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The mission of this magazine is to transform the expanding information on cancer research into understandable language to the curious and the informed.



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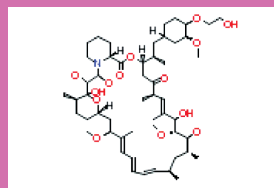
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Glossary



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## Metastatic Melanoma: A Murine Study Confirming an Immune Response with PV-10

*Why would a small animal study attract the attention of key metastatic melanoma researchers? Why would a xanthene dye originally developed to color wool fibers an intense shade of red cause tumors to regress even though they have not directly been treated with it?*

The questions are intertwined, and several threads of the Rose Bengal story lead to research presented at the 65<sup>th</sup> Annual Cancer Symposium of the

Society for Surgical Oncology (SSO) recently in Orlando, FL.

First granted a patent in 1885 as part of a new family of wool dyes combining halogens with fluorescein, Rose Bengal found its first medical use in 1914 when adding it to another dye, Victoria Yellow, was shown to fight pneumococcal infections (Feenstra RPG, Tseng CG. *Arch Ophthalmol* 1992; 110:984-993). Subsequently it was used

mostly for staining, as an intravenous assay for impaired liver function, and as a food dye.

A little noted 1980s Japanese study revealed, contrary to its intention of finding tumorigenicity for the food dye, a dose-dependent survival increase (Ito A, *et al. J Natl Cancer Inst* 1986 Jul; 77(1): 277-281). A 1990s literature search by Provectus scientists looking for a laser-activated photodynamic therapy agent

with antineoplastic activity turned up Rose Bengal as a possible candidate.

Provectus researchers were exploring intralesional therapy, which had been pioneered in the 1970s through BCG therapy (Mastrangelo MJ, Bellet RE, Berkelhammer J, *et al. Cancer* 1975; 36:1305-1308). Research into therapy with Bacille Calmette-Guérin (BCG) had suggested in addition to effective local ablation, induction of systemic host immune anti-tumor activity in regional and distant uninjected metastases through a systemic adjuvant response. Severe adverse events, however, have limited enthusiasm for BCG.

High rates of recurrence and metastasis among significant percentages of patients with locally advanced melanoma, despite locoregional therapy with surgery and/or radiation have kept interest in intralesional therapy alive.

After Provectus scientists modified Rose Bengal and subjected it to further pre-clinical testing, intralesional injections of PV-10 in Phase I research among 20 patients with stage III-IV melanoma produced promising results (locoregional control in 75% of lesions directly injected and 55% in non-injected “bystander” lesions). In Phase II research among 80 patients, locoregional control of injected lesions was achieved with PV-10 in 71% of patients, and locoregional control of bystander lesions was achieved in 55% (21/38). Regression of bystander lesions strongly correlated with response in target lesions.

Still, the traverse from the finding of an apparent association with a strategy such as intralesional treatment with PV-10 to then showing remission of bystander lesions to be an actual effect of PV-10 treatment can be long. It requires a plausible and testable mechanism of action hypothesis. In the specific case of PV-10, it required going back to the laboratory and conducting animal studies.

The regression of tumors injected directly with PV-10, explained Paul Toomey, MD, H. Lee Moffitt Cancer Center & Research Institute, Tampa,

FL, at a presentation of his research poster at the 65<sup>th</sup> Society of Surgical Oncology 2012 Annual Symposium in March (*Ann Surg Oncol* 2012; 19(1): S125) ensues when PV-10 accumulates in the cell membrane of cancer cells. It subsequently enters lysosomes, leading to acute lysosomal destruction.

Merrick Ross, MD, professor of surgery at MD Anderson Cancer Center, Houston, TX, in an interview at the recent HemOnc Melanoma and Cutaneous Malignancies Symposium in New York City, noted further that while PV-10 is excluded from normal cells, its accumulation in the lysosomal membrane triggers lysosomal release and complete autophagy of tumor cells relatively quickly—within 30-60 minutes of the injection. Importantly, this acute necrosis of the treated tumor does not denature tumor antigens, allowing acute exposure of antigenic tumor fragments to antigen-presenting cells.

To find evidence for a specific trigger to an immune system anti-tumor response to administration of PV-10, despite having already achieved impressive rates of locoregional control in humans in both injected and in “bystander” lesions (including remote visceral lesions) in Phase I and II research, investigators headed back to the lab to do more animal studies.

Dr. Toomey and colleagues undertook two murine studies. In the first, B16-F10 melanoma cells were injected subcutaneously (s.c.) and administered intravenously (i.v.) to establish a solitary flank tumor and multiple lung metastases. On day 7, the s.c. tumor was treated with intralesional PV-10 or intralesional phosphate buffered saline (PBS).

In the second study, after B16-F10 cells were implanted in bilateral flanks, the resulting right tumor was injected with PV-10 or PBS on day 7. Twenty-one days after treatment with intralesional PV-10, in the mice bearing a solitary flank tumor with lung metastases, s.c. tumor size decreased significantly ( $p < 0.05$ ).

After 25 days, mice with bilateral flank tumors had significant regression of the

tumors injected with PV-10 ( $p < 0.05$ ). Also in the mice treated with PV-10, the size of the untreated bystander flank tumor declined nonsignificantly ( $p = 0.11$ ).

Furthermore, mice treated with PV-10 had significantly fewer induced lung metastases than mice treated with PBS after 21 days ( $p < 0.05$ ), and 3 out of 5 mice treated with PV-10 had 3 or fewer lung metastases, compared with more than 250 lung metastases in the untreated mice.

In addition, investigators collected splenocytes on day 14, analyzed them by flow cytometry and co-cultured them with B16-F10 melanoma cells prior to an interferon- $\gamma$  enzyme-linked immunosorbent assay (ELISA). Interferon- $\gamma$  is a cytokine that is critical for innate and adaptive immunity for tumor control and against intracellular bacterial infections.

The analysis, in a comparison of the mice treated with PV-10 vs. those treated with PBS, showed tumor-specific interferon- $\gamma$  production to be significantly higher ( $p = 0.05$ ) in the PV-10-treated mice. The evidence suggesting that the reaction was tumor-specific, Dr. Toomey said, was that co-culture with MC-38, a colorectal cancer tumor, did not increase interferon- $\gamma$ . Larger future studies with the intent of confirming a lasting immune, tumor-specific central memory response will be conducted.

A further analysis looking at T-cell subsets, surprisingly found no difference between control and treated mice.

Dr. Toomey concluded that the studies confirm both direct effects of PV-10 chemoablation on injected melanoma lesions as well as a systemic response that leads to regression of synchronous lung metastases. “Intralesional PV-10 treatment leads to the induction of tumor-specific immunity,” he said.

“Interferon- $\gamma$  is thought to be the quintessential cytokine mediating an immune response to melanoma,” noted Robert Andtbacka, MD, assistant professor of surgical oncology, University of Utah

School of Medicine, and Huntsman Cancer Institute, in an interview. "The fact that restimulating splenocytes with the tumor produced a lot more interferon- $\gamma$  is very promising," he added.

"While Dr. Toomey's research needs to be confirmed on a much larger scale," said Donald L. Morton, MD, chief of the melanoma program at John Wayne Cancer Institute, Santa Monica, CA, in an interview, "the findings are interesting and promising."

Dr. Morton is widely recognized as a pioneer of surgical oncology research in general, of vaccine therapy for melanoma and of intralesional therapy. His research from 1974 with BCG (Morton DL, *Ann Surg* 1974 October; 180(4): 635-641) demonstrated 90% control of injected lesions and regression of uninjected lesions in 17%.

BCG continues to be used infrequently in metastatic melanoma, Dr. Ross noted in his HemOnc Symposium review of intralesional therapies, because of inconsistent efficacy, allergic reactions, disseminated infections and local injection reactions.

### Inherent PV-10 advantages?

"While there does appear to be a systemic response with BCG, and some tumor-associated antibody titers are increased, the precise mechanism has not been elucidated," Dr. Morton said. He noted that BCG does not work well with tumors larger than 2 cm in diameter, because unlike PV-10, it has no direct cytotoxic effects.

"With BCG," said Amod A. Sarnaik, MD, assistant professor of cutaneous oncology at Moffitt Cancer Center, in an interview at the SSO meeting, "there is a greater recruitment of the innate arm of the immune system – and you get destruction of the basement membrane, and a kind of 'bomb crater' effect with a lot of necrosis and side effects." Abscesses, possibly containing viable tumor cells, can form. The PV-10-induced antigen response is more like a "surgical strike" in that only a subpopulation of cells is targeted for destruction, and immune

cells that remodel or repair an immune reaction are recruited, Dr. Sarnaik said. The lack of disfiguring results with PV-10 may account for interest among head and neck cancer investigators.

Among other intralesional therapies reviewed at the HemOnc symposium, interleukin-2 (IL-2) has demonstrated high overall response rates (Radny P *et al. Br J Cancer* 2003; 89(9):1620-1626) with only grade 1 and 2 toxicities. While IL-2 produces the strongest regression of local tumors among intralesional therapies, Dr. Toomey said, treatment with IL-2 is very time-intensive and costly, and no systemic effects have been observed.

The importance of a systemic effect, Dr. Toomey explained, is because of the likely existence of microscopic levels of metastases that cannot be visualized and treated directly. "The hope is that if you have a lasting immune response, you'll end up with longer disease-free survival and overall survival," he said.

Dr. Andtbacka, who presented a session at the HemOnc Melanoma Symposium on treatment of systemic disease with intralesional therapy, compared response rates among agents currently under investigation (see Figure). Response rates among non-injected systemic lesions were highest for PV-10, Allovectin-7 and OncoVEX<sup>GM-CSF</sup>.

options include use of targeted therapies, monoclonal antibodies or TIL (tumor infiltrating lymphocytes) therapy.

Because some patients are likely to have partial responses to single agents, strategies using combinations of agents that target different mechanisms should be tested, for example stimulating the immune system with a human monoclonal antibody like ipilimumab and directly attacking the tumor with PV-10. "By going after two at the same time you might be able to increase efficacy," Dr. Andtbacka suggested.

Dr. Andtbacka affirmed also that stronger local response rates with IL-2, an agent without systemic effects, suggest that combinations of intralesional agents merit study. The other researchers interviewed all concurred that combinations of agents would be an inevitable subject of study.

A Phase III randomized trial of PV-10 is in planning stages, with enrollment scheduled for late 2012 in Australia, the US and Europe. PV-10 will be compared with dacarbazine and temozolomide. The trial will include 250-300 subjects with stage IIB and IIIC melanoma and will have progression-free survival as its primary endpoint.

Dr. Toomey's study, while taking an essential step in the process of elucidating

**Table.** Intralesional injection response rates

Intralesional therapy	Injected lesions	Non-injected lesions	Non-injected systemic lesions
IL-2	70-97%	0%	0%
BCG	45-91%	11-17%	0%
Allovectin-7	19%	19%	21%
OncoVEX <sup>GM-CSF</sup>	26%	23%	18%
PV-10 (Rose Bengal)	49%	37%	35%

### Next steps

Once a mechanism is identified and confirmed to be operant in humans, as well, the next step, Dr. Toomey said, is to see if it can be enhanced, for example by taking dendritic cells and co-culturing them with tumor antigens and then injecting them back into the patient. Other

PV-10's immune effects, is still a minimally-powered animal study. An array of questions central to the future promise of PV-10 are opened subsequent to it.

- Does the PV-10-induced immune response last?
- Are multiple injections useful?

- Why do some patients respond to PV-10 and others not? Would retreatment help non-responders?
- Would pre-neoadjuvant use (prior to surgery) be beneficial?
- Front-line therapy?

Clinical trials are needed to address these and other questions. Given the unmet need in melanoma, the impetus is a strong one to pursue intral-

esional therapy research. Dr. Ross underscored that unlike chemotherapy, because drug concentrations are high only locally, intralesional therapy produces limited toxicities. Candidates include about 8-15% of patients with primary melanomas, those high-risk patients with injectable thick, ulcerated lesions and positive sentinel lymph nodes. This group is subject to significant morbidity and

greater than a 50% risk for distant disease and death.

Investigators and Provectus, the PV-10 manufacturer, have been in consultation with the US Food and Drug Administration regarding clinical trial design. An accelerated approval track for PV-10 is possible.

“There is no question that there is a clinical need for this sort of treatment,” Dr. Sondak stated. □