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MELANOMA (RJ SULLIVAN, SECTION EDITOR)

Novel Immunotherapy Combinations

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Abstract

Purpose of Review The last decade witnessed an explosion in immunotherapeutic agent approvals for various malignancies. The success of immune checkpoint inhibitors (CTLA-4 and PD-1/PD-L1) in melanoma quickly sprung to other cancer types and are considered the emerging face of oncology.

Recent Findings Antibodies to CTLA-4 were first to enter the field, quickly followed by PD-1/PD-L1 inhibitors. Combination anti-CTLA4 and anti-PD-1/PD-L1 therapies were investigated, and after demonstrating improved responses, rapidly gained approval. Certain tumor types previously considered non-immunogenic also demonstrated durable responses which has been a remarkable discovery. However, not all tumor types respond to immunotherapies and it is widely recognized that tumor-specific immune inflammatory status predicts the best responders. Ongoing translational work indicates specific upregulation in additional immune checkpoints that circumvent response to anti-CTLA4 and anti-PD-1/PD-L1 antibodies.

Summary Here, we provide a comprehensive review of promising therapies on the horizon with unique combinations designed to overcome resistance or expand the pool of treatment responders.

Keywords Immune checkpoint inhibitors · Melanoma · Tumor microenvironment · CTLA-4 · PD-1 · Immunotherapy combinations

Introduction

The immune checkpoint inhibitors (ICI) have revolutionized the discipline of Oncology. Their impact can be underscored by the 2018 Nobel Prize in Medicine to James Allison and Tasuku Honjo [1], for the discovery of cytotoxic T- lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein receptor (PD-1) and ligand (PD-L1) respectively. Monoclonal antibodies (mAbs) to CTLA-4 and PD-1/PD-L1 gained initial approvals in cutaneous melanomas and rapidly sprung to other tumor types. Historically, metastatic or locally advanced cutaneous melanomas were treated with cytotoxic chemotherapies [2–5] with response rates generally less than 20% and 5-year survival rate of <10% with single agent chemotherapy [6]. Indeed, dacarbazine (DTIC) originally approved for treatment of metastatic melanoma in 1975 remained the standard of care until 2011. The role of immunotherapy has long been known in the treatment of melanoma, with approval of high-dose interleukin (IL)-2 in 1998 [7]. High dose IL-2 provided benefit to a small minority of patients but the responses are durable [8].

Cutaneous Melanoma as a Precursor to Immunotherapy Explosion

The treatment landscape for metastatic cutaneous melanoma dramatically changed with initial approval of ipilimumab in 2011, an anti-CTLA-4 mAb, followed soon by pembrolizumab and nivolumab (PD-1 inhibitors). Initial ipilimumab approval was based on two randomized, double-blind, phase III clinical trials that showed response rates (RR) of 11–15%, median overall survival (mOS) of 11.2–15.7 months [9, 10] and long-term 5-year OS rate of 18% in contrast to 9% with single-agent DTIC

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[11•, 12•]. This was the first treatment to ever demonstrate improved overall survival in melanoma patients, followed shortly by the BRAF inhibitor, vemurafenib [13]. Grade 3 or 4 immune-related adverse events (irAEs) were observed in 10–38% of patients receiving ipilimumab which led to comparison of high-dose vs. low-dose ipilimumab where frequency of severe irAEs decreased to 14% with comparable efficacy [14•]. In comparison to ipilimumab, pembrolizumab, a PD1 inhibitor, demonstrated higher response rates (37% vs. 13%), 2-year progression-free survival (PFS) rate (31% vs. 14%) and 2-year OS rates (55% vs. 43%) in treatment-naïve patients [15••, 16••]. When compared to DTIC in treatment-naïve patients, nivolumab produced higher response rate (27% vs. 10%) with improvement in mOS (37.5 vs. 11.2 months) [17•, 18••]. Similarly, nivolumab compared to ipilimumab demonstrated improved RR (45% vs 19%), mOS (36.9 vs. 19.9 months) [19••, 20••]. Both pembrolizumab and nivolumab had lower incidence of irAEs in comparison to ipilimumab, but the long-term cumulative treatment-related toxicities are not substantially different [15••, 16••, 19••, 20••].

The success of single-agent immunotherapy logically extended to testing combination of anti-CTLA-4 and anti-PD-1 therapies. In CheckMate-067, a comparison between nivolumab/ ipilimumab combination vs. ipilimumab alone demonstrated higher RR (58% vs. 19%), mPFS (11.5 vs. 6.9 months) and 4-year survival rate (37% vs. 9%) [19••]. However, an increased incidence of treatment-related grade 3–4 toxicities was observed in combination arm (59% vs. 28%) [19••]. While a statistical comparison between combination nivolumab/ ipilimumab and nivolumab alone was not performed, descriptive analyses showed a trend towards higher RR and PFS with combination therapy. The National Comprehensive Cancer Network (NCCN) guidelines for cutaneous melanoma endorse first line combination nivolumab/ ipilimumab as a category 1 recommendation in metastatic melanomas, however their use must be limited to patients who have limited comorbidities and accept the elevated risk of irAEs [21•].

Mechanisms of Tumor Immunotherapy

Interest in immunotherapy to treat cancer can be dated back to late 1800s when William Coley first inoculated malignant sarcomas with erysipelas [22]. Our understanding of immune surveillance of cancer has substantially increased over the last two decades. Tumor cells quickly evolve to utilize a vast array of regulators to evade destruction by immune system. Cytotoxic T-lymphocytes (CD8+ T cells, CTLs) are the principal immune effectors that recognize tumor neoepitopes packaged in the groves of major histocompatibility complex (MHC) by the antigen presenting cells (APCs). T cell receptor (TCR)-MHC-neoantigen complex interaction activates a variety of pathways that produce immunologic memory as well as activation, proliferation and expansion of CTL pool leading to destruction of tumor cells. As with most things in physiology, the immune-cancer interaction is heavily regulated by checkpoints and co-stimulatory pathways, whose balance determines the fate of immunologic outcome. These checkpoints are an important component of the immune system in that they dampen the immune response to prevent autoimmunity. TCR-MHC interaction is dependent upon additional co-stimulatory interaction between CD28 on T cells and B7–1/2 (CD80 and CD86) on APCs for T cell activation. CTLA-4 functions as an off switch that captures and removes B7–1/2 from APCs, thus making these unavailable for interactions with CD28 [23]. CTLA-4 expression is upregulated in CTLs after activation through tumor-released factors, resulting in immune evasion [24•]. PD-1 similarly suppresses T cell activity after binding to its ligands (PD-L1 and PD-L2) secreted by APCs and tumor cells [24•, 25]. PD-1/PD-L1 interactions promote apoptosis of antigen-primed CTLs and reduces apoptosis of regulatory T cells (T_{regs} or $FOXP3^+$ T-cells) [26, 27•]. An essential component of immune system, T_{regs} promotes tolerance to self-antigens and prevent autoimmunity. T_{regs} make up to 20–30% of tumor infiltrating lymphocytes in the tumor microenvironment (TME), indeed a higher T_{regs} level or T_{reg} to $T_{effector}$ ratio portends poor prognosis for various cancers [28–30].

Novel Combinations to Overcome Immune Evasion Due to Additional Checkpoints

Beyond CTLA-4 and PD-1, there are various other T cell associated inhibitory molecules that include LAG-3, TIM-3, TIGIT, VISTA, B7-H3 and, A2aR/CD73 [31•]. These inhibitory pathways are adjunct immune-regulators that have sparked immense interest in the research community to find the next breakthrough in immunotherapy [32•]. Indeed, specific antagonists targeting these pathways are currently under development, either alone or in combination with anti-CTLA-4/ anti-PD-1/PD-L1 backbone (Table 1). These compounds are still in early phase of development with some displaying early evidence of promising activity. LAG-3 (lymphocyte activation gene-3, CD223) is expressed by effector and regulatory T cells and inhibits the activation of $CD4^+$ T cells by binding to peptide-MHC complex thereby promoting $CD8^+$ T-cell exhaustion [33•, 34]. BMS-986016 is an anti-LAG-3 mAb and is under clinical development with nivolumab in heavily pre-treated melanoma patients, where it demonstrated an ORR of 16% and disease control rate (DCR) of 46% with benefit observed in PD-1 refractory patients [35••]. TIM-3 (T cell immunoglobulin and mucin domain-3) is expressed on macrophages and natural killer (NK) cells where it directly inhibits CTLs [36•]. MBG453, TSR-022 (anti-TIM-3 mAbs) are currently in Phase I clinical development in combination with PD-1 inhibitors (NCT02608268 and NCT02817633) [37, 38]. TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a part of CD28 superfamily, expressed by T cells, exerts its immunosuppressive effects by release of IL-10 and inhibition

Table 1 Summary of Ongoing Clinical Trials of Combination Immunotherapies in Metastatic Melanoma

Drugs	Therapeutic targets	Phase of Development	Clinical Trial Identifier	Efficacy	Toxicities
BMS986016 + Nivolumab	Anti-LAG-3 Anti-PD-1	Ib/II	CA224–020 NCT01968109	ORR 16% DCR 45%	Any grade 46% Gr ¾ 9%
MBG453 + PDR001	Anti-TIM-3 Anti-PD-1	Ib/II	NCT02608268 CMBG453X2101	–	–
TSR-022 + TSR-042	Anti-TIM-3 Anti-PD-1	Ib	NCT02817633	–	–
OMP-313 M32 + Nivolumab	Anti-TIGIT Anti-PD-1	Ib	NCT03119428 313 M32–001	–	–
CA-170	Anti-VISTA Anti-PD-L1/L2	Ib	NCT02812875	–	–
Enoblituzumab + Ipilimumab	Anti-B7-H3 Anti-CTLA-4	Ib	NCT02381314 CP-MGA271–02	–	–
Enoblituzumab + Pembrolizumab	Anti-B7-H3 Anti-PD-1	Ib	NCT02475213 CP-MGA271–03	PR 7.7% SD 38.5% PD 46.2%	Any grade 86.5% Grade 3 36%
MDG009 (Orlotamab) + MGA012	B7-H3 x CD3 Dual-Affinity Re-Targeting protein Anti-PD-1	Ib	NCT03406949 CP-MGD009–02	–	–
CPI-444 + Atezolizumab	Anti-A2aR Anti-PD-L1	Ib	NCT02655822 CPI-444-01	6-mo DCR 35% PR 11% mPFS 5.9 mo 20-mo OS rate 88%	Mild (only 5/30 AEs were grade 3/4)
IPI-549 + Nivolumab	Anti-PI3Kγ Anti-PD-1	1b	NCT02637531	2 PRs; 40% on study >= 12 weeks	Most Gr ½ AEs; 2 DLTs
TTI-621 + Pembrolizu/ Durvalu/ Avelu/ Atezolizu (mab)	Anti-CD47 Anti-PD-1/L1	Ib	NCT02890368	–	–
PF-04518600 + Utomilumab	OX40 agonist 4-1BB agonist	Ib	NCT02315066	4/9 patients with best ORR of SD	Mild; no DLT or Gr ¾ AEs
Utomilumab + Avelumab	4-1BB agonist Anti-PD-L1	Ib/2	NCT02554812	–	–
MEDI6383 + Durvalumab	OX40 agonist Anti-PD-L1	Ib	NCT02221960	–	–
GSK3174998 + Pembrolizumab	OX40 agonist Anti-PD-1	Ib	NCT02528357	–	–
TRX518 + Avelumab	GITR agonist Anti-PD-L1	Ib	NCT03861403 TRX518–004	–	–
BMS-986156 + Nivolumab	GITR agonist Anti-PD-1	Ib	NCT02598960	–	Gr 3 lung infection, lipase/AST elevation; Gr 4 CPK elevation
MK-4166 + Pembrolizumab	GITR agonist Anti-PD-1	Ib	NCT02132754	–	–
INCAGN01876 and Nivolumab or Ipilimumab	GITR agonist Anti-PD-1 Anti-CTLA-4	Ib	NCT03126110	–	–
GWN323 + PDR001	GITR agonist Anti-PD-1	Ib	NCT02740270	–	–
JTX-2011 and/or Nivolumab/Pembrolizumab Ipilimumab	ICOS agonist Anti-PD-1 Anti-CTLA-4	Ib/II	NCT02904226	–	–
GSK3359609 + Tremelimumab	ICOS agonist Anti-CTLA-4	Ib	NCT03693612	–	–
Utomilumab + Pembrolizumab	4-1BB agonist Anti-PD-1	Ib	NCT02179918	26% ORR	No DLTs
Utomilumab + Mogamulizumab	4-1BB agonist Anti-CCR4	Ib	NCT02444793	Terminated	Terminated
Utomilumab + PF-04518600	4-1BB agonist OX40 agonist	Ib	NCT02315066	4/9 patients with best ORR of SD	Mild; no DLT or Gr ¾ AEs
Utomilumab + Avelumab	4-1BB agonist Anti-PD-L1	Ib	NCT02554812	–	–
Varilumab + Glembatumumab vedotin	CD27–70 agonist ADC	II	NCT02302339	–	–
CP-870893 + Tremelimumab	CD40 agonist Anti-CTLA-4	Ib	NCT01103635	ORR 27.3%; 9.1% CR; 18.2% PR. mPFS 3.2 mo; mOS 23.6 mo.	2 DLTs (Gr 3 colitis); 1 uveitis, hypophysitis, 1 g 3 CRS
APX005M + Nivolumab	CD40 agonist Anti-PD-1	Ib/II	NCT03123783	2/12 PR; 3 SD; 7 PD	–
APX005M + Nivolumab + Cabiralizumab	CD40 agonist Anti-PD-1 CSF1R inhibitor	Ib	NCT03502330	–	–
APX005M +	CD40 agonist	Ib	NCT02706353	–	–

Table 1 (continued)

Drugs	Therapeutic targets	Phase of Development	Clinical Trial Identifier	Efficacy	Toxicities
Pembrolizumab	Anti-PD-1				
NEO-PV-01 + Nivolumab + Adjuvant + APX005M	Neoantigen vaccine Anti-PD-1 Poly-ICLC (adjuvant) CD40 agonist	Ib	NCT03597282	–	–
Selicrelumab + Atezolizumab	CD40 agonist Anti-PD-L1	Ib	NCT02304393	–	–
Selicrelumab + Emactuzumab	CD40 agonist CSF1R inhibitor	Ib	NCT02760797	–	–
Selicrelumab and Vanucizumab Or Bevacizumab	CD40 agonist Anti-angiopoiten-2 Anti-VEGF	Ib	NCT02665416	–	–
BMS-986205 + Nivolumab + Ipilimumab	IDO inhibitor Anti-PD-1 Anti-CTLA-4	Ib	NCT02658890	–	–
Epocadostat + Pembrolizumab Vs. Pembrolizumab alone	IDO inhibitor Anti-PD-1	III	NCT02752074	PFS rate 37% in both groups; ORR 34.2 vs. 31.5	Gr ¾ AEs in 21.8% vs. 17% (TERMINATED)
Indoximod with Ipilimumab Or Nivolumab/Pembrolizumab	IDO inhibitor Anti-CTLA-4 Anti-PD-1	Ib/II	NCT02073123	ORR 55.7%; mPFS 12.4 mo; CR 18.6%	Well-tolerated
GDC-0919 + Atezolizumab	IDO inhibitor Anti-PD-L1	Ib	NCT02471846	4/45 with PR and 24% with SD	1 DLT (G3 spesis), 34% G3 AEs
GR-MD-02 + Ipilimumab	Galectin-3 inhibitor Anti-CTLA-4	Ib	NCT02117362	–	–
INCB001158 + Pembrolizumab	Arginase inhibitor Anti-PD-1	Ib/II	NCT02903914	–	–
DS-8273a + Nivolumab	Anti-TRAIL DR5 Anti-PD-1	Ib	NCT02983006	–	–
ATRA + Ipilimumab	All trans retinoic acid Anti-CTLA-4	II	NCT02403778	–	–
Lacnotuzumab + Spartalizumab	Anti-MCSF Anti-PD-1	Ib/II	NCT02807844	1 PR; 19% SD; irPR or irSD 27%	>30% all grade AWEs; >= 10% grade3 AEs
Pexidartinib + Pembrolizumab	CSF1R inhibitor Anti-PD-1	Ib/II	NCT02452424	–	–
ARRY-382 + Pembrolizumab	CSF1R inhibitor Anti-PD-1	Ib/II	NCT02880371	–	–

Abbreviations: A2aR = adenosine A2A receptor; AE = adverse events; B7-H3 = B7 homolog-3; CCR4 = C-C chemokine receptor type 4; CR = complete response; CSF1R = colony stimulating factor 1 receptor; CTLA-4 = cytotoxic T lymphocyte associated protein 4; DCR = disease control rate; DLT = dose-limiting toxicity; GITR = glucocorticoid-induced TNFR-related protein; Gr = grade; ICOS = inducible T-cell co-stimulator; IDO = indoleamine 2,3 dioxygenase; LAG-3 = lymphocyte activation gene 3; MCSF = macrophage colony stimulating factor; mo = months; mPFS = median progression free survival; mOS = median overall survival; ORR = objective response rate; PD = progressive disease; PD-1 = programmed death-1; PR = partial response; PI3Kγ = phosphoinositide 3 kinase γ; SD = stable disease; TIM-3 = T cell immunoglobulin and mucin domain containing-3; TIGIT = T cell immunoreceptor with Ig and ITIM domain; TRAIL DR5 = TNF-related apoptosis-inducing ligand death receptor 5; VEGF = vascular endothelial growth factor; VISTA = V domain of immunoglobulin suppressor of T cell activation

of interferon (IFN)-γ and IL-17 [34, 39]. An anti-TIGIT antibody (OMP-313 M32) is also in early phase development [40]. Similarly, VISTA (V domain immunoglobulin suppressor of T cell activation), overexpressed in tumor cells that simultaneously inhibits CTLs and activates APCs [41]. CA-170 is a dual inhibitor of VISTA and PD-L1/L2 and undergoing phase I clinical trial [42].

B7-H3 (B7 homolog-3, CD276) also inhibits T cell activation and is overexpressed in melanomas and other tumors [43•]. Enoblituzumab (a humanized B7-H3 mAb) in combination with pembrolizumab was well tolerated and demonstrated tumor shrinkage rate of 2–69% across several tumor types including melanomas in the interim report [44]. CD39 and CD73 (ectonucleotidases) are overexpressed in the TME where they promote degradation of ATP to adenosine allowing it to bind its receptor (A2aR) on a variety of immune cells (T-cells, NK cells, APCs) promoting immunosuppression [31•, 45•]. This adenosinergic pathway is particularly upregulated in the hypoxic TME under the influence of HIF-1α [46•]. CPI-444 (A2aR inhibitor), in combination with atezolizumab (anti-PD-L1), demonstrated DCR of 45% [47•], mature data on efficacy outcomes is awaited.

Targeting the Immune co-Stimulatory Pathways

An alternate strategy to boost immune targeting of tumors involves activation of co-stimulatory pathways that include: OX40, CD40, GITR, ICOS and, 4-1BB [31•, 32•]. Agonists that activate these pathways are also in clinical development (Table 1). OX40 (CD134, member of tumor necrosis factor receptor [TNFR] superfamily) when bound to its ligand (OX40L) results in activation, proliferation and survival of cytotoxic T-cells and represses regulatory T-cells [48•, 49•]. Similarly, 4-1BB (CD137) is another member of TNFR superfamily and works in synergy with OX40 [50, 51•]. PF-8600 (OX40 agonist) and utomilumab (4-1BB agonist) combination in a phase 1 clinical trial showed best ORR in 4/9 patients [52•]. Other OX40 agonists are still under early phase development in combination with PD-1 inhibitors (NCT02221960 and NCT02528357) [53, 54]. GITR (glucocorticoid-induced TNFR-related gene) is upregulated in activated T-cells, endothelial cells and APCs. Upon ligand binding, GITR activates effector T-cells, attenuates suppressor activity of regulatory T-cells, thus overstimulating the immune system [55]. Several GITR agonistic mAbs are currently undergoing early stage clinical development, in combination with anti-PD-1 or anti-CTLA-4 antibodies [56–60]. CD40 (another member of TNFR superfamily), expressed on DCs, B cells and activated T-cells, signaling results in maturation of DCs, promotes B cell signaling and isotype switching priming them for antigen presentation [61]. APX005M (CD40 agonist) combination with nivolumab demonstrated 2 partial responses, 3 patients with stable disease as best overall response out of 12 patients with melanoma [62••]. Nanostring analysis revealed high proportion of tumor infiltrating lymphocytes and increased INF-γ inducing cytokines in paired tumor biopsies supporting biologic synergy of this combination [62••].

Novel Combinations to Enhance Immune Recognition of Tumors

An important pre-requisite for immune recognition of tumors is abundance of non-self neoepitopes determined by somatic mutation burden in the tumor genome (tumor mutational burden or TMB). Indeed, tumors with high TMB are more likely to respond to ICIs [63]. TMB alone, however, is not a perfect biomarker of response to immunotherapy. In fact, some cutaneous melanomas have a high TMB, but exhibit a phenotype of T cell exclusion owing to ß-catenin gain-of-function mutation that in turn results in ligand-independent activation of the WNT pathway with transcriptional activation or repression of target genes (such as TGF-ß) [64•, 65•, 66•, 67•]. In addition, tumors with a deficient mismatch repair (MMR) machinery lose the integrity of their genomic replicative fidelity and result in abundance of neoepitopes [68•]. The deficient MMR tumors have an intrinsically inflamed T cell phenotype and predicts response to ICIs, the basis of first tissue-agnostic approval of pembrolizumab [69••]. The so-called cold tumors that exhibit a T cell exclusion phenotype due to lack of neoantigens or immunosuppressive TME can also be primed with autologous cancer vaccines. Neoantigens are isolated from the tumor tissue and injected back to the patient, this results in expansion of their T cell repertoire. In combination with checkpoint inhibitors or other immune adjuvants, this approach can generate an effective T cell response primed to tumor-specific neoantigens. Indeed, a personal neoantigen vaccine, NEO-PV-01 in combination with nivolumab showed promising preliminary efficacy with 5 partial responses, 5 patients with stable disease, the responses were durable and the combination was well tolerated [70••]. In addition to cancer vaccines, oncolytic viruses such as talimogene laherparepvec (T-VEC, herpes simplex virus-1), when injected intratumorally, can also induce T-cells leading to immune-mediated destruction. A phase II study of T-VEC with ipilimumab showed an ORR of 39% compared to 18% in ipilimumab arm alone [71•]. In addition to response in injected lesions, responses were also observed in distant lesions [71•]. The incidence of ≥ grade 3 AEs was 45% in the combination arm compared to 35% with ipilimumab alone [71•]. Similarly, combination of T-VEC and pembrolizumab showed promising efficacy in a phase Ib study, where it demonstrated ORR of 48–57% and an impressive CR rate of 14–24% [72••]. Grade ¾ AEs occurred in 33% of patients. A phase III, randomized, double-blind study of T-VEC and pembrolizumab is ongoing [73].

Novel Combinations to Overcome the Immunosuppressive Tumor Microenvironment

Beyond tumor genomic profile driving recognition of tumor-associated antigens, TME defines the CTL access to tumors. TME comprises of tissue-specific extracellular matrix that comprises of cancer-associated fibroblasts (CAFs), integrins, adipose cells, components of the blood and lymphatic system, neuroendocrine cells and immune-inflammatory cells [74•, 75•]. TME performs a variety of functions that includes escape from tumor growth suppressors, promoting invasion and metastasis, resisting apoptosis, promotion of immunosuppressive adenosinergic pathway and angiogenesis facilitating nutrient supply, maintaining proliferative niche, tumor acidosis with stimulation of autophagy and immunosuppression, evasion from immune-mediated destruction and, tumor genomic instability [45•, 74•, 75•, 76•, 77•, 78]. CAFs in particular are central players in the immunosuppressive pathways, by release of soluble factors such as FGF2, VEGF, TGF-ß, HGF, CXCL-12, IL-1, tenascin C, MCP-1 among many others [79–82]. The immune inflammatory cells in the TME include both components of the adaptive (T and B-cells) and innate immune systems (DCs, NK cells, macrophages etc.) [83].

An immunosuppressive environment is promoted by T_{regs} and myeloid-derived suppressor cells (MDSCs) by release of cytokines and chemokines by the tumor cells [84–86]. In addition, macrophages within the TME develop into M2 subtype (called tumor associated macrophages or TAM) that promote tumor growth, angiogenesis, invasion, metastasis and resistance to therapy [87, 88]. This transformation of macrophages from the pro-inflammatory and tumoricidal (M1 subtype) to TAMs is thought to be a consequence of continuous presence of growth factors such as colony-stimulating factor-1 (CSF1 or macrophage CSF) from tumor cells as well as CD4+ derived cytokines such as IL-4, IL-13 and IL-10 in the TME [89•]. CSF1 receptor (CSF1R) on macrophages mediates survival of TAMs and has emerged as an attractive target to inhibit tumor-promoting signaling [89•, 90]. In addition to TAMs, CSF1R expression is also detected in other myeloid cells within TME such as MDSCs. Once activated, the TAMs effectively suppress immune effector functions either by directly inhibiting effector T-cells or indirectly by activation of T_{regs}, intratumoral DCs or Th2-helper T-cells. A variety of small molecules and mAbs directed at CSF1R or its ligand (such as cabiralizumab, Five Prime Therapeutics) are in clinical development, either alone, in combination with ICIs or other immune pathway inhibitors.

Together with T_{regs} and MDSCs, TAMs result in a cascade of transcellular events that inhibit CTLs and promote immune tolerance. Many of these functions are achieved through release of soluble factors such as: IDO, TGF-ß, PI3Kγ and, CD47. Indeed, a promising strategy is to target these soluble factors to promote tumor directed immune attack. Indolamine 2,3-dioxygenase (IDO) is an enzyme that converts tryptophan to kynurenines and is overexpressed in many tumor types [91•, 92•, 93•]. Kynurenines produce an immunosuppressive mileu by promoting T_{regs} and suppressing CTLs [91•]. IDO inhibition with epocadostat combined with pembrolizumab demonstrated an impressive ORR of 55% in melanomas in phase I clinical trial [94••]. However, the phase III trial failed to demonstrate superiority of epocadostat + pembrolizumab combination over pembrolizumab alone [95••]. Indeed, this failure has reinvigorated a more careful approach to testing novel immunotherapy combinations, ideally driven by biomarker signatures. Transforming growth factor (TGF)-ß plays a central role in immunosuppression, is consistently found to be upregulated in so-called cold tumors and associated with poor survival [96]. Specific inhibitors of TGF-ß are under development and offer a promising approach in overcoming cancer immune evasion.

Novel Combination Immunotherapies with Targeted Agents

In addition to T cell inflamed phenotype, cutaneous melanomas also harbor key driver mutations (*BRAF*^{V600E}, *NRAS*, *MITF*, *PTEN*, *PREX2*, *TERT* and c-*KIT*) [97•]. Targeted therapies that inhibit these pathways, particularly a combination of BRAF/MEK inhibitor in *BRAF* mutated melanomas have demonstrated durable responses. In addition, targeting *BRAF* oncogenic pathways is known to increase expression of antigens on melanoma cells, induction of PD-L1 expression and inhibition of immunosuppressive cytokines resulting in a more favorable TME [98••, 99]. In this context, combination immunotherapy with these targeted agents is a promising approach, and clinical trials are ongoing, although cumulative toxicity remains a major concern.

Conclusions

The foregoing is a brief review of combinatorial immunotherapy strategies that have the potential to stimulate the immune system to destroy tumor cells. Removing the brakes from immune checkpoints and/ or activation of co-stimulatory pathways accelerates T cell effector functions. Tumors with an inherently high burden of neoepitopes attract a robust immune response. Finally, the TME activates a variety of immunosuppressive pathways, often driven by tissue hypoxia. Therapeutic targets within the TME include soluble immunosuppressive factors, antagonizing inhibitory chemokines (CXCL-12) and inhibition of TAM recruitment (CSF1R inhibitors). Antibodies to CTLA-4 and PD-1/L1 checkpoints have demonstrated impressive clinical responses leading to their approval in frontline setting for various malignancies. At the same time, other immunomodulatory agents are needed to move the field forward, likely in synergy with existing immunotherapies. In this context, expert consensus is to use approved therapies (CTLA-4 or PD-1/L-1) as the backbone for testing safety and efficacy of newer agents [98••]. Cutaneous melanomas have been at the forefront of immunotherapy revolution, however, the durable responses seen with ICIs and novel immunotherapy combinations have a direct translational relevance as they provide the basis for potential to expand application to other tumor types.

Compliance with Ethical Standards

Conflict of Interest Babar Bashir declares that he has no conflict of interest.
Melissa A. Wilson has received compensation from Bristol-Myers Squibb for service on an advisory board.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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