

RESEARCH ARTICLE

Results of a phase II, open-label, non-comparative study of intralesional PV-10 followed by radiotherapy for the treatment of in-transit or metastatic melanoma

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BACKGROUND: In-transit and recurrent dermal or subcutaneous melanoma metastases represent a significant burden of advanced disease. Intralesional Rose Bengal can elicit tumor selective ablation and a T-cell mediated abscopal effect in untreated lesions. A subset of patients in a phase II trial setting received external beam radiotherapy to their recurrent lesions with complete or partial response and no significant acute radiation reaction.

METHODS: An open-label, single-arm phase II study was performed to assess the efficacy and safety of PV-10 followed by hypofractionated radiotherapy. Patients had in-transit melanoma metastases suitable for IL therapy and radiotherapy.

RESULTS: Fifteen patients were enrolled and thirteen completed both treatment components. The overall response rate was 86.6% and the clinical benefit was 93.3% on an intention to treat analysis (CR 33.3%, PR 53.3%, SD 6.7%). The median follow up duration was 19.25 months. Size of metastases (<10 mm) predicted lesion complete response (74.6%). Treatment was well tolerated with no associated grade 4 or 5 adverse events.

CONCLUSIONS: The combination of PV-10 and radiotherapy resulted in lesion-specific, normal tissue-sparing, ablation of disease with minimal local or systemic adverse effects.

KEYWORDS

in-transit, melanoma, metastases, PV-10, radiotherapy, trial

1 | BACKGROUND

The incidence of cutaneous melanoma has steadily increased by 3-8% annually over the past 30 years and now presents a lifetime risk of up to 1 in 53 in the U.S. and 1 in 25 in Australia.¹ In-transit and recurrent dermal, and subcutaneous melanoma metastases are an advanced form of disease (\geq Stage IIIb) associated with significant morbidity stemming from both disease-related functional impairment and treatment side-effects.^{2,3}

For localized melanoma metastases complete surgical excision is accepted as standard treatment. However, the location, size, number, and distribution of metastases, and patient co-morbidities mean that

surgery is not always reasonable or appropriate. Regional perfusion or infusion with melphalan or other chemotherapeutic agents may be considered for limb-constrained deposits, while systemic chemotherapy, immunotherapy, and radiation are commonly considered treatment options.^{1,4} Given that melanoma is genetically diverse and inherently immunogenic there is now wide availability of a systemic therapies targeting MAP kinase (BRAF-mutated melanoma) and anti-CTLA-4, and anti-PD-1 inhibitors.⁵⁻⁸

PV-10 is a sterile, non-pyrogenic 10% solution of Rose Bengal (RB, 4,5,6,7-tetrachloro-2,4,5,7-tetraiodofluorescein disodium) that has previously been investigated for the IL chemoablation of melanoma metastases.⁹ PV-10 may elicit both selective local and systemic anti-tumor responses, including a T-cell mediated abscopal effect in untreated lesions. In a Phase II trial of 80 patients with Stage III-IV melanoma, treatment yielded a 51% objective response rate (ORR) and complete response in 50% when all disease was injected.¹⁰

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Radiotherapy is an important modality in the treatment of patients with recurrent, in-transit or metastatic melanoma requiring local therapy. The optimal dose and fractionation schedule for remains controversial and is often selected based on the site of disease, volume of disease, extent of metastatic disease, and patient preference. It is widely accepted that melanoma has a variable radiosensitivity with fractionation sensitivity characteristic of a late-responding normal tissue.¹¹ In the largest clinical radiobiological study of radiotherapy in the treatment of recurrent and metastatic melanoma the complete response rate was 48% with a significant relationship between dose per fraction ≥ 4 Gy and response.¹²

Based on the clinical observation of a synergistic benefit using IL PV-10 followed by radiotherapy the radiobiological rationale may involve radio-sensitization and augmentation of the host's immune response.¹³ In this Phase II study it was hypothesized that the combination of IL PV-10 and radiotherapy would result in lesion specific, normal tissue sparing, ablation of melanoma tumors with minimal potential for local or systemic adverse effects.

2 | MATERIALS AND METHODS

2.1 | Study design

A Phase II open-label, non-comparative study was conducted using PV-10 and hypofractionated radiotherapy for patients with clustered in-transit or recurrent dermal and subcutaneous melanoma metastases within an encompassable treatment field confined to one body part. Patients were considered for treatment if they were over the age of 18 years, not pregnant or lactating and had histopathologically/cytologically confirmed melanoma metastases that were >2 mm in measurable diameter. Both Stage III and IV disease were eligible for treatment provided disease could be encompassed by a reasonable radiotherapy volume, had failed or were not suitable for other loco-regional therapies and were not better suited to systemic treatments as determined through discussion at an expert multidisciplinary meeting. Patients were excluded if they received local treatments (including the investigational agent) or systemic cancer therapies within 4 weeks, anti-tumor vaccines within 6 weeks or regional chemotherapy within 12 weeks of commencing trial treatment.

2.2 | Treatment protocol

The study was approved by the Princess Alexandra Hospital Human Research Ethics Committee and all patients provided informed, written consent. PV-10 was dispensed as a sterile, non-pyrogenic 10% concentration of Rose Bengal saline solution. This was administered by IL injection to uniformly infiltrate up to a maximum of 20 non-visceral lesions with the total dose limited to 1500 mg RB (i.e., 15 mL PV-10 solution). The dosage delivered by IL injection was calculated using a standardized algorithm based on tumor volume.¹⁰ Prior to tumor injection, local anaesthetic was infiltrated around (but not into) the lesions. Lesions were injected using a fanning technique with a fine gauge needle to optimize intra-tumoral PV-10

distribution. Patients were treated with PV-10 during the trial and were monitored for immediate post-treatment AEs with follow-up performed on days 1 and 7, and week 4. Provided patients did not develop progressive disease, radiotherapy was commenced within 6-10 weeks of initial PV-10 treatment. All identified cutaneous soft tissue lesions were treated with PV-10 in this study and there were no non-visceral or nodal "bystander lesions". Lesions outside of an encompassable radiation field were classified as 'non-target lesions' and were treated using PV-10 alone.

Radiotherapy was delivered by either photons or electrons using a hypofractionated regimen of 30 Gy in 6 fractions (5 Gy/fraction) at 2 fractions/week over 3 weeks. Planning Target Volume 30 Gy (PTV30) was defined as the Gross Tumor Volume (GTV) with a minimum 5 mm margin. The volume of tissue planned for radiotherapy was determined by computed tomography (CT) planning with the beam arrangement at the discretion of the treating radiation oncologist. For dermal, subdermal and in-transit disease, tissue equivalent material (bolus) was applied to ensure the full radiotherapy dose on the skin surface and appropriate dose constraints to any adjacent critical structures was observed in all cases.

Patients were reviewed regularly following XRT administration with clinic visits on weeks 2, 6, 12, and 24 after the completion. These visits included evaluation of study lesions with photo-documentation and lesion measurement using calipers or ultrasound assessment; CT documentation of visceral lesions (in Stage IV subjects at 12 weeks post-XRT only); assessment of local and systemic toxicity signs and symptoms; changes to concurrent medication and other medical conditions. At 36 weeks following the last radiotherapy treatment, patients had their final study assessment of their disease status.

2.3 | Presentation of findings and analysis criteria

Planned accrual for this study was 25 patients, however, due to slow recruitment the study was closed early. Patient medical records including clinical photographs and lesion measurements were independently reviewed then compared. The findings were recorded using a standardized data proforma, entered on to a secure institutional database with the major datapoints collated within Microsoft Excel and analyzed using STATA v13.0 statistical software. In order to provide a useful tool for clinical practice, an intention to treat statistical analysis was performed including an assessment of categorical variables and treatment responses. Survival outcomes were calculated using the Kaplan-Meier survival estimate. Response Evaluation Criteria in Solid Tumors (RECIST) was used to assess the primary outcomes. Standard thresholds for percentage change in sum of longest diameters were used to define complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). All lesions specified at baseline were followed over the course of the study. When eschar or ulceration were reported, a standard query was used to ascertain lesion status which was then measured at the next available time point of the follow-up schedule. Adverse events (AEs) were assessed using the CTCAE V3.0.

TABLE 1 Patient demographics and key characteristics

Patient	Age and gender	Primary lesion	Overall disease stage	Previous treatments	No. lesions treated	Best overall response (treated lesions)	In-field recurrence/progression
1	82 F	7.00 mm ulcerated nodular melanoma left forearm	IIIb	None	10	SD	N
2	44 M	5.06 mm ulcerated nodular melanoma left leg	IIIb	None	2	CR	Y
3	82 M	Unknown primary	IIIb	Surgery	3	PR	Y
4	71 F	Melanoma (NA) right leg	IIIb	Surgery	2	CR	N
5	53 M	2.10 mm ulcerated nodular right forearm	IIIb	Surgery	20	PR	N
6	74 M	2.00 mm non-ulcerated nodular left leg	IIIc	Surgery	6	PD ^a	Y
7	63 M	5.80 mm non-ulcerated nodular left thigh	IIIb	Surgery	3	CR	N
8	79 M	3.20 mm ulcerated mixed melanoma left leg	IIIb	Surgery	3	CR	N
9	80 M	7.80 mm non-ulcerated desmoplastic melanoma scalp vertex	IV	Surgery	7	CR	Y
10	50 F	1.20 mm ulcerated melanoma right leg	IIIc	Surgery, ILI	10	PR ^b	N
11	86 M	4.50 mm non-ulcerated nodular melanoma left shoulder	IIIb	Surgery	5	PR	Y
12	73 M	1.50 mm ulcerated SSM left scalp vertex	IIIc	Surgery	10	PR	N
13	69 F	2.00 mm non-ulcerated SSM right thigh	IIIb	Surgery	4	PR	Y
14	85 F	3.20 mm non-ulcerated nodular melanoma right leg	IIIb	Surgery, DPCP	12	PR	Y
15	80 M	0.95 mm non-ulcerated nodular left forearm	IIIb	ILI, DPCP	15	PR	Y

^aPatient rapidly developed loco-regionally progressive disease following PV-10 treatment and did not receive radiotherapy.

^bPatient presented with progressive systemic disease during interval between PV-10 treatment and scheduled radiotherapy and therefore did not receive radiotherapy. Prior to this event the patient experienced a partial response in the treated lesions.

2.4 | Outcomes

The primary outcomes in this study were the best overall response rate (CR + PR) and clinical benefit (CR + PR + SD) of in-field target lesions as determined by RECIST. This outcome measure was used to assess the treatment effect and thereby provide an indication of the overall clinical utility of sequential IL PV-10 and radiotherapy. Secondary objectives were patients' best overall response, time to best response, melanoma-specific survival, and treatment-related adverse events (toxicity). The effect of disease volume (lesion size) on best overall clinical response was also investigated.

3 | RESULTS

3.1 | Patients

A total of 15 patients were enrolled prospectively and treated between January 2011 and September 2015 (Table 1). The median age was 74 years (range 53–86 years) and the majority were male ($n = 10$).

All patients had in-transit disease (AJCC Stage IIIB+) after a median of one previous loco-regional therapy. Thirteen patients completed both treatment modalities as per protocol, while two developed rapidly progressive disease (one locally and the other distant) after undergoing PV-10 alone. A total of 98 target lesions were treated with both PV-10 and radiotherapy while five non-target lesions were treated using PV-10 alone due to their location outside a planned radiation field (Fig. 1).

3.2 | Melanoma and in-transit characteristics

The clinical and pathological characteristics of patients' primary melanoma are presented in Table 2. The median time to develop in-transit disease was 14.7 months and the median follow-up duration was 19.25 months. All patients had nodular lesion morphologies and a number also displayed mixed bulky and papular disease types (refer to Table 2).

The median number of treated lesions was 6 (range 2–20) and diameters ranged between 2 and 55 mm (median 18 mm). There were total of 103 lesions in 15 patients, 98 target, and 5 non-target lesions.

TABLE 2 Primary and in-transit melanoma characteristics

Characteristics	Number (%)
Breslow thickness (mm)	
Mean	3.56 mm
<1.0	1 (6.7)
1.01-2.0	4 (26.7)
2.01-4.0	3 (20.0)
>4.0	5 (33.3)
NA	2 (13.3)
Primary anatomical location	
Head and neck	2 (13.3)
Trunk	1 (6.7)
Upper limb	3 (20.0)
Lower limb	8 (53.3)
Unknown	1 (6.7)
Histopathological subtype	
SSM	2 (13.3)
Nodular	8 (53.3)
Desmoplastic	1 (6.7)
Other	1 (6.7)
NA	3 (20.0)
Ulceration	
Present	6 (40.0)
Absent	7 (46.7)
NA	2 (13.3)
Mitoses	
Median	6
<1/mm ²	3 (20.0)
2-5/mm ²	3 (20.0)
>5/mm ²	7 (46.7)
NA	2 (13.3)
Lymphovascular invasion	
Present	2 (13.3)
Absent	10 (66.7)
NA	3 (20.0)
Microscopic satellites	
Present	3 (20.0)
Absent	10 (66.7)
NA	2 (13.3)
BRAF mutation status	
Positive	3 (20.0)
Negative	8 (53.3)
NA	4 (26.7)
ITM morphology	
Macular / papular (epidermotropic)	0 (0)
Nodular	7 (46.7)
Mixed bulky and nodular	3 (20.0)
Mixed papular and nodular	5 (33.3)
ITM pigmentation	

(Continues)

TABLE 2 (Continued)

Characteristics	Number (%)
Amelanotic	3 (20.0)
Melanotic	2 (13.3)
Mixed	5 (33.3)
NA (subcutaneous)	5 (33.3)
Lesion diameter	
0-5 mm	25 (24.3)
5-10 mm	42 (40.7)
10-20 mm	31 (30.1)
>20 mm	5 (4.9)

BRAF status testing was performed in eleven patients with eight (72.7%) carrying the wild-type allele and three (27.2%) positive for the mutation (V600E/K).

3.3 | Treatment response and disease outcomes

The overall patient best response rates were: CR 33.3%, PR 53.3%, SD 6.7%, and PD 6.7%. The overall response rate of patients was 86.6% (CR + PR) and the clinical benefit was 93.3% (CR + PR + SD) on an intention to treat (ITT) analysis. Individual per lesion responses were also recorded on an ITT basis (refer to Table 3) and included: 66 CR (64.1%), 22 PR (21.3%), 6 SD (5.8%), and 9 PD (8.7%). Lesion diameters and responses were stratified into 0-5.0, 5.01-10.0, 10.01-20.0, and >20.01 mm subgroups with the majority of lesions less than 10 mm in diameter ($n = 67$). In this category, there were 50 complete responses observed in 67 treated lesions (74.6%). The effect of maximum lesion diameter on treatment response is presented in Table 3. Five non-target lesions were treated with PV-10 alone and four developed a complete response, these were all in the 5.01-10.0 mm size category.

The median time to best response was 3.8 months, the median duration of CR 8.1 months, and overall loco-regional progression rate 80%. The median time to in-field recurrence was 1.9 months from the best response (Fig. 1). The median time to develop distant disease was 20 months from the date of in-transit disease. The melanoma-specific mortality rate was 46.7% and the median melanoma-specific survival was 30.6 months from the time of PV-10 treatment. The median melanoma-specific survival was 41.7 months from the date of the primary melanoma diagnosis. From the time of treatment, the 12-, 24-, and 36-month melanoma-specific survival rates were 77%, 54%, and 40%, respectively on the Kaplan-Meier survival analysis.

3.4 | Toxicity

All patients were able to complete the recommended treatments. All patients experienced at least one adverse event during the study (grade 1 or 2), there was one treatment-associated grade 3 event, no grade 4 AEs, and no patient withdrew due to an AE (Table 4). The most common complaint was transient pain at the injection site (87%) followed by localized edema (73%), and blistering (20%). No patients

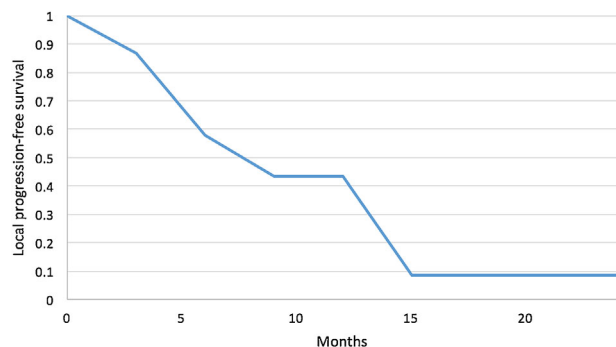


FIGURE 1 Time to local progression from treatment commencement. Kaplan-Meier estimate of patients with local progression-free survival following PV-10 treatment

developed treatment-related phototoxicity or permanent lymphedema. There was one case of cellulitis following radiotherapy that was successfully managed with a short course of antibiotics.

4 | DISCUSSION

Patients with in-transit or recurrent dermal and subcutaneous metastatic melanoma may have a long survival and often pose

management challenges due to the local effects of uncontrolled dermal and subdermal disease, the proliferation of new lesions and the various treatment options available.^{2,14,15} The NCCN guidelines highlight that there is no consensus approach, with treatment guidelines including: surgical excision, local ablation, IL therapy, regional chemotherapy, and systemic therapy.¹⁶ A phase 2 study of IL PV-10 showed durable local control with a high lesion control rate and toxicity predominantly confined to the injection site.¹⁰ Radiotherapy is another well recognized treatment modality in the management of this disease.^{12,17} This study demonstrates that a combined regimen produces high rates of robust lesion control without a clinically significant increase in toxicity. Compared to previous studies with PV-10 alone, combined therapy using PV-10 and XRT may improve clinical outcomes through a reduction in tumor volume associated with a more durable ORR secondary to improved CR and PR.¹⁰

In this study, the overall patient best ORR was 86.6% (where CR 33.3%, PR 53.3%), with the clinical benefit being 93.3% on an ITT analysis. This compares favourably with results from a phase 2 trial where patients were treated with IL PV-10 alone. In the previous study the overall patient best ORR was 51% (CR 26%) on an ITT analysis, while the per lesion ORR was 71% (CR 50%) when all lesions were treated.¹⁰ The ORR of 86.6% is also favorable when compared with other IL therapies such as Bacille Calmette-Guerin (BCG)

TABLE 3 Clinical best response stratified according to maximum lesion size (intention to treat)

Maximum diameter (mm)	Total number	Target lesions	Non-target lesions	Lesion outcome—best response			
				CR	PR	SD	PD
0-5.0	25	25	0	20 (80%)	2 (8%)	2 (8%)	1 (4%)
5.01-10.0	42	38	4	30 (72%)	9 (21%)	2 (5%)	1 (2%)
10.01-20.0	31	30	1	14 (45%)	9 (29%)	1 (3%)	7 (23%)
>20.01	5	5	0	2 (40%)	2 (40%)	1 (20%)	0
Totals	103	98	5	66 (64%)	22 (21%)	6 (6%)	9 (9%)

Lesion diameters and responses were stratified into 0-5.0 mm, 5.01-10.0 mm, 10.01-20.0 and >20.01 mm subgroups. As maximum lesion diameter increased there was a commensurate decrease in the observed complete response rate of treated lesions. The majority of lesions were less than 10 mm in diameter ($n = 67$). In this category, there were 50 complete responses (74.6%) and on bivariate analysis lesions less than 10 mm in maximum diameter were significantly more likely to develop a complete response ($P = 0.005$).

TABLE 4 Adverse events (CTCAE v3.0) at least probably related to PV-10 and radiotherapy treatment

System organ class adverse event-preferred term	CTCAE grade			Total (%) $n = 15$
	1	2	3	
Administration site conditions and general disorders				
Injection site pain	5	7	1	13 (86.7)
Injection site swelling	7	2	0	9 (60.0)
Injection site blistering	1	2	0	3 (20.0)
Injection site erythema	0	3	0	3 (20.0)
Injection site ulceration/bleeding	0	1	0	1 (6.7)
Peripheral lymphedema	1	3	0	4 (26.7)
Cellulitis	1	0	0	1 (6.7)
Nausea	1	0	0	1 (6.7)
Distant edema	0	1	0	1 (6.7)

There were no photosensitivity reactions and no Grade IV or V adverse events.

immunotherapy (ORR 45%) and Talimogene Laherparepvec (T-VEC), a herpes simplex virus type 1-derived oncolytic immunotherapy (ORR 26.4%).^{18,19} The overall response rate obtained in this study was similar to following isolated limb infusion (ORR 75%) and isolated limb perfusion (ORR 90%), accepting that these patients often have more extensive and bulky disease.^{20,21} Objectively, this treatment appeared to produce inferior results in terms of complete response and time to local progression compared to ILP.²¹ Irrespective, compared to PV-10 alone these findings suggest there is a modest advantage in CR and ORR when combining IL PV-10, and radiotherapy for clustered disease.¹¹

The median time to best response in this study was 3.8 months which is consistent with the published literature of IL therapy. The median duration of a complete response was 8.1 months and also compares favourably with other IL therapies.^{19–21} On bivariate analysis lesion size ≤ 10 mm predicted both CR and ORR (*P*-values 0.005 and 0.039, respectively, Fisher's exact test). The CR of 74.6% for target lesions ≤ 10 mm suggests that the combination of IL PV-10 and XRT may be particularly useful in patients with multiple, low volume lesions encompassable in a XRT field.

In the phase II study of IL PV-10 alone the best ORR (CR + PR) was 33% in adjacent non-target, bystander lesions. Patients treated with IL T-VEC had an ORR of 15% in distant visceral metastatic lesions.¹⁹ This indicates that there is a locoregional and systemic immune effect and is consistent with data suggesting that IL PV-10 also activates T-cells responsible for the bystander effect on untreated lesions.^{22,23} In addition to the proposed primary chemoablation of neoplastic cells through lysosomal disruption and rapid autolysis, IL PV-10 may promote a secondary immunomodulatory mechanism that results in a tumor-specific functional immune response.^{24,25}

In keeping with the variable natural history in patients with recurrent loco-regional and metastatic melanoma, the overall mortality rate was 47.7% with the median melanoma-specific survival from the time of primary melanoma diagnosis being 41.7 months. From the time of PV-10 treatment the 12, 24, and 36 month estimated melanoma-specific survival rates were 77%, 54%, and 40%, respectively, with a median melanoma-specific survival length of 30.6 months. Although expected, the survival length of this group (predominately patients with stage III disease) exceeds that of the patients treated on the phase III study of IL T-VEC.¹⁹

The combination of IL PV-10 and radiotherapy was well tolerated with the rate of grade III/IV toxicity being 6.7%. Most patients experienced transient pain at the injection site and localized edema which is consistent with the experience using IL PV-10 alone¹⁰ and similar to other IL therapies.^{18,19} The rate of systemic effects such as fever, chills, nausea, and flu-like symptoms was low and with no patients experiencing any life-threatening AEs, the combination appears safe.

The development of systemic targeted agents and immunotherapies has brought significant improvements in outcomes for patients with metastatic melanoma.^{6–9} Despite the response rates of these agents and impact on overall survival, the data is limited in patients with regional (stage III) disease.^{5,6} Given that patients with in-transit or recurrent dermal and subcutaneous metastases may

experience a long survival length, the significance of local tumor control becomes more relevant. The addition of radiotherapy to IL PV-10 to treat localized clusters of recurrent melanoma is safe, easily administered on an outpatient basis and is an attractive option in this selected cohort of patients. The role of combining this type of therapy with systemic agents is worth assessing in future studies.

5 | CONCLUSION

For patients with localized in-transit or dermal and subcutaneous melanoma metastases the combination of IL PV-10 and XRT resulted in lesion specific, normal tissue sparing, ablation of melanoma tumors. This treatment provided a high response rate however limited duration of complete response. There were tolerable local adverse effects with limited systemic adverse effects. The combination offers another approach when treating patients with this difficult clinical presentation of disease.

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