EDITORIAL

Special Focus: Viral-immunotherapies and vaccines in melanoma

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Melanoma Management

Vaccine therapy + dasatinib for the treatment of patients with stage IIIB–IV melanoma

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Melanoma remains a significant health concern worldwide, with disease incidence continuing to rise over the past four decades [1]. In the USA alone in 2016, the American Cancer Society estimates that 76,380 new cases of melanoma will be diagnosed, with 10,130 patients succumbing to this disease [1]. Although early stages of the disease are surgically curable and adjuvant therapy of high-risk disease is moderately effective in preventing recurrence, advanced-stage, metastatic melanoma has historically exhibited poor durable response rates to existing therapy options and high disease-associated fatality rates [2]. The landscape of first-line treatment options for advanced-stage IIIB–IV melanoma patients has been radically altered over the past decade with the advent of targeted small molecule inhibitors

(SMIs; i.e., BRAFi, MEKi) and immune checkpoint inhibitors (ICB; i.e., antagonist antibodies against CTLA4, PD1 or PD-L1) [3]. SMIs have demonstrated profound, yet often transient, antitumor efficacy in the clinical setting, with the majority of treated patients ultimately developing treatment-refractory progressive disease [3]. Although superior rates of durable clinical response have been noted with ICBs and when combining ICBs such as ipilimumab (anti-CTLA4) and nivolumab (anti-PD1), such trials have also evidenced high rates (>50% incidence grade 3–4 for ipilimumab/nivolumab) and severity of immune-related toxicities, including autoimmune colitis [4]. Developing research for patient biomarkers predictive of durable objective clinical response or reduced risk for pathologic autoimmune sequelae

KEYWORDS

adjuvant • dasatinib • dendritic cells • immunotherapy • melanoma • T-cells receptor • tumor microenvironment • tyrosine kinase inhibitors • vaccine

“...there remains an urgent need to develop second-line treatment options for the many advanced-stage melanoma patients that have or will fail current small molecule inhibitor and/or immune checkpoint inhibitor-based clinical trials.”

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should lead to more restrictive inclusion criteria and patient safety monitoring, allowing for an optimal clinical benefit-to-toxicity ratio to be realized for existing first-line and evolving combination approaches utilizing these agents. Despite optimism for such developing therapies, there remains an urgent need to develop second-line treatment options for the many advanced-stage melanoma patients that have or will fail current SMI- and/or ICB-based clinical trials [5].

Melanoma vaccine: promise & limitations

The frequent, but often short-lived, clinical benefits of ICB in the melanoma setting provide optimism for the curative potential of the patient's immune system, despite a consensus perception of its dysfunctional state in patients with progressive disease [6]. In particular, there remains great enthusiasm for therapeutic vaccines designed to promote the activation and expansion of tumor-reactive T-cells (and B cells producing protective antibodies), and for adoptive transfer approaches implementing enriched populations of endogenous or genetically-engineered anti-tumor T-cells (i.e., adoptive cell therapy; ACT) [3,5]. Melanoma vaccines have evolved a rich history in the clinical setting over the past 20 years, demonstrating a high-degree of safety [7,8]. However, a meta-analysis of 56 Phase II/III melanoma vaccine trials (involving a total of 4375 patients) revealed that while vaccines were commonly effective in promoting tumor antigen-specific T-cell responses as measured in the peripheral blood of patients, they did not provide better overall disease control or extend overall survival versus standard of care [8].

So why have not melanoma vaccines performed better in the clinic? Clear limitations in such approaches include omnipresent concerns for immune checkpoint and regulatory cell (i.e., myeloid-derived suppressor cells [MDSC], tumor-associated macrophages [TAM] and regulatory T-cells [Treg])-mediated immunosuppression of antitumor T-cell responses *in vivo*. An additional limitation relates to the inability of vaccine-induced (melanoma-reactive) T-cells to be effectively recruited into tumor sites throughout the body in sufficient numbers to manifest clinically meaningful benefit(s) to the patient [3,6,7]. Indeed, the presence of persistent vaccine depots in the patient's dermis serves as a sink to preferentially outcompete the tumor microenvironment (TME) in recruiting

vaccine-induced T-effector cells [9]. The physical milieu of the TME (i.e., hypoxia, acidosis, high interstitial fluid and mechanical pressure) is also hostile to antitumor T-cell survival and function [10]. Furthermore, T-effector cells exhibit a primary dependency on glycolysis as a source of cellular energy, an addiction that is not sustainable in the poorly perfused, nutrient-deficient TME [10,11].

Vaccines targeting melanoma-associated blood vessels

An additional possible reason for the limited clinical success of vaccines targeting melanoma-associated antigens reflects the heterogeneity in antigenic phenotypes among cancer cell subpopulations within a given patient lesion, as well as across multiple lesions that may exist in a single patient. More specifically, tumor cell subpopulations are known to exhibit deficiencies in their expression of vaccine-targeted antigens, of MHC class I/II antigen-presenting molecules or of components in the antigen processing machinery, with such cells having clear survival advantages in the face of vaccine-induced immune surveillance [5,7]. Indeed, such clonal subpopulations of tumor cells are believed to serve as the foundation of immunotherapy-resistant progressive disease in previously treated patients [5,7].

A theoretical means by which to promote anti-tumor immunity, while coordinately circumventing the immune-phenotypic heterogeneity of melanomas, involves the development of vaccines targeting tumor-associated stromal cell populations, such as (myo)fibroblasts, vascular pericytes and vascular endothelial cells (VECs) [12,13]. We have recently shown that vaccination of HLA-transgenic mice against tumor blood vessel-associated antigens (peptides) results in therapeutic immunity against melanoma under conditions that are nonpermissive for direct T-cell recognition of tumor cells [12]. Instead, these vaccines elicit CD8⁺ T-effector cell targeting of tumor (but not normal tissue) associated blood vascular pericytes and/or VEC [12]. Since the vaccine-inclusive peptides (derived from tumor vascular antigens) can be recognized by CD8⁺ T-cells isolated from the blood of HLA-A2⁺ melanoma patients after *in vitro* stimulation [12], this vaccine strategy was amenable to translation into clinical trials for the treatment of HLA-A2⁺ patients with advanced-stage melanoma.

Therapeutic ‘vascular normalization’

Based on core findings by Jain and colleagues [14], we believe that vaccine efficacy may be improved by the inclusion of agents that promote therapeutic vascular normalization (TVN), leading to the improved delivery of antitumor T-cells into the TME, with coordinate support for their sustained functionality *in vivo*. TVN results in the trimming of abundant nonfunctional venules within the tumor mass, maturation of vascular pericytes and VEC, improved tissue perfusion/oxygenation, and elimination of acidosis and high IFP in the TME [14]. A broad range of in-clinic drugs have now been demonstrated to promote at least transient TVN, including VEGF/VEGFR antagonists, tyrosine kinase inhibitors and even the anti-malarial drug chloroquine [14–17]. Interestingly, recent reports that integrate immune monitoring as a therapeutic end point have shown that TVN is associated with improved T-effector cell recruitment into tumors (based on upregulated expression of the CXCL9, CXCL10, CXCL11 and/or CCL5 chemokines in the TME), the removal of suppressor MDSC, TAMs and Treg in the TME, and a reduction in the expression of hypoxia-responsive immune checkpoint molecules (i.e., PD-L1) that inhibit tumor-infiltrating T-cell activity/survival [13–15]. Unfortunately, anti-angiogenic agents when used as monotherapies exhibit high rates of acquired drug-resistance (based on the activation of compensatory pro-angiogenic signaling pathways), leading to only transient clinical benefits for several months [14].

Dasatinib as a vaccine ‘adjuvant’ to promote TVN

Dasatinib (BMS-354825; NSC-732517) is a potent, broad spectrum ATP-competitive inhibitor of five critical oncogenic tyrosine kinases/kinase families: BCR-ABL, SRC, KIT, PDGFR and ephrin receptor kinases, known to impact tumor growth and metastasis [18]. Although dasatinib monotherapy has previously manifested disappointing clinical activity in patients with advanced-stage melanoma patients [19], we observed that it functions well as an adjuvant to specific vaccination in the MO5 murine melanoma model [16]. Hence, dasatinib + vaccine combined therapy promoted: superior expansion and melanoma recruitment of therapeutic T-cells (via loco-regional production of CXCL9, CXCL10 and

CXCL11, reduced tumor hypoxia and MDSC and Treg presence in the TME, evidence of ‘broadening/spreading’ in the anti-tumor T-cell repertoire (to include specificities that were not included in the vaccine formulation) and extended overall survival [16].

Development of combined vaccine therapy + dasatinib for the treatment of patients with stage IIIB–IV melanoma

As our preclinical data support:

- The ability of dasatinib to favorably condition the host for improved responsiveness to vaccination, and to better direct and sustain vaccine-induced T-effector cells into/within the TME via TVN [16]; and
- The therapeutic efficacy of vaccine targeting tumor-associated blood vessels under conditions where the immune system need not directly recognize tumor cells [12].

We developed a pilot clinical trial at the University of Pittsburgh Cancer Institute combining these modalities for the treatment of patients with advanced stage melanoma (NCT01876212).

This is an open single-center, prospective randomized Phase II study to determine the activity and safety of intradermal administration of Type-1, autologous dendritic cells (DCs) loaded with a mixture of six tumor vascular antigen (DLK1, EPHA2, HBB, NRP1, RGS5, TEM1)-derived peptides combined +/- daily oral administration of dasatinib (70 mg BID) applied as an immune adjuvant/conditioning agent [19]. This trial will evaluate the activity, safety and immune effects of the combination vaccine in up to 28 HLA-A2⁺ patients (≥18 years of age) with measurable, advanced stage IIIB–IV melanoma. Based on possible efficacy for dasatinib among patients with c-kit mutant melanomas [20], these patients will be excluded from study. Randomization will also be stratified to balance the two study treatment cohorts based on melanoma *BRAFV600E* status.

The primary objective of this study is to evaluate the effects of combination therapy of dasatinib and vaccine on specific immune response rates against the six tumor-associated vascular antigens. Secondary objectives include the evaluation of the objective clinical response rate, overall survival, progression-free survival and exploratory immunological

“This trial will evaluate the activity, safety and immune effects of the combination vaccine in up to 28 HLA-A2⁺ patients...”

end points, including the number of CD8⁺ T-cells, MDSC/Treg regulatory cells and blood vessels in melanoma biopsies, and the level of CXCL10 chemokine in patient serum pre- versus post-treatment.

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