7, no. 23, 2016.

[28] Q. Zhang, K. Tian, J. Xu et al., "Synergistic effects of cabozantinib and EGFR-specific CAR-NK-92 cells in renal cell carcinoma," *Journal of Immunology Research*, vol. 2017, 2017.

[29] H. Yu, J. Pan, Z. Guo, C. Yang, and L. Mao, "CART cell therapy for prostate cancer: status and promise," *OncoTargets and Therapy*, vol. 12, pp. 391–395, 2019.

[30] K. A. Schalper, "PD-L1 expression and tumor-infiltrating lymphocytes: revisiting the antitumor immune response potential in breast cancer," *OncoImmunology*, vol. 3, 2014.

[31] R. J. Motzer, N. M. Tannir, D. F. McDermott et al., "Nivolumab plus Ipilimumab versus Sunitinib in advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 378, no. 14, pp. 1277–1290, 2018.

[32] K. Ghate, E. Amir, M. Kuksis et al., "PD-L1 expression and clinical outcomes in patients with advanced urothelial carcinoma treated with checkpoint inhibitors: a meta-analysis," *Cancer Treatment Reviews*, vol. 76, pp. 51–56, 2019.

[33] M. Weinstock and D. McDermott, "Targeting PD-1/PD-L1 in the treatment of metastatic renal cell carcinoma," *Therapeutic Advances in Urology*, vol. 7, no. 6, pp. 365–377, 2015.

[34] B. Huard, P. Prigent, M. Tournier, D. Bruniquel, and F. Triebel, "CD4/major histocompatibility complex class II

interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins," *European Journal of Immunology*, vol. 25, no. 9, pp. 2718–2721, 1995.

[35] C. J. Workman and D. A. A. Vignali, "Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223)," *The Journal of Immunology*, vol. 174, no. 2, pp. 688–695, 2005.

[36] A. C. Anderson, D. E. Anderson, L. Bregoli et al., "Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells," *Science*, vol. 318, no. 5853, pp. 1141–1143, 2007.

[37] J. Fourcade, Z. Sun, M. Benallaoua et al., "Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+T cell dysfunction in melanoma patients," *The Journal of Experimental Medicine*, vol. 207, no. 10, pp. 2175–2186, 2010.

[38] G. L. Sica, I.-H. Choi, G. Zhu et al., "B7-H4, a molecule of the B7 family, negatively regulates T cell immunity," *Immunity*, vol. 18, no. 6, pp. 849–861, 2003.

[39] J. Kleeff, M. Loos, D. M. Hedderich, and H. Friess, "B7-H3 and its role in antitumor immunity," *Clinical and Developmental Immunology*, vol. 2010, 2010.

[40] E. D. Thompson, M. Zahurak, A. Murphy et al., "Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma," *Gut*, vol. 66, no. 5, pp. 794–801, 2016.

[41] A. Kitano, M. Ono, M. Yoshida et al., "Tumour-infiltrating lymphocytes are correlated with higher expression levels of PD-1 and PD-L1 in early breast cancer," *ESMO Open*, vol. 2, 2017.

[42] P. A. Ott, Y.-J. Bang, S. A. Piha-Paul et al., "T-Cell-Inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028," *Journal of Clinical Oncology*, vol. 37, no. 4, pp. 318–327, 2019.

[43] C. Pfannstiel, P. L. Strissel, K. B. Chiappinelli et al., "The tumor immune microenvironment drives a prognostic relevance that correlates with bladder cancer subtypes," *Cancer Immunology Research*, vol. 7, no. 6, pp. 923–938, 2019.

[44] L. Chen and D. B. Flies, "Molecular mechanisms of T cell co-stimulation and co-inhibition," *Nature Reviews Immunology*, vol. 13, no. 4, pp. 227–242, 2013.

[45] W. Zou, J. D. Wolchok, and L. Chen, "PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations," *Science Translational Medicine*, vol. 8, Article ID 328rv4, 2016.

[46] S. Kim, J. Koh, D. Kwon et al., "Comparative analysis of PD-L1 expression between primary and metastatic pulmonary adenocarcinomas," *European Journal of Cancer*, vol. 75, pp. 141–149, 2017.

[47] Q. F. Manson, W. A. M. E. Schrijver, N. D. ter Hoeve, C. B. Moelans, and P. J. van Diest, "Frequent discordance in PD-1 and PD-L1 expression between primary breast tumors and their matched distant metastases," *Clinical & Experimental Metastasis*, vol. 36, no. 1, pp. 29–37, 2019.

[48] A. Lopez-Beltran, V. Henriques, A. Cimadamore et al., "The identification of immunological biomarkers in kidney cancers," *Frontiers in Oncology*, vol. 8, 2018.

[49] M. Callea, L. Albiges, M. Gupta et al., "Differential expression of PD-L1 between primary and metastatic sites in clear-cell renal cell carcinoma," *Cancer Immunology Research*, vol. 3, no. 10, pp. 1158–1164, 2015.

[50] S. Spranger, R. Bao, and T. F. Gajewski, "Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity," *Nature*, vol. 523, no. 7559, pp. 231–235, 2015.

[51] W. Peng, J. Q. Chen, C. Liu et al., "Loss of PTEN promotes resistance to T cell-mediated immunotherapy," *Cancer Discovery*, vol. 6, no. 2, pp. 202–216, 2016.

[52] R. D. Schreiber, L. J. Old, and M. J. Smyth, "Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion," *Science*, vol. 331, no. 6024, pp. 1565–1570, 2011.

[53] G. C. Prendergast, W. P. Malachowski, J. B. DuHadaway, and A. J. Muller, "Discovery of Ido1 Inhibitors: from bench to bedside," *Cancer Research*, vol. 77, no. 24, pp. 6795–6811, 2017.

[54] S. Mariathasan, S. J. Turley, D. Nickles et al., "TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells," *Nature*, vol. 554, no. 7693, pp. 544–548, 2018.

[55] D. Bedognetti, T. L. Spivey, Y. Zhao et al., "CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2," *British Journal of Cancer*, vol. 109, no. 9, pp. 2412–2423, 2013.

[56] S. Spranger, "Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment," *International Immunology*, vol. 28, no. 8, pp. 383–391, 2016.

[57] A. J. Templeton, M. G. McNamara, B. Šeruga et al., "Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis," *Journal of the National Cancer Institute*, vol. 106, 2014.

[58] A. Passardi, E. Scarpi, L. Cavanna et al., "Inflammatory indexes as predictors of prognosis and bevacizumab efficacy in patients with metastatic colorectal cancer," *Oncotarget*, vol. 7, pp. 33210–33219, 2016.

[59] B. Hu, X.-R. Yang, Y. Xu et al., "Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma," *Clinical Cancer Research*, vol. 20, no. 23, pp. 6212–6222, 2014.

[60] A. Farolfi, M. Petrone, E. Scarpi et al., "Inflammatory indexes as prognostic and predictive factors in ovarian cancer treated with chemotherapy alone or together with bevacizumab. A multicenter, retrospective analysis by the MITO group (MITO 24)," *Targeted Oncology*, vol. 13, no. 4, pp. 469–479, 2018.

[61] L. Rossi, M. Santoni, S. J. Crabb et al., "High neutrophil-to-lymphocyte ratio persistent during first-line chemotherapy predicts poor clinical outcome in patients with advanced urothelial cancer," *Annals of Surgical Oncology*, vol. 22, no. 4, pp. 1377–1384, 2015.

[62] C. Lolli, O. Caffo, E. Scarpi et al., "Systemic immune-inflammation index predicts the clinical outcome in patients with mCRPC treated with abiraterone," *Frontiers in Pharmacology*, vol. 7, 2016.

[63] M. Chovanec, Z. Cierna, V. Miskovska et al., "Systemic immune-inflammation index in germ-cell tumours," *British Journal of Cancer*, vol. 118, no. 6, pp. 831–838, 2018.

[64] A. K. A. Lalani, W. Xie, D. J. Martini et al., "Change in Neutrophil-to-lymphocyte ratio (NLR) in response to immune checkpoint blockade for metastatic renal cell carcinoma," *Journal for ImmunoTherapy of Cancer*, vol. 6, no. 1, 2018.

[65] M. Racioppi, L. D. Gianfrancesco, M. Ragonese, G. Palermo, E. Sacco, and P. F. Bassi, "Can Neutrophil-to-Lymphocyte ratio predict the response to BCG in high-risk non muscle

invasive bladder cancer?," *International Brazilian Journal of Urology*, vol. 45, no. 2, pp. 315–324, 2019.

[66] R. J. Motzer, T. Powles, M. B. Atkins et al., "IMmotion151: a randomized phase III study of atezolizumab plus bevacizumab vs sunitinib in untreated metastatic renal cell carcinoma (mRCC)," *Journal of Clinical Oncology*, vol. 36, no. 6, p. 578, 2018.

[67] B. I. Rini, E. R. Plimack, V. Stus et al., "Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 380, no. 12, pp. 1116–1127, 2019.

[68] R. J. Motzer, K. Penkov, J. Haanen et al., "Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 380, no. 12, pp. 1103–1115, 2019.

[69] R. J. Motzer, B. Escudier, D. F. McDermott et al., "Nivolumab versus everolimus in advanced renal-cell carcinoma," *New England Journal of Medicine*, vol. 373, no. 19, pp. 1803–1813, 2015.

[70] *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines ®) NCCN.Org NCCN Guidelines for Patients ®*, http://www.nccn.org/patients, 2019.

[71] J. E. Rosenberg, J. Hoffman-Censits, T. Powles et al., "Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial," *The Lancet*, vol. 387, no. 10031, pp. 1909–1920, 2016.

[72] A. G. Robertson, J. Kim, H. Al-Ahmadie et al., "Comprehensive molecular characterization of muscle-invasive bladder cancer," *Cell*, vol. 171, pp. 540–556.e25, 2017.

[73] T. Powles, J. P. Eder, G. D. Fine et al., "MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer," *Nature*, vol. 515, no. 7528, pp. 558–562, 2014.

[74] P. Sharma, M. Retz, A. Siefker-Radtke et al., "Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial," *The Lancet Oncology*, vol. 18, no. 3, pp. 312–322, 2017.

[75] A. B. Apolo, J. R. Infante, A. Balmanoukian et al., "Avelumab, an anti-programmed death-ligand 1 antibody, in patients with refractory metastatic urothelial carcinoma: results from a multicenter, Phase Ib study," *Journal of Clinical Oncology*, vol. 35, no. 19, pp. 2117–2124, 2017.

[76] C. Massard, M. S. Gordon, S. Sharma et al., "Safety and efficacy of durvalumab (MEDI4736), an anti-programmed cell death ligand-1 immune checkpoint inhibitor, in patients with advanced urothelial bladder cancer," *Journal of Clinical Oncology*, vol. 34, no. 26, pp. 3119–3125, 2016.

[77] M. M. Wattenberg, L. Fong, R. A. Madan, and J. L. Gulley, "Immunotherapy in genitourinary malignancies," *Current Opinion in Urology*, vol. 26, no. 6, pp. 501–507, 2016.

[78] A. M. Martin, T. R. Nirschl, C. J. Nirschl et al., "Paucity of PD-L1 expression in prostate cancer: innate and adaptive immune resistance," *Prostate Prostate Cancer and Prostatic Diseases*, vol. 18, pp. 325–332, 2015.

[79] C. D. Fankhauser, A. Curioni-Fontecedro, V Allmann et al., "Frequent PD-L1 expression in testicular germ cell tumors," *British Journal of Cancer*, vol. 113, pp. 411–413, 2015.

[80] Z. Cierna, M. Mego, V. Miskovska et al., "Prognostic value of programmed-death-1 receptor (PD-1) and its ligand 1 (PD-L1) in testicular germ cell tumors," *Annals of Oncology*, vol. 27, no. 2, pp. 300–305, 2016.

[81] N. Adra, L. H. Einhorn, S. K. Althouse et al., "Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: a Hoosier Cancer Research Network Study GU14-206," *Annals of Oncology*, vol. 29, no. 1, pp. 209–214, 2018.

[82] J. Bellmunt, S. A. Mullane, L. Werner et al., "Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma," *Annals of Oncology*, vol. 26, pp. 812–817, 2015.

[83] S. R. Dannenmann, J. Thielicke, M. Stöckli et al., "Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma," *Oncoimmunology*, vol. 2, Article ID e23562, 2013.

[84] C. Granier, C. Dariane, P. Combe et al., "Tim-3 expression on tumor-infiltrating PD-1+CD8+ T cells correlates with poor clinical outcome in renal cell carcinoma," *Cancer Research*, vol. 77, no. 5, pp. 1075–1082, 2017.

[85] Y.-R. Piao, L.-Z. Piao, L.-H. Zhu, Z.-H. Jin, and X.-Z. Dong, "Prognostic value of t cell immunoglobulin mucin-3 in prostate cancer," *Asian Pacific Journal of Cancer Prevention*, vol. 14, no. 6, pp. 3897–3901, 2013.

[86] Y. Piao and X. Jin, "Analysis of Tim-3 as a therapeutic target in prostate cancer," *Tumor Biology*, vol. 39, pp. 1–6, 2017.

[87] Y. Liu, L. Vlatkovic, T. Saeter et al., "Is the clinical malignant phenotype of prostate cancer a result of a highly proliferative immune-evasive B7-H3-expressing cell population?," *International Journal of Urology*, vol. 19, no. 8, pp. 749–756, 2012.

[88] G. Chavin, Y. Sheinin, P. L. Crispen et al., "Expression of immunosuppresive B7-H3 ligand by hormone-treated prostate cancer tumors and metastases," *Clinical Cancer Research*, vol. 15, no. 6, pp. 2174–2180, 2009.

[89] P. L. Crispen, Y. Sheinin, T. J. Roth et al., "Tumor cell and tumor vasculature expression of B7-H3 predict survival in clear cell renal cell carcinoma," *Clinical Cancer Research*, vol. 14, no. 16, pp. 5150–5157, 2008.

[90] A. E. Krambeck, R. H. Thompson, H. Dong et al., "B7-H4 expression in renal cell carcinoma and tumor vasculature: associations with cancer progression and survival," *Proceedings of the National Academy of Sciences*, vol. 103, no. 27, pp. 10391–10396, 2006.

[91] R. H. Thompson, X. Zang, C. M. Lohse et al., "Serum-soluble B7x is elevated in renal cell carcinoma patients and is associated with advanced stage," *Cancer Research*, vol. 68, no. 15, pp. 6054–6058, 2008.

[92] S. A. Boorjian, Y. Sheinin, P. L. Crispen et al., "T-Cell coregulatory molecule expression in urothelial cell carcinoma: clinicopathologic correlations and association with survival," *Clinical Cancer Research*, vol. 14, no. 15, pp. 4800–4808, 2008.

[93] D. Lorente, J. Mateo, A. J. Templeton et al., "Baseline neutrophil-lymphocyte ratio (NLR) is associated with survival and response to treatment with second-line chemotherapy for advanced prostate cancer independent of baseline steroid use," *Annals of Oncology*, vol. 26, no. 4, pp. 750–755, 2015.

[94] G. Schepisi, A. Farolfi, V. Conteduca et al., "Immunotherapy for prostate cancer: where we are headed," *International Journal of Molecular Sciences*, vol. 18, no. 12, p. 2627, 2017.

[95] K. Hu, L. Lou, J. Ye, and S. Zhang, "Prognostic role of the neutrophil-lymphocyte ratio in renal cell carcinoma: a meta-analysis," *BMJ Open*, vol. 5, 2015.

[96] A. J. Templeton, J. J. Knox, X. Lin et al., "Change in neutrophil-to-lymphocyte ratio in response to targeted therapy

for metastatic renal cell carcinoma as a prognosticator and biomarker of efficacy," *European Urology*, vol. 70, no. 2, pp. 358–364, 2016.

[97] G. Schepisi, M. Santoni, F. Massari et al., "Urothelial cancer: inflammatory mediators and implications for immunotherapy," *BioDrugs*, vol. 30, no. 4, pp. 263–273, 2016.

[98] U. De Giorgi, M. Mego, E. Scarpi et al., "Relationship between lymphocytopenia and circulating tumor cells as prognostic factors for overall survival in metastatic breast cancer," *Clinical Breast Cancer*, vol. 12, no. 4, pp. 264–269, 2012.

[99] S. Saroha, R. G. Uzzo, E. R. Plimack, K. Ruth, and T. Al-Saleem, "Lymphopenia is an independent predictor of inferior outcome in clear cell renal carcinoma," *Journal of Urology*, vol. 189, no. 2, pp. 454–461, 2013.

[100] U. De Giorgi, K. Rihawi, M. Aieta et al., "Lymphopenia and clinical outcome of elderly patients treated with sunitinib for metastatic renal cell cancer," *Journal of Geriatric Oncology*, vol. 5, no. 2, pp. 156–163, 2014.

[101] D. Y. Heng, W. Xie, M. M. Regan et al., "External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study," *The Lancet Oncology*, vol. 14, no. 2, pp. 141–148, 2013.

[102] U. De Giorgi, G. Procopio, D. Giannarelli et al., "Association of systemic inflammation index and body mass index with survival in patients with renal cell cancer treated with nivolumab," *Clinical Cancer Research*, vol. 25, no. 13, pp. 3839–3846, 2019.

[103] C. Lolli, U. Basso, L. Derosa et al., "Systemic immune-inflammation index predicts the clinical outcome in patients with metastatic renal cell cancer treated with sunitinib," *Oncotarget*, vol. 7, pp. 54564–54571, 2016.

[104] H.-C. Jan, W.-H. Yang, and C.-H. Ou, "Combination of the preoperative systemic immune-inflammation index and monocyte-lymphocyte ratio as a novel prognostic factor in patients with upper-tract urothelial carcinoma," *Annals of Surgical Oncology*, vol. 26, no. 2, pp. 669–684, 2019.

[105] L. Fan, R. Wang, C. Chi et al., "Systemic immune-inflammation index predicts the combined clinical outcome after sequential therapy with abiraterone and docetaxel for metastatic castration-resistant prostate cancer patients," *The Prostate*, vol. 78, no. 4, pp. 250–256, 2018.

[106] E. D. Kwon, C. G. Drake, H. I. Scher et al., "Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial," *The Lancet Oncology*, vol. 15, no. 7, pp. 700–712, 2014.

[107] T. M. Beer, E. D. Kwon, C. G. Drake et al., "Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer," *Journal of Clinical Oncology*, vol. 35, no. 1, pp. 40–47, 2017.

[108] J. N. Graff, J. J. Alumkal, C. G. Drake et al., "Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer," *Oncotarget*, vol. 7, pp. 52810–52817, 2016.

[109] A. Hansen, C. Massard, P. A. Ott et al., "Pembrolizumab for patients with advanced prostate adenocarcinoma: preliminary results from the KEYNOTE-028 study," *Annals of Oncology*, vol. 27, 2016.

[110] D. Robinson, E. M. Van Allen, Y.-M. Wu et al., "Integrative clinical genomics of advanced prostate cancer," *Cell*, vol. 161, no. 5, pp. 1215–1228, 2015.

[111] M. Nilbert, M. Planck, E. Fernebro, Å. Borg, and A. Johnson, "Microsatellite instability is rare in rectal carcinomas and signifies hereditary cancer," *European Journal of Cancer*, vol. 35, no. 6, pp. 942–945, 1999.

[112] G. Iyer, F. Audenet, S. Middha et al., "Mismatch repair (MMR) detection in urothelial carcinoma (UC) and correlation with immune checkpoint blockade (ICB) response," *Journal of Clinical Oncology*, vol. 35, no. 15, p. 4511, 2017.

[113] C. C. Pritchard, C. Morrissey, A. Kumar et al., "Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer," *Nature Communications*, vol. 5, 2014.

[114] B. Nghiem, X. Zhang, H.-M. Lam et al., "Mismatch repair enzyme expression in primary and castrate resistant prostate cancer," *Asian Journal of Urology*, vol. 3, no. 4, pp. 223–228, 2016.

[115] J. L. Mohler, E. S. Antonarakis, A. J. Armstrong et al., "Prostate cancer, version 2.2019, NCCN clinical practice guidelines in oncology," *Journal of the National Comprehensive Cancer Network*, vol. 17, no. 5, pp. 479–505, 2019.

[116] R. S. Herbst, J.-C. Soria, M. Kowanetz et al., "Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients," *Nature*, vol. 515, no. 7528, pp. 563–567, 2014.

[117] D. N. Rodrigues, P. Rescigno, D. Liu et al., "Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer," *Journal of Clinical Investigation*, vol. 128, pp. 4441–4453, 2018.

[118] P. S. Hegde, V. Karanikas, and S. Evers, "The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition," *Clinical Cancer Research*, vol. 22, no. 8, pp. 1865–1874, 2016.

[119] M. Conti, V. Tazzari, C. Baccini, G. Pertici, L. P. Serino, and U. De Giorgi, "Anticancer drug delivery with nanoparticles," *In Vivo*, vol. 20, pp. 697–702, 2006.