Review

For reprint orders, please contact: reprints@futuremedicine.com

Melanoma Management

Topical and intralesional therapies for in-transit melanoma

Michael A Henderson*,[1]

[1]Division of Cancer Surgery, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria 3000, Australia

*Author for correspondence: Tel.: +61 385 597 666; Fax: +61 385 597 709; Michael.Henderson@petermac.org

This report surveys the role of topical and intralesional agents in the management of in-transit melanoma. The extent and progression of in-transit disease is highly variable and many patients can have a protracted period of locoregional control. These agents are useful in the management of patients who have progressed beyond local surgical excision in whom more aggressive therapies, such as isolated limb infusion or use of talimogene laherparepvec, are not appropriate or have failed. In general, these agents are modestly effective and associated with frequent but only minor toxicity. As the mechanism of action of many of these agents includes initiation of a local immune response, combinations with immune checkpoint inhibitors are currently being explored.

First draft submitted: 3 March 2019; Accepted for publication: 8 May 2019; Published online: 2 September 2019

Keywords: diphenylcyclopropenone • electrochemotherapy • imiquimod • in-transit melanoma • rose bengal • T-VEC

In-transit metastases (ITM) are a form of locoregional spread peculiar to melanoma with a unique biology and natural history. The heterogeneous nature of the presentation and clinical course of ITMs has bedeviled an understanding of this condition and its management. Traditionally, these lesions were defined as dermal or subcutaneous deposits occurring usually in a limb between the primary site and the draining lymph node basin, and were distinguished from local or satellite lesions which were defined as dermal or subcutaneous recurrences located within 5 cm of the site of the primary melanoma. ITMs are now understood to represent arrest of tumor emboli in the dermal or subcutaneous lymphatics and share a common etiology with local and satellite recurrence. In the most recent iteration of the American Joint Committee on Cancer staging system (8th edition), local recurrence, satellite lesions and ITM are considered together and have similar prognostic implications and outcomes [1]. As virtually all reports of ITM exclude local or satellite recurrences, they will not be considered further in this review.

This report will describe topical and intralesional agents in the management of in-transit recurrence. The overall management of ITMs and other treatments is considered elsewhere in this single focus issue on the multidisciplinary management of melanoma. In general, the role of topical and intralesional agents is first – management of small-volume disease where surgery is not appropriate, or second – palliation of progressive or extensive disease when standard treatments have failed. These agents although not as effective as standard therapies, such as systemic therapy or isolated limb infusion, continue to have a role even in the era of effective targeted and immune checkpoint inhibitor therapies [2]. In general, they are well tolerated with minimal toxicity and can benefit patients with ITM particularly as these patients may experience prolonged periods of disease control without developing systemic disease. A feature of many of these agents has been the recognition that uninjected adjacent lesions may also respond, the so-called bystander effect. Talimogene laherparepvec (T-VEC), Bacillus Calmette–Guérin (BCG), interferon, IL-2 and imiquimod are all considered appropriate treatments in the current US National Cancer Centre Network Guidelines, although all the recommendations except for T-VEC are based on limited data (category 2B) [3]. The recent Australian Guidelines provide similar recommendations but also include intralesional Rose Bengal and topical diphenylcyclopropenone (DCP) [4].

Future Medicine

Topical agents

Diphenylcyclopropenone

The DCP is a contact sensitizer used in general dermatology most notably for alopecia areata. Intermittent case reports from as early as 1989 described successful use of DCP in the management of ITM, but the first objective report was from Damien and colleagues at the Melanoma Institute Australia who reported a series of 50 patients in 2009 with a further update in 2014 [5,6]. Overall 46% of treated patients had a complete response and a further 38% a partial response. Among patients who had a response but subsequently recurred, reintroduction of DCP was successful. Patients with thin low burden disease rather than bulky or subcutaneous melanoma deposits had a much greater chance of a complete or partial response (93 vs 63%). Read and colleagues from the Queensland Melanoma Project reported a prospective series of 53 patients [7]. In this well-defined group, the response states were somewhat reduced compared with the Sydney experience, the complete response rate was 22%, the partial response rate was 38% and 22% had stable disease. The time to complete response was prolonged at a mean of 10 months. The median follow-up was 21 months and the overall survival (OS) at 3 years was 51%. Response to DCP treatment was more likely to occur in patients with predominantly epidermotrophic rather than bulky or subcutaneous disease, females and patients who had a marked local reaction to the DCP application. Other reports have confirmed these findings [8,9].

As has been demonstrated in the initial studies, induction of a local inflammatory response by sensitization to DCP is necessary and the extent of the response is related to successful treatment. Treatment usually commences with low-dose DCP (0.00001%) cream rapidly increasing to stronger strengths (0.01%). Treatment is given daily to several times per week for up to 24–48 h at a time [10]. Patients are monitored and the dose adjusted as necessary with the aim to induce a rapid cutaneous hypersensitivity reaction. Side effects include an excessive cutaneous hypersensitivity reaction, blistering, regional lymphadenopathy and occasionally generalized eczema [6].

The mechanism of action of DCP is likely complex and currently not well understood. DCP induces a delayed hypersensitivity reaction associated with local cytokine production and both T cell and dendritic cell infiltration resulting in lymphocyte mediated tumor destruction [11]. Ongoing experience with DCP suggests that a proportion of patients will develop a bystander reaction in adjacent lesions. In the study by Damian, four patients who developed regional lymphadenopathy achieved a complete response with ongoing DCP treatment [6]. As with other intralesional and topical agents, the possibility of modulating and expanding an established immune response has been considered and case reports of successful use of DCP combined with immune checkpoint inhibitor therapy (anti-PD-1) have been reported [12]. This is clearly an area that is likely to receive ongoing attention. The DCP therapy has not been compared with other therapies for ITM; however, a randomized study of DCP versus imiquimod is currently underway [13]. A final issue which needs to be considered is the potential for the development of hypersensitivity among carers responsible for application of this agent or direct patient care.

Imiquimod

Imiquimod is a topically applied agent which is also approved for the treatment of superficial basal cell carcinoma and lentigo maligna. The major mechanism of action is toll-like receptor agonist activity leading to extensive cytokine production with induction of a predominantly local immune response [14]. Data from case reports suggest a response may be expected in up to 50%, but prolonged control is unusual [10,15–17]. Activity appears to be dependent on inducing a clinically significant inflammatory response which may limit use particularly in frail patients; otherwise, toxicity is minimal [10]. At the current time, imiquimod has a very limited role except possibly for palliation of ITM unresponsive to other therapies. More recently, interest in combining imiquimod with other agents, for example, BCG, intralesional IL-2, isolated limb infusion or carbon dioxide ablation have been reported [18,19].

Intralesional agents

Bacillus Calmette–Guérin

The BCG is the oldest intralesional agent used for ITM and is mentioned here only for completeness sake as it is rarely used due to its potentially disastrous toxicity and inferior results compared with other treatment modalities [20]. The early experience with BCG in the management of patients with ITM was encouraging. Morton *et al.* in a series of patients with disseminated disease demonstrated a 90% response rate in injected cutaneous lesions and a local bystander effect in 17% [21]. The initial enthusiasm for intralesional BCG was reduced with further experience, and the long-term results of a randomized trial of injected BCG for patients with stage I–III melanoma which

failed to demonstrate a survival advantage [22]. In addition, the potential biohazards associated with the handling and administration of a live organism as well as the risk of major adverse events (AEs) including death due to disseminated tuberculosis and disseminated intravascular coagulation as well as less severe toxicities, including fever, chills, arthropathies, pneumonitis and hepatitis, have led to its almost complete abandonment as a single agent therapy [23–25]. In a small study, intralesional BCG combined with imiquimod was associated with minimal toxicity and reasonable outcomes (56% complete response rate) but given the comments noted above and the availability of safer and potentially more effective agents, it is unlikely this strategy will be pursued.

Interleukin-2

IL-2 is a naturally occurring cytokine produced during T cell and natural killer cell activation as part of the normal immune response [26]. Based on an understanding of its role in the immune response, IL-2 is used in a small number of specialized centers in the management of patients with widespread melanoma. A small proportion (10–15%) of patients will achieve an enduring complete response but at the cost of frequent and significant morbidity [27]. Intralesional IL-2 was introduced in an attempt to minimize systemic side-effects and generally is well tolerated. The initial experience suggested high rates of local control. Two Phase II studies evaluated IL-2 in patients with subcutaneous metastases (stage III and IV). Radny and colleagues reported a complete response rate of 63% and a partial response rate of 21% in 24 patients injected two- to three-times per week for 1–57 weeks with 0.6–1.0 million international units [28]. Weide and colleagues reported on 48 patients with a complete response rate of 69% [29]. Patients with cutaneous rather than subcutaneous ITM and those without evidence of metastatic disease responded much better to intralesional IL-2. In both series, toxicity was very common but was limited to grade 1–2. Most patients experienced a local reaction at the site of injection including local pain, fever, malaise and occasionally nausea. The results from a systematic review of six observational studies of intralesional IL-2 demonstrated less impressive results [30]. Among injected lesions, the complete response rate was 78% but among patients treated only 50% achieved a complete response rate. A bystander effect was seen in 54% of adjacent uninjected lesions. There was considerable heterogeneity in the dose and dosing schedule in these studies. Response rates appeared to be related to the dose and the presence of distant disease. Toxicity was common (73%) but minimal and predominantly local reactions and flu-like symptoms.

The major issue with IL-2 treatment from the patient's perspective is the burdensome treatment regime with the need for multiple attendances. Consensus has not yet been achieved in defining the treatment protocol, dose to be injected, dose scheduling or duration of treatment. The drug is expensive and not available widely outside North America.

A potentially effective strategy to improve response to agents such as IL-2 is the use of recombinant fusion proteins linking cytokines with known activity, such as IL-2 and tumor necrosis factor, to an antibody fragment engineered to localize to tumor cells. A combination of L19IL2 and L19TNF is currently under evaluation as are other combinations such as recombinant fusion proteins with chemotherapeutic agents and isolated limb infusion [31].

Rose bengal

Rose bengal, a soluble dye, is related to the dye fluoriscine used extensively in ophthalmology. Rose bengal has been used both topically in ophthalmology to identify conjunctival and corneal defects and for many years was used as a test of excretory liver function typically in a 2% solution administered intravenously. The tumorcidal effects of rose bengal were identified during screening of novel agents for photodynamic therapy. Rose Bengal is now marketed as a 10% solution, PV-10 (Provectus Biopharmaceuticals, TN, USA).

The mechanism of action of rose bengal is twofold: it appears to be taken up preferentially by tumor cells and stored in the lysosomes leading to cell autolysis. This occurs rapidly within 30–60 min and presumably leads to the second mechanism of action, exposure of multiple cell antigens triggering a local immune response [32]. Rose bengal is associated with a significant bystander effect on adjacent deposits of tumor and even distant metastases in a portion of patients.

Thompson and colleagues reported a Phase I trial of rose bengal in 11 patients [33]. The overall response rate was 69 and 27% of adjacent nontarget lesions responded. This increased to 44% among patients who had a marked response in the injected lesions. A Phase II study of 80 patients (62 stage III, 18 stage IV) found an overall response rate of 51% and a complete response rate of 26% [34]. The median duration of response was 4 months and 8% of patients had a continuing complete response at 12 months. The median time to response was 1.9 months (1–2

treatments). Overall the treatment was very well tolerated, all patients experienced toxicity related to the injection site. In all but 15% of patients who developed grade 3 toxicity, it was minor (grade 1,2). The quality of life over 3 months of the study was unchanged. Responses were related to the burden of untreated disease, no patient with stage IV disease achieved a complete response suggesting that residual untreated disease impaired the immune response to treatment. A small study of rose bengal followed by radiotherapy found a higher overall response rate (87%), but the complete response rate (27%) was similar to the Phase I and II and other reports [35]. At the present time, there is an ongoing Phase III trial comparing intralesional PV-10 versus systemic chemotherapy or intralesional oncolytic viral therapy for unresectable Stage III/IV disease.

Electrochemotherapy

Electrochemotherapy is a technique particularly suited to ITM by increasing the local tumor destructive effect of chemotherapy. Under heavy sedation or general anesthesia, the probe array is inserted into the dermal or subcutaneous metastasis for a short period. Relatively high intensity pulsed electrical current is delivered to the lesion in the setting of concurrent bolus systemic and/or intralesional injection of either bleomycin or cis-platinum. The electrical current disrupts the melanoma cell membrane (electroporation) facilitating increased entry of the chemotherapeutic agent into tumor cells by a factor of approximately 80 for cisplatin and 8000 for bleomycin. The role of any local and/or systemic immune response to electrochemotherapy is not well understood, but the major effects of electrochemotherapy appear to be due to local tissue destruction.

Initial reports indicated high response rates, 56 and 85% and complete response rates of 72 and 56% [36,37]. A meta-analysis of 22 studies of electrochemotherapy based on only 150 patients with melanoma ITM reported a complete response rate of 57% and overall response rate of 81% [38]. No difference in effectiveness was found between bleomycin and cisplatin. Intralesional chemotherapy was more effective than systemic bleomycin or cis platinum. Toxicity was minimal apart from minor local wound discomfort. A registry study of 151 patients found a complete response rate of 58% with minimal toxicity and at 12 months 67% of patients were still alive [39]. No long-term survival data are available, but repeat electrochemotherapy appears to be of benefit [39]. Bystander effects due to electrochemotherapy with bleomycin have not been documented to date although some adjacent lesions may show a response possibly due to injected bleomycin.

Talimogene laherparepvec

T-VEC is the only approved intralesional therapy for ITM by both the US FDA and European Commission based on the results of a randomized Phase II trial, the OPTIM study (OncovexGM-CSF Pivotal Trial in Melanoma). T-VEC is an oncolytic immunotherapy based on the herpes simplex virus type 1. The virus has been modified first to preferentially replicate in and lyse cancer cells, and second to produce granulocyte–macrophage colony stimulating factor resulting in a specific immune response by enhancing antigen presentation and cytotoxic T cell responses [40].

The OPTIM Study randomized 436 patients with inoperable stage III or IV disease to either intralesional T-VEC or subcutaneously injected GM-CSF [41]. The durable response rate defined as a complete or partial response lasting more than 6 months was significantly higher in the T-VEC group (16 versus 2%). The overall response rate was also significantly higher in the T-VEC group (26 versus 6%), but median OS was not different (26 versus 6%, $p = 0.051$). Patients, who were treatment naive or who were Stage IIIB, IIIc or IVM1a, compared with more advanced disease experienced both a superior durable response rate and OS. Serious AEs were very uncommon and limited predominantly to cellulitis. Minor AEs, grade 1,2 fever, malaise and local injection site symptoms were common. The median time to response was 4.1 months and a significant proportion of patients experienced an increase in size of the injected lesion(s) before a treatment effect was seen. (A total) Total 64% of injected lesions had a greater than 50% reduction in size. Among uninjected nonvisceral metastases 34% responded as did 15% of visceral metastases [42].

Overall these results are the best reported in the management of melanoma ITM. The results are durable and among responders the OS is increased. The significant bystander effect highlights the importance of a systemic immune response and has provided the impetus to further enhance local and distant responses by combining T-VEC with systemic immune checkpoint inhibitor therapy. A randomized trial of combination T-VEC and ipilimumab in 198 patients with stage IIIB-stage IV melanoma reported an improved objective response rate (39 vs 18%). In the T-VEC and ipilimumab arm 52% of the noninjected metastases responded [43]. However, progression free survival

Table 1. This table compares currently recommended intralesional and topical therapies for in-transit melanoma.

Agent		Number of patients	Population	CR %	Response rate of noninjected lesions	Outcome	Ref.
DCCP	Phase II	54	ITM Stage 3b,c	22	12%	PFS 12 m Survival 3 year 51%	[7]
IL-2	Systematic review	140	Stage 3b,c + Stage IV	78	Nil	Not reported	[30]
Electrochemotherapy + Bleomycin	Registry	151	Stage 3b,c + Stage IV	58	34%	Survival 12 m 67%	[39]
Rose bengal	Phase II	80	III b,c Refractory IV	26	33%	PFS 4 m	[34]
T-VEC	Phase II (versus GM-CSF)	295	III b,c Unresected M1a	16	34% loco-regional, 15% visceral	Median overall survival 23 months versus 19 months, p = 0.051	[42]

Major reports and/or systematic reviews are included.
CR: Complete response rate; DCP: Diphenylcyclopropenone; PFS: Progression free survival; Rose bengal 10% (PV-10); T-VEC: Talimogene laherparepvec.

was similar between the two treatment arms. Toxicity was common, grade 3 and above 45 and 35%, respectively, for the two treatment groups.

Combinations of intralesional & topical agents with systemic therapy

Given the observation that no more than one third of patients treated with topical and intralesional therapies will develop a complete response, enhancement of currently available treatments is an active area of research. The unprecedented success of mitogen-activated phosphokinase pathway inhibitors and immune checkpoint inhibitors for patients with metastatic melanoma has prompted investigation of combined therapy with topical and intralesional agents. Although the exact mechanism of action of most of the agents discussed in this review is not completely understood, most are known to initiate and/or promote local immune responses. Enhancement of the immune response is an active area of research in immune checkpoint therapy and the potential for a synergistic effect with topical and intralesional agents leading to local and distant bystander effects are a developing strategy. Early results from the combination of ipilimumab and TVEC as described above appear in the early stages to provide some support for this strategy. In addition to T-VEC, other agents under evaluation combined with checkpoint inhibitor therapy include DCP, rose bengal and electrochemotherapy (as well as isolated limb infusion and radiotherapy).

Conclusion

A summary of studies of current intralesional and topical agents for in-transit melanoma is shown in Table 1. Assessment of these agents has focused on objective response rates to direct application usually assessed over very short time periods. OS, local and regional control, disease-free survival and progression free survival are variably reported, but when documented the length of follow-up is usually short, in the order of less than 12 months. As documented in both the US NCCN and Australian clinical practice guidelines the strength of the evidence guiding the management of ITMs is poor. Only T-VEC has been the subject of a randomized trial, virtually all the remaining reports are small, observational or early phase studies. Making sensible comparisons between these agents is not possible. T-VEC would appear to be the agent of choice as although the complete response rate of 16% is the lowest compared with other therapies, the considerable locoregional and distant bystander effect is unmatched. Furthermore, like rose bengal when responses occur, they are durable. Several factors confound the interpretation of the role of topical and intralesional agents in the management of patients with ITM. The natural history of ITM ranging from an indolent course of infrequent local recurrences to rapid progression with regional and distant metastases can make rational selection of appropriate therapies, for example, isolated limb infusion or systemic therapy a challenge. Local tumor burden and the presence of metastatic disease are repeatedly identified in the reports of topical and intralesional agents for ITN as indicating a poor response, but no other markers among patients with favorable outlook exist. Topical and intralesional agents as discussed in this review are characterized by relatively high rates of response generally exceeding 50% in lesions directly treated with only minor toxicity. All rely on some form of tumor destruction for their efficacy, but increasingly interest has focused on bystander effects due to induction of an effective immune response. Strategies to enhance both local and systemic antitumor effects are under development. For the present, the role of topical and intralesional therapies will remain under evaluation but

increasingly will be used in combination with systemic therapies, for example, anti-PD-1. The major indications for topical and intralesional agents apart from T-VEC appear to be for patients either unable or unwilling to receive systemic therapy or undergo isolated limb infusion. Both rose bengal and DCP are easy to administer, relatively effective and have minimal toxicity and should be considered for these patients as well as those who have failed standard management.

Future perspective

The modest results of current therapies argue that novel strategies for the management of ITM will be required to advance patient care. Given the highly variable course of ITM, from the occasional isolated small recurrence to extensive progressive disease with regional and distant recurrence, the need for effective markers of tumor behavior is vital.

The combination of topical and intralesional therapies with immune checkpoint inhibitors is an evolving strategy to enhance local and systemic immune responses which will hopefully lead to improved survival. The details of this strategy are likely to be worked out over the next few years as mature results from current studies become available. Immunotherapy for melanoma is a rapidly developing space and in the medium term most advances for the management of patients with ITM are likely to follow from improvements in the management of patients with metastatic disease.

Executive summary

- Patients with intransit melanoma (ITM) may experience prolonged periods of local disease without regional or distant metastasis. Intralesional and topical agents may be of value in managing these patients.
- Topical and intralesional agents for the most part are associated with reasonable rates of control and only minor toxicity.

Topical agents

Diphenylcyclopropenone (DCP)

- The DCP is a contact sensitizer used extensively in general dermatology and induces a local inflammatory response.
- Overall approximately 50% of patients will experience a complete or partial response. In addition, there is a modest local bystander effect and several cases of distant bystander effect have been reported.

Imiquimod

- Also used in the management of superficial basal cell carcinoma and lentigo maligna, imiquamod induces a local immune response.
- Based on very limited data approximately 50% of patients respond to topical application; however, the durability of the response appears to be limited.

Intralesional agents

Bacillus Calmette–Guérin (BCG)

- Intralesional BCG has a minimal role in the management of ITM due to its relative lack of activity compared with other agents and potentially catastrophic adverse events including disseminated tuberculosis.

Interleukin 2

- Unlike systemic administration, intralesional injection is associated with much less severe toxicity with up to 50% of patients developing a complete response and a local bystander effect in approximately 50% of responders.

Rose bengal

- Intralesional rose bengal as a 10% solution is an effective and well-tolerated treatment with approximately 50% of patients responding. Bystander effects are common and toxicity is minimal.

Electrochemotherapy

- A unique strategy to control ITM is electrochemotherapy. Electrical current delivered directly to ITM results in disruption of the cell membrane enhancing entry of cisplatin or bleomycin into the cell.
- Limited results indicate a response rate of over 50% with intralesional chemotherapy. The procedure is well tolerated and can be repeated as needed.

Talimogene laherparepvec

- Talimogene laherparepvec is an oncololytic immunotherapy based on the herpes simplex virus which has been engineered to produce granulocyte macrophage colony stimulating factor and lyse tumor cells.
- The local control rate is low (16%), but a local bystander effect was noted in one third of patients and a distant bystander effect on visceral metastases in 15%.

Combined topical & intralesional agents and systemic therapy

- A common feature of many of the topical and intralesional agents is induction of a local inflammatory response and in some cases both local and distant bystander effects.

- Combinations of several of these agents; for example, rose bengal, talimogene laherparepvec with immune checkpoint inhibitor therapy are currently under evaluation. Early results suggest that this may be an effective strategy.

Conclusion

- Topical and intralesional agents are associated with modest response rates but minimal toxicity.
- Although short-term results are encouraging, there is relatively little information on long-term local control or survival.
- The major role for these agents is patients with limited disease no longer suitable for simple surgical excision or following failure of standard isolated limit infusion or systemic therapy.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Author contribution

MA Henderson was solely responsible for this report.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Gershenwald JE, Scolyer RA, Hess KR *et al.* Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA. Cancer J. Clin.* 67(6), 472–492 (2017).
2. Nan Tie E, Henderson MA, Gyorki DE. Management of in-transit melanoma metastases: a review. *ANZ. J. Surg.* 67, 472 (2018).
3. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Cutaneous Melanoma (2019). www.nccn.orgprofessionalsphysicianglspdfcutaneousmelanoma.pdf;
4. Cancer Council Australia Melanoma Guidelines Working Party. Clinical practice guidelines for the diagnosis and management of melanoma. (2019). httpswiki.cancer.org.auaustraliawikiindex.phpoldid https://wiki.cancer.org.au/australia/Guidelines:Melanoma
5. Damian DL, Shannon KF, Saw RP, Thompson JF. Topical diphencyprone immunotherapy for cutaneous metastatic melanoma. *Australas. J. Dermatol.* 50(4), 266–271 (2009).
6. Damian DL, Saw RPM, Thompson JF. Topical immunotherapy with diphencyprone for in transit and cutaneously metastatic melanoma. *J. Surg. Oncol.* 109(4), 308–313 (2014).

• **Largest study of diphenylcyclopropenone in the management of in-transit metastases highlighting moderate activity and bystander effects.**

7. Read T, Webber S, Tan J *et al.* Diphenylcyclopropenone for the treatment of cutaneous in-transit melanoma metastases – results of a prospective, non-randomized, single-centre study. *J. Eur. Acad. Dermatol. Venereol.* 31(12), 2030–2037 (2017).
8. Moncrieff M, Fadhil M, Garioch J. Topical diphencyprone for the treatment of locoregional intralymphatic melanoma metastases of the skin; the 5-year Norwich experience. *Br. J. Dermatol.* 174(5), 1141–1142 (2016).
9. Yeung C, Petrella TM, Wright FC, Abadir W, Look Hong NJ. Topical immunotherapy with diphencyprone (DPCP) for in-transit and unresectable cutaneous melanoma lesions: an inaugural Canadian series. *Expert Rev. Clin. Immunol.* 13(4), 383–388 (2017).
10. Veverka KK, Jakub JW, Baum CL. Responses to topical diphenylcyclopropenone as an adjunct treatment for in-transit melanoma: a tertiary referral center experience. *Dermatol. Surg.* 44(12), 1501–1508 (2018).
11. Gulati N, Suarez-Farinas M, Fuentes-Duculan J *et al.* Molecular characterization of human skin response to diphencyprone at peak and resolution phases: therapeutic insights. *J. Invest. Dermatol.* 134(10), 2531–2540 (2014).
12. Gulati N, Garcet S, Fuentes-Duculan J *et al.* Molecular profiling of immune activation associated with regression of melanoma metastases induced by diphencyprone. *J. Invest. Dermatol.* 136(10), 2101–2103 (2016).
13. Read T, Webber S, Thomas J *et al.* Protocol for the TIDAL Melanoma Study: topical imiquimod or diphenylcyclopropenone for the management of cutaneous in-transit melanoma metastases-a Phase II, single centre, randomised, pilot study. *BMJ Open* 7(10), e016816 (2017).
14. Walter A, Schafer M, Cecconi V *et al.* Aldara activates TLR7-independent immune defence. *Nat. Commun.* 4, 1560 (2013).

15. Steinmann A, Funk JO, Schuler G, den Driesch von P. Topical imiquimod treatment of a cutaneous melanoma metastasis. *J. Am. Acad Dermatol.* 43(3), 555–556 (2000).
16. Bong AB, Bonnekoh B, Franke I, Schon MP, Ulrich J, Gollnick H. Imiquimod, a topical immune response modifier, in the treatment of cutaneous metastases of malignant melanoma. *Dermatology* 205(2), 135–138 (2002).
17. Sisti A, Sisti G, Oranges CM. Topical treatment of melanoma skin metastases with imiquimod: a review. *Dermatol. Online J.* 21(2) pii: 13030/qt8rj4k7r6 (2014).
18. Kibbi N, Ariyan S, Faries M, Choi JN. Treatment of in-transit melanoma with intralesional bacillus Calmette-Guerin (BCG) and topical imiquimod 5% cream: a report of 3 cases. *J. Immunother.* 38(9), 371–375 (2015).
19. Fujimura T, Kambayashi Y, Sato Y *et al.* Successful treatment of nivolumab-resistant multiple in-transit melanomas with ipilimumab and topical imiquimod. *Case Rep. Oncol.* 11(1), 1–5 (2018).
20. Yang J, Jones MS, Ramos RI *et al.* Insights into local tumor microenvironment immune factors associated with regression of cutaneous melanoma metastases by mycobacterium bovis Bacille Calmette–Guerin. *Front. Oncol.* 7, 61 (2017).
21. Morton DL, Eilber FR, Holmes EC *et al.* BCG immunotherapy of malignant melanoma: summary of a seven-year experience. *Ann. Surg.* 180(4), 635–643 (1974).
22. Agarwala SS, Neuberg D, Park Y, Kirkwood JM. Mature results of a Phase III randomized trial of bacillus Calmette–Guerin (BCG) versus observation and BCG plus dacarbazine versus BCG in the adjuvant therapy of American Joint Committee on Cancer Stage I-III melanoma (E1673): a trial of the Eastern Oncology Group. *Cancer* 100(8), 1692–1698 (2004).
23. Cohen MH, Elin RJ, Cohen BJ. Hypotension and disseminated intravascular coagulation following intralesional bacillus Calmette–Guerin therapy for locally metastatic melanoma. *Cancer Immunol. Immunother.* 32(5), 315–324 (1991).
24. Robinson JC. Risks of BCG intralesional therapy: an experience with melanoma. *J. Surg. Oncol.* 9(6), 587–593 (1977).
25. Karakousis CP, Douglass HOJ, Yeracaris PM, Holyoke ED. BCG immunotherapy in patients with malignant melanoma. *Arch. Surg.* 111(6), 716–718 (1976).
26. Liao W, Lin J-X, Leonard WJ. Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity* 38(1), 13–25 (2013).
27. Atkins MB, Lotze MT, Dutcher JP *et al.* High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J. Clin. Oncol.* 17(7), 2105–2116 (1999).
28. Radny P, Caroli UM, Bauer J *et al.* Phase II trial of intralesional therapy with interleukin-2 in soft-tissue melanoma metastases. *Br. J. Cancer.* 89(9), 1620–1626 (2003).
29. Weide B, Derhovanessian E, Pflugfelder A *et al.* High response rate after intratumoral treatment with interleukin-2: results from a Phase II study in 51 patients with metastasized melanoma. *Cancer* 116(17), 4139–4146 (2010).
30. Byers BA, Temple-Oberle CF, Hurdle V, McKinnon JG. Treatment of in-transit melanoma with intra-lesional interleukin-2: a systematic review. *J. Surg. Oncol.* 110(6), 770–775 (2014).

- **Systematic review of intralesional IL-2 confirming local activity but highlighting the great variation in dosing schedules.**

31. Weide B, Neri D, Elia G. Intralesional treatment of metastatic melanoma: a review of therapeutic options. *Cancer Immunol. Immunother.* 66(5), 647–656 (2017).
32. Toomey P, Kodumudi K, Weber A *et al.* Intralesional injection of rose bengal induces a systemic tumor-specific immune response in murine models of melanoma and breast cancer. *PLoSONE* 8(7), e68561 (2013). https://doi.org/10.1371/journal.pone.0068561
33. Thompson JF, Hersey P, Wachter E. Chemoablation of metastatic melanoma using intralesional rose bengal. *Melanoma Res.* 18(6), 405–411 (2008).
34. Thompson JF, Agarwala SS, Smithers BM *et al.* Phase II Study of intralesional PV-10 in refractory metastatic melanoma. *Ann. Surg. Oncol.* 22(7), 2135–2142 (2015).

- **Detailed study of intralesional rose bengal (PV10) confirming significant rates of local and bystander response with minimal toxicity.**

35. Lippey J, Bousounis R, Behrenbruch C *et al.* Intralesional PV-10 for in-transit melanoma – A single-center experience. *J. Surg. Oncol.* 114(3), 380–384 (2016).
36. Byrne CM, Thompson JF, Johnston H *et al.* Treatment of metastatic melanoma using electroporation therapy with bleomycin (electrochemotherapy). *Melanoma Res.* 15(1), 45–51 (2005).
37. Mir LM, Glass LF, Sersa G *et al.* Effective treatment of cutaneous and subcutaneous malignant tumors by electrochemotherapy. *Br. J. Cancer.* 77(12), 2336–2342 (1998).
38. Mali B, Jarm T, Snoj M, Sersa G, Miklavcic D. Antitumor effectiveness of electrochemotherapy: a systematic review and meta-analysis. *Eur. J. Surg. Oncol.* 39(1), 4–16 (2013).

- **Meta-analysis of the small number of cases reported in the literature confirming modest activity with minimal symptoms.**

39. Kunte C, Letule V, Gehl J *et al.* Electrochemotherapy in the treatment of metastatic malignant melanoma: a prospective cohort study by InspECT. *Br. J. Dermatol.* 176(6), 1475–1485 (2017).

40. Kohlhapp FJ, Kaufman HL. Molecular pathways: mechanism of action for talimogene laherparepvec, a new oncolytic virus immunotherapy. *Clin. Cancer Res.* 22(5), 1048–1054 (2016).

41. Andtbacka RHI, Kaufman HL, Collichio F *et al.* Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol.* 33(25), 2780–2788 (2015).

•• **The only randomized study of topical therapy, significant local and distant bystander effects were reported.**

42. Andtbacka RHI, Ross M, Puzanov I *et al.* Patterns of clinical response with talimogene laherparepvec (T-VEC) in patients with melanoma treated in the OPTiM Phase III clinical trial. *Ann. Surg. Oncol.* 23(13), 4169–4177 (2016).

43. Chesney J, Puzanov I, Collichio F *et al.* Randomized, open-label Phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. *J. Clin. Oncol.* 36(17), 1658–1667 (2018).

• **First report of combination of immune checkpoint inhibitor therapy and intralesional therapy, talimogene laherparepvec.**