From personalized to patient-specific treatment of metastatic melanoma

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Metastatic melanoma remains a highly lethal disease with 5-year survival rates ranging between 10 and 15% during 1990–2009 [1,2]. However, there is reason to believe that we are on the verge of improving long-term survival rates thanks to the emergence of new therapies that target specific enzymes associated with altered signal transduction [3,4], and checkpoint molecules that effect suppression of anti-tumor immune responses [5–8]. However, even with these therapies most metastatic melanoma patients die within 1–2 years after starting treatment. It is anticipated that genomic analysis will identify additional therapeutic targets that are common to melanoma patients, and that molecular diagnostics will help guide therapeutic decisions, making it possible to practice what is commonly referred to as ‘personalized medicine’, or the misnomer ‘precision medicine’.

Despite recent advances, the major challenge for cancer therapy continues to be the interpatient heterogeneity associated with each individual’s malignant genome and pharmacogenomics [9]. By taking advantage of our increasing understanding of these molecular domains, we may be able to overcome some aspects of cancer heterogeneity with ‘personalized medicine’.

However, for many patients, the optimum outcomes may only be achieved with ‘patient-specific’ cancer therapy that goes beyond ‘personalized’ cancer therapy.

Personalized medicine versus patient-specific therapy

‘Personalized medicine’ implies that management and treatment are based on the results of tests, increasingly molecular tests, which are prognostic of outcome or predictive of clinical benefit. Prognostic markers are most useful for anticipating disease recurrence while predictive markers are associated with clinical benefit from a specific therapy. Thus, in metastatic melanoma, the V600E *B-RAF* mutation (present in about 50% of patients with metastatic melanoma) is predictive of an objective tumor response in over 50% of patients whose tumors have the mutation when treated with enzyme inhibitors vemurafenib or dabrafenib. When an anti-MET therapy is added to block an alternative signal transduction pathway that is frequently associated with resistance to the BRAF inhibition and characteristic cutaneous toxicity, the response rates are even higher, progression-free survival longer and toxicity decreased [3,4].

KEYWORDS

- autologous adoptive cell therapy
- autologous dendritic cell therapeutic vaccines
- autologous tumor cell lines
- melanoma
- patient-specific cancer therapy
- personalized cancer therapy
- tumor-associated antigens
- tumor-infiltrating lymphocytes

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The limitation of personalized cancer therapy is the finite armamentarium that is currently available. Consequently, there are only limited combinations and permutations of therapeutic agents with which to ‘personalize’ or ‘individualize’ a therapeutic approach. Therefore, at this time ‘personalized’ or ‘precision’ therapy is not synonymous with ‘patient-specific’ therapy.

Patient-specific therapy implies a therapeutic product that is unique and specific for each patient. There is no menu from which to choose. The best examples are autologous cell-based immunotherapies. Each therapeutic product is genetically unique to that individual. Some believe that diagnostic tests or ‘immune signatures’ will be at least partially predictive for the likelihood of benefit from such patient-specific therapies [10]. Patient-specific therapies almost certainly will be used in combination with agents that are chosen from other treatment options based on their mechanisms of action.

The most developed patient-specific approaches are autologous adoptive cell therapies, and autologous anti-cancer vaccines. Both have been associated with long-term survival in a significant subset of patients, and longer 5-year survival rates than are associated with other anti-melanoma therapies. Both are derived from the patient’s own tumor and rely on recognition of unique antigens resulting from non-synonymous mutations present in each specific patient’s cancer [11]. Exomic analyses combined with computer analyses and immunologic assays have confirmed the therapeutic benefit associated with immune recognition of such patient-specific mutations [12–14]. Some are unique cancer-driving mutations, but most result in proteins that do not facilitate malignancy. These so-called ‘passenger mutations’, however, may result in unique antigens that can be recognized by the host’s immune system. Neither of these approaches is melanoma specific, but perhaps melanoma patients are more likely to benefit from a patient-specific approach because of the high number of nonsynonymous mutations associated with that malignancy [11].

• Patient-specific adoptive cell therapy

A variety of autologous lymphocyte populations have been utilized as possible cell therapies [15], but the best known of these are tumor infiltrating lymphocytes (TILs) [16]. The T-cell cytokine IL-2 is critical for selection and propagation of TIL *in vitro*, and the most successful reports of

TIL therapy have included high doses of IL-2 as part of the therapy. TIL can be cultured from a melanoma metastasis about 70% of the time [17,18]. Regressions of metastatic melanoma have been seen following TIL treatment with cells of either CD4⁺ helper or CD8⁺ cytotoxic phenotypes. Objective response rates of 24 and 34% were reported respectively for a multicenter TIL trial [19], and for patients treated at the US National Cancer Institute (NCI) [20], with some patients experiencing long-term survival. In these trials, doses of 10–200 billion TILs were typically infused with IL-2. In addition to high doses of IL-2, these treatment protocols usually included pretreatment with cyclophosphamide to decrease suppressor effects that today might be attributed to Tregs.

The NCI subsequently focused on subclones of TILs which were selected for their cytotoxicity and/or immune reactivity to autologous or allogeneic tumor cells. Such cells are often referred to as antigen-specific TILs. Therapy with these cells evolved to include nonmyeloablative lymphocyte depletion with fludarabine and cyclophosphamide chemotherapy, with or without total body irradiation, infusion of several million antigen-restricted TILs and high-dose IL-2. It was estimated that such antigen-specific TIL could be generated for about 67% of patients [21]. In terms of feasibility, 55% of tumors (at least 2 cm in diameter) were converted into an antigen-specific treatment product (189/344) and 27% (93/344) were treated with 5–20 million cells [22]. An objective tumor response and a complete response were reported for 56% (53/92) and 22% (20/92), respectively. The median survival for all patients was about 17 months and the projected 5-year survival rate was 29%. The vast majority of the long-term survivors were patients who experienced complete remissions. Other studies have shown that the antigenic targets of these TILs are unique to each patient, and often target antigens associated with passenger rather than driver mutations [23].

• Patient-specific autologous vaccines

Given interpatient tumor heterogeneity and the limited therapeutic benefits anticipated from immunizing against only one or a few shared antigens, it is doubtful that allogeneic cell lines or vaccines consisting of only one or a few antigens will be successful in mounting a clinically beneficial immune response that can provide long-term control of a patient’s metastatic

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melanoma [24]. An autologous tumor approach assures that unique antigens resulting from nonsynonymous mutations have the potential to be targeted [14,25]. Approaches that rely on bulk autologous tumor have been plagued by difficulties in producing a prespecified product, and may be limited by immunosuppressive cells and factors that are present in tumor masses [24]. These same issues may limit approaches that involve incubating whole autologous tumor samples with antigen presenting cells, or direct injection of inflammation-inducing agents into the inhibitory environment that is associated with *in vivo* depots of tumor [24]. It may be more effective to utilize short-term cell lines derived from autologous tumors, an approach that offers the advantages of focusing on antigens associated with self-renewal and proliferation in an environment that is free of inhibiting cytokines or Tregs that may impair antigen uptake by antigen presenting cells [25]. Because of *in vivo* suppressive factors, there likely is an advantage to loading antigens onto dendritic cells *ex vivo* rather than depending on endogenous loading.

Patient-specific therapeutic vaccine approaches using antigens from autologous tumor cell lines have been used in a series of

trials in metastatic melanoma. Treatment-related toxicities associated with the 6-month series of subcutaneous injections of these products have been minimal, and mostly consisted of minor localized injection site reactions. A 74-patient trial, in which patients were treated with injections of autologous irradiated tumor cells along with various adjuvants, was associated with a 3/35 tumor response and a 5-year overall survival rate of 29% [26]. Subsequently, 54 patients were treated with autologous dendritic cells loaded with antigens from autologous irradiated tumor cells, and then suspended in the adjuvant GM-CSF at the time of each injection [27]. There were 0/15 objective responses in patients with measurable disease per RECIST (Response Evaluation Criteria in Solid Tumors), but the 5-year overall survival rate was projected at 54%. A 42-patient randomized trial of these two approaches (i.e., irradiated tumor cells or autologous antigen-loaded dendritic cells with GM-CSF) demonstrated a much better survival for patients treated with the antigen-loaded dendritic cells [28]. A pooled analysis of 72 patients treated with the antigen-loaded dendritic cell product confirmed a survival advantage for patients who had no evidence of disease at the

Table 1. Summary of issues for patient-specific immunotherapy products.

Issues	TIL	DC-TC
Therapeutic product	Lymphocytes selected from clones that react with tumor	Dendritic cells loaded with antigens from autologous tumor cell line (DC-TC)
Procedures	Excision of tumor	Excision of tumor and if successful, then leukapheresis from which dendritic cells generated
Key autologous biological materials	Metastatic tumor	Metastatic tumor and peripheral blood mononuclear cells
Tumor-related product	Expanded to 5–20 million cells (57%)	Expanded to 130 million cells (50%)
Additional manufacturing step	None	Generation of dendritic cells and antigen loading (1 week, 100% success)
Fastest time from tumor harvest to possible treatment	12 weeks	12 weeks
Flexibility for timing of treatment	Need to administer when TIL are prepared	Individual doses are cryopreserved
Proportion treated [†]	27% (92/344)	26% 98/384
Preconditioning therapy	Nonmyeloablative chemotherapy ×5 days ± TBI	None
Cotherapy	High-dose intravenous bolus intravenous IL-2 every 8 h up to 14 doses	Subcutaneous GM-CSF as an adjuvant with each dose
Route of administration	Intravenous	Subcutaneous
Toxicity	Severe to life-threatening side effects for both chemo-TBI and high-dose IL-2	Minor local injection site reactions
Frequency of Rx	One time	Weekly ×3, then monthly ×5
Median survival	17 months (n = 92)	46 months (n = 72)
5-year survival	29%	48%

[†]Number treated divided by number of tumors originally received that might have been used for this purpose.

DC-TC: Dendritic cell–tumor cell; Rx: Treatment; TBI: Total body irradiation; TIL: Tumor-infiltrating lymphocyte.

time of vaccine therapy, as well as for those who had nonmeasurable and measurable disease [29].

• Remaining questions for patient-specific therapies

Several general questions have been raised regarding these two patient-specific approaches. These include, feasibility: how reliably can the patient-specific products be generated?; practicality: how long does it take to produce the product?; logistics: what is the feasibility of manufacturing and shipping of cell products?; convenience: when and where can the treatment be administered?; toxicity: what side effects are produced by the cells themselves or other components of therapy?; efficacy: what is the benefit of treatment in terms of disease control and/or long-term survival? and; cost: what is the cost of goods to manufacture a specific product for each patient, and associated medical costs related to administration and concomitant therapy? These issues are summarized and addressed in Table 1.

The therapies described above are patient-specific immunotherapy approaches that go beyond personalized medicine, and provide a personal, rather than personalized, treatment product. They are potentially synergistic therapies, since one potentially enhances endogenous tumor-associated antigen recognition, and the other consists of lymphocytes that recognize those tumor-associated antigen. Issues and concerns remain to be addressed, but our current understanding of tumor immunology makes these approaches more attractive than ever. Because of the unintentional patient selection of the patients who eventually were treated with these products, only randomized trials can demonstrate a relative survival benefit for a population of patients treated with these patient-specific therapies. For successful

commercialization, efficient manufacturing of these products will have to be established.

Future perspective

Over the next 5–10 years we can expect that the focus of outcome measurement for metastatic melanoma patients will increasingly be on long-term survival rather than just response rates and progression-free survival. To date, the best 5-year survival rates have been associated with patient-specific immune cell therapies. Technical and methodological advances will permit reliable manufacturing of such patient-specific cell products for testing in randomized clinical trials for potential commercial application. These approaches will be integrated with therapies that target aberrant intracellular transduction pathways and other immunotherapies that enhance autologous immune responses.

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RO Dillman served as Chief Medical Officer for California Stem Cell, Inc. (CSC) CA, USA which in 2011 acquired the intellectual property associated with the autologous vaccines described in this paper. In May 2014, Caladrius Biosciences, Inc., NY, USA, acquired CSC. RO Dillman is now an employee of Caladrius Biosciences, Inc. and owns stock in that company. He and/or other immediate family members have stock in other companies that make antimelanoma products, including Bristol-Myers Squibb, GlaxoSmithKline and Pfizer. RO Dillman serves as a consultant to Prometheus, Inc., CA, USA, and participates in a speakers' bureau for Genentech. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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