The expression “bench to bedside” describes the progression from basic laboratory and animal research into disease and therapeutic processes through to “translational” studies that test human clinical applications suggested by those initial investigations. Sometimes, though, the arrow points the other way — “from bedside to bench,” for example, when promising clinical findings of apparent health benefits demand more granular and comprehensive understanding. That is the case in a Phase 1 study of a treatment for metastatic melanoma being conducted under Amod Sarnaik, MD, of the Experimental Therapeutics Program at the H. Lee Moffitt Cancer Center and a faculty member at the University of South Florida.

The therapy under investigation is intralesional PV-10 (Provectus Pharmaceuticals). PV-10 was developed from Rose Bengal, a xanthene dye combining halogens with fluorescein, patented in the 1880s. Before its antineoplastic potential was discovered and developed, it was used medicinally to fight eye infections, for staining, as an intravenous assay...
for impaired liver function, and as a food dye.

Back in October, 2012, Phase 2 metastatic melanoma data on use of intravesional PV-10 presented at ESMO (European Society for Medical Oncology) 2012 Congress in Vienna, Austria, showed an objective response rate (ORR) of 51%, and a disease control rate of 69% in target melanoma lesions. The other finding, which lies behind some of the intensifying interest in PV-10, was a 61% ORR in bystander (uninjected) lesions among patients who had complete or partial responses in their target lesions. The bystander lesion ORR in patients with nonresponsive target lesions was 18%. Of deep interest, as well, were case studies showing potential stasis or regression of untreated visceral lesions in patients following PV-10 treatment of their cutaneous lesions.

While PV-10 is known to be excluded from normal cells, it accumulates in the lysosomal membranes of cancer cells. There it subsequently triggers lysosomal release and complete autolysis of tumor cells relatively quickly—within 30-60 minutes of the injection. Importantly, this acute necrosis of the treated tumor does not appear to denature tumor antigens, potentially allowing acute exposure of antigenic tumor fragments to antigen-presenting cells.

Murine evidence

In murine research, Paul Toomey, MD, also of Moffitt, and colleagues used B16-F10 melanoma cells to establish a solitary subcutaneous flank tumor and multiple lung metastases in one study, and in a second study, bilateral flank tumors. When investigators administered a subcutaneous PV-10 injection to the flank tumor in the first study and a PV-10 injection in one of the tumors in the second, tumor size was reduced significantly. A bystander effect was clearly apparent in the first study in that PV-10-treated-mice had 3 or fewer lung metastases as compared with more than 250 in each of the untreated mice. Also, tumor-specific interferon (IFN)-γ production was significantly higher (p = 0.05) in the PV-10-treated mice. IFN-γ is a cytokine that is critical for innate and adaptive immunity for tumor control. The overall conclusion, Dr. Toomey said, was that “intravesional PV-10 treatment leads to direct chemoablation of melanoma lesions and to a systemic response.”

Seeking an immune cell infiltrate

To find direct evidence of such a systemic immune response is part of the motive behind heading back to the bench—although this time involving human subjects. “A further impetus toward teasing out the precise mechanism of how PV-10 can exert a systemic immune response in patients,” said Dr. Sarnaik in an interview, “is to allow us to rationally combine PV-10 treatment with some of the exciting emerging immunotherapies for metastatic melanoma.”

The focus at Moffitt, Dr. Sarnaik continued, is on discerning the presence of immune cell infiltrate in untreated tumors after PV-10 injections into other lesions. “We are really interested in harnessing immune cell infiltrate as a form of treatment,” he said, noting also that while creating cancer vaccines has been thought of traditionally as one of the Holy Grails of cancer research, cancer vaccines have turned out to be not strong enough to generate an adequate immune response.

Adoptive cell transfer

The strategy of adoptive cell transfer potentially overcomes the weak vaccine response. With adoptive cell transfer, antigen-specific effector cells are taken from the patient’s tumor and expanded ex vivo under laboratory conditions favoring growth of T-lymphocytes and then re-infused to the patient. This precludes the need to provide antigens or to activate antigen-presenting cells.

In melanoma, T-cells from the tumor are cultured from tumor resection specimens in the presence of interleukin-2. A second strategy infuses peripheral blood T-cells that have been genetically engineered to express tumor-antigen-specific T-cell receptors.

While adoptive cell transfer offers the advantage that enough T cells can be obtained for infusion in all patients, the T-cell receptors transduced into the T cells have a limited antigen-specificity. The strategy works, Dr. Sarnaik said, only about half the time. “We generate large numbers of T-lymphocytes, but we don’t have control over their quality. We think one of the limitations is that the T cells you get out of the tumor just aren’t good enough.” PV-10, however, does cause an immune response, suggesting that a combination treatment may improve the quality of the T-lymphocytes and have a greater impact on the disease.

When Shari Pilon-Thomas, PhD, also a Moffitt researcher, demonstrated that T-lymphocytes recovered from mice treated with PV-10 do appear to be of a higher quality, as evidenced by stronger tumor reactivity, the stage was set for Dr. Sarnaik’s current 15-patient pilot study. In it, one of two resectable melanoma tumors is injected with PV-10. Both are removed several weeks later. Serum is assessed before and after treatment to look for changes in the infiltration of immune cells. In patients with an immune response, PV-10 therapy can be continued.

“This is a straightforward study that will give a yes or no answer,” Dr. Sarnaik said.

Investigators will monitor carefully for known PV-10 adverse events, such as strong sun sensitivity, and interactions with diuretics, older psychiatric medications and some topical agents. Because drug concentrations are high only locally, intrallesional therapy produces limited toxicities. The study will be completed in a year, with data analysis requiring another 6 months. First results may be ready in a year, however.
If the hypothesis that PV-10 will produce a better immune cell infiltrate is borne out, that would justify testing of combination treatments, Dr. Sarnaik said. Likely candidates are adoptive cell therapy, approved drugs like ipilimumab that boost immune response, or PD-1-blocking antibodies (none approved yet).

**What kind of therapy is PV-10?**

Echoing Dr. Sarnaik, Eric Wachter, PhD, Provectus chief technology officer, said that he hopes that the findings of Dr. Sarnaik’s study will point toward rational judgments about combining PV-10 with other documented therapies. “We then might want to try two or more orthogonal therapies to stress tumor cells from several different angles simultaneously, for example an immune therapy plus a metabolic therapy (e.g., a kinase inhibitor), or in a rationally designed sequence.” In a hepatocellular carcinoma model, he added, PV-10 showed significant potential for synergy with 5-fluorouracil. Provectus recently initiated clinical testing of PV-10 with the multikinase inhibitor sorafenib, again bringing in two therapies with divergent mechanisms of action.

Which category does PV-10 fall into? “I think we are getting a clearer picture of how it might be classified, but it has features of several previously unrelated categories, such as of adoptive cell transfer and vaccination,” Dr. Wachter said. “PV-10 initially reduces tumor burden through chemoablation—but then activates the immune system bringing in capacities completely orthogonal to the ablative tumor destruction,” he added.

“Amod Sarnaik’s work may give us the molecular basis for closing the loop on one of the founding concepts for going into the clinic in the first place,” Dr. Wachter commented. “Back in the preclinical days at Provectus, Craig Dees, PhD, theorized that ablation of tumors with PV-10 might lead to unmasking of tumor antigenic material. I don’t think he anticipated that it would work as well as it does.”

**Further testing**

A Phase 3 randomized trial comparing PV-10 with dacarbazine and temozolomide monotherapy is expected to begin enrolling patients in Australia, the US and Europe later this year. The trial will include approximately 200 subjects with stage IIIB and IIC melanoma and will have progression-free survival as its primary endpoint.

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1) Sanjiv Agarwala, M.D., abstract #1137P, “Immuno-chemoablation of metastatic melanoma with intralesional rose bengal.”