

Chemoablation of metastatic melanoma using intralesional Rose Bengal

John F. Thompson^{a,b}, Peter Hersey^{a,c} and Eric Wachter^d

To study the effects of intralesional Rose Bengal for chemoablation of metastatic melanoma. Twenty-six target lesions in 11 patients with locoregionally recurrent disease were injected with the agent PV-10 (10% w/v Rose Bengal in saline) at a dose of 0.5 ml/cc lesion volume. An additional 28 untreated lesions were observed for potential bystander effect. The treatment was well tolerated and an objective response was observed in 12 target lesions, with an additional seven lesions remaining static over at least 12 weeks of observation. In this dose-evaluation study, response rate was dose dependent, increasing to 69% after higher dose injections. Nontarget lesions exhibited a 27% objective response rate that increased to 44% in patients exhibiting a positive response of target lesions. These findings indicate that intralesional Rose Bengal is nontoxic and could benefit patients with metastatic

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^aSydney Melanoma Unit, Sydney Cancer Centre, Royal Prince Alfred Hospital, Camperdown, ^bDiscipline of Surgery, The University of Sydney, Sydney, ^cNewcastle Melanoma Unit, Newcastle Misericordiae Hospital, Newcastle, New South Wales, Australia and ^dProvectus Pharmaceuticals, Inc., Knoxville, Tennessee, USA

Correspondence to Professor John F. Thompson, MD, FRACS, FACS, Sydney Melanoma Unit, Sydney Cancer Centre, Gloucester House, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia
Tel: +61 2 9515 7185; fax: +61 2 9550 6316;
e-mail: john.thompson@smu.org.au

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Introduction

The incidence of cutaneous melanoma has steadily risen by 3–8% annually over several decades and now represents a lifetime risk of one in 53 for men in the United States and one in 25 for men in Australia [1]. Disease staging is predicated on tumour thickness, followed by regional nodal metastasis and finally by the presence of distant metastases [2]. The American Joint Committee on Cancer stage III metastatic melanoma is characterized by the existence of one or more nodal, intransit or satellite metastases, whereas stage IV disease is characterized by the existence of one or more distant metastases [3,4]. Five-year survival for stage III patients ranges from approximately 24 (gross nodal disease) to 70% (microscopic nodal disease); survival drops to approximately 10% for stage IV patients [2].

Standard treatment of primary lesions is wide area excision, whereas lymphadenectomy is applicable for accurate staging and treatment of regional nodal metastases. Regional perfusion or infusion of melphalan or other chemotherapeutic agents may be used to treat regional limb metastases [5], whereas systemic chemotherapy, radiation therapy and palliative surgery are the most common approaches for management of advanced metastatic disease [1]. The observation that melanoma may be susceptible to immunologic response has led to efforts to develop autologous and allogeneic vaccines [6–8] as well as in-situ treatment methods capable of stimulating an antitumour immune response [9–11].

Rose Bengal (RB) disodium is a well-known intravenous diagnostic agent that undergoes rapid hepatic excretion in unmetabolized form [12,13], but in a new formulation it may also be used for chemoablation of localized tumours. Ten percent (w/v) RB in saline (PV-10) is a sterile, nonpyrogenic saline solution of RB suitable for intralesional (IL) administration. In-vitro and in-vivo studies have shown that RB exhibits preferential uptake into lysosomes of cancer cells but is excluded from normal cells, resulting in selective necrosis in multiple melanoma lines (including MM200, Igr3, SK-Mel-28 and Me 4405) [14,15]. Ablation of melanoma tumours from several cell lines (A375 and B16F10) in murine models demonstrate the potential of PV-10 to elicit selective tumour ablation with minimal adverse effects. Localized increases in mononuclear tumour-infiltrating lymphocytes suggest that tumour necrosis releases antigens to nearby antigen-presenting cells, facilitating presentation of antigenic targets to T-cells and B-cells. Collateral destruction of granulocytes observed at the margins may precipitate chemokine release and local inflammation, potentially serving an adjuvant role in the promotion of a specific antitumour response. Such response has been observed in murine hepatocellular carcinoma homografts, where ablation of injected target tumours correlates closely with spontaneous rejection of untreated nontarget tumours. These features demonstrating the potential of PV-10 to elicit selective tumour ablation and systemic bystander response with minimal adverse effects motivated us to investigate the therapeutic potential of PV-10 in patients with stage III metastatic melanoma.

Materials and methods

Patients

The trial was conducted under the auspices of the Therapeutic Goods Administration (Australia), Clinical Trials Notification Scheme and an Investigational New Drug Application with the US Food and Drug Administration, and the study protocol and informed consent forms were reviewed and approved by local ethics committees. Written informed consent was obtained from each patient before study participation. Patients with Eastern Cooperative Oncology Group performance status 0–2, a life expectancy of at least 6 months, and at least two histologically or cytologically confirmed measurable melanoma lesions (American Joint Committee on Cancer stage III, each target lesion ≤ 3 cm in maximum diameter) were eligible for participation. All patients had normal haematopoietic, renal, hepatic and thyroid function. Patients receiving radiation therapy, chemotherapy, local treatment or other investigational agents within 4 weeks of study participation, or an antitumour vaccine within 12 weeks, were excluded, as were patients with acute concurrent illness. Premenopausal females who were pregnant, lactating, or not using contraception were also excluded.

Clinical protocol

Eleven patients were enrolled in the study and each received a single IL injection of PV-10 into one to three target lesions at a dose of 0.5 ml/cc lesion volume (V_L) during a single treatment session. V_L was calculated according to the equation $V_L = (\text{length} \times \text{width} \times \text{height}) / 2$, where length, width and height are maximum lesion dimensions in centimetres. Alternatively, for lesions where height could not be accurately determined, V_L was calculated according to the equation $V_L = 4/3\pi r^3$, where r is maximum radius. A 25-gauge needle was used for injection of large lesions, otherwise a 26-gauge or 30-gauge needle on a tuberculin syringe was used to allow precise injection with minimal leakage from puncture sites when the needle was withdrawn. Injection was performed using a fanning technique to slowly and uniformly infiltrate the lesion with PV-10 as the needle tip was inserted to the margin, then withdrawn along multiple tracks. In addition, one to three nontarget lesions were designated at screening and remained untreated in each patient to assess potential bystander effect.

Patients were monitored for locoregional or systemic adverse effects and study lesions were followed for at least 12 weeks after PV-10 injection to assess objective response. Long-term outcomes were determined using follow-up data from the Sydney Melanoma Unit and Newcastle Melanoma Unit databases.

Statistical analysis

All patients were included in the safety and response analyses. Descriptive statistics were used to tabulate the

incidence, frequency and severity of all adverse events relative to their relationship with the study treatment. Changes in vital signs and laboratory test values after treatment were compared with pretreatment baseline levels. Descriptive statistics were also used to describe objective response rate (ORR) of treated and nontreated study lesions. Response rate data were analysed using the χ^2 and Fisher's exact tests. Potential correlation between various response parameters was examined using the Pearson's product moment correlation method. Laboratory and vital signs data were analysed using the Kruskal–Wallis one-way analysis of variance on ranks method. In addition, the paired t -test was used to analyse safety data for changes at each follow-up interval versus baseline for those data exhibiting normal distribution. For those data of nonnormal distribution, the Wilcoxon signed rank test was used. Long-term outcome data were analysed using the Kaplan–Meier survival method. The statistical power of each test was the same ($\alpha = 0.050$).

Results and discussion

All 11 patients (with 26 target lesions and 28 nontarget lesions in total, median age 83 years, range 75–86 years) received PV-10 injections into their target lesions (median V_L is 0.29 cm³, range 0.02–12.8 cm³) at single treatment sessions. Ten patients had locoregionally recurrent disease confined to a single lower extremity whereas the 11th patient had locoregionally recurrent disease of the head and neck. The mean dose of PV-10 per patient was 1.6 ml (160 mg RB) with a range of 0.11–4.5 ml (11–450 mg RB) whereas the mean dose injected into an individual lesion was 0.66 ml (range 0.01–3.8 ml). Patient characteristics, response to treatment and outcome are summarized in Table 1.

In almost all lesions the injected dose of PV-10 seemed to be largely retained within the lesion, with minimal leakage occurring from the needle puncture site. In some cases there was visible evidence of diffuse migration of PV-10 to surrounding tissue within several minutes after injection. In general, lesions were very deeply stained and exhibited high levels of retained red dye for up to several weeks (Fig. 1, a2 and a3).

Adverse effects

PV-10 administration was well tolerated in all patients, with no serious adverse events observed in the study cohort. No treatment discontinuations were observed and all patients completed a minimum of 12-week post-treatment evaluation (mean follow-up 15.4 \pm 1.5 weeks). The most common adverse effects were transient mild-to-moderate pain at the treatment site (reported by eight patients), local inflammation around the treatment site (observed in four patients) and treatment site pruritus (reported by three patients). One patient experienced a mild photosensitivity reaction in the treated limb after

Table 1 Patient characteristics, response to treatment and outcome

Study lesion location, initial size (maximum diameter, millimetre) and outcome at the end of study interval								
Patient number, age (years) and sex	Target lesions			Nontarget lesions			Subsequent history	Current status
	Location	Size	Outcome	Location	Size	Outcome		
0001, 75 F	Right thigh	13.0	CR	Right thigh	15.0	SD	Ex ITM+DTIC; SD	Alive at 30 months
	Right lower leg	16.0	CR	Right thigh	10.0	PD		
	Right lower leg	20.0	CR	Right lower leg	17.0	SD		
0002, 77 F	Left lower leg	10.1	SD	Left lower leg	7.4	CR	Regional node Ex (1+); NSR	Alive at 34 months
	Left lower leg	7.2	PR	Left lower leg	8.1	PR		
0003, 82 F	Left lower leg	12.7	CR	Left lower leg	6.4	PR	ILI; died with MM (SD)	Dead at 12 months
	Left midleg	8.9	PD	Left upper leg	7.7	PD		
	Left lower leg	7.8	PD	Left thigh	9.5	PD		
0004, 84 F	Left thigh	7.3	PD	Left thigh	5.7	PD	Ex ITM; ILI; XRT; death from MM (PD)	Dead at 9 months
	Left mid leg	6.2	PD	Left thigh	5.2	PD		
0005, 83 F	Left midleg	6.1	PD	Left midleg	5.1	PD	Ex ITM; NSR	Alive at 31 months
	Left thigh	9.5	SD	Left midleg	4.1	SD		
	Left lower leg	23.1	PR	Left lower leg	5.7	SD		
0006, 78 F	Left lower leg	9.2	CR	Left foot	6.8	PD	Ex ITM; ILI; SD	Alive at 27 months
	Right midleg	18.0	SD	Right midleg	7.8	PD		
	Right lower leg	20.0	SD	Right lower leg	6.5	ND		
0007, 83 M	Right lower leg	6.0	ND	Right lower leg	3.6	ND	Ex ITM; ILI; death from MM (PD)	Dead at 8 months
	Right knee	6.0	SD	Right knee	9.6	SD		
	Right knee	6.0	PD	Right lower leg	6.7	PD		
0008, 76 M	Right lower leg	16.5	SD	Right lower leg	8.8	PD	Ex ITM; PD	Alive at 28 months
	Right knee	26.8	SD	Right knee	15.4	SD		
	-	-	-	-	-	-		
0009, 84 F	-	-	-	-	-	-	SD	Alive at 29 months
	Left knee	22.0	CR	Left lower leg	45.0	SD		
	Left foot	29.0	CR	Left lower leg	13.0	SD		
0010, 84 M	-	-	-	-	-	-	Death from MM (PD)	Dead at 9 months
	Right midleg	6.2	CR	Right thigh	8.0	PD		
	Right midleg	6.6	PR	Right midleg	6.6	PR		
0109, 86 M	-	-	-	-	-	-	NSR	Alive at 28 months
	Left cheek	17.0	CR	Left ear	36.0	CR		
	-	-	-	Left upper neck	27.0	CR		
	-	-	-	Left lower neck	9.0	CR		

DTIC, dacarbazine; Ex, excision; F, female; ILI, isolated limb infusion; ITM, intransit metastasis; M, male; MM, metastatic melanoma; ND, not done; NSR, no sign of recurrence; 1+, one positive node; PD, progressive disease; SD, stable disease; XRT, radiation therapy.

exposure to sunlight several days after treatment. Only one systemic adverse event was reported (mild insomnia secondary to injection site pain). No clinically significant changes or abnormalities in vital signs, haematology values or blood chemistry, including those for thyroid function were observed.

Clinical response

Of the 26 target lesions treated with PV-10, 25 were evaluable at final follow up. Nine target lesions (36%) showed complete response (CR) based on the Response Evaluation Criteria in Solid Tumors criteria (i.e. disappearance of lesion, histologically negative residual mass, or negative contrast-enhanced computed tomography scan at final follow up) [16], three (12%) showed partial response (PR), seven (28%) showed stable disease and six (24%) showed progressive disease, for an ORR of

48%. A similar rate was noted for overall response analysed per patient [55% ORR based on 27% CR, 27% PR, 27% stable disease (SD) and 18% progressive disease (PD)].

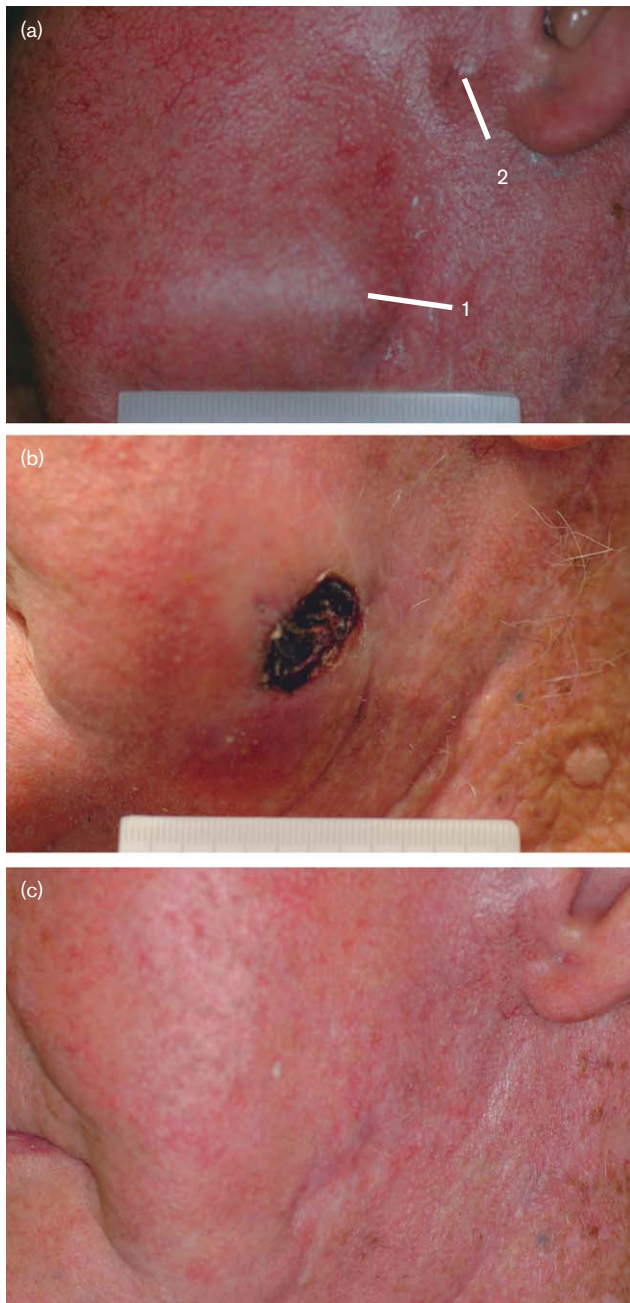
Statistically significant differences in response rate were noted for target lesions receiving 0.2 ml or greater doses of PV-10 (13 lesions; 69% ORR consisting of 62% CR, 8% PR, 31% SD and 0% PD) relative to that of smaller lesions receiving lower doses (12 lesions; 25% ORR consisting of 8% CR, 17% PR, 25% SD and 50% PD). This differential response suggests a threshold dose is necessary to establish an adequate IL depot of PV-10 for effective chemoablation.

Of 28 nontarget lesions assessed at screening, 26 were evaluable at final follow-up. Nontarget lesions exhibited an individual ORR of 27% (15% CR, 12% PR, 31% SD and 42% PD) that when analysed on a per patient basis also

Fig. 1



Response in cutaneous lesions of a lower extremity. a1, b1 and c1: pretreatment photographs showing target (a1 and b1) and nontarget (c1) lesions immediately before injection with PV-10 (10% w/v Rose Bengal in saline) into lesions a1 and b1. Scale gradations 1 mm. a2–c2: response at day 7 postinjection. a3–c3: response at week 4. a4–c4: response at week 12. On the basis of lesion measurements at week 12, a4 graded complete response (CR), b4 graded CR and c4 graded stable disease. Patient 0009 ultimately achieved long-term stable disease.

Fig. 2

Response in head and neck lesions. (a) Pretreatment photograph showing locations of (1) subcutaneous target lesion (1.7 cm maximum diameter) and (2) nontarget lesion (3.6 cm maximum diameter). Two additional nontarget lesions were located in neck (below bottom edge of image). Scale gradations 1 mm. (b, c) Responses at weeks 4 and 27, respectively. Patient 0109 exhibited no evidence of disease on computed tomography or clinical examination at month 10 and has remained disease free for 28 months.

equalled 27% (9% CR, 18% PR, 45% SD and 27% PD). A strong positive correlation was noted between response of target and nontarget lesions, consistent with a possible bystander effect (correlation coefficient 0.803, $P = 0.003$,

$N = 11$). Notably, patients experiencing a positive objective response of target lesions exhibited a significantly higher rate of response in their nontarget lesions (44% ORR) than those with a negative target lesion response (0%).

Lesions exhibiting CR or PR were characterized by an early onset of localized coagulative necrosis progressing to eschar within several days of injection. This was followed by gradual involution of the remaining tumour mass that appeared to continue beyond 12 weeks, with progressive improvement noted during longer-term follow-up in several patients (Figs 1 and 2). This suggests that extended follow up could result in an increased ORR over that observed with the current cohort. In contrast, lesions exhibiting disease progression demonstrated marked growth by 8 weeks, providing a clear basis for planning potential retreatment or alternative therapy of non-responsive lesions.

Long-term outcome

At the time of preparation of this report, seven of the 11 patients (64%) enrolled in the trial remained alive, with three deaths attributed to underlying disease progression. These data are summarized in Table 1. The mean survival time for all patients was estimated at 27.3 ± 4.2 months according to the Kaplan–Meier analysis. For those patients who had died from disease progression, the median survival period until death was 8.8 months. The median follow-up time for those still alive was 29.1 months. These durations are likely to increase, possibly by substantial amounts, since the majority of patients remained alive at their most recent follow up. Eight patients required one or more subsequent procedures to treat their metastatic disease, with seven patients achieving an overall status of SD (four patients) or no evidence of recurrence (three patients) at their most recent follow up.

Comparison with other intralesional therapies

The preliminary efficacy and side-effect results from single IL treatment sessions with PV-10 compare favourably with those of other IL regimens for melanoma. Localized ablation of melanoma in similar patient populations using IL cisplatin [11] or IL cisplatin with electroporation [17] has yielded superficially comparable results in treated lesions. For example, Serša *et al.* [17] reported a 38% ORR (19% CR + 19% PR) after a single electrochemotherapy treatment of 82 small lesions (median V_L 0.06 cm^3) in 10 patients whereas Oratz *et al.* [11] reported 53% ORR (47% CR + 7% PR) after a median of five treatments each of 244 small lesions (median V_L is 0.02 cm^3) in 25 patients. Although, the authors did not provide sufficient detail to quantitatively compare effectiveness in larger tumours, the ORR reported by Oratz *et al.* decreased from 53% for lesions of median diameter 0.6 cm to 44% for a subgroup having a

median diameter 3.0 cm. In neither study was a significant effect on nontreated lesions reported.

Byrne *et al.* [18] and Gaudy *et al.* [19] reported substantial local efficacy in cutaneous lesions upon a single electrochemotherapy treatment with IL bleomycin (72% CR + 5% PR in 54 treated lesions in 19 patients and 36% CR + 10% PR in 30 treated lesions in 12 patients, respectively, with mean diameters of 1.0 ± 1.0 cm, range 0.3–5.0 cm and 1.1 ± 0.5 cm, range 0.3–2.6 cm, respectively). As with cisplatin, response was generally reduced in larger tumours, and no effect was reported for nontreated lesions.

IL interferons (IFN) have yielded mixed results; with von Wussow *et al.* [20] reporting a 45% ORR (31% CR + 14% PR) in injected lesions and a transient response in nontreated lesions (18% ORR for 2–18 months) in 51 patients receiving INF- α three times weekly. This regimen produced significant toxicity, with 95% of patients reporting flu-like symptoms (fever, headache, chills and myalgia) and 26% experiencing dose-limiting toxicity (lethargy, fatigue). Shorter-term regimens with IL IFN- γ produced no evidence of efficacy in a study by Nemunaitis *et al.* [21] (13 patients administered IFN- γ daily for 5 days), whereas Khorana *et al.* [22] observed only transient responses in 11 patients receiving IFN- γ weekly, for 3 weeks, with 10 patients experiencing disease progression and one achieving SD after 5 weeks follow-up. Side effects were comparable with those for IFN- α .

Radny *et al.* [23] reported an objective response in 83% of patients (62% CR + 21% PR) receiving 2–3 weekly IL treatments with interleukin-2, including complete response in 209 of 245 treated lesions (85% CR). Repetitive treatment of the 24 patients continued over 2–57 weeks (mean treatment interval 11.5 ± 14.4 weeks) and produced flu-like symptoms in 58% of patients. Green *et al.* [24] reported a similar outcome in 13 patients treated with topical imiquimod and interleukin-2 injected one to three times weekly (41% CR + 10% PR among treated lesions). The authors noted that although new lesions did appear during the treatment course, some patients experienced a marked slowing of the appearance of new cutaneous lesions.

IL allovectin-7 (an HLA-B7/ β 2-microglobulin DNA-liposome complex) yielded 60% PR in 10 patients after a single injection by Waddill *et al.* [10], but Stopeck *et al.* [25] achieved only 8% ORR (one CR + three PR) in 52 patients with large lesions (≥ 1 cm and $\leq 5 \times 5$ cm) administered six IL injections over 9 weeks; two patients in this cohort achieved an overall PR that the authors concluded could be evidence of a systemic response. In a multicentre phase 2 study of 77 advanced patients

(92% stage IV disease at entry, 57% having cutaneous/nodal disease only), however, Gonzalez *et al.* [26] reported 9.1% ORR (two CR + five PR having a duration of 4 months or longer) for allovectin-7 administered in up to three cycles of six weekly IL doses, with a median duration of response of 4.8 months in patients responding to treatment.

In one of the earliest reports on this topic, Storm *et al.* [9] reported transient responses in patients receiving IL Bacille Calmette Guerin (BCG) every 2–3 weeks (median of four treatments, range 2–14 treatments), with 10 patients achieving CR (mean duration 20 months) and 10 PR (mean duration 11 months). In a later review of data from 15 trials of IL Bacille Calmette Guerin, Tan and Ho [27] found 19% CR and 26% PR, with extended survival in 13% of stage III patients.

In contrast to the IL treatments outlined above, a single treatment with PV-10 resulted in successful chemoablation in 48% of injected tumours (having a median volume of 0.29 cm^3 , approximately 10-fold greater than that of Serša or Oratz), and approached 70% (62% CR + 8% PR) in larger lesions receiving 0.2 ml or greater doses of PV-10. This robust response was associated with generally mild and transient adverse effects. Perhaps most interestingly, PV-10 chemoablation resulted in an apparent bystander effect in untreated tumours in 27% of patients (consisting of one instance each of patients achieving a CR or PR in their untreated tumours in the group of six patients who achieved an objective response in their injected target lesions, along with one PR in a patient achieving SD in her target lesions). Such results, if proved reproducible, could comprise a significant advantage of PV-10 chemoablation over these other local therapies in terms of combined potential for local effectiveness, generally mild adverse effect profile and systemic benefit. The apparent safety of PV-10 treatment supports potential use of additional rounds of therapy in patients failing to respond fully to a single treatment, which could further increase efficacy.

Conclusion

PV-10 treatment was easily administered and well tolerated, eliciting a robust response in a majority of patients. The benign local and systemic adverse effect profile observed in this trial is consistent with earlier human experience with RB [12,13] and preclinical observations with PV-10, [14] and supports safety of retreatment of nonresponsive or partially responsive lesions to maximize ORR and long-term patient outcome. Further study is under way to assess the role of treatment of larger numbers of lesions (up to 20 per patient) and retreatment of new or PR lesions as an approach for potential locoregional control of metastatic disease. It is

expected that the results will also help to elucidate the bystander effect observed in this initial study cohort.

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