Rose Bengal for melanoma treatment: will it translate to the clinic?

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Before addressing translation of PV-10 (rose Bengal) research findings to the clinic, a prior justifiable question that has been raised by some melanoma experts deserves mention: Why even bother with intralesional therapy, given the new highly active systemic immunotherapies (anti-CTLA-4, anti-PD-1, anti-PD-L1) on the market and in development?

Among strong justifications for ongoing interest in intralesional therapies is the fact that about 10% of patients with locally advanced melanoma develop in-transit cutaneous metastases [1]. Also, local/satellite/in-transit lesions represent about the same percentage of primary melanomas [1]. Many of these lesions are accessible to injection, and treating them may be clinically important not only because of the potential to alleviate such symptoms as ulceration, bleeding, infection and pain, but also because many of these lesions remain localized for a considerable period of time and successful local regional therapy may provide durable responses and defer systemic therapy until it is inevitable.

While the new systemic immunotherapies are a groundbreaking advance,

response rates are far from absolute, often not durable in the case of BRAF-mutated disease, and for most patients cure remains elusive. Serious and in some cases fatal adverse events have been reported, and the risk of significant morbidities related to uncontrolled locoregional disease is high. In the Phase III EORTC 18071 trial of ipilimumab in patients with stage III melanoma, 5/471 (1.1%) patients died of ipilimumab-induced colitis [2]. The overall response rates with pembrolizumab, an anti-PD-1 agent, in one trial [3] were weaker for stage III patients than for M1a and M1b, raising some doubt about systemic immunotherapies as treatment for cutaneous disease. Other patients, owing to age or comorbidities, will be ineligible for systemic immunotherapies.

Specific interest in intralesional therapies arises in part because melanoma is characterized by a propensity to cutaneous, subcutaneous and nodal metastases, and by lymphocyte infiltration of tumors. Direct injection of various agents has been demonstrated to affect tumor shrinkage, and with some agents there is strong suggestion of an indirect stimulation of a

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systemic immune response by modifying the tumor's antigenic milieu.

The putative mechanisms of such immune stimulation have been studied extensively with PV-10, a water-soluble derivative of rose Bengal disodium that has a long history as a diagnostic tool in ophthalmological and hepatic pathologies. The suggestion of immune response comes from the so-called 'bystander' effects in uninjected melanoma lesions when the 10% sterile, nonpyrogenic saline solution of PV-10 is injected and produces chemical ablation of targeted tumors. While excluded from normal cells, PV-10 transits the plasmalemma of tumor cells, accumulates in lysosomes, stimulates lysosomal release and quickly causes complete autolysis. The hypothesis is that acute exposure of antigenic tumor fragments to antigen-presenting cells leads to the observed bystander effect.

Animal research addressed the question as to whether the injected drug was traveling to other sites to cause tumor ablation or rather was inducing a T-cell response. In a murine model, PV-10 induced fivefold increases in IFN- γ , a cytokine critical for innate and adaptive immunity (including tumor control) and for activating macrophages [4]. Responses in untreated lesions occurred only when responses were observed in injected lesions, and the bystander responses were generally delayed. Furthermore, the induced immunity was tumor specific.

At last year's ASCO annual meeting, data from a pilot study [5] enrolling eight patients with dermal and/or subcutaneous metastatic melanoma, the Moffitt Cancer Center showed intralesional PV-10 to be associated with a significant increase in circulating cytotoxic CD8⁺ T cells ($p = 0.03$). Pretreatment, two-study lesions in each patient were sampled by biopsy. One of the two lesions was injected with intralesional PV-10; then both residual sites were completely excised 1 to 2 weeks after PV-10 injection. Tumors were compared before and after treatment to determine pathologic complete response (pCR). In four of the eight patients, PV-10 led to pCR in the post-treatment biopsies of both PV-10-injected and uninjected study lesions. At least partial regression was observed in all patients. The metastatic disease in six of the eight patients had been refractory to previous ipilimumab, anti-PD-1 and/or vemurafenib (BRAF) therapy. Four of these six patients had pCRs in both the injected and uninjected lesions.

Research was presented at the same ASCO session by Igor Puzanov and colleagues for T-VEC, an HSV-1-derived oncolytic immunotherapy that also produces both local and systemic effects in melanoma. The virus selectively replicates within tumors, rupturing them and producing GM-CSF, enhancing systemic, specific antitumor immune responses. Preliminary data from the Phase Ib/II study [6] of T-VEC combined with ipilimumab in stage IIIB–IV M1c melanoma suggest much higher complete and overall response rates with the combination than have been seen with either agent alone. Flow cytometry showed a higher increase in the number of activated CD8 T cells in patients with disease control.

The 80 patients with measurable stage III–IV melanoma in a multicenter, international Phase II trial of PV-10 had a median of six prior interventions. Over a 16-week period, they received PV-10 injections in up to 20 cutaneous and subcutaneous lesions up to four-times. The overall response rate in the intent-to-treat population was 51% (CI: 40–63%), with a 26% complete response rate (CI: 17–37%). Among the subgroup of patients who had all existing melanoma lesions injected with PV-10, the overall response rate by patient was 71%. Half of the patients achieved complete responses. When a further group of 26 patients with monitoring of one to two uninjected bystander tumors was added to the analysis, complete responses were achieved in 232 of the 363 injected lesions for a 64% complete response rate. In the uninjected bystander lesions, complete responses were achieved in ten of 28 patients (36%).

Some have downplayed intralesional treatment of advanced locoregional disease based on the fact that mortality ensues not from it, but from metastatic tumors in the internal organs. The evidence that intralesional therapies can activate a systemic immune response, however, suggests a role for them in reducing the likelihood that distant disease will develop. For the surgical oncologist, treating cutaneous or subcutaneous lesions with PV-10 before excising them, beyond making resection easier or even possible for some that are borderline resectable, adds stimulation of a systemic response to the potential benefit.

The upcoming Phase III trial of PV-10 will enroll patients in whom systemic immunotherapies have failed or who are ineligible for them

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because they are BRAF wild-type, or because of comorbidities or age. Observations on time to response in the PV-10 Phase II study helped suggest the Phase III design. They demonstrated that responses were already complete in the majority of lesions after only one or two injections, a time range when chemotherapy responses are not expected. Chemotherapy with dacarbazine or temozolomide, the standard next option for this population, will be the comparator therapy in the Phase III trial.

The trial will include 225 patients with locoregional disease. They will be randomized 2:1 to intralesional PV-10 or one of the two chemotherapy agents. Differences in response should emerge quickly. The primary end point will be progression-free survival using standard RECIST 1.1 criteria, and the secondary end points will include complete response rate and overall survival. Patient-reported outcomes characterizing the relationship between complete response and symptoms of locally advanced cutaneous melanoma such as pain and bleeding will also be documented.

The most likely application of PV-10 and other intralesional therapies, many agree, will be in combination with systemic immunotherapies. The low toxicities found with PV-10 especially, but generally with all of the intralesional injectables (usually transient injection site inflammation), make combining them with immune checkpoint inhibitors

(anti-CTLA-4 or anti-PD-1) or targeted small molecules (BRAF and MEK kinase inhibitors) easy and attractive. The preliminary findings from the T-VEC/ipilimumab trial noted above are encouraging.

Beyond melanoma, evaluations of PV-10 in treatment of primary and metastatic tumors of the liver are ongoing. Testing of PV-10 with the newer systemic immunotherapies is being planned actively, as well.

Many unanswered questions remain. How well will PV-10 combine with systemic immunotherapies? How strong, in fact, are the systemic immune responses evoked by local tumor ablation, and how extensive and valuable for patients are the quality of life improvements? Of course, only future clinical trials can further resolve these and other uncertainties. The promise suggested by the research to date is compelling – and if confirmed, translating these benefits to the clinic is a high priority.

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