

Evidence for Systemic Effects Moves PV-10 Toward Further Clinical Trials

At the same time that current research projects are solidifying and reinforcing the evidence for PV-10's systemic antitumor effects, explorations of PV-10 in potentially powerful synergistic combinations are underway.

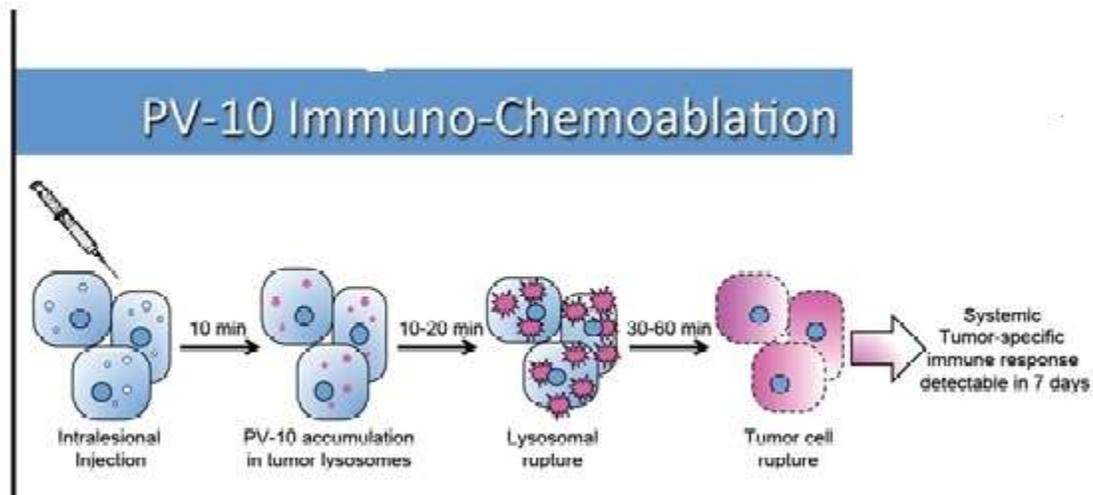
Recent prior reports detailed the initial supportive evidence for PV-10's immunological effects. When a Phase 2 trial in metastatic melanoma revealed a 61% overall response rate (ORR) in bystander (non-injected) lesions in those patients who had complete or partial responses in the lesions receiving intralesional PV-10 injections^{1,2} and when case studies showed stasis or regression in untreated visceral lesions of patients whose cutaneous lesions were injected with PV-10, it stimulated interest in looking more closely at just *how* PV-10 was impacting non-injected tumors.

Is PV-10 acting systemically?

The investigational focus of Shari A. Pilon-Thomas, PhD, Moffitt Cancer Center Immunology Program member and assistant professor at University of South Florida, is enhancing immune responses to tumors in patients with melanoma. Patients with metastatic melanoma have multiple suppressive mechanisms that prevent the establishment of effective anti-tumor immunity.

Dr. Pilon-Thomas's overriding question with respect to PV-10, she said in an interview at the American Association for Cancer Research (AACR) 2013 Annual Meeting, was as follows: "Is it just because you inject the drug and it goes everywhere and then kills tumor cells at other sites? Or is injecting PV-10 inducing a T-cell response, such that T-cells travel throughout the body and kill tumors in their various locations?" (Figure 1)

Figure 1.



In a poster presentation at the AACR 2013 meeting³, she pointed to evidence suggesting that an immune-mediated process underlies PV-10 responses in untreated lesions. First, responses in untreated lesions occurred only when responses had occurred in injected lesions, and second, “bystander” lesion responses typically occurred in a delayed fashion compared to responses in injected lesions.

In one murine model, one of two induced bilateral flank tumors (via subcutaneous [s.c.] injections of MT-901 breast cancer cells) was treated with PV-10 or placebo. Thirty days later, mean tumor size in the treated tumors was about 100 mm² as compared with about 300 mm² for the placebo treated tumors ($p < 0.001$). Bystander lesion size was reduced to about 220 mm² vs. 300 mm² ($p < 0.05$).

Furthermore, no placebo-treated mice survived beyond ~35 days, while 20% of PV-10-treated mice were alive at 60 days ($p < 0.01$). Also, production of IFN- γ in response to MT-901 was significantly greater (~1000 pg/ml versus <200 pg/ml, $p < 0.05$) in PV-10-treated mice. IFN- γ is a cytokine critical for innate and adaptive immunity (including tumor control) and for activating macrophages.

A second murine model evaluated solitary flank tumors (B16 melanoma cells via s.c. injection) and multiple lung metastases introduced intravenously. Seven days after tumor induction, the s.c. flank tumor was treated with intralesional PV-10 or placebo, and after another 7 days, investigators collected splenocytes and then separated out T-cells from them.

Dr. Pilon-Thomas reported that PV-10-treated mice had significantly fewer lung metastases (~75 versus 200, $p < 0.01$), and that their s.c. tumors were significantly smaller (mean ~20 mm² versus ~120 mm², $p < 0.05$).

IFN- γ production in the extracted splenocytes was enhanced in PV-10-treated mice (~2700 pg/ml versus ~1600 pg/ml, $p < 0.001$), making them significantly more cytotoxic to B16 tumors ($p < 0.001$).

Importantly, Dr. Pilon-Thomas noted also, when investigators injected PV-10 into the tumor-free flank of treatment-naïve mice with the B16 melanoma lung metastases, just as one would with a vaccine, it produced no effect on the lung metastases. “That indicates that you need to inject PV-10 into a tumor to produce a systemic effect,” she said.

In an adoptive transfer experiment, B16 tumor-bearing mice were treated with low-dose total body radiation (600 rad) to destroy peripheral immune cells,

thereby making space for an injection of 10 million of the T-cells purified from the spleens of the B16 tumor-bearing mice treated with intralesional PV-10. When investigators subsequently measured tumor size, they found that by 30-days T-cell-treated tumors were significantly smaller than placebo-treated tumors (mean $\sim 150 \text{ mm}^2$ versus $\sim 320 \text{ mm}^2$, $p < 0.01$).

“The cells we took from mice treated with PV-10 were indeed activated against B16 melanoma tumors. We demonstrated that by showing that they could be transferred to untreated mice with the same tumor and produce an antitumor response. This is the definitive way to test for a tumor-specific T-cell response.” Against MC-38 adenocarcinoma tumors, the T-cells from treated B16 mice had no significant effect. “This is a very specific response to the B16 tumor,” Dr. Pilon-Thomas said.

Local treatment evokes specific immune response

How does local chemoablation of a cutaneous lesion with PV-10 lead to transferable immunity? “We think that when you inject PV-10 into a tumor, it destroys the tumor, releasing tumor fragments that are then taken up by immune cells. The immune cells travel to the lymph nodes where they ‘educate’ or activate T-cells which can in turn travel anywhere in the body. The key question intriguing researchers is, ‘How can we maximize this process?’” Dr. Pilon-Thomas said.

Dr. Pilon-Thomas concluded, “These murine studies confirm that PV-10 chemoablation results in both a direct effect on injected lesions as well as a systemic response that leads to regression of uninjected subcutaneous and lung lesions. Intralesional PV-10 treatment leads to the induction of tumor-specific immunity.”

Translational medicine

In Phase 2 testing of intralesional PV-10 in metastatic melanoma, patients received up to 4 doses over 16 weeks. Locoregional control of disease, a measure that includes those with complete and partial responses plus those with stable disease, was reported in 69% of patients⁴. Among those patients who had all of their tumor burden treated, 54% had complete responses.

Among patients entering the study with stage IV disease and extensive tumor burden inaccessible to PV-10 injections, no complete responses were reported, however.

That limitation was apparent, as well, in murine research at Moffitt Cancer Center, in which a single PV-10 injection into a flank tumor reduced B16 melanoma lung metastases, but not in untreated B16 flank tumors. In the less aggressive MT-901 breast cancer model, untreated bystander tumors were reduced significantly.

The difference in response of untreated tumors in these two models points to the challenge posed by aggressive, high disease burden tumors, noted Provectus Chief Technology Officer, Eric Wachter, PhD. The finding appears to mirror the disease progression that occurred rapidly in a number of the stage IV patients participating in the Phase 2 trial, prior to repeat dosing with PV-10. Systemic immuno-stimulatory effects of PV-10, which may take days or weeks to develop, may not catch up to such aggressive disease progression. “What we learn from leveraging the clinical data with these new nonclinical results highlights the crucial role translational medicine can play in clinical development,” observed Dr. Wachter in an interview. While more frequent dosing could potentially improve outcomes, for patients with extensive tumor burden inaccessible to PV-10 injections a combination therapy strategy may be attractive, he said.

PV-10 favorable for combination therapy

Because PV-10 is a small molecule immuno-chemoablative agent, when it is injected directly into tumors, it is cleared rapidly from blood circulation, minimizing the potential for systemic side effects. Almost all adverse events reported with PV-10 are local injection site side effects such as erythema, edema, pain, and blistering.

The potential for complementary therapy aimed at temporarily arresting tumor growth in disease inaccessible to intralesional PV-10 and/or amplifying the tumor-specific immune responses induced by PV-10 ablation has been explored in murine and human clinical trials. Prior to commencing clinical investigation of a sorafenib + PV-10, a combination of PV-10 with 5-fluorouracil in a murine hepatocellular carcinoma (HCC) model revealed a longer time to progression in untreated tumors and suggested a synergistic effect⁵.

Dr. Wachter presented two murine studies at AACR 2013 of PV-10 in combination with 9H10, the murine analog of the anti-CTLA-4 therapy, ipilimumab. Ipilimumab (Yervoy®) is the first therapy approved by the FDA to clearly demonstrate a survival benefit in patients with metastatic melanoma.

In the first murine study, a solitary flank tumor and multiple lung metastases induced respectively via s.c. injection and introduction to systemic circulation of B16-F10 tumor cells were treated with a single injection of PV-10 (50-100 µL, based on tumor size) or vehicle (saline) to the flank lesion. The ipilimumab analogue, 9H10, was administered intraperitoneally (100 µg on day 0, followed by 50 µg on days 3 and 6).

When lung metastases were counted 19 days later, the mean was 84.0 in mice treated with intralesional vehicle and systemic 9H10 as compared with 3.2 in mice receiving PV-10 + 9H10 (p=0.004). Dr. Wachter noted that it was not possible to determine whether or not there was any PV-10/9H10 synergy, given that the reductions in lung metastases were comparable to previous tests of PV-10 alone. "Because the PV-10 response was extremely robust, it is hard to demonstrate an addition benefit from 9H10. We're repeating these studies with additional controls to test further for synergistic effects," Dr. Wachter said.

Evaluation of the flank tumors revealed that tumor size increased in the vehicle plus 9H10 group (from a mean of 13 mm² at treatment onset to 194 mm² on day 11. Flank tumors in the PV-10 + 9H10 combination arm, of similar size at treatment onset, were no longer measurable on day 11, consistent with prior studies.

Dr. Wachter concluded that the results corroborate previously reported data showing robust suppression of synchronous lung metastases with PV-10 ablation of an established flank tumor. He noted, though, that the magnitude of the effect of PV-10 alone made it impossible to unequivocally confirm a synergy between PV-10 and 9H10. The likelihood of any interference between the component therapies, given the suppression of lung metastases and absence of apparent toxicities, is minimal.

The second study attempted to discriminate the systemic effect of PV-10 alone from its combination with CTLA-4 blockade with 9H10 while also ensuring safety of the combination. Again using B16 melanoma tumors, the most aggressive model, investigators combined CTLA-4 blockade at 3 dose levels with PV-10. Examples of successful treatment of established B16 tumors in murine models, Dr. Wachter noted, are rare. Efficacy measures included number of lung metastases, tumor area, and survival.

In each of the three dosing groups, tumors were induced in both flanks via B16-F10 tumor cell injections. Once both tumors were palpable, the larger tumor was treated with PV-10 (50-100 µL based on tumor size) or vehicle (saline) control and 9H10 or saline was delivered intraperitoneally.

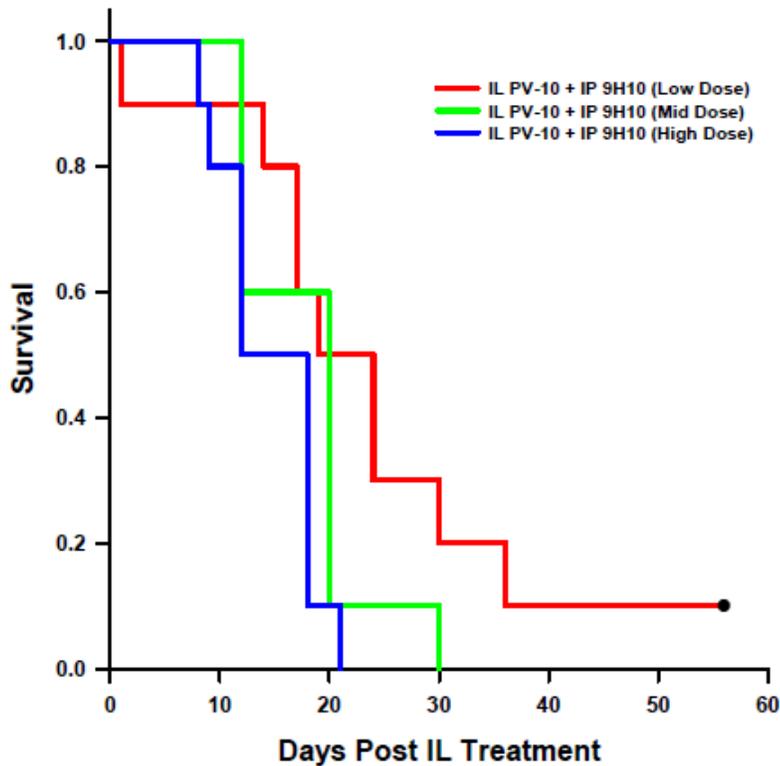
In the low-dose 9H10 group at 17 days post-treatment, mean total tumor burden was ~310 mm² for the PV-10 group and ~120 mm² for the PV-10 + 9H10 group. While total tumor volume in both of these groups was significantly smaller than that in the placebo and 9H10 alone groups, the difference between the intralesional PV-10 and combination group was not significant.

In the uninjected flank lesion, tumor volumes were significantly lower in the PV-10 + 9H10 group and in the 9H10 + saline group than in the PV-10 alone group on days 12,14, and 17.

Overall survival was longest in the PV-10 + 9H10 combination arm ($p=0.052$ versus PV-10 alone, $p=0.01$ versus placebo alone, $p=0.13$ versus 9H10 alone).

Analysis of all three dose levels revealed longer survival in the lowest dose 9H10 + PV-10 group (median 21.5 days), with shorter and shortest survival in the mid-dose (20.0 days) and high-dose arms (15.0 days), respectively. The difference favoring survival in the low-dose arm compared to the high-dose arm was significant ($p=0.03$). Median PV-10 plus placebo survival (low=17.0 days, mid=15.0 days, high=18.0 days) was longer than placebo survival in general (low=15.5 days, mid=9.0 days, high=12.0 days), and longer than that for the PV-10 plus high-dose 9H10 combination (15.0 days). “Variability in response across dose levels,” Dr. Wachter speculated, “may be due to toxicity of 9H10 at elevated doses, with additional differences arising from the effect of baseline tumor burden.” He suggested further that higher levels of 9H10 may be compromising immune competence, impairing responses both to 9H10 and PV-10.

Comparison of Bilateral Flank Model Results: Despite delay in initiation of therapy until onset of palpable tumors in a very aggressive melanoma model, PV-10 and PV-10 + 9H10 exhibited robust response in both injected and uninjected tumors.



In general, the combination of PV-10 + 9H10 was favored, while PV-10 yielded increased survival over saline in each case. At the high dose level of 9H10, PV-10 proved to be more effective than the combination.

The analysis showed as well that for placebo alone and PV-10 alone, the relationship between uninjected tumor burden and survival was linear. In contrast, at the mid-dose of 9H10 with PV-10, despite a higher tumor burden, there was a marked improvement in survival (but with a nonlinear response pattern), while at high 9H10 dose with PV-10, toxicity apparently negated any survival benefit.

In phase 2 and 3 clinical trials, Dr. Wachter pointed out, the higher doses of ipilimumab were associated with an increase in immune-related adverse effects, presumably mirroring the dose response seen in murine models with 9H10.

Dr. Wachter concluded, “Examples of successful treatment of established B16 tumors in murine models are rare. These results demonstrate that when all existing tumor is accessible for injection, PV-10 is highly effective both in animal models and clinically in cancer patients. Given that tumor ablation with PV-10 induces tumor-specific immunity, the combination of PV-10 with CTLA-4 blockade has important potential for synergy.”

Speaking further in an interview, he said, “I think the case has been made successfully for PV-10’s role as a potent stimulator of specific anti-tumor activity. This is evident in clinical data from Phase 1 and 2 testing, where regression of untreated bystander tumors correlated with ablation of tumors, and in these nonclinical mechanism studies. And, our recent murine studies show that this stimulation works robustly in combination with CTLA-4 blockade.”

Further studies designed to confirm the apparent synergy are underway, including one with only the low 9H10 dose/ PV-10 combination. A phase 1/2 anti-CTLA-4 dose escalation trial with PV-10 is warranted, Dr. Wachter said. Similarly, models for kinase inhibitors and an analogue for vemurafenib are being sought. Vemurafenib, like PV-10, rapidly reduces tumor burden.

PV-10 murine research demonstrated unambiguously, Dr. Wachter noted, that tumor burden is a critical variable in predicting response to a combination therapy. It has been suggested that earlier research into therapeutic melanoma vaccines faltered because tumor burden grew beyond the immune system’s capacity for control before the vaccine could develop its full effect. “We think that the combination of PV-10 with something like a kinase-inhibitor has the potential to dial back or reduce tumor burden even better than an anti-CTLA-4 agent while the systemic PV-10 immunologic effect is developing. The kinase inhibitor would do the early work against visceral disease until PV-10 can catch up and take the baton across the finish line.”

While the PD-1 And PD-L1 drugs will be interesting candidates for combinations, because none are approved, testing is currently impractical.

¹ [Back to Phase 1: Understanding Systemic Effects of PV-10](#). Cancer Watch Volume 22, Issue Number 2, February 2013.

² Sanjiv Agarwala, M.D., "Immuno-chemoablation of metastatic melanoma with intralesional rose bengal." Abstract #1137P, ESMO 2012.

³ S.Pilon-Thomas, Intralesional injection with PV-10 Induces a systemic anti-tumor response in murine models of breast cancer and melanoma. Abstract #1248, AACR 2013.

⁴ E Wachter, Combination of PV-10 immuno-chemoablation and systemic anti-CTLA-4 antibody therapy in murine models of melanoma. Abstract #4755, AACR 2013.

⁵ C. Dees, "Generation of an antitumor response and immunity using a small molecule drug (PV-10)." Abstract #1452582, SITC 2012.