

## SPECIAL REPORT

Special Focus: Viral-immunotherapies and vaccines in melanoma

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# Melanoma Management



## Advances in the development of intralesional therapies for melanoma

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### Practice points

- The management of in-transit metastases is a unique challenge in melanoma.
- The first intralesional therapy, heat-killed bacteria known as Coley's toxins, was the earliest form of immunotherapy.
- Recent development of intralesional therapies has provided additional options for clinicians to tackle this challenge.
- Talimogene laherparepvec, a herpes-based oncolytic viral injectable therapy, is the first US FDA approved intralesional therapy for advanced melanoma.
- Other intralesional therapies including PV-10 (Rose Bengal), IL-12 plasmid electroporation and Cocksackievirus A21 have shown promising clinical results.
- There has been some evidence of a bystander effect where there are responses in noninjected tumor lesions; however, there are few responses in visceral lesions.
- The combination of intralesional and systemic therapy is being explored to enhance the immune response in melanoma.

Advances in immune therapy have changed the landscape of advanced melanoma treatment. Intralesional therapy is an important type of immune therapy due to its efficacy and safety, especially in the setting of locoregional metastases. These therapies induce frequent responses in injected lesions as well as distant nontreated lesions through a 'bystander' effect of priming an antitumor immune response. The culmination of nearly a century of innovation has led to the approval of the first US FDA approved intralesional therapy for melanoma in talimogene laherparepvec. Numerous efforts to combine intralesional therapies with systemic immune checkpoint inhibitors are ongoing, whereby a synergistic effect may continue to improve outcomes for patients.

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As the deadliest form of skin cancer, melanoma will account for over 10,000 deaths annually in the USA alone [1]. Despite the historical poor prognosis for metastatic disease, there are now promising systemic immune therapy options such as immune checkpoint therapy. These innovative treatments have improved the overall survival (OS) in advanced melanoma from a 5-year OS of less than 10% in 2008 to over 30% for patients treated with anti-PD-1 [2]. In some cases, however, advanced melanoma blurs the lines between locoregional and disseminated disease, posing challenging clinical dilemmas. In these situations, locoregional metastases develop between the primary melanoma and the draining lymph-node basin, known as in-transit metastasis. These types of metastasis have been reported to occur in 4% of melanoma and up to 8% in patients with primary melanoma

### KEYWORDS

- intralesional therapy
- melanoma • talimogene laherparepvec

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≥1 mm [3]. In many cases, these in-transit lesions are technically resectable, but operations would cause substantial morbidity with minimal chance of cure. Historic options for these lesions included limb perfusion, systemic therapies or surgery, which were all associated with suboptimal safety and efficacy. However, the recent development of effective intralesional therapy appears to particularly benefit patients with extensive locoregional disease with minimal toxicity. This approach has led to the development of talimogene laherparepvec (T-VEC), the first US FDA approved intralesional therapy for melanoma. In this review, we will discuss the history of the development of intralesional therapies, current effective therapies and the future direction of these injectable therapies (see [Table 1](#) for summary of current agents).

#### Historic intralesional agents (Coley's toxins, *Bacillus Calmette–Guérin*, IL-2, granulocyte macrophage colony-stimulating factor)

The advent of cancer immunotherapy and intralesional therapy began with observations of spontaneous regressions after infections in the 19th century. WB Coley attempted to leverage this observation by using heat-killed cultures of *Streptococcus pyogenes* and *Serratia marcescens*, better known as Coley's toxins, for intratumoral injection in sarcoma patients in 1891 [4]. Although Coley's toxins had some success, the underlying mechanism was unknown at the time and met with skepticism by his contemporaries [5,6]. The use of Coley's toxins eventually became unfavorable as it was considered illegal to use as a 'new drug' by the FDA in 1963 and was added to the 'unproven methods' list by the American Cancer Society in 1965 [7].

*Bacillus Calmette–Guérin* (BCG) is a live strain of *Myobacterium bovis* that was first administered in humans in 1921 as a vaccine against tuberculosis. Using the principle that BCG could trigger a nonspecific immune response to tumors in murine models, BCG was used as a form of intralesional therapy in melanoma beginning in the 1970s [8,9]. Early success was seen in locally injected lesions and distant metastases in stage II–III melanoma patients with some trials reporting regression of 90% of injected lesions and 17% of noninjected lesions [10,11]. In a Phase III trial (ECOG1673), over 700 patients with stage I–III melanoma were randomized to receive adjuvant BCG injections in the regional lymph node drainage area alone or in combination with intravenous dacarbazine [12]. With 30 years of follow-up, the mature results of this study were conclusively negative with no improvement in OS or disease-free survival with the addition of BCG. Toxicity was generally mild, with the exception of frequent punctate abscesses and occasional cases of anaphylaxis. This study thus concluded that intralesional BCG does not play a role in adjuvant therapy for melanoma.

GM-CSF plays a critical role in the expansion of antigen-presenting cells, such as dendritic cells (DCs), in the tumor microenvironment which then promotes antitumor cytotoxic T-cell responses [13,14]. Several early studies have evaluated direct injection of GM-CSF into melanoma lesions with modest response and tolerable side effect profile [15,16]. Currently, GM-CSF is being used in distinct ways as adjunctive therapy incorporated into other treatment regimens (discussed below).

IL-2 has been a mainstay of systemic melanoma therapy for many years, becoming the first FDA-approved immunotherapy for

**Table 1. Summary of intralesional therapy in melanoma.**

Agent	Type or mechanism	Phase	n	ORR in injected lesions (%)	ORR in bystander lesions (%)	CR rate (%)	PFS (months)	OS (months)	Grade 3–4 AE (%)	Trial information
Plasmid IL-12	Plasmid DNA	2	29	33	62	11	NR	NR	0	NCT01502293
PV-10 (Rose Bengal)	Xanthene dye	2	80	51	33	26	8.2	NR	15	NCT00521053
Coxsackievirus 21	Oncolytic virus	2	57	28	NR	14	4.2	27	0	NCT01227551
Talimogene laherparepvec	Oncolytic virus	3	436	26	15	11	8.2 (TTF)	23	11	NCT00769704

AE: Adverse event; CR: Complete response; NR: Not reported; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; TTF: Time-to-treatment failure.

metastatic melanoma in 1998. Recombinant IL-2 was shown to enhance immune responses against cancer cells in melanoma mouse models [17]. Systemic IL-2 therapy, while showing durable responses (approximately 6–8%) lasting over 10 years in some patients, is restricted to patients with excellent performance status with preserved organ function [18]. As an intralesional therapy, IL-2 has shown to be well tolerated with only low-grade flu-like symptoms and local injection-side adverse events (AEs) [19]. Response rates for injected lesions have generally exceeded 80%, but did not induce a significant bystander effect in noninjected lesions [19,20]. Randomized trials have not evaluated injectable IL-2, and it is not commonly used currently.

### Velimogene aliplasmid

Velimogene aliplasmid or Allovectin is an investigational plasmid DNA-based immunotherapy. It was designed with a bicistronic plasmid (VCL-1005) encoding two transgene proteins, an MHC class I heavy chain (HLA-B7), and the light chain common to class I molecules  $\beta$ 2-microglobulin and formulated in a cationic lipid suspension [21]. Based on this design, intralesional administration of velimogene aliplasmid was thought to stimulate both innate and adaptive immune responses [21].

A Phase II dose-escalation study was conducted to evaluate the safety and efficacy of this intralesional therapy in patients with stage IIIB/C and IV M1a/b melanoma [22]. Of the 127 patients evaluated for efficacy, 11.8% had an objective response with a median duration of response of 13.8 months in those with stage IV melanoma. Responses in noninjected target lesions were seen in 21% of the patients.

The subsequent Phase III Allovectin Immunotherapy for Metastatic Melanoma trial included 390 patients with stage IIIB–IV M1a/b melanoma randomized 2:1 to velimogene aliplasmid or chemotherapy in the form of dacarbazine or temozolomide [23]. The trial failed to meet its primary end point of response rate at 24 weeks with velimogene aliplasmid (4.6%) as compared with the chemotherapy arm (12.3%,  $p = 0.01$ ). Duration of response among velimogene aliplasmid responders was marginally longer but this did not reach statistical significance ( $p = 0.066$ ), and median OS appeared shorter (18.8 vs 24.1 m,  $p = 0.49$ ). Given these results, further development of velimogene aliplasmid was halted.

### Plasmid IL-12 electroporation

IL-12 plays an important role in antitumor response by expanding the T-helper-1 response and subsequent production of IFN- $\gamma$ , leading to the proliferation of cytotoxic natural killer and T cells in preclinical models [24,25]. Furthermore, it was shown that IL-12 could improve antitumor immune responses in human melanoma cells *in vitro* by enhancing the levels of HLA class I/II and ICAM-1 expression [26]. However, early trials were largely unsuccessful due to the severe toxicity and narrow therapeutic window involved with systemic therapy [27,28]. It was not until the advancements in intratumoral electroporation with gene transfer of plasmid IL-12 that this cytokine could be deployed in melanoma [29]. Intratumoral electroporation involves the delivery of an electrical field by an electrode directly to a lesion, increasing the permeability of the cell membrane transiently to the cytotoxic agent [30].

A Phase II clinical trial in patients with stage IIIB–IV M1a melanoma revealed an objective response rate (ORR) of 33% in 27 evaluable patients with a 11% complete response (CR) rate [31]. There was a notable bystander effect with regression of noninjected lesions in 62% of the patients. The treatment was well tolerated with no grade 3–4 drug-related AEs and transient pain (57%) and inflammation (17%) at the treatment site being the most common side effect. An expansion protocol has been planned to evaluate treatment frequency, and a combination study with pembrolizumab is also ongoing.

### PV-10 (Rose Bengal disodium 10%)

PV-10 is a sterile, nonpyrogenic solution derived from Rose Bengal disodium, a red-dark xanthene-based dye developed for use in liver and ophthalmological diagnostic studies [32,33]. Mouse models have shown that PV-10 has direct cytotoxic effects on cancer cells, by lysozyme-induced apoptosis and necrosis [34,35]. Additionally, it appears that the necrosis does not cause the denaturation of tumor antigens [36]. In 11 patients with metastatic melanoma, a Phase I trial demonstrated that intralesional injection of PV-10 can induce regression in both injected (OR: 48%) and noninjected lesions (OR: 27%) [37]. The single-arm Phase II trial, involving 80 patients with stage III/IV disease, demonstrated an ORR of 51% (CR in 26%) with 8% of patients having no evidence of disease

after 1 year [38]. An OR in bystander lesions was seen in 33% of patients. In terms of safety, the treatment was well tolerated with predominately mild-to-moderate injection site-related AEs. Approximately 15% of patients had at least one grade 3 AEs, which were largely injection site related such as pain. A Phase III clinical trial comparing PV-10 with either systemic chemotherapy (dacarbazine, temozolomide) or T-VEC in stage IIIB/C melanoma patients is currently ongoing (NCT02288897). Additionally, a Phase Ib/II trial evaluating the combination therapy of intralesional PV-10 with pembrolizumab in patients with metastatic melanoma with at least one injectable lesion (NCT02557321).

Based on the clinical data, there seems to be an immunologic basis for these responses given the responses in nontreated bystander lesions. In patients with regression of injected lesions, there appeared to be increased circulating CD3<sup>+</sup> T cells ( $p = 0.03$ ) in peripheral blood as compared with pretreatment samples [39]. T cells from these samples also produced increased levels of IFN- $\gamma$  in response to autologous tumor after treatment, which implies antigen-specific T-cell activation and proliferation. In addition, murine studies revealed that PV-10 treatment led to elevated serum levels of HMGB1 protein, a damage-associated molecular pattern molecule, crucial in the activation of DCs [40]. The authors demonstrated increased infiltration of DCs in the lymph nodes and proliferation of tumor-specific CD8<sup>+</sup> T cells in mice after intralesional PV-10 treatment, which suggests that PV-10 plays a critical role in activation of DCs and induction of a systemic antitumor immune response. Elevated serum HMGB1 levels in patients 7–14 days after intralesional PV-10 also suggest an analogous mechanism in humans.

#### Coxsackievirus A21 (CAVATAK)

The coxsackievirus A21 (CVA21) is a naturally occurring ‘common cold’ enterovirus, which has been shown to have potent oncolytic activity in both *in vitro* and *in vivo* models of cancer [41]. This activity appears related to specific viral capsid interactions with surface expressed virus receptors such as ICAM-1 and decay-accelerating factor [41]. These receptors are upregulated on the surface of melanoma cells compared with normal tissue, causing CVA21 to preferentially infect these cancer cells and cause oncolysis. CAVATAK is a proprietary formulation of genetically unmodified CVA21 that can

be given intralesionally in patients.

A Phase I trial (NCT00438009) demonstrated that even two intralesional injections of CVA21 were sufficient to cause regression or stabilization of injected melanoma lesions. A subsequent Phase II trial evaluated 57 patients with unresectable stage IIIC–IV M1c melanoma [42]. Patients were treated with up to ten series of multi-intralesional CVA21 injections. The primary end point of the trial was met with 38.6% of patients experiencing immune-related progression-free survival (irPFS) at 6 months with a median irPFS of 4.2 months. The overall response rate by irRECIST was 28.1% with a  $\geq 6$ -month durable response rate (DRR) of 19%. Median OS was 26 months with a 1-year survival of 75.4%. In terms of safety, the most common AEs were grade 1 fatigue, fevers, chills and local injection site reactions. No grade 3 or 4 drug-related AEs were observed. A subsequent study was performed to evaluate the nature of the systemic responses by collection of sequential tumor biopsies of both injected and noninjected lesions in 13 patients [43]. The samples were assessed for levels of viral replication and evidence of viral-induced immune activation within the tumor microenvironment as well as serum levels of viral loads, anti-CVA21 neutralizing antibody and levels of immune-inflammatory cytokines. In five of the six cases, there were increases in CD8<sup>+</sup> infiltrates within the tumor microenvironment with elevated expression of PD-L1<sup>+</sup> cells. Four patients were also noted to have increased immune cell infiltrates after failing treatment with single or double immune checkpoint blockade. The authors concluded that the findings provided a strong rationale for investigation of intralesional CVA21 administration in sequential or concurrent treatment with checkpoint inhibitors. Currently, there are several Phase I clinical trials exploring the use of intralesional CVA21 in combination with ipilimumab (NCT02307149) or pembrolizumab (NCT02565992).

#### Talimogene laherparepvec

T-VEC is another oncolytic virus based on a genetically modified herpes simplex virus type 1 (HSV-1). The modifications include the deletion of viral genes *ICP34.5*, which enhances the oncolytic properties by providing tumor-selective replication and reduces neurotropism, and *ICP47*, which increases the level of antigen presentation in HSV-infected cells [44,45]. Instead, a coding sequence

for GM-CSF was inserted, promoting antigen-presenting cell recruitment and enhancing the tumor-specific T-cell response (Figure 1) [13].

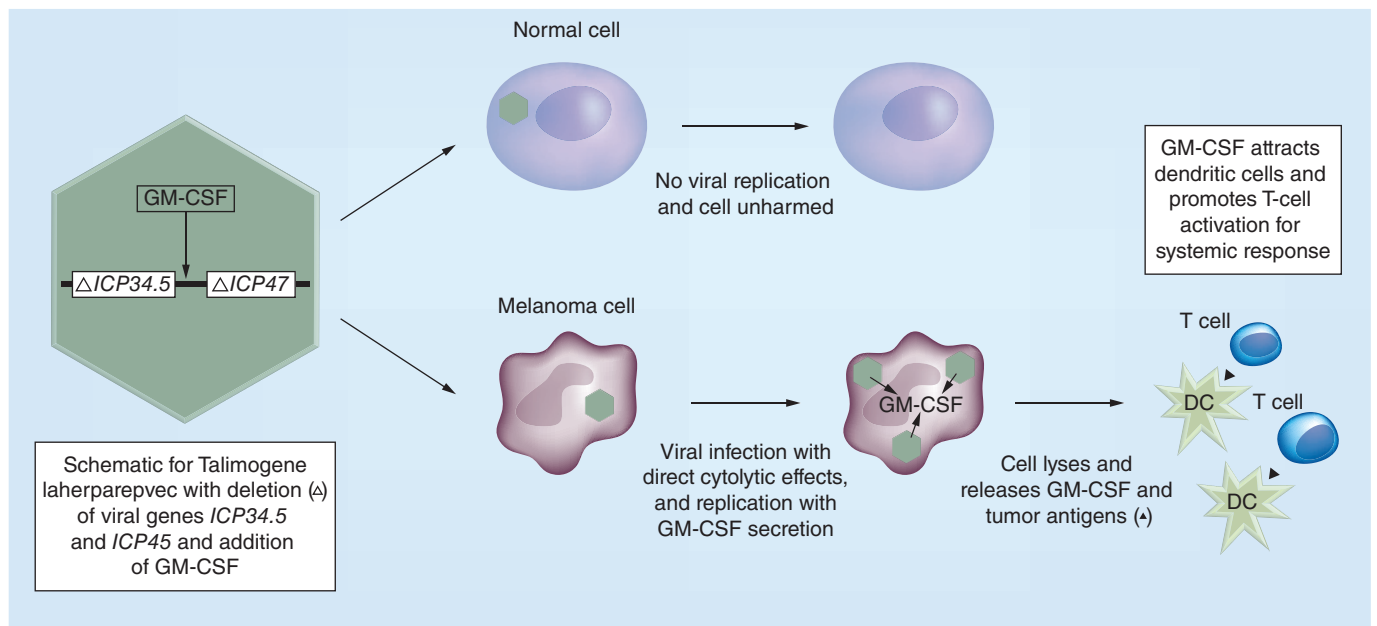
The Phase I trial proved that T-VEC was generally well tolerated with the only AEs being local inflammation, erythema and fever [46]. These effects were more noticeable in patients that were HSV seronegative. Post-treatment biopsies also revealed areas of tumor necrosis or apoptosis that stained strongly for HSV and expression of GM-CSF. A multidose regimen every 2–3 weeks was instituted in the subsequent trials to account for the side effects in the seronegative patient, which allowed for eventual seroconversion and less toxicity.

The single-arm Phase II trial evaluated the efficacy of T-VEC every 3 weeks in 50 patients with unresectable or metastatic melanoma [47]. The ORR was 26% with 92% with durable responses maintained for 7–31 months. Regressions were seen in both injected and non-injected lesions including visceral lesions. At 1- and 2-year OS was 58 and 52%, respectively.

The subsequent Phase III trial, OPTiM, randomly assigned 436 patients in a 2:1 ratio to either intralesional T-VEC or subcutaneous GM-CSF in patients with stage IIIB–IV melanoma [48]. The DRR, the primary end point defined as the proportion of patients with a response lasting 6 months or more, was significantly higher in the T-VEC arm as compared

with the patients who received GM-CSF (16.3 vs 2.1%,  $p < 0.001$ ). Key secondary end points such as ORR (26.4 vs 5.7%) were significantly higher in the T-VEC arm with a CR rate of 10.8%, but median OS had a nearly statistically significant trend toward benefit with T-VEC (23.3 vs 18.9 months,  $p = 0.051$ ). Among patients that responded in the T-VEC arm, the median time to response was 4.1 months, but more than half had pseudoprogression ( $\geq 25\%$  increase in the size of lesions or appearance of new lesions) before achieving a response, a similar phenomenon seen in other immune therapies [49,50]. Further exploratory analyses revealed that the differences in DRR between T-VEC and GM-CSF were more pronounced in stage IIIB/C (33 vs 0%) and IV M1a (16 vs 2%) as compared with stage IV M1b (3 vs 4%) and IV M1c (7 vs 3%). These key differences favoring in patients with more locoregional disease (stage IIIB/C and IV M1a) in the T-VEC group were also noted in OS (HR: 0.57; 95% CI: 0.4–0.8). Patients who were previously untreated also had a survival benefit (HR: 0.50; 95% CI: 0.35–0.73).

Based on these studies, monotherapy with T-VEC has shown to be an effective intralesional agent in advanced melanoma, especially in stage III or stage IV M1a disease. It is the first FDA-approved oncolytic viral therapy for the treatment of melanoma.



**Figure 1. Proposed mechanism for Talimogene laherparepvec.**  
DC: Dendritic cell.



### Future perspective

A major issue surrounding the development of intralesional therapies is the difficulty in determining response rates and PFS. As these agents modulate the immune response, immune-related pseudoprogression may frequently be observed. Furthermore, injected and noninjected lesions may have discordant growth in many cases. Another major concern surrounding intralesional therapy surrounds distant, including visceral metastases. While noninjected lesions may respond, patients with stage IV M1c disease (nonlung visceral metastases) had extremely low response rates to T-VEC and other intralesional injections. Thus, combination therapies or novel methods of delivery are major priorities.

Based on the underlying mechanism, efficacy and tolerable side effect profile, intralesional therapy combined with a systemic immune therapy seems to be an intriguing strategy supported by preclinical studies [51,52]. Several trials combining intralesional therapy with immune checkpoint inhibitors have been previously mentioned. Of note, a Phase Ib/II trial of T-VEC combined with ipilimumab revealed an ORR in 10 out of 18 patients with stage IIIB–IV M1c melanoma, a 33% CR rate and a median PFS of 10.8 months without any dose-limiting toxicities (DLTs) [53]. Results from a randomized study of ipilimumab + T-VEC versus ipilimumab alone are pending (NCT01740297). Another randomized study, the Phase I/III MASTERKEY-265 (NCT02263508), compares the combination of T-VEC with pembrolizumab with pembrolizumab alone in stage IIIB–IV M1c melanoma patients. Results from the Phase Ib trial revealed no DLTs with confirmed ORR of 10 out of 21 (56%) evaluable patients and a 14% CR rate [54]. The Phase III portion is now enrolling patients.

In addition to combination T-VEC therapy, trials with combinations of other intralesional agents have been initiated. Interim data presented at AACR 2016 of the Phase Ib trial of CVA21 and ipilimumab (NCT02307149) revealed no DLTs and only one grade 3 AE with the best ORR of four out of six evaluable patients with stage IIIC/IV melanoma [55]. A Phase Ib, multicenter trial evaluating the safety and tolerability of combination CVA21 and pembrolizumab is ongoing (NCT02565992). Similarly, a Phase Ib/II trial of combination PV-10 and pembrolizumab dosed the first patient in January 2016 (NCT02557321). Finally, a single-center Phase II clinical trial evaluating the efficacy of

intratumoral plasmid IL-12 and pembrolizumab is also underway (NCT02493361).

Besides intralesional agents for melanoma in the metastatic or unresectable setting, investigators have been exploring the potential for these agents in the neoadjuvant setting. Given the high risk of recurrence and death with locoregional melanoma, there is strong rationale to see if the use of neoadjuvant therapy will outweigh the risks in delaying surgery. A Phase II trial comparing neoadjuvant T-VEC of resectable IIIB–IV M1a melanoma with surgery alone will help to determine the efficacy of this treatment [56].

Last, the role of intralesional therapy has expanded outside the realm of advanced melanoma. As seen from the benefits of systemic immunotherapy in other solid tumor types, multiple trials have been initiated. These include liver-directed therapy with T-VEC in hepatocellular carcinoma (NCT02509507), PV-10 in hepatocellular carcinoma (NCT00986661) and neuroendocrine tumors metastatic to the liver (NCT02693067) as well as CVA21 and pembrolizumab in non-small-cell lung cancer and bladder cancer (NCT02043665).

### Conclusion

The advancement of injectable therapy for melanoma has evolved over the past several decades from the early days of Coley's toxins in the 1890s. As the basis for immunotherapy has been elucidated over the past several years, the role of intralesional therapy may expand in both monotherapy and combinatorial strategies. Given its particular efficacy in advanced stage III and stage IV M1a melanoma, intralesional therapy currently plays an important role in local management where surgery or regional chemotherapy may not be amenable. However, as seen by the bystander effect on noninjected and systemic disease, intralesional therapy may be useful in augmenting current systemic immunotherapy in advanced melanoma.

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 • of interest; •• of considerable interest

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