Intralesional treatment for advanced melanoma: what's on the horizon?

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Practice points

- Melanoma incidence continues to rise, and most skin cancer deaths are caused by melanoma.
- Despite the recent advances in systemic immunotherapies, ineligible populations, incomplete response rates, serious toxicities and extreme cost leave an unmet need.
- The potential role for intralesional therapies in metastatic melanoma remains controversial.
- In-transit metastases represent a clinically significant opportunity for intralesional therapies.
- Adjuvant systemic host responses suggest use as a neoadjuvant therapy.
- Initial clinical trials of combined intralesional and systemic immunotherapies suggest synergistic effects.
- Intralesional therapies will be an additional tool for clinicians.

Advances in treatment of melanoma with systemic immunotherapies continue, with promising findings for anti-PD-1 agents combined with ipilimumab. Still, an unmet need persists because of populations ineligible for systemic immunotherapies, incomplete cure/response rates, toxicities and extreme costs. Also, potential for effective use of intralesional therapies remains, especially for local regional disease, but also for benefits of local ablation and adjuvant systemic host tumor-specific responses. Clinical trials of T-VEC, PV-10, CAVATAK and electroporation with plasmid IL-12 have demonstrated favorable, durable responses. Initial experience combining T-VEC, the agent furthest along in testing, with ipilimumab revealed higher complete and overall response rates than with either agent alone. Intralesional therapies may offer a treatment tool in the growing therapeutic armamentarium against this lethal disease.

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New therapies spur melanoma interest

Heightened interest in melanoma is occasioned by a few converging factors. Certainly contributing is the amazing emergence of effective systemic immunotherapies. They have lifted melanoma from the shadows of a lack-of-survival-benefit obscurity in just a few years to a current status in which complaints can be heard that dramatic immunotherapy research findings are attracting an excessive share of the limelight. The fact that melanoma incidence, while constituting a mere 2% of skin cancers, continues to rise (as it has over at least the last 30 years) and causes most skin cancer deaths [1] likely counts heavily, as well. Interest in intralesional therapies for melanoma, while still at

KEYWORDS

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a nascent stage, is being fed by impressive clinical research and maturing data suggesting that several strategies have potential utility among subsets of melanoma patients.

The round of clinical trial results recently published or presented at prominent oncology meetings (American Association for Cancer Research [AACR]/American Society of Clinical Oncology [ASCO]), at the same time as demonstrating the enormous promise of systemic immunotherapies, leaves doors open to possible roles for effective local therapies.

• Systemic immunotherapy advances & limitations

The new immunotherapy era began in 2011 with the introduction and approval of ipilimumab, an anti-CTLA-4 antibody that enables T-cell activation. For decades previously, dacarbazine chemotherapy and high-dose IL-2 were the only US FDA approved agents for advanced disease. Both drugs are limited in their efficacy and application and are without any randomized trials proving their benefit. As the first drug of any kind to improve overall survival in patients with advanced melanoma in a randomized clinical trial, ipilimumab quickly became the standard of care. The FDA approval of ipilimumab [2] was based on the Phase III MDX010-20 trial's finding of improved median overall survival of 10 months with ipilimumab (3 mg/kg intravenous [iv.]) as compared with 6 months for tumor vaccine in patients with unresectable or metastatic melanoma. All had received at least one prior systemic treatment for melanoma.

Ipilimumab was also tested in the adjuvant setting for surgically resected, high-risk melanoma in the European Organization for Research and Treatment of Cancer (EORTC) 18071/CA184-029 trial. Stage III melanoma patients with lymph node involvement who had undergone complete resection received adjuvant ipilimumab (10 mg/kg every 3 weeks $\times$ 4, followed by every 3 months $\leq$ 3 years). Recurrence was reduced by about 25% as compared with placebo [3] (34.8 vs 46.5%; hazard ratio [HR]: 0.75; $p = 0.0013$).

Toxicity was the trade-off, with three of five (1.1%) treatment-related deaths due to colitis in the ipilimumab arm (two gastrointestinal perforations), 37.6% of patients experiencing endocrine disorders (hypophysitis, hypothyroidism), and hepatic issues in 25.1%. Nearly half of ipilimumab-treated patients discontinued because

of adverse events, and at a median of 31 weeks, endocrine effects had resolved in only 56% of patients. It should be noted that ipilimumab has not yet received regulatory approval for adjuvant therapy of melanoma.

In September of 2014, pembrolizumab, a highly selective humanized monoclonal IgG4 antibody directed against PD-1 on the cell surface, received FDA approval for use among patients already treated with ipilimumab, or among patients with *BRAF* V600 mutations, after ipilimumab and a *BRAF* inhibitor. Pembrolizumab prevents binding and activation of PD-L1 and PD-L2. Pembrolizumab had been granted breakthrough therapy designation by the FDA.

In the KEYNOTE-001 study [4] of pembrolizumab at either the currently recommended dose of 2 mg/kg or at 10 mg/kg among 173 patients whose advanced disease had progressed after prior therapies, the overall response rate (ORR) was 24% in the 2 mg/kg group and 26% for the combined pembrolizumab groups. Median progression-free survival in the 2-mg/kg group was 22 weeks (95% CI: 12–36) and 14 weeks (95% CI: 12–24) in the 10-mg/kg group (HR: 0.84; 95% CI: 0.57–1.23).

Grade 3/4 adverse events were reported in only 12% of patients; grade 3/4 potentially immune-mediated events including at 1% frequency pneumonitis, rash, diarrhea and hypophysitis, led to treatment discontinuation in only four patients [5].

Later in 2014, the FDA granted accelerated approval for nivolumab, a PD-1 inhibitor, for unresectable or metastatic melanoma patients who had prior ipilimumab treatment, and for patients with *BRAF* V600 mutations after treatment with both ipilimumab and a *BRAF* inhibitor [6]. The approval came subsequent to a 370-patient trial demonstrating a 32% (120/370) ORR for nivolumab. Responses lasted $\geq$ 6 months in about a third of these patients. Patients had been assigned 2:1 to nivolumab or chemotherapy (investigator's choice). The ORR for chemotherapy was 11%. Importantly, grade 3/4 adverse events were reported in only 9% of patients assigned to nivolumab as compared with 31% in the chemotherapy arm.

In the Phase III KEYNOTE-006 trial [7], pembrolizumab was compared with ipilimumab in the front-line setting among 834 patients with advanced melanoma. Subjects were randomized 1:1:1 to pembrolizumab (10 mg/kg)

every 2 weeks or every 3 weeks or four doses of ipilimumab (3 mg/kg) every 3 weeks. The primary end points were progression-free and overall survival.

The estimated 12-month survival rates were 74.1, 68.4 and 58.2%, respectively, for the three treatment groups (HR pembrolizumab every 2 weeks: 0.63; 95% CI: 0.47–0.83; $p = 0.0005$; HR pembrolizumab every 3 weeks: 0.69; 95% CI: 0.52–0.90; $p = 0.0036$). Response rates were improved with pembrolizumab for both every 2-week and every 3-week administration (33.7%/32.9%, respectively), as compared with ipilimumab (11.9%; $p < 0.001$ for both comparisons).

Higher grade (3–5) adverse events were lower in the two pembrolizumab groups than in the ipilimumab group (13.3%/10.1% vs 19.9%).

CheckMate 067 [8,9], a trial of first-line nivolumab with or without ipilimumab versus ipilimumab alone in 945 treatment-naïve advanced melanoma patients, showed longer median progression-free survival for the nivolumab/ipilimumab combination than for either nivolumab or ipilimumab alone (11.5 vs 6.9 and 2.9 months, respectively). HRs were 0.42 and 0.57 for nivolumab/ipilimumab and nivolumab versus ipilimumab ($p < 0.00001$). Overall response rates were 57.5% (95% CI: 52.0–63.2) for nivolumab + ipilimumab, 43.7% (95% CI: 38.1–49.3) for nivolumab alone and 19.0% (95% CI: 14.9–23.8) for ipilimumab alone. Response rates were higher in those with PD-L1 expression of 5% or higher. It should be noted that the trial was not statistically powered to discern a benefit of combination therapy over nivolumab, and overall survival data of the three treatment arms are not yet available.

Of note, the rate for discontinuations attributed to treatment-related adverse events was 36.4% for the nivolumab/ipilimumab arm, 7.7% for the nivolumab arm and 14.8% for the ipilimumab arm. Responses were reported among a third of patients discontinuing the combination regimen.

At the recent ASCO 2015 annual meeting, Michael Atkins, MD, of Georgetown University Medical Center the plenary session discussant called pembrolizumab and nivolumab, as monotherapies and in combination with ipilimumab, new standards of care in advanced melanoma. At that same session, however, Leonard Saltz, MD, of Memorial Sloan–Kettering Cancer Center, in a talk entitled ‘Perspectives on value’, suggested

that the era in which we can afford the luxury of focusing purely on clinical benefit devoid of cost considerations is over. Based on respective current per milligram prices of US\$28.78 and US\$157.46 for nivolumab and ipilimumab, the total treatment regimen cost in Checkmate 067 was in the hundreds of thousands of dollars, about 4000-times the price of gold. The discussions made necessary by such a reality, Dr Saltz said, will be ‘unpleasant’ and ‘uncomfortable’.

With this as background, it is clear that much work remains to be done and ‘one size does not fit all’. Current interest in intralesional therapies persists and is, in fact, blossoming as these remarkable systemic immunotherapy breakthroughs are still accompanied by less than perfect response rates, sometimes serious toxicities and dizzying cost spirals.

• Rationale for intralesional therapy

Melanoma’s tendency to develop cutaneous, subcutaneous and nodal metastases naturally lends itself to a strategy of direct cutaneous interventions [10–12] with the potential to ablate or shrink tumors. But melanoma’s relationship to the immune system, as exemplified by lymphocyte infiltration of tumors, suggests an immune system attempt to eliminate the tumor. The notion that intratumoral injections of therapeutic agents might modify the tumor’s antigenic milieu and stimulate a systemic immune response was supported by a case study of inoculations with Bacille Calmette–Guérin (BCG) published in 1975 [13]. The subject, a 77-year-old male, had 17 cutaneous lesions, all of which resolved by 8 months after BCG inoculations. It was the >50% resolution of his lung metastasis that evidenced an adjuvant systemic host response. That case study followed from animal research [14,15] showing BCG to heighten host immune responses against transplanted murine experimental tumors, and another report from human clinical research of melanoma regression in 5/8 injected lesions and in two uninjected nodules [16].

Interest in BCG was dampened by subsequent reports of anaphylactic reactions and mortality traced to disseminated BCG [17], in addition to lack of impact on outcomes and high rates of punctate abscesses [18]. Interest in intralesional therapies, however, has continued, despite the array of agents in the novel systemic immunotherapies pipeline, and despite viewpoints colored by the high systemic immunotherapy

response rates, and in the newest agents, much reduced toxicities.

Why consider intralesional therapies when systemic treatments are available for a systemic disease?

The fact remains that melanoma is a systemic disease, and patients die of distant metastases and not regional involvement. There continues, therefore, to be an ongoing debate on the utility of intralesional therapy for melanoma.

Local/regional disease can be clinically significant

In transit' metastases, in a clinical trial [19] including 11,614 patients with single primary cutaneous melanomas, developed in 505 patients. Median primary tumor thickness was 2.95 mm. Ulceration was evident in 39.4% of primary tumors. The in-transit melanoma rates in patients with primary melanomas <1 mm or ≥1 mm were 0.4 and 7.8%, respectively, with a rate of 7.2% for patients who underwent sentinel node biopsy. The in-transit metastases rates for sentinel node-positive and sentinel node-negative patients were 21.6 and 4.7%, respectively. Treatment remains a challenge, as responses to a variety of systemic therapies administered for in-transit metastases have been limited. While in some patients, in-transit disease progresses rapidly to distant disease and early mortality, for other patients, in-transit metastatic disease may remain indolent even for decades. In-transit metastases do allow direct access to tumors for intralesional injections, and for this latter group, strategies to ablate local disease and to control local symptoms such as pain and bleeding are valuable.

All patients may not be candidates for or benefit from systemic therapy

As noted above, while major advances have been made in the systemic therapy of advanced melanoma, these treatments come with toxicity that can sometimes be considerable. Furthermore, several contraindications for systemic immunotherapy exist including autoimmune disease and other medical comorbidities that are more common in elderly patients. The lower toxicities of interleukin (IL) therapies may allow them to be considered for such patients. Also, despite the successes of systemic therapy, most patients are still not cured and may need other options after systemic options are exhausted. Those that have

a significant component of disease accessible for IL injections may benefit from such an approach.

Local therapy may have systemic effects

Several of the newer intralesional agents that have completed or are a part of ongoing clinical trials have a significant systemic effect in addition to their local/regional effects. Putting this feature to good use, intralesional therapies in this setting may use the tumor as a mechanism to incite a specific immune response to distant metastatic sites.

The tumor lysis that occurs after IL injection with an oncolytic virus such as talimogene laherparepvec (T-VEC) and with a chemical ablator like PV-10 are examples. Selective viral replication in tumor tissue after T-VEC injection with subsequent increased production of GM-CSF leads to tumor cell rupture and an oncolytic effect, like a multivalent vaccine, releasing antigens specific to the tumor. Functional deletion of two key genes (ICP34.5 and ICP47) from HSV-1 is thought to deprive tumors of proteins through which they usually circumvent normal response to infection.

In Phase Ib/II research among previously untreated advanced melanoma patients randomized to T-VEC plus ipilimumab versus T-VEC alone, analysis revealed increases from baseline after T-VEC treatment in total and activated CD8 T cells in peripheral blood, with further increases after ipilimumab administration. Also, increases in CD4 T cells expressing ICOS (inducible T-cell costimulator – indicating upregulated CTLA-4 blockade) followed ipilimumab treatment. Importantly, patients with disease control after T-VEC had >1.4-times increases in activated CD8 T cells, while 4/5 patients with tumor growth did not have such increases [20].

PV-10 is a sterile, nonpyrogenic 10% solution of Rose Bengal disodium, a small molecule fluorescein derivative with a long history as a hepatic and ophthalmic diagnostic, and with an established safety history. When injected directly into tumors, it transits the plasmalemma of cancer cells, accumulating in lysosomes and triggering their release with complete autophagy within 30–60 min.

After observing consistent increases in anti-tumor T cells following PV-10 injections in a murine model, Sarnaik *et al.* [21] demonstrated increases in circulating cytotoxic CD8⁺ T cells in a small study among eight patients with dermal and/or subcutaneous metastatic melanoma

treated with PV-10. Six of eight patients had metastatic disease refractory to previous ipilimumab, anti-PD-1 and/or vemurafenib therapy. Two study lesions in each patient were sampled by biopsy pretreatment; one of the two lesions was injected with intralesional PV-10, then both residual sites were completely excised 1–2 weeks after PV-10 injection. To determine pathologic complete response (pCR), investigators compared tumors before and after treatment. Intralesional PV-10 was associated with an increase in circulating cytotoxic CD3⁺/CD8⁺ T cells (paired t-test; $p = 0.008$). Pre- and post-PV-10 treated peripheral blood mononuclear cells from one patient were restimulated with autologous tumor *in vitro*. Compared to pretreatment, PV-10 treatment produced an increase in tumor-specific interferon-gamma release by ELISA. Also, pCRs were achieved in four of the eight patients with disease refractory to prior treatment. The authors concluded that treatment with intralesional PV-10 can lead to systemic antimelanoma immunity and pCR in injected and uninjected lesions including treatment-refractory tumors.

Potential for combination with systemic agents

The rationale for combining intralesional therapy with systemic therapy lies in the separately demonstrated benefits of both modalities, their potential synergy and their nonoverlapping toxicities. Synergy could occur when the immune stimulation effected after intralesional agents such as T-VEC or PV-10 promote the release and presentation of tumor-derived antigens is combined with the systemic effects of checkpoint blockade with agents that target CTLA-4 and PD-1.

The second oncolytic virus demonstrating promising clinical findings is coxsackievirus A21 (CVA21: CAVATAK™). CVA21 is a naturally occurring common cold ICAM-1-targeted RNA virus. With a number of cancers including melanoma, surface ICAM-1 is upregulated. Tumor cells lysed by CVA21, in animal models and in *in vivo* xenografts, induced a secondary systemic host-generated antitumor immune response. Low levels of ICAM-1 expression in normal cells limit the capacity of CVA21 to infect them.

The mechanism is similar to that for T-VEC. CVA21 replicates rapidly in tumor cells and ruptures them, releasing progeny virus and tumor antigens. In nearby tumor cells, replicated viruses repeat the oncolytic process. Cytokine

release attracts immune cells, and dendritic cells present tumor antigens to mediate a tumor specific immune response.

In the Phase II CALM study [22] of intratumorally-delivered CVA21 in patients with stage IIIC and stage IV malignant melanoma, there was preliminary evidence of biomarker activity (IL-8, γ -IFN) indicating possible host antitumor immune activity in patients with objective tumor responses.

Electroporation with plasmid IL-12

IL-12 augments adaptive and innate immune responses, but systemic IL-12 can cause significant toxicity [23], which is avoided through electroporation. *In vivo* electroporation uses a brief, controlled electric charge to facilitate macromolecule cell entry of plasmid IL-12 and to achieve high levels of IL-12 protein expression. IL-12 expression stimulates a local proinflammatory process, leading to a targeted immune response and lymphocyte education [24]. A Phase I trial included seven stage IVa, five stage IVc, one stage IVb, nine stage IIIC and two stage IIIB lesions. While the numbers were small, there did not appear to be any correlation between disease stage and response.

The trial revealed dose proportional increases in IL-12 protein levels, tumor necrosis and lymphocytic infiltrates. It also found responses in distant lesions in 4/19 patients, 3 of which were CRs. Systemic toxicity was minimal.

Other intralesional therapies have reached Phase II testing, including IFN- α 24, adenovirus-expressing IL-2 [25], GM-CSF [26], BCG, several other strategies including IL-2 (GM-CSF, with topical imiquimod and retinoid) [27], KORTUC II and velimogene aliplasmid (AlloVectin-7). The failure of velimogene aliplasmid to meet primary end points in its Phase III AIMM trial [28] led to discontinuation of its development program. The primary end point of response rate at ≥ 24 weeks was lower in the velimogene aliplasmid group at 4.6% than it was for its comparator, intravenous DTIC or oral temozolomide at 12.3% ($p = 0.010$). The most promising agents going forward, at this time, are T-VEC, PV-10, CVA21 and electroporation with IL-12.

Current state of the science with selected IL agents in development

• T-VEC

Among intralesional therapies, T-VEC is farthest along in the development process. In October

2015 the FDA approved T-VEC for treatment of melanoma lesions in the skin and lymph nodes.

The Phase III OPTiM [29] trial tested T-VEC against GM-CSF in 436 patients with unresected stage IIIB-IV melanoma and regional metastases. Patients were randomized 2:1 to intralesional T-VEC (initially $\leq 4 \text{ ml} \times 10^6 \text{ pfu/ml}$ then after 3 weeks, $\leq 4 \text{ ml} \times 10^8 \text{ pfu/ml}$ every 2 weeks) or subcutaneous GM-CSF ($125 \text{ } \mu\text{g/m}^2\text{qd} \times 14 \text{ days}$ q28d).

The primary end point of durable response rate (complete or partial response lasting continuously for at least 6 months) was reported in 3/141 patients (2.1%) in the GM-CSF group and in 48/295 (16.3%, $p < 0.0001$) of the T-VEC group. The durable response rate was highest among patients with stage III and stage IVM1a disease devoid of spread to distant organs. In patients with stage IIIB/c melanoma, 33% of those treated with T-VEC demonstrated a durable response, as compared with no responses among patients receiving GM-CSF treatment. In patients with stage IVM1b melanoma and stage IVM1c, durable response rates were 3 and 8%, respectively, for T-VEC as compared with 4 and 3%, respectively, for GM-CSF.

The overall response rate was also higher for T-VEC at 26.4% as compared with 5.7% for GM-CSF ($p < 0.0001$). The complete response (CR) rate was 10.8% for T-VEC and 0.7% for GM-CSF, and time to treatment failure was longer with T-VEC at 8.2 months (95% CI: 6.5–9.9) months versus 2.9 (95% CI: 2.8–4.0) months.

An analysis of lesion-level responses [30] among 2116 lesions showed $\geq 50\%$ tumor area decreases in 64% of injected lesions, 34% of uninjected nonvisceral lesions and in 15% of uninjected visceral lesions. Decreases were 100% in 47% of injected lesions, 22% of uninjected nonvisceral lesions and 9% of uninjected visceral lesions. At the patient level, overall response (OR) and CR, respectively, for injected were 33 and 15%, for uninjected nonvisceral were 18 and 6%, and for uninjected visceral were 14 and 3%.

In the intention-to-treat population, while the improvement in the secondary end point of overall survival (OS) with T-VEC did not achieve statistical significance, it closely approached it. Median OS was 23.3 months (95% CI: 19.5, 29.6) for T-VEC and 18.9 months (95% CI: 16.0–23.7) for GM-CSF (HR: 0.79 (95% CI: 0.62–1.00; $p = 0.051$), a 4.4-month difference.

In OPTiM, T-VEC was well tolerated, with cellulitis (2.1%) as the only grade 3/4 adverse event reported at $\geq 2\%$. Fatigue, the most common adverse event overall, was reported at 50.3 versus 36.2% in the GM-CSF arm. Chills and pyrexia (48.6%/42.8%), the next most common, also were reported more frequently for T-VEC than for GM-CSF (8.7%/8.7%).

The logic of combining intralesional therapies with the impressive emerging systemic immunotherapies has been at the forefront of recent melanoma strategic thinking. A Phase Ib study [31] of single-dose T-VEC added to the CTLA-4-blocking antibody ipilimumab evaluated safety and efficacy in 19 patients with previously untreated, unresected stage IIIB-IV melanoma. Patients received intralesional T-VEC at weeks 1 and 4 and every other week following. Starting at week 6 they received 3 mg/kg ipilimumab every 3 weeks for a maximum of four infusions.

While no dose-limiting toxicities were reported in the evaluation period, two patients had grade 3/4 immune-related adverse events, including hypophysitis, adrenal insufficiency and diarrhea in one patient and elevated amylase and lipase in another.

The objective response rate was 56%; CRs were reported in a third of patients and the disease control rate was 72%. Total and activated CD8 T cells increased after T-VEC and further after ipilimumab, with greater increases observed in patients with disease control. Median time to response was 5.3 months.

In a further analysis of this study population [32], investigators reported $\geq 50\%$ regression in 74% of index lesions, and complete regression in 31%. Impressively, 52% of uninjected index lesions regressed by half or more and 35% regressed completely. Regression was observed in both uninjected nonvisceral and visceral lesions. Out of nine responders, eight had responses lasting ≥ 6 months (44% durable response rate).

While median PFS and OS were not reached, 12- and 18-month PFS were both 50% and 12- and 18-month OS were 72.2 and 67%, respectively.

While truly preliminary, the study data suggest higher CR and OR rates than with either agent alone. An ongoing Phase II study of the same strategy versus ipilimumab alone is enrolling 70 patients per arm and will have OS as its primary end point.

Additional evaluation of T-VEC in combination with a systemic immunotherapy is being

conducted in a Phase Ib study [33] with the immune checkpoint inhibitor pembrolizumab in patients with previously untreated, unresected stage IIIB–IV melanoma.

• PV-10

Phase I testing of PV-10 included 20 subjects with stage III–IV melanoma. They received a single PV-10 dose injected intralesionally. Treatment was well tolerated and produced durable objective responses at 12–24 weeks in 40% of subjects (20% CR + 20% partial response [PR]). Locoregional disease control, defined as objective response + stable disease (SD), was observed in 75% of subjects. Among those with uninjected ‘bystander’ lesions, 15% achieved an OR. Bystander responses correlated strongly with responses in patients’ injected lesions.

Our Phase II study [34] included 80 subjects (median age: 70.0 years; range: 33–97 years) with stage III–IV metastatic melanoma from seven centers in Australia and USA. Patients received up to four PV-10 courses treating up to 20 cutaneous or subcutaneous lesions. Objective responses were robust, with 25% achieving a CR, 26% a PR, 18% SD and locoregional disease control in 69% (82% of evaluable subjects). Among 35 patients with evaluable, untreated bystander lesions, a CR was achieved in 31%, PR in 9% and SD in 20%, giving a locoregional control rate of 60%. Again, responses in injected lesions correlated strongly with bystander responses.

A comparison of stage III subjects (n = 62) demonstrated better responses than in stage IV subjects (n = 18), with CRs in target and bystander lesions for stage III in 32%/33%,

respectively, as compared with 0%/11% for stage IV. Locoregional disease control was achieved in 79%/53% for stage III target and bystander lesions, respectively, and in 33%/42% in stage IV. Differences for stage III and stage IV disease in objective response ($p = 0.014$) and in locoregional control ($p < 0.001$) were highly significant.

Findings among the 28 patients who had all of their lesions treated with PV-10 were very favorable, with a 50% CR, 21% PR and 11% SD rate for a locoregional disease control rate of 82% [35]. In this group, mean PFS was 9.8 months.

PV-10 was well tolerated, with adverse events predominantly mild to moderate, locoregional and transient, with no grade 4 or 5 adverse events.

We found PV-10 to be suitable for repeat treatment to achieve disease control and to maximize long-term outcome. Also, it was quickly evident when patients were nonresponsive, allowing us to avoid delaying our transition to alternate therapy.

Enrollment for PV-10's Phase III trial, which is planned to include 225 patients with locally advanced cutaneous melanoma, began in April 2015. The international multicenter, open-label, randomized controlled trial compares single-agent intralesional PV-10 (every 4 weeks) 2:1 versus systemic chemotherapy (every 4 weeks) consisting of investigator's choice of either dacarbazine or temozolomide. Patients are *BRAF* V600 wild-type and have failed or are not otherwise candidates for ipilimumab or another immune checkpoint inhibitor. Cross-over is allowed upon documented progressive disease in the comparator arm. The primary efficacy end point is PFS.

Table 1. Systemic immunotherapy trials.

Agent	Phase	n	OR (%)	Grade-3–4 adverse events (%)	Additional findings	AJCC disease substage
Ipilimumab + dacarbazine	3	502	10.9	56.3	Median OS 11.2 months/PD 44.4%	M1a = 14.8% M1b = 25.6% M1c = 57.2%
Pembrolizumab	1b	173	24 [†]	12	2 mg/kg; PFS 22 weeks/12-month OS 58%	M1a = 11% M1b = 22% M1c = 65%
Pembrolizumab	3	834	33	~12	6-month PFS 45%/OS 87%	M1a–c ~ 90%
Nivolumab	3	370	32	9	6-month PFS nivolumab 32%; chemotherapy 11%	NA
Nivolumab + ipilimumab	3	945	57.5	55	Median PFS 11.5 months	M0–M1b = 42.4% M1c = 57.6%

[†]See additional findings column.

AJCC: American Joint Committee on Cancer; NA: Not available; OR: Objective response; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival.

Table 2. Intralesional therapy trials.

Agent	Phase	n	OR (%)	Grade-3–4 adverse events (%)	Additional findings	AJCC disease substage
T-VEC	3	436	26.4	2.1	OS 23.3 months	IIIB: 8%, IIIC: 22% IVa–c: 70%
T-VEC + ipilimumab	1b	19	56	10.5	18-month PFS 50%/18-month OS 67%	IVM1b/c: 58%
PV-10	1	20	40 [†] /15 (bystander)	0	Durable reponse	NA
PV-10	2	80	51	0	Mean PFS stage III ≥9.7 months/ stage IV ≥3.1 months; mean OS stage III ≥12.6 months/stage IV ≥7.3 months	IIIB: 47.5% IIIC: 30.0% IVM1a–c: 22.5%
PV-10	2	28 [†]	71	0	Patients with all lesions injected; mean PFS 9.8 months	NA
CVA21	2	57	28.1	0	Immune-related PFS at 6 months 36.5%/median OS 26 months	IIIC: 42.1A% IV: 57.9%
Plasmid IL-12	2	30	31.0	0.3	Median PFS 3.1 months	IIIB/C: 63.3% IVM1a–c: 37.7%

The intralesional therapies as compared with the systemic ones have response rates at least as high with lower rates of higher grade toxicities.

[†]See additional findings column.

NA: Not available; OR: Objectiveresponse; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival.

• CVA21

The Phase II CALM study enrolled 57 patients with stage IIIC and IV melanoma, all with at least one injectable lesion, receiving ten series of multi-intratumoral CVA21 injections. Similar to other intralesional therapies, higher grade toxicities were not an issue.

The primary end point of investigator-assessed immune-related PFS at 6 months (CR + PR + SD) was 38.6% (22/57). ORR was 28.1% (16/57, with eight Cr and eight PR and median OS was 26 months (95% CI: 16.7–not reported). The 1-year survival rate was 75.4% (43/57). Median time to response onset was 3.4 months.

Andtbacka *et al.* [36] noted that responses were observed in injected lesions, noninjected nonvisceral lesions and in distant noninjected visceral lesions.

CVA21 activity in combination with ipilimumab is currently being investigated and combinations of CVA21 with other checkpoint inhibitors are in planning stages.

Plasmid IL-12 electroporation

In Phase II trial in 30 patients with unresected stage IIIB/C or stage IV melanoma, Algazi *et al.* [37] reported CR in 4 patients (13.3%), PR in five (16.7%) and SD in five (16.7%) for an ORR of 31% and a disease control rate of 48%.

Regression of nontreated tumors supported that a systemic antitumor response was induced, and increases in IL-12, IFN inducible genes

and natural killer cells [38] were consistent with a pharmacodynamic IL-12 effect. No serious adverse events were reported, and except for grade 3 pain with electroporation in one patient, all adverse events possibly or definitely related to treatment were grade 1 and 2.

Overall picture

If we look at the clinical trial evidence for both systemic and intralesional therapies in a broad sweep, knowing fully well the limitations of juxtaposing trials that may be widely disparate, two features stand out in sharp outline (see [Tables 1 & 2](#)).

Future prospects: what's on the horizon?

In spring of 2015, members of the FDA's Oncologic Drugs Advisory Committee (ODAC) and Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) voted 22 to 1 to recommend T-VEC approval as a treatment for patients with advanced melanoma. The FDA approved talimogene laherparepvec (T-VEC/Imlygic) on 27 October 2015. This is a major milestone in the ongoing journey of intralesional agents. Of course, it remains to be seen whether this strategy will be embraced by clinicians who treat melanoma, particularly in the front-line setting.

Ongoing combination trials

In the coming months to years, data on combinations therapies with TVEC, PV-10 and others will become available. If successful, this may provide additional therapeutic options for any

patient with melanoma who has distant disease and at the same time has a lesion available for IL injections.

Neoadjuvant therapy

A logical further extension of application for the intralesional approach is to consider it prior to surgical resection in surgically resectable disease. While there may be inherent risks to consider in delaying surgery, in this strategy the patient's autologous tumor stimulates a systemic immune response that can persist after surgical removal of the tumor, a response that would not occur if we simply removed the tumor. In other words, we make the tumor an ally.

Indeed, a Phase II, multicenter, randomized, open-label trial with TVEC [39] in patients with resectable stage IIIB, IIIC or IVM1a melanoma is enrolling patients. It is evaluating the efficacy and safety of neoadjuvant T-VEC followed by surgery compared with surgery alone. The primary end point is recurrence-free survival from the time of randomization to the date of the first local or distant recurrence of melanoma or death due to any cause. Biomarker evaluations will assess correlations between baseline intratumoral CD8⁺ cell density in injected melanoma lesions and clinical outcomes. About 50 sites in Australia, Brazil, Europe, Russia and the USA will participate.

Conclusion & future perspective

There is no doubt that with the recent onslaught of successful Phase III trials in melanoma and

the resulting FDA approvals, we will have a wealth of options for our patients. The role of intralesional therapy however remains to be defined. As a monotherapy its use is likely to be restricted to those who have progressed on other therapies or are not able to receive them for reasons such as co-morbidity or availability. Ongoing combination trials will clarify the role of intralesional therapies in conjunction with agents such as checkpoint inhibitors. Early trials of systemic immunotherapies with intralesional therapies strongly suggest synergies with response rates higher than for the individual components. Ongoing and planned trials will tell us if survival and recurrence rates are also favorably affected.

There remains much work to be done. With choices come decisions, and we will need to tailor our therapeutic approach to individual patients. Intralesional therapy may provide yet another tool to offer patients with this still devastating disease.

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