


Intralesional PV-10 for the treatment of in-transit melanoma metastases—Results of a prospective, non-randomized, single center study

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Background: Patients with in-transit melanoma metastases frequently experience high rates of recurrence, limited overall survival and reduced quality of life. After promising results within a Phase II, multi-center study, PV-10 treatment was continued at our institution for patients with in-transit disease.

Methodology: An open-label, non-randomized, prospective study was performed at the Princess Alexandra Hospital, Queensland, Australia. Patients were treated with PV-10 in accordance with the treatment protocol established during a previous Phase II study. The primary outcome was the complete response of treated lesions.

Results: Forty-five patients were enrolled over a total of 82 treatment episodes from July 2008 to December 2015. With sequential PV-10 treatments the complete response rate was 42% and overall response rate 87% on an intention to treat analysis. The median follow-up duration was 22 months and the median overall survival was 25 months from first PV-10 treatment. Having fewer than 15 metastases at the time of treatment was associated with a complete response ($P = 0.03$).

Conclusions: Intralesional PV-10 provided rapid lesion-specific ablation of melanoma metastases with well-tolerated local effects and minimal systemic adverse events. This therapy should be considered for patients with multiple accessible deposits within the spectrum of low to moderate disease volume.

KEYWORDS

intralesional, in-transit, melanoma, metastases, PV-10, Rose Bengal

1 | BACKGROUND

Patients with in-transit melanoma metastases represent a clinically important group with locoregional recurrence. These patients face significant challenges related to the high rates of recurrence, reduced quality of life and potential to develop systemic disease.^{1–3} For limited

locoregional intralymphatic metastases, when feasible, complete surgical excision remains the recommended treatment. However, various factors such as tumor volume, anatomical location, tissue distribution, and medical comorbidities may restrict patients' suitability for surgery. Consequently, numerous less invasive therapies are now available to the surgical oncologist.^{4–6}

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Recently, immunotherapies including CTLA-4 and PD-1 checkpoint inhibitors and targeted BRAF and MEK signal transduction inhibitors have been utilized for the treatment of advanced disease.^{7–10} While effective, these systemic therapies produce variable responses with a low rate of complete response, have significant documented toxicity profiles, and high health-related costs. The ideal timing for commencement and duration of treatment are also not defined. Multiple directed locoregional therapies have been investigated for the treatment of in-transit disease, and these have been repeatedly reported as safe, relatively efficacious and may improve progression-free survival.¹¹ Such locoregional treatments therefore remain relevant, providing an important intermediate therapeutic option for patients unsuitable for surgery.

Intralesional (IL) therapies involve a high dose of a biologically active agent directly administered to the site of disease. These can provide an effective, well-tolerated and convenient option for the serial treatment of metastases to achieve a sustained reduction in disease burden.¹² 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 been evaluated in both the in vitro and in vivo settings for the treatment of melanoma.^{13,14} PV-10 causes direct tumor lysis, promotes selective lymphocyte-mediated tumor destruction and is associated with a quantifiable local and systemic anti-tumor response, including a T-cell regulated abscopal effect in untreated lesions.^{15–18} PV-10 has been investigated both as a single agent and in combination therapy with radiotherapy.^{14,19}

In a multi-center Phase II trial of 80 patients with Stage III-IV melanoma, PV-10 treatment produced a 51% overall response rate (ORR) and complete response (CR) in 50% when all disease was injected.²⁰ In another case series, IL PV-10 yielded an ORR of 52%, a complete response rate of 26% and bystander response in 50% of patients with non-target lesions.²¹ Based on this clinical evidence, PV-10 treatment was continued at our institution after the completion of the Phase II trial, using the existing trial protocol in a selected cohort of patients.

It was hypothesized that IL PV-10 would provide disease-specific ablation of melanoma metastases without significantly compromising the treatment site and with limited systemic adverse effects. The purpose of this study was to further assess the clinical efficacy, safety profile and treatment outcomes of patients receiving IL PV-10 chemoablation therapy for the treatment of in-transit melanoma metastases. The aim was to identify predictors of treatment response and investigate the association with disease phenotype so that patients could be more suitably selected for PV-10 treatment.

2 | METHODOLOGY

2.1 | Study design

An open-label, non-comparative, non-randomized, prospective study was conducted using IL PV-10 for patients with accessible dermal and subcutaneous in-transit melanoma metastases. Patients were considered for treatment if they were over the age of 18 years, had

histopathologically or cytologically confirmed metastases and measurable lesions >2 mm in diameter. Patients with both (AJCC 7th Ed.) Stage III and IV disease were eligible for treatment provided they had failed or were not suitable for other locoregional therapies and were not better suited to systemic treatments as determined through discussion at a multidisciplinary meeting.

2.2 | Treatment protocol

Treatment under the Medicare Special Access Scheme (SAS) was approved by the appropriate human research ethics committee and hospital executive and all patients provided informed, written consent. All clinically identified in-transit melanoma metastases were designated as “target lesions” then treated using PV-10. There were no “by-stander” or “non-target lesions” in this study. PV-10 was dispensed as a sterile, non-pyrogenic solution of 10% concentration Rose Bengal. Following local anaesthetic infiltration, PV-10 was administered by intralesional injection. A fanning technique was used with a fine gauge needle to uniformly infiltrate target lesions and optimise the intratumoral distribution. The total dosage of PV-10 was limited to 1500 mg (ie, 15 mL of PV-10 solution) and was calculated using a standardized volumetric algorithm developed by Provectus Biopharmaceuticals and previously employed during the Phase II study.

Patients were monitored for immediate post-treatment adverse events (AEs) with additional short-term follow-up on days 1 and 7. Patients were reviewed regularly with clinical visits during weeks 4, 8, and 12 following each PV-10 treatment. These visits included photodocumentation of study lesions, assessment of adverse events, and changes to concurrent medications and other medical conditions. Other clinical evaluation included a physical examination, laboratory tests, evaluation of study lesions by a senior clinician with assessment of the best overall response and radiological surveillance of non-observable regional and visceral lesions in accordance with the treatment protocol. If a complete response was not achieved and patients did not develop progressive disease, further PV-10 therapy was considered 4 weeks after treatment and this therapeutic approach was continued to facilitate multiple, consecutive PV-10 treatments. Following the final PV-10 treatment, patients were assessed regularly at 3 monthly intervals within the outpatient setting until the time of progressive disease or death.

2.3 | Presentation of findings, analysis criteria, and statistics

Patient medical records, including clinical reports, photodocumentation and lesion measurements were independently reviewed, compared and analyzed. Study findings were recorded using a standardized data proforma, entered on to a secure institutional database and the major data points collated within Microsoft Excel and analyzed using STATA v13.0 statistical software. An intention to treat (ITT) statistical analysis was performed including stratification per patient and per treatment episode. The relationships between continuous variables and complete response were assessed using Student's (independent)

t-tests, Mann-Whitney U Tests or univariable (logistic regression) analysis. Variables with dichotomous outcomes were compared using Fisher's exact test, based on the study's small sample size. A Multivariate Logistic Regression analysis was subsequently performed to assess the relative influence of co-variables on complete response. Whenever possible the Response Evaluation Criteria in Solid Tumors (RECIST) were used to assess the response rates.²² Standard thresholds for percentage change in sum of longest diameters were used to define patients' best overall responses as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). All lesions specified at baseline were followed over the course of the study. Adverse events (AEs) were assessed using the Common Terminology for Adverse Events (CTCAE) V3.0.

2.4 | Outcomes

The primary outcome was the complete response rate of target lesions as determined by the senior clinician. This outcome measure was used to assess the treatment effect and thereby provide an indication of the overall clinical utility of serial IL PV-10 therapy. Secondary objectives were the overall response rate (CR + PR), clinical benefit (CR + PR + SD), time to best response, disease-free survival (DFS), progression-free survival (PFS), overall survival (OS) and treatment-related adverse events or toxicity. The best response rates were determined by a senior clinician and trials nurse during clinical review at weeks 4, 8, or 12 following PV-10 treatment. The durable response rate (DRR) was also assessed based on an objective response lasting more than 6 months from the time of first PV-10 treatment. The effect of disease phenotype (including lesion size, number and morphology) on best complete response was also investigated.

3 | RESULTS

3.1 | Patients

A total of 45 patients were enrolled prospectively and treated within a total of 82 treatment episodes between July 2008 and December 2015 (Table 1). The median age was 76 years (range 51-90 years) with a slight male predominance ($n = 27$). All patients had satellite or in-transit melanoma metastases (AJCC 7th Ed. Stage IIIB+) and the most frequent site of disease was the lower limb (84.6%). Two patients had distant non-cutaneous metastases at the time of first PV-10 treatment (Stage IV). Patients were treated with PV-10 after a median number of two other directed locoregional therapies. Twenty-nine patients were re-treated as per protocol 4-12 weeks following the first treatment event. There were a median number of two PV-10 treatments (range 1-4) in this cohort and the median total volume of PV-10 administered per treatment was 2.1 mL (range 0.2-15.0 mL).

3.2 | Melanoma and in-transit characteristics

The characteristics of patients' primary melanoma are presented in Table 2. The median time from primary melanoma diagnosis to develop

TABLE 1 Patient demographics and disease characteristics^a

Characteristics	Number (%)
Gender	
Male	27 (60.0)
Female	18 (40.0)
Age (years)	76.1 (Median)
Anatomical location	
Head and neck	1 (2.2)
Upper limb	3 (6.6)
Lower limb	41 (91.2)
Phenotype	
Macular/papular (epidermotropic)	2 (4.4)
Nodular	20 (44.4)
Bulky disease	1 (2.2)
Mixed	22 (49.0)
Tumour pigmentation	
Amelanotic	13 (28.9)
Melanotic	17 (37.8)
Mixed	6 (13.3)
NA (subcutaneous)	9 (20.0)
Median maximum tumor diameter (mm)	18 [range 4-50]
Median number of lesions treated	6 [range 1-31]
<15 lesions	67
≥15 lesions	15
Median number of PV-10 treatments	2 [range 1-4]
Median total volume of PV-10 per treatment episode (mL)	2.1 mL
Treatment Line	
1st	5 (11.1)
2nd	29 (64.5)
3rd	10 (22.2)
4th	1 (2.2)
Stage (AJCC 7th edition)	
Stage IIIB	21 (46.7)
Stage IIIC	22 (48.9)
Stage IV	2 (4.4)

^aAt the time of first PV-10 treatment.

in-transit disease was 26.7 months (range 1.1-124.4 months). There were a total of 401 lesions in the 45 patients, with a median number of six lesions (range 1-31) at the time of first PV-10 treatment. Overall, the maximum diameters ranged between 7 and 50 mm (median 18 mm). The majority of patients (93.3%) had a nodular component to their lesion morphology type. Twenty-six patients (57.8%) had regional lymph node involvement and of these eight patients (30.1%) had positive regional lymph nodes diagnosed prior to the development of

TABLE 2 Primary tumor characteristics

Characteristics	Number (%)
Breslow thickness (mm)	
Mean	3.00 mm
<1.0	0
1.01-2.0	14 (31.1)
2.01-4.0	16 (35.6)
>4.0	9 (20.0)
NA	6 (13.3)
Clark level	
I and II	0
III	4
IV	28
V	7
NA	6
Histopathological subtype	
Superficial spreading	8 (17.8)
Nodular	10 (22.2)
Desmoplastic/spindle cell	6 (13.3)
Acral lentiginous	3 (6.6)
Other	6 (13.3)
NA	12 (26.8)
Ulceration	
Present	19 (42.2)
Absent	19 (42.2)
NA	7 (15.6)
Mitoses	
Median	4
<1/mm ²	7 (15.6)
2-5/mm ²	13 (28.8)
>5/mm ²	13 (28.8)
NA	12 (26.8)
Lymphovascular invasion	
Present	4 (8.8)
Absent	29 (64.5)
NA	12 (26.7)
Microscopic satellites	
Present	6 (13.3)
Absent	30 (66.7)
NA	9 (20.0)
Tumour stage	
T1b/T2a	7 (15.6)
T2b/T3a	12 (26.7)
T3b/T4a	13 (28.8)
T4b	7 (15.6)
NA	6 (13.3)

(Continues)

TABLE 2 (Continued)

Characteristics	Number (%)
BRAF mutation status	
Positive	2 (4.4)
Negative	23 (51.1)
NA	20 (44.5)

in-transit disease and has previously undergone lymphadenectomy. The median interval from the diagnosis of primary melanoma to the development of metastatic disease within the regional lymph nodes was 29.9 months (0.8-146.8 months). There were 20 patients who developed distant metastases at a median of 41.9 months (range 1.6-126.7 months) from the diagnosis of in-transit disease and 74.3 months (range 14.7-321.6 months) from primary treatment. BRAF status testing was performed on twenty-five patients with twenty-three (92%) carrying the wild-type allele and two (8%) positive for V600E/K mutations.

3.3 | Treatment response and disease outcomes

The median follow-up was 21.9 months from the first PV-10 treatment. Using multiple, consecutive treatments the best overall responses obtained per patient were: 19 CR (42.2%), 20 PR (44.4%), 3 SD (6.7%), and 3 PD (7.7%) with the ORR 86.6% and CB 93.3%. On a per treatment episode analysis ($n = 82$) the best response rates were: CR 30.5%, PR 47.6%, SD 9.8%, and PD 12.1%. The ORR of patients was therefore 78.1% (CR + PR) and the clinical benefit (CB) was 87.9% (CR + PR + SD). The best overall response per treatment episode is presented in Table 3. Within 67 of the treatment episodes (81.7%) there were fewer than 15 lesions treated with PV-10 resulting in 24 complete responses in this category, compared with one CR observed in patients with more than 15 lesions ($P = 0.03$).

The median time from treatment to best response was 1.1 month (range 0.4-27.7 months), while the median time to in-field recurrence was 2.3 months (range 0.8-39.7 months) from the best response. The rates of local PFS at 3-, 6-, 12-, and 24-months were 47.7%, 24.2%, 8.1%, and 5.4% respectively (Kaplan-Meier survival estimate) and the median duration was 2.3 months (mean 5.0 months) (Figure 1). In complete responders, the median DFS was 2.1 months (mean 4.9 months). The median OS was 48.4 months from the diagnosis of in-transit melanoma. During the study, the mortality rate was 48.9% and the median overall survival was 25.1 months from the first PV-10 treatment. The median OS was 66.2 months from the date of the primary melanoma diagnosis. From the time of PV-10 treatment the 12-, 24-, 36-, and 48-month OS rates were 90.4%, 84.8%, 68.1%, and 64.5%, respectively (Kaplan-Meier survival estimate).

3.4 | Exploratory analysis

An exploratory analysis of variables predictive of complete response was performed (Table 4). On univariate and multivariate analysis there

TABLE 3 Patients' best overall response per treatment episode

Overall best response	First treatment	Second treatment	Third treatment	Fourth treatment
Complete response	13	8	3	1
Partial response	24	12	3	-
Stable disease	3	4	1	-
Progressive disease	5	5	-	-
Total	45	29	7	1

was no clear relationship between complete response and patients' gender, age at PV-10 commencement, time to develop in-transit disease, primary melanoma characteristics, lesion morphology, maximum lesion size, or total PV-10 volume per treatment. There was a non-significant trend toward significance for clinical benefit (CR + PR + SD), in younger patients treated with PV-10 ($P = 0.07$) although not for complete response ($P = 0.15$). Blistering or ulceration at the treatment site did not significantly correlate with a complete response ($P = 0.57$). The total number of treatments, total number of lesions and AEs did not significantly influence patients' best overall response on multivariate analysis.

3.5 | Toxicity

Every patient experienced at least one AE during the study (CTCAE grade 1 or 2). There were three treatment-associated grade 3 events—treatment site ulceration, cellulitis, and photosensitivity reaction. There were no grade 4 or 5 AEs and no patient withdrew due to an AE (Table 5). The most common complaint was injection site oedema (62.2%), followed by transient pain (29.3%) and blistering (18.3%) localized to the treatment site. No patients developed treatment-related phototoxicity or permanent lymphoedema. There were two cases of cellulitis that were successfully managed with a short course of oral antibiotics and two photosensitivity reactions that required no active treatment.

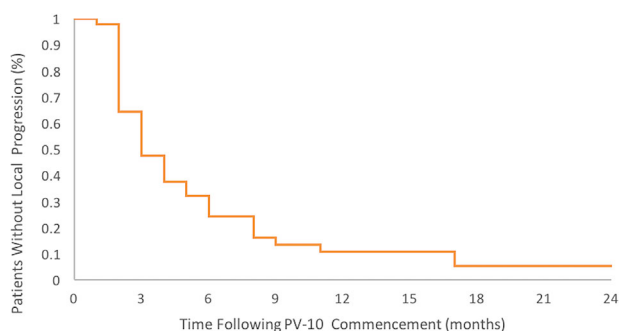


FIGURE 1 Time to local progression following PV-10 commencement. For all patients following the first PV-10 treatment episode and including re-treatments, the 3-, 6-, 12- and 24-month local PFS rates were: 47.7%, 24.2%, 8.1%, and 5.4% respectively (Kaplan-Meier survival estimate)

4 | DISCUSSION

In-transit melanoma is often characterized by a poly-recurrent pattern of disease progression with variable treatment responses and considerable morbidity. Intralesional PV-10 has previously been reported as a successful therapy for patients with stage III and IV melanoma (Supplementary Table S1).^{14,20,21} This prompted further investigation into predictors of treatment response in order to improve patient selection. The current study was conducted to further evaluate the efficacy and safety profile of PV-10 and assess important patient and disease factors correlating with complete response.

In this study, the per treatment episode ORR was 78.1% (where CR 30.5%, PR 47.6%), and the clinical benefit was 87.9%. Thompson et al reported on the outcomes of patients treated using PV-10 with complete and partial response rates of 26% and 25%, respectively on an ITT per lesion analysis. Given a substantial increase in CR to 50% and mean progression-free survival of 9.8 months observed when all disease was injected, it was suggested that treatment response was co-dependent on the untreated disease burden. Consequently, in this study all clinically apparent disease was treated using PV-10 therapy. With multiple, consecutive interventions, the ORR increased to 86.6% (CR 42.2%, PR 44.4%) and the mean PFS duration was 5.0 months. These results complement both the Phase II results (ORR 71% with all treated lesions) and a parallel study where the ORR was 52%.^{20,21} The findings also corroborate the Phase I study results where the complete response rate was 27% and ORR was 55%, with a median follow-up duration of 28 months.¹⁴

We report a cohort of patients with similar demographics and disease characteristics to those previously described.^{20,21} Key features of this patient sub-group include an elderly population with primary melanomas of predominately intermediate Breslow thicknesses, high mitotic count, and without ulceration or lymphovascular invasion. Few patients had melanoma with BRAF mutations (8%) and the majority presented with in-transit disease and developed distant metastases following an extended latency period after their primary melanoma diagnosis (median times 26.7 months and 74.3 months). This low rate of BRAF mutation may be partially accounted for by the older age of patients (median age 76 years) and sampling bias, as only 55% of patients underwent testing, often when they developed systemic disease. Patients experienced long overall survival (median 48.4 months from in-transit diagnosis) and while this likely reflects selection of a less aggressive disease type for PV-10 treatment it may have also been influenced by the availability of checkpoint immunotherapies and targeted systemic therapies.

There also appeared to be a disproportionate quantity of disease involving the lower limbs (84.6%) compared to the expected distributions.²³ Prior to the availability of IL PV-10 at our institution, such patients would have been considered for isolated limb infusion (ILI).²⁴ Throughout the study period there was a trend away from this modality and the proportional use of ILI versus PV-10 decreased from

TABLE 4 Exploratory analysis of variables predictive of complete response (ITT analysis per treatment episode)

Variable	Value	Complete response (CR) [n = 25]	Incomplete response (PR + SD + PD) [n = 57]	P-value
Age	Mean (SD)	72.1 (9.5)	75.5 (9.9)	0.15 ^a
	Median (IQR)	71.5 (66.8-78.5)	77.9 (71.4-82.5)	
Gender	Male	14	34	0.81 ^b
	Female	11	23	
Time from primary to in-transit melanoma	Mean (SD)	29.8 (20.5)	30.6 (30.1)	0.84 ^c
	Median (IQR)	22.7 (17.1-34.3)	24.6 (4.7-37.1)	
Number of lesions	Mean (SD)	6.9 (5.4)	9.7 (7.4)	0.14 ^c
	Median (IQR)	6 (4-9)	7 (4-14.5)	
Number of lesions	<15	24	43	0.03b
	≥15	1	14	
Lesion morphology	Epidermotropic	2	4	0.70 ^b
	Nodular	8	25	
	Bulky (≥20 mm)	0	1	
	Mixed	15	27	
Maximum lesion size (mm)	Mean (SD)	13.4 (8.3)	15.2 (7.4)	0.19 ^c
	Median (IQR)	10.0 (6.5-20)	14.0 (10-19)	
PV-10 volume (mL)	Mean (SD)	3.2 (4.1)	3.4 (3.5)	0.24 ^c
	Median (IQR)	1.6 (0.6-4)	2.2 (1.3-3.95)	
PV-10 treatment site AEs (Blistering or ulceration)	Yes	4	13	0.57 ^b
	No	21	44	

SD, standard deviation IQR; interquartile range.

^aStudent's (Independent) t-test.

^bFisher's Exact test.

^cMann-Whitney-U test.

26% in 2008 to 14% in 2016. This therapeutic strategy involved preferential PV-10 use, as an intermediate treatment option for patients with non-resectable metastases, while ILI was reserved for patients with progressive disease or numerous bulky deposits.

In a separate Phase II study, performed at our institution, a combined regimen of PV-10 followed by XRT for patients with localized metastases yielded an ORR of 86.6% (where CR 33.3%, PR 53.3%) with a mean DFS of 12.2 months.¹⁹ A comparative analysis of the current study's mean DFS (4.9 months) with the results of Foote et al (12.2 months) indicates that the combination of PV-10 and radiotherapy yields a significant benefit in terms of improved DFS ($P = 0.047$). The mechanism underpinning this improved DFS may be radiotherapy-induced destruction of sub-clinical microscopic intra-lymphatic metastases that persist in the region following PV-10 treatment alone. This concept is further supported by the limited local PFS duration of 2.3 months (median) to in-field recurrence in the present study. These outcomes suggest that a combined therapeutic approach should be considered in patients with clustered disease, encompassable by a radiotherapy field, when a complete response with PV-10 alone is not achieved.

While the ORR of 78% in this series is favorable when compared with other intralesional therapies such as T-VEC (26%), Bacille

Calmette-Guerin (BCG) (45%), and interleukin-2 (82%), the short duration of effect necessitated multiple treatment episodes.²⁵⁻²⁷ In this study the durable response rate was 21.6% and this is promising when benchmarked against the DRR of 16.1% reported using T-VEC therapy.²⁶ Here, the ORR obtained using sequential PV-10 treatments was similar to those reported for isolated limb infusion and hyperthermic isolated limb perfusion (75% and 90%, respectively), while recognizing that such therapies are frequently employed in the setting of more advanced disease burden.²⁸⁻³⁰ Advantages of PV-10 therapy compared with other intralesional agents include the robust response rates, low side effect profile and reduced treatment frequency.

In keeping with the variable natural history of locoregionally recurrent disease, the overall mortality was 48.9% and there was a 75.6% rate of locoregional progression following each treatment episode. Despite a modest survival advantage reported for certain patient sub-groups, there is currently no substantial evidence that directed locoregional therapies extend melanoma-specific survival.^{30,31} This study further supports this observation with a median OS time of 25 months following the first PV-10 treatment. The relative survival lengths of patients in this study, despite high locoregional recurrence rates and short PFS intervals underscore the need for

TABLE 5 Adverse events (CTCAE v3.0) related to PV-10 treatment

Adverse event—preferred term	CTCAE grade			Total (%) N = 82
	1	2	3	
Administration site conditions and general disorders				
Injection site/peripheral oedema	28	23	0	51 (62.2)
Injection site pain	17	7	0	24 (29.3)
Injection site blistering	10	5	0	15 (18.3)
Injection site erythema	9	1	0	10 (12.2)
Injection site ulceration	0	1	1	2 (2.4)
Injection site pruritis	1	0	0	1 (1.2)
Cellulitis	1	0	1	2 (2.4)
Photosensitivity reaction	1	0	1	2 (2.4)
Distant oedema	0	1	0	1 (1.2)

N.B. There was one Grade IV adverse event: A DVT involving left brachial and axillary vein following PV-10 administration to the left forearm. This complication was managed with anticoagulation therapy and regular clinical surveillance.

further in vivo investigation of the “functional immune response” described with PV-10 treatment.^{1,32}

On bivariate analysis, fewer than 15 lesions at the time of treatment was a predictor of a complete response ($P = 0.03$, Fisher's exact test, adjusting for treatment clustering). The CR of 35.8% per treatment episode in this group suggests that sequential PV-10 treatment may be particularly useful for patients with accessible metastases and low to moderate disease volume. Other predictors of complete response such as >0.2 mL PV-10 per lesion, bystander lesion response, younger age and blistering have also been described (Supplementary Table S1).^{14,19–21} Outcomes stratified by lesion morphology and maximum size were not significant in this study, although other data suggest that low volume metastases, particularly individual lesion diameters <10 mm are significantly associated with complete response.^{19,20} Here, lesion morphology as a covariate may have been confounded by recruitment given that most patients selected for treatment had nodular lesions.

Overall, PV-10 treatment was well tolerated with a predominately locally limited side-effect profile consistent with the reported literature.^{19–21} Despite multiple retreatment episodes there was a low rate (4.9%) of grade III/IV toxicity. While most patients developed swelling and transient pain at the injection site (CTCAE grades 1 or 2), in contrast to previous data, there was no correlation between blistering, ulceration or treatment severity and complete response.¹⁹ The toxicity profile of PV-10 treatment is low compared with more invasive and potentially toxic therapies such as ILI or ipilimumab.^{7,28} The PV-10 treatment regimen also involved fewer treatment episodes and there is a significant reduction in treatment-related costs compared with systemic agents used for advanced disease.

Recent studies have focused on describing the functional immune response associated with PV-10 related tumor destruction.^{15–18} In vivo evidence demonstrates CD8+ T lymphocyte

mediated tumor regression with melanoma-specific dendritic cell activation and the induction of a systemic anti-tumor immune response.^{17,32} Further translational studies are required to quantify the immune-provocative effects of PV-10 and investigate the long-term clinical significance. Combining PV-10 and systemic immunotherapies may produce additive or synergistic effects and this should be evaluated within further clinical trials.¹⁵ Despite the success of systemic therapies the utility of these treatments in patients with stage III disease is restricted by available data and cost. Considering patients with in-transit disease may experience prolonged overall survival, directed locoregional therapies therefore remain relevant.

Limitations of this study include a selected cohort of predominately elderly patients with co-morbidities and disease rendering them unsuitable for surgery. Within the broader context, the treatment regimens were individualized in terms of the treatment frequency and this may reduce reproducibility. The provision for early retreatment (from week 4) may have also influenced the accuracy of calculating the time to best response and response rates. Considering the median times to best response (1.1 month) and in-field recurrence (2.3 months), it seems sensible to wait 12 weeks post-intervention to retreat. Based on these results, by this time point most patients should have experienced a response and may benefit from further therapy prior to in-field or locoregional progression. At the study's commencement, RECIST had not been formally revised to include specifications for assessing in-transit disease. Ergo, while RECIST was used as a reference, the best response was determined clinically by the senior treating clinician. Ultrasound or other imaging modalities could have been employed to more objectively measure the response of subcutaneous or distant metastases. Finally, in the future studies priority should be placed on changes in progression-free and disease-free survival while objectively determining the effect of treatment on patients' health-related quality of life.

5 | CONCLUSIONS

PV-10 provided rapid lesion-specific ablation of melanoma metastases with reasonable response rates. Serial treatment using PV-10 alone produced satisfactory overall response rates without a commensurate increase in toxicity. The results of this study further substantiate previous reports that PV-10 is a relatively non-invasive, well-tolerated and efficacious therapy for multiple accessible in-transit melanoma deposits. Synthesising these findings with those of other studies, ideally patients with fewer than 15 metastases less than 10 mm in diameter should be selected for PV-10 treatment. Other factors including PV-10 volume >0.2 mL per lesion, injecting all accessible metastases and a robust early treatment response also appear to portend better treatment outcomes.

ETHICS AND CONSENT

The submitted study was performed in accordance with appropriate ethical standards. procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

CONFLICTS OF INTEREST

The investigational agent PV-10 was provided free of commercial charge by Provectus Biopharmaceuticals. Dr Tavis Read was the recipient of a Junior Research Fellowship funded by the Queensland Government. The preliminary results of this study were presented on 3rd May 2016 at the Royal Australasian College of Surgeons' Annual Scientific Congress held in Brisbane, Australia.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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