EXPERT OPINION

Intralesional therapy for metastatic melanoma

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Introduction: Intralesional therapy for metastatic melanoma has some advantages over systemic therapy. Local drug administration allows for delivery of an increased concentration of the agent and reduced systemic exposure, thereby increasing local efficacy and limiting toxicity. Moreover, since *in vivo* tumor nodules contain the tumor antigens, this tumor tissue may serve as an autologous vaccine to induce systemic immunity. This so-called 'bystander effect', where uninjected distant lesions exhibit a response, has been reported in select intralesional therapy trials.

Areas covered: This review will give an overview of the working mechanisms, clinical evidence and side effects for available intralesional and topical therapies and summarize the most recent developments in this field.

Expert opinion: The ideal treatment approach for locoregionally advanced melanoma should be multidisciplinary and tailored to the patient, taking into consideration patient-related, tumor-related factors (such as location, tumor burden, mutation status) and previous treatments received. It will likely not be a single therapy, but rather a combination of injectable treatments, regional perfusions and systemic therapies.

Keywords: in-transit melanoma, intralesional, locoregional recurrence, melanoma

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1. Introduction

Melanoma is the most deadly form of skin cancer, with an estimated 76,100 cases and 9710 deaths in the USA in 2014 [1]. Locoregional metastases are not infrequent and when diagnosed pose present a unique clinical challenge. Locoregional metastatic melanoma is defined as metastases that arise between the primary melanoma and the draining lymph node basin and develops in 2 to 10% of all melanoma patients [2]. Traditionally, locoregional recurrent disease is subdivided into two entities: satellite metastasis, located < 2 cm from the primary, and in-transit metastasis, located > 2 cm from the primary melanoma, both considered locoregional recurrent disease. The landscape for the treatment of stage III and IV metastatic melanoma has changed dramatically over the past few years, with FDA approval of many new drugs (ipilimumab, trametinib, dabrafenib, vemurafenib and pegylated interferon) [3]. However, topical therapy and intralesional injections used in the treatment of locoregional disease have been just as innovative and are promising treatment modalities in selected patients, both due to their efficacy and tolerability.

For patients with limited locoregional disease where all disease can be fully resected, surgical resection is still the standard of care. For patients with more extensive disease confined to the limb, treatment with isolated limb infusion (ILI) or hyperthermic isolated limb perfusion (HILP) are options. Both ILI and HILP are associated with significant overall response rates (ORR), ranging from 50 to 90% [4,5]. However, both procedures can be associated with regional toxicity, involve a surgical procedure (HILP is an open procedure via an incision in the groin/axilla with open surgical exposure of the vessels at the root of the extremity, while ILI is percutaneous access to the vessels) and are obviously not suitable for disease outside of the extremities.

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Article highlights.

- An in depth review of clinical trial and outcomes data associated with intralesional therapies for metastatic melanoma.
- A review of mechanisms of action, side effects of intralesional therapies used for metastatic melanoma.

This box summarizes key points contained in the article.

Radiotherapy is mainly of use to treat individual lesions or localized clusters and therefore has limited benefit when there is extensive locoregional disease. Wide field irradiation of a limb is usually contraindicated due to considerable morbidity [6].

Intralesional therapy is far from a new concept. Coley reported regression of locally advanced tumors after injection of mixed bacterial toxins in 1893, even before Handley advocated wide excision as the treatment of choice for melanoma in 1907 [7,8]. This type of local therapy has several advantages over systemic therapy, as local drug administration allows for delivery of an increased concentration of the agent and reduced systemic exposure, thereby increasing efficacy and lowering toxicity [9,10]. Moreover, since *in vivo* tumor nodules contain the tumor antigens, this tumor tissue may serve as an autologous vaccine to induce systemic immunity. This 'bystander effect,' where uninjected distant lesions exhibit a response, has been reported in select intralesional therapies [11-14]. This is a significant observation, since it potentially induces systemic tumor reduction by using locally delivered therapy.

Although technique may vary, generally lesions are injected either in the clinic (for palpable/visible lesions) or ultrasound-guided using a 25 – 30 gauge needle via a fanning technique. The needle is moved in different directions within the same lesion and preferably via the same needle stick so as to not have many needle tracks in the tumor where intralesional injectate can leak out. Tumor response can be assessed by caliper measurements, ultrasound or cross-sectional imaging such as MRI or CT scans [15]. General guidelines are that the smaller the bulk of tumor, the more likely it is to regress under treatment and that cutaneous lesions are more receptive than subcutaneous lesions [16-18]. Most studies use an intralesional volume of 1 ml or less to minimize the risk of local side effects [16].

Many agents have been used for intralesional therapy throughout the years, but only few of those have progressed through stage I, II and III clinical trials. This review will give an overview of the working mechanisms, clinical evidence and side effects for available intralesional and topical therapies and summarize the most recent developments in this field.

2. Intralesional therapies

2.1 Allovectin-7

Allovectin-7 is an immunotherapeutic agent, containing plasmid DNA that encodes for the HLA B7 antigen [19]. It causes immune responses against the alloantigen and attracts T cells and macrophages that recognize and destroy both injected and noninjected lesions. As Allovectin-7 is considered gene therapy, Phase I studies initially concentrated on HLA B7-negative patients. However, later studies demonstrated no correlation between HLA-status and response rate.

Four Phase I trials assessing Allovectin-7 were conducted in small patient groups ranging from 5 to 17 patients with response rates up to 50% (**Table 1**) [20-23]. Allovectin-7 use thereafter progressed to four Phase II trials. VCL-1005-201- was the first Phase II trial to explore the agent, injecting 124 patients with 4 injections of 10 μg each, of whom 38 had melanoma [19]. Of the 25 patients with melanoma that were evaluable after therapy, 7 responded. Partial response (PR) and complete response (CR) rates were not specified. Bedikian *et al.* enrolled 133 patients in a dose-escalation/efficacy study to receive 0.5 – 2 mg Allovectin-7 weekly during 6 weeks [16]. A total of 2 mg was taken as the highest dose since this was the maximum dose that could be formulated in a 1 ml volume. In six patients treated with < 2-mg dose of Allovectin-7, no responses were seen. Efficacy data were available for the 127 patients treated at the highest dose, of whom 3% reached CR and 9% PR. Stopeck *et al.* recruited 52 patients for up to 6 injections with 10 μg per injection at week 1, 2, 3, 4, 8 and 9, of whom 51 were evaluable [24]. They included visceral lesions. Nine patients had a response (2% CR, 16% PR). Maximum tumor response was achieved at a median of 11 weeks. Gonzalez *et al.* evaluated 77 patients after 10 μg once a week, for 6 weeks, up to 3 cycles. CR and PR were 3 and 7%, respectively [25]. Median survival was 14.0 months.

There are two Phase III trials evaluating Allovectin, neither of which reached their end points. Richards *et al.* randomized 202 patients to either systemic dacarbazine alone every 28 days (n = 104) or to intralesional Allovectin-7, added on days 3 and 10 to the systemic dacarbazine cycle (n = 98) [26]. Response rates were 11.6 and 13.2%, respectively, and the addition of Allovectin-7 showed no significant difference in survival (9.24 vs 10.75 months) or time to progression (1.6 vs 1.9 months). In the second Phase III trial, 390 patients got randomized between Allovectin or physician's choice of chemotherapy (dacarbazine or temozolomide) in a 2:1 fashion (Clinical trial no. NCT00395070). The trial was ended when the end points of 1) a difference in objective response rate at > 24 weeks and 2) overall survival were not met. Side effects included paresthesias, asthenia, myalgias, fatigue, injection site pain, rigors and flu-like symptoms.

2.2 Bacille Calmette-Guerin

Bacille Calmette-Guerin (BCG) is a live attenuated strain of *Mycobacterium bovis*. The theory behind this therapy is to stimulate an immune response in the lesions treated by injection of the agent in patients who have mounted an immune reaction, as BCG has been demonstrated as a nonspecific immunostimulant in animal models. Twenty-seven patients

Table 1. Key publications in the development of intralesional and topical therapies.

Author	Year	No. melanoma pts for analysis	Treatment	Dosing regimen	Dosing interval	Duration treatment	CR (%)	PR (%)	SD (%)	PD (%)	Bystander effect
Stopeck *et al.* [24]	2001	51	Allovectin-7	10 μg	Week 1, 2, 3, 4, 8 and 9	Up to 6 cycles	2	16	24	59	No
Gonzalez *et al.* [25]	2006	77	Allovectin-7	10 μg	1×/week for 6 weeks	Up to 3 cycles	3	7	23	68	No
Bedikian *et al.* [16]	2010	127	Allovectin-7	0.5 – 2 mg	1×/week	6 weeks	3	9	25	63	Yes
Kidner *et al.* [68]	2012	19	BCG and Imiquimod	3×10^6 CFU, 5%	Once every 2 weeks, 5 – 7 days/week	2 injections, titrated to local inflammation	56	11	33	0	No
Karakousis *et al.* [98]	1976	8	BCG	0.1 ml of 4×10^{11} to 9×10^{11} viable organisms per milliliter)	Once	once	75	0	0	25	No
Byrne *et al.* [34]	2005	19	Bleo + EP vs. Bleo vs. EP	1 unit per ml tumor volume	4, 8 or 12 Weeks	4, 8 or 12 weeks	72	5	18	5	No
Heller *et al.* [33]	1998	12	Bleo + EP vs. Bleo vs. EP	0.025 units, 1250 V/cm	Once	once	89.3	9.5	1.2	0	No
Mir *et al.* [31]	1998	20	Bleo + EP	18 or 27 units/m^2, 1300 V/cm	Once	once	52.8	39.4	7.7	0	No
Damian *et al.* [73]	2009	50	DPCP	Cream, 0.00001 – 10%	Once weekly	NR	46	38	18* (SD/PD)		Yes
Ridolfi *et al.* [39]	2001	16	GMCSF, IL-2	150 ng, 3,000,000 IU	Every 21 days	6 cycles	0	13	69	19	No
Boyd *et al.* [50]	2011	39	IL-2	10.4 MIU	Biweekly	4 cycles	51	31	18* (SD/PD)		No
Weide *et al.* [18]	2010	48	IL-2	0.3 – 6.0 MIU	3×/week	1 – 32 weeks	69	NR	NR	NR	No
Florin *et al.* [64]	2012	5	Imiquimod and 5-FU	Imiquimod: NR 5-FU: 5%	5×/week	NR	20	80	0	0	No
Agarwala *et al.* (abstract) [57]	2013	80	PV-10	10% Rose Bengal Dye	Week 1, 8, 12, 16	Up to 4 cycles	24	25	22	29	Yes
Agarwala *et al.* (abstract) [56]	2012	20	PV-10	10% Rose Bengal Dye	Once	1 cycle	20	20	35	25	Yes
Senzer *et al.* [40]	2009	50	TVEC	10^6 PFU 1st dose then 10^8 PFU thereafter	First interval: 3 weeks Then every 2 weeks	Up to 24	16	10	24	50	Yes
Andtbacka *et al.* (abstract) [59]	2013	295	TVEC	10^6 PFU 1st dose then 10^8 PFU thereafter	First interval: 3 weeks Then every 2 weeks	NR	11	15	74* (SD/PD)		No
		141	GM-CSF	125 μg/m^2	Daily × 14 days Every 4 weeks	NR	1	5	94* (SD/PD)		No

*Responses were not split out.

BCG: Bacille Calmette-Guerin; Bleo: Bleomycin; CR: Complete response; DPCP: Diphencyprone; EP: Electrophoresis; MIU: Million individual units; NR: Not reported; PD: Disease progression; PFU: Plaque forming units; PR: Partial response; SD: Stable disease; TVEC: Talimogene laherparepvec.

with lower extremity, local recurrence after primary tumor wide excision and complete lymphadenectomy were treated with intralesion injection of BCG [27]. Twenty (74%) had complete or transient disease control, with 14 (52%) alive at 1.5 – 55 months follow-up. Of those who had no response to BCG, nine underwent HILP with melphalan with a 78% response rate and 56% were alive at 2 – 65 months.

Seigler *et al.* treated 160 patients with metastatic melanoma with BCG in a four-stage approach [28]. First, patients were evaluated for immune sensitivity to BCG. Second, booster therapy with BCG was administered to produce a delayed hypersensitivity reaction. Third, lymphocytes were harvested from the patient, layered on tumor cell samples and transferred back to the patient to achieve adoptive immunity. Fourth, the patient was actively vaccinated with an inoculum of tumor cells with BCG as an adjuvant to augment antitumor immune responsiveness. Seventy patients were evaluated for antitumor-specific responsiveness, with 31 patients demonstrating an increased response and they were more reactive as they progressed through the stages of treatment. When 62 patients were evaluated for cell-mediated immunity, 43 of them demonstrated a prolonged response. The mean percentage of tumor lysis observed was 60%. The 19 patients who failed to develop any immunity against melanoma all developed diffuse melanoma and died from disease. Furthermore, when BCG triggered a positive tuberculosis skin test, those patients were found to have BCG-induced tumor lysis [28].

Despite promising early results, BCG fell largely out of favor due to its side effect profile. Because it utilizes a live attenuated strain of *M. bovis*, it is no surprise that complications have included fevers, chills, diaphoresis, arthralgias, malaise, angioedema in tuberculin-positive patients, axillary lymphadenopathy, pneumonitis, BCG granulomas, granulomatous hepatitis, and a disseminated intravascular coagulation (DIC) rate as high as 12% [29]. Accordingly, patients treated with BCG require close monitoring. It is recommended that reactions after BCG should be treated with fluids, antituberculosis therapy, antihistamines and steroids [30]. Furthermore, given the potential severe reactions, including DIC, prophylactic antihistamines and isoniazid should be considered [30].

2.3 Electrochemotherapy

Electrochemotherapy (ECT) utilizes brief, high-intensity pulsed electrical current to enhance the uptake of chemotherapeutic agents, vaccines and/or genes into cells [31,32]. Bleomycin is most commonly used as the chemotherapy agent at a dose of 0.025 units with 1250 V/cm [33]. Following promising reports in numerous small series of the response to ECT in metastatic melanoma, there have been multiple Phase II trials. A recent meta-analysis reported a detailed summary of the use and results of ECT in metastatic melanoma and other cutaneous metastatic lesions [32].

A Phase II trial in 19 patients comparing bleomycin injection alone versus bleomycin plus ECT demonstrated a 72% CR, 5% PR rate, 18% stable disease (SD) rate and 5% progression of disease (PD) rate over 12 weeks compared to a 32% response rate in bleomycin injection alone [34]. Another small Phase II trial in 15 patients with 28 skin lesions utilizing ECT and bleomycin demonstrated interesting results not only in melanoma, but also in basal cell carcinoma, squamous cell carcinoma and breast cancer [35]. The authors reported an ORR at 8.6 months of 98% for all these lesions treated, although it remains unclear how these responses are measured. In metastatic melanoma, 23% of the lesions demonstrated CR, 61.5% PR, and 15.3% SD/PD. A recent study that included 291 tumors (basal cell carcinoma, melanoma and squamous cell carcinoma) in 50 patients treated with bleomycin plus ECT demonstrated a 56.4% CR rate [31]. Of those cases, there were 142 metastatic melanoma lesions treated in 200 patients producing a 92.2% ORR with 52.8% CR and 39.4% PR at 30 days. With multiple small studies that include multiple tumor histologies, it is difficult to draw conclusions based on the results of these studies alone.

In 2013, a large meta-analysis was reported that analyzed the treatment of 1,894 tumors in 44 studies with ECT [32]. They reported the results of ECT alone, bleomycin alone, cisplatin alone, and ECT with bleomycin or cisplatin. In all tumor subtypes, the reported CR and ORR were 59.4 and 84.1%, respectively, as measured by WHO/RECIST/scans or biopsy measurements, compared to anywhere from 0 to 19% response rates in those treated with either modality alone. In 150 patients with 922 metastatic melanoma lesions, the CR and ORR were 56.8 and 80.6%, respectively. While these reports are promising, adequately powered prospective studies with long-term follow-up are needed to determine which patients may benefit from this modality.

2.4 GMCSF

GMCSF functions by two mechanisms. First, injection of the lesions causes direct destruction of the cancer cells [36]. Second, GMCSF attracts dendritic cells to the dying cancer cells and induces tumor-specific immunity [36]. The implication of this dual mechanism is that it both has the potential to treat the injected lesion while triggering a systemic immune response to the melanoma. Immunologic studies that compared antimelanoma antigen responsiveness in tumor T cells between those treated and not treated with GMCSF demonstrated increased antigen recognition in the treated group [36]. Furthermore, injection with GMCSF demonstrated a decrease in T-regulator and T-suppressor cells, decreased myeloid-derived suppressor cells and decreased regulatory suppression of the immune response to cancer [36]. These mechanisms were the basis for proceeding with clinical trials to determine to what extent such findings correlate with outcomes.

In a small Phase I study of seven patients treated with escalating doses of GMCSF until response or progression

were evaluated for the effect on both tumor T cells as well as clinical response and toxicity [37]. The authors found similar results with regard to tumor immune response, for there was an increased humoral response: CD4, CD8, lymphocyte, histiocyte and eosinophil tumor infiltrate. In those seven patients, three demonstrated a mixed response, two with the largest tumor burden failed, one demonstrated PR with resection of the remaining lesions to become disease free, and one demonstrated CR. There was no long-term follow-up reported. The only toxicity reported was flu-like symptoms. Another small Phase I trial exploring the use of intralesional GMCSF in 13 patients treated with GMCSF demonstrated PR in 3 patients and those patients were found to have a higher T-cell tumor infiltration and IL-2 receptor expression than the patients who did not respond to therapy [38]. Accordingly, other trials investigated these mechanisms and clinical outcomes.

Ridolfi *et al.* reported a Phase I – II study that evaluated intralesional GMCSF followed by intralesional IL-2 treatment for metastatic melanoma [39]. Out of 16 patients, 4 demonstrated a response to treatment and 9 had SD. Similarly, the only toxicity reported was flu-like symptoms. Follow-up was 3 – 6 months. Senzer *et al.* reported a Phase II trial in stage IIIC and IV melanoma utilizing intralesional GMCSF [40]. Out of 50 patients, the ORR was 26%. Ten out of 50 patients had SD for > 3 months and 2 of 50 had CR at 24 months. The overall 1- and 2-year survival reported were 58 and 52%, respectively. Again, the only toxicity reported was flu-like symptoms. To further address these mixed results, a study was reported in an animal model to attempt to transfect tumor cells with YKL-GB that would express co-stimulatory molecules that would potentially enhance immune response to these tumor cells [41]. This study demonstrated that transfection of both GMCSF and B7-1 genes into those tumors by intralesional injection resulted in increased T-cell infiltration and increased systemic levels of co-stimulatory molecules. Further clinical trials are needed to assess the effectiveness of these treatments, to predict which tumors may respond and how to potentially improve response in refractory lesions in order to determine which patients may benefit.

2.5 IL-2

IL-2, discovered in 1976, is an immune-modulating glycoprotein produced by T-lymphocytes. IL-2 stimulates T-cell proliferation, induces activation of cytotoxic T lymphocytes and NK cells [42].

Systemic IL-2 has been FDA-approved for the treatment of metastatic melanoma since 1998, but its response rates have been low (10 – 15%) at the cost of high morbidity. Regarding locoregional treatment both perilesional and intralesional IL-2 use has reported. Intralesional IL-2 has been proven more efficient. The use of intralesional IL-2 was first described by Adler in 1984 in a series of seven patients, of whom three had melanoma [43]. Adler used IL-2-cultured lymphoid cells (i.e., autologous lymphoid cells cultured *in vitro* in the presence of IL-2). Two patients did not respond. The third patient had CR in 4 of 10 lesions, PR in 1, SD in 2 and PD in 2 lesions; however, it is noted that this patient received radiotherapy just before IL-2 to an unspecified number of lesions. IL-2 has been given in various other vectors, such as adenovirus, IL-2-cultured lymphoid cells and xenogeneic monkey fibroblasts (Vero cells), as well as in combination with other treatments, that is, systemic dacarbazine, topical imiquimod and retinoid cream [17,43-47].

Dehesa *et al.* treated 244 lesions in 7 patients with $0.6 - 6 \times 10^6$ units of IL-2 per lesion [48]. The vast majority of patients (6 out of 7, 86%) had a CR. The remaining patients achieved a PR. Radny *et al.* treated 24 patients in a Phase II trial, of whom 23 were available for analysis and 20 responded (63% CR, 21% PR) [49].

Boyd *et al.* treated 39 patients with human recombinant IL-2 [50]. The authors divided a mean of 10.4 ml of a 5 μg/ml solution of IL-2 over the in-transit lesions in biweekly sessions. The mean number of injections was 5 (range 1 – 7). Twenty patients (51%) experienced CR, 12 (31%) PR and 7 (18%) had no response. Overall survival was improved in complete responders as opposed to partial responders (80% 5-year-survival vs 33%, $p = 0.012$).

Weide *et al.* evaluated 48 patients in a Phase II trial after injection of 0.3 – 6.0 million units, depending on lesion size, for 3 times a week up to 32 weeks [18]. Sixty-nine percent of patients achieved CR. PR, SD and PD were not given for the treated patients, but as for the 894 treated lesions, 79% achieved CR, 1% PR, 16% SD and 4% PD.

Flu-like symptoms following injections are very common (85%) [50]. IL-2 intralesional therapy has demonstrated a high local response rate; however, intralesional IL-2 use is labor intensive with intralesional injections 2 – 3 times per week needed, and it is very expensive. Additionally there are no bystander effects reported with intralesional IL-2 therapy despite it being an immune type treatment.

2.6 PV-10 (Rose Bengal)

PV-10 is a 10% solution of Rose Bengal, a water-soluble xanthine dye (**Figure 1**) [51,52]. It is thought that xanthine dyes can react with visible and ultraviolet light to create singlet oxygen, a key mediator of phototoxic reactions. The safety profile of Rose Bengal disodium has been well established, since it has been used for decades as an intravenous diagnostic agent for assessing hepatic function, reports being published as early as the 1920s [53]. It is still used by ophthalmologists as a diagnostic aid and as a food coloring in Japan. PV-10 is being developed for treatment of both melanoma and liver tumors and works through selective uptake in the lysosomes of cancer cells where it induces autolysis via a light-independent mechanism [54,55].

In a mouse model, PV-10 led to an increased tumor-specific IFN-γ secretion, suggesting that PV-10 can induce a systemic antitumor immune response that is responsible for

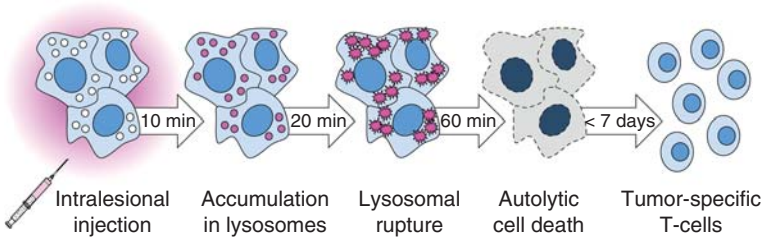

Figure 1. Mechanism of action of PV-10. Intralesional injection of PV-10 (10% Rose Bengal disodium) leads to rapid uptake by cancer cells, where accumulation in lysosomes precipitates lysosomal rupture and autolytic cell death. The resulting debris are implicated in release of tumor antigens, recruitment of dendritic cells and induction of a tumor-specific reactivity in circulating T cells.

the bystander effect observed both nonclinically and clinically. The mechanism is currently unclear, but PV-10 has to be injected in a tumor lesion to induce a systemic effect, as injection into the flank away from tumor had no effect on distant lesions [55]. Intralesional PV-10 is associated with recruitment of dendritic cells to draining lymph nodes and an increase in circulating cytotoxic $CD3^+/CD8^+$ T cells [13]. Responses have been reported in patients refractory to previous systemic ipilimumab, anti-PD1 and vemurafenib [13].

Thompson *et al.* injected 26 lesions in 11 patients with a single injection of 0.5 ml/cc lesion volume of PV-10, treating 1 – 3 lesions per patient [52]. CR and PR were achieved in 27 and 27% of patients, respectively. Target lesions receiving > 0.2 ml doses of PV-10 had a larger response rate than lesions receiving less (69 vs 25%), suggesting that a threshold dose is needed. In 27% of patients a bystander effect was seen, which strongly correlated with the response of the target lesion.

In another Phase I study, 20 patients underwent injection with PV-10 once per lesion, for up to 20 lesions per patient. CR was achieved in 20%, PR in 20% [56]. A bystander effect was seen in 15%. The same group conducted a Phase II trial, injecting up to 20 lesions per patient in 80 patients with PV-10 at day 0 and again after 8, 12 and 16 weeks if needed [57]. The patients were refractory to a median of six previous treatments. Twenty-six percent of patients achieved CR, 25% PR, the majority of which responded after 1 – 2 injections. Response was substantially better when all baseline disease was treated with 50% CR reported. No CR was seen in stage IV disease. The most important adverse effect was locoregional blistering in 40% of patients, strongly correlated with a better outcome. A bystander effect was observed in 40% of the 35 evaluable patients (31% CR, 9% PR), both in visceral and cutaneous lesions, and was strongly correlated with the response of injected lesions. These results prompted an expanded access availability of the trial with over a 100 melanoma patients treated so far. A Phase III trial is currently pending.

PV-10 has a half-life of 5.9 h and is associated with a low morbidity. The majority of patients experience some local side effects, such as injection site discomfort (up to 80%), vesicles, edema, skin discoloration, inflammation and pruritus around the treatment site [51].

2.7 Talimogene laherparepvec

Talimogene laherparepvec (TVEC), previously known as OncoVEX, is an immune-enhanced, oncolytic HSV-1 virus, with a deletion of the ICP47 gene, thereby enabling antigen presentation and enhancing virus growth and replication in tumor cells (**Figure 2**) [40,58]. Additionally, the coding sequence for neurovirulence factor ICP34.5 is replaced by that for the human cytokine GM-CSF, enhancing immune response to tumor antigens and thereby theoretically initiating a systemic antitumor immune response [40,58]. Oncolytic viruses are designed to selectively replicate in tumors, thereby infecting and destroying cancer cells due to direct effects on the metabolic processes in the cell as well as inducing immune responses that target the cancer cell. This is believed to be aided by activation of NF-κB and release of chemokines and cytokines from the cancer cell. Systemic administration of these agents is limited by immune responses of the host; however, they are suitable for intralesional injection.

Senzer *et al.* conducted a single-arm Phase II trial in 50 patients with stage III (n = 10) or stage IV (n = 40) patients, with intratumoral injection of up to 4 ml of 10^6 pfu/ml of either TVEC or GM-CSF followed 3 weeks later by 4 ml of 10^8 pfu/ml, every 2 weeks, for up to 24 treatments [40]. The protocol allowed injection of cutaneous, subcutaneous and nodal lesions with or without ultrasound guidance. Response rate by RECIST was 26% (8 CR, 5 PR). Overall survival was 58% at 1 year and 52% at 24 months. Subsequently, Andtbacka *et al.* presented Phase III study results at ASCO in 2013 [59]. The Phase III study (OPTiM) included 436 patients randomized in a 2:1 fashion to intralesional TVEC or subcutaneous GMCSF, respectively. 295 patients were randomized to TVEC and 141 to GMCSF. TVEC was given using the same regimen as the Phase II trial. Results showed a 26% ORR (PR/CR) for TVEC with a highly significant difference in durable response rate, defined as a PR or CR for 6 months or more, between the arms (26.4% [TVEC] vs 2.1%[GMCSF], respectively, $p < 0.0001$). The treatment was especially effective in stage

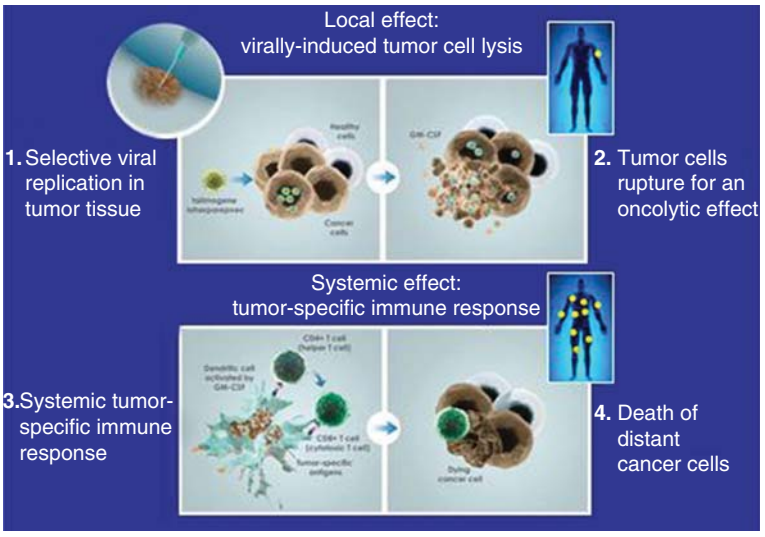

Figure 2. TVEC — an HSV-1-derived investigational oncolytic immunotherapy designed to produce both local and systemic effects.

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TVEC: Talimogene laherparepvec.

IIIB/C melanoma, with durable response rates in 33% of patients versus 0% for GMCSF. Six patients were converted from unresectable to resectable disease. The most common side effects were fatigue, chills and pyrexia. Grade 3 – 4 AEs were rare (< 3%). Overall survival results (primary analysis) were presented at ASCO 2014 and showed a trend toward statistical significance ($p = 0.051$). In stage IIIB/C and IVM1a patients, a survival benefit was demonstrated (41.1 months on TVEC vs 21.5 months on GMCSF, $p < 0.001$) [60]. The effect was stronger when TVEC was given as first-line therapy as opposed to second-line or later therapies.

The same group published an SSO 2014 abstract reporting results of a lesion level analysis in 3,219 lesions in 286 patients: 2,043 injected, 1022 uninjected non-visceral and 156 uninjected visceral lesions [61]. More than 50% reduction in tumor size was seen in 64% of the injected, 32% of the uninjected non-visceral and 16% of the uninjected visceral lesions. These findings indicate a robust bystander effect and thus a systemic immune response from the local injection of TVEC. A Phase Ib study of TVEC in combination with ipilimumab suggested a higher CR rate (6/18 patients, 33%) for the combination than for either agent alone [61].

3. Topical therapies

Topical agents have shown some success, although their use is restricted to superficial lesions. Imiquimod is a toll-like receptor agonist that has been FDA-approved for treatment of genital warts, keratosis and superficial basal cell carcinomas. Since its first use in metastatic melanoma in the year 2000 several case reports and small case series have been reported [62-65]. Treatment protocols vary highly, with imiquimod application ranging from once-weekly to twice-daily and from 2 to 88 weeks [66]. So far evidence is mainly anecdotal and largely conducted in patients with lentigo maligna, with the largest case series reported by Junkins-Hopkins [67]. Daily or twice-daily application of the cream has led to reported regression in > 90% of patients with lentigo maligna [66]. Data in melanoma are limited and mainly include a small case series (n = 5) treated with imiquimod and 5-Fluorouracil (5-FU), eliciting a response in 44 out of 45 lesions [64]. Combined treatments with BCG and IL-2 have also been reported [45,68].

Topical diphencyprone (DPCP) cream is a synthetic contact sensitizer, which is mainly used in the treatment of alopecia and warts [69,70]. Its mechanism in melanoma is not completely understood. It has been proven effective in small series of patients with metastatic melanoma, with the majority of results coming from the same research group [71-76]. The largest trial to date was conducted by Damian *et al.*, who included 58 patients, of whom 50 received more than a month of DPCP. Of these 50 patients, 46% achieved CR and 46% achieved PR [73].

4. Other

A great many other therapies have been tested for intralesional therapy. Sporadic studies have reported responses for IL12, cisplatin, B7-1, human monoclonal antibodies, toll-like receptor 9 agonist, dendritic cells (either alone or in combination with radiotherapy/local hyperthermia), fotemustine, IFN-α, IFN-β and topical therapies including retinoic acid, 5-FU and dinitrochlorobenzene [41,64,66,77-97]. An ongoing Phase II study investigating the intralesional use of

CAVATAK, an oncolytic virus that is better known as Coxsackievirus 21 (a 'common cold' virus) is aiming for enrollment of 63 patients after the interim efficacy end point of > 3 objective responses had been achieved [59].

5. Conclusion

Intralesional therapy is an attractive option when the standard of care, surgical resection to render patients no evidence of disease, is not feasible. The ideal agent should have a low toxicity profile, be easy to administer, lead to fast responses and trigger a systemic immune response, thereby creating a bystander effect. There are many intralesional compounds available, producing a broad range of local response rates with reports of systemic bystander effects seen in the studies using PV-10 and TVEC. This bystander effect is reported in up to 40% of patients.

6. Expert opinion

Over the few past decades, numerous agents have been tested for intralesional therapy with varying success; however, never have developments been as promising as now. Up until recently, only agents with inconsistent efficacy, that is, BCG, interferon, interleukin and GM-CSF, were used. Use of these agents has been supported more by small case series than prospective controlled trials. That changed with the introduction of intralesional PV-10, Allovectin-7 and TVEC. Unfortunately, Allovectin did not meet its primary end point in the Phase III trial. TVEC is a promising agent, met its Phase III trial objectives and shows a survival benefit in selected patient groups. The Phase II results of the PV-10 trials are also promising with a Phase III being planned. Intralesional injections are particularly attractive due to the low toxicity profile, the fact that they are generally very well tolerated, that injections can be done as an outpatient under local anesthesia (if any anesthesia is needed), and that they have a modest response rate that is durable in many cases. The fact that some intralesional treatments are associated with a systemic bystander effect (PV-10 and TVEC) is a particularly attractive aspect of these locally delivered treatments that appear to trigger a systemic immunotherapy reaction. The ideal treatment approach should be individualized based on the extent of disease, tumor characteristics, such as BRAF status and disease-free interval, and patient characteristics including age and comorbidities. The approach for locoregionally advanced melanoma should therefore be multidisciplinary and tailored to the patient. The ideal approach takes into account related characteristics, including age and comorbidities. The ideal approach should be discussed in a multidisciplinary setting and tailored to the patient. It will likely not be a single therapy, but rather a combination of injectable treatments, regional perfusions and systemic therapies.

Declaration of interest

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