# Research Highlight #2001

Dr. Paolo Provenzano
University of Minnesota

## Defining the barriers to effective anti-tumor immunity in poor prognosis cancers and engineering cell therapies to break them down.

Dr. Provenzano's research program focuses on poor prognosis cancers including pancreatic cancer, which has one of the lowest survival rates of all cancers, as well as the poor prognosis subgroup of metastatic breast cancer. His lab takes a two-pronged approach to their research. The first prong is defining the barriers, both chemical and physical, to effective anti-tumor immunity—in other words, defining why certain areas of a tumor do not allow infiltration of T cells. This is a concept that Dr. Provenzano referred to as immune exclusion.

> "A T cell therapy or cytotoxic T cell can only be effective if the T cell can meet its target—the cancer cell—in order to do killing. If they don't meet, if there's a barrier region of the tumor, where the T cells move all around, but they don't seem to move in and through that area, then that's not going to be an area where either a natural immune approach or molecular therapy will be effective."

The second prong to his research is engineering cell-based immunotherapies for poor prognosis cancers. In addition to defining what the barriers are, Dr. Provenzano works to design and engineer cell-based therapies to better move through sample tumor environments. In his recent publication in the journal *Nature Communications* (Tabdanov et al., 2021), it is shown that manipulation of the microtubule-contractility axis, either pharmacologically or with genome engineering, resulted in engineered T cells that can more effectively move through the 3D matrix of a tumor sample. This is evidence that engineering cells to move through tumor microenvironments more efficiently could be an important strategy for enhancing the efficacy of immune therapeutics. Building off this work, Dr. Provenzano shared how they are currently approaching this arm of their research.

> "We're working to use genome engineering approaches on therapeutic T cells, where our approaches are not only to recognize some specific antigen in the tumor, but also to be able to surmount the physical barriers in the tumor, and in some cases, chemical barriers as well, more effectively. One of the major readouts in both defining what those barriers are and seeing how well our engineering approach is going is multiphoton imaging platforms."

To execute the two-pronged approach, Dr. Provenzano relies on multiphoton as a core technology in his lab. He shared some reasons as to why multiphoton is such a crucial tool in his research.

> "We build around multiphoton because it provides the depth resolution that we need and it allows us to image collagen plus three other fluorophores simultaneously without switching laser wavelengths. It's extremely non-toxic, versus confocal, which still has a high degree of toxicity, especially for some of the bright fluorophores we use. And so it allows us to do long-term imaging at depth."

### ABOUT THE RESEARCHER

Dr. Paolo Provenzano is an associate professor in the Department of Biomedical Engineering at the University of Minnesota. He received his B.S. in Mechanical Engineering and M.S. and Ph.D. in Biomedical Engineering from the University of Wisconsin-Madison. Before joining the University of Minnesota, he was a DOD Postdoctoral Fellow at the University of Wisconsin and a Research Associate at the Fred Hutchinson Cancer Research Center.

### More from Dr. Provenzano

**Recent Publications:**

Tabdanov, E. D., et al. (2021). Engineering T cells to enhance 3D migration through structurally and mechanically complex tumor microenvironments. *Nature Communications*, 12(1). https://doi.org/10.1038/s41467-021-22985-5

**Lab Website:**

Laboratory for Engineering in Oncology

> "Something that I've found really exciting is the applicability of the microscope in immune mechanobiology work"
>
> –Dr. Provenzano

Dr. Provenzano then explained one of the most common experiments his lab currently runs with long-term live imaging using the Ultima multiphoton system.

> "I would say long term imaging of T cell dynamics in live tumor slices is probably the biggest assay that we're most frequently running today. We've optimized the protocol to keep slices of tumor alive for up to a week, so we can either take engineered cell therapies or T cells directly from that mouse and expand the culture. Then we can do live imaging of the T cells interacting inside of that tumor, then they'll infiltrate, and we can visualize them moving over hours to a day. And in that time, we can either just let it be, or sometimes we can add drugs or other things to perturb the system."

## Looking to the future of barrier definition and cell-based therapies

In addition to sharing about his current work, Dr. Provenzano shared some near-term goals for his research program regarding cell-based therapies.

> "We're going to use the knowledge we've gained and design next generation cell-based therapies, mostly in the immune space, that can recapitulate some of the successes that immune therapies have seen in liquid tumors, like blood cancers, where transformed cells and T cells can interact more freely because they're not in a complex tumor and microenvironment. We're trying to do those same kinds of things but make them more successful in solid tumors, and I think we'll make great progress in that area in the next five plus years.

Dr. Provenzano also provided insight on the future of this field more broadly, including a need for increased efforts in imaging and quantitative modeling of data.

> "Therapeutic T cells, like CAR T cells, and other engineered T cells, have been imaged to a much smaller degree than normal immune tracking dynamics, and so that's an area we need to learn a lot more for sure. I would say the other thing is the quantitative aspect of imaging approaches the modeling of the imaging data, trying to put those pieces together in terms of structure, function, relationships, not just chemical, but mechanical. Those things have not been really put together in great detail in the immune field."

Defining immune exclusion beyond chemical factors in these poor prognosis cancers is another area that remains under-explored. There has been a dominance in the field for years that immune exclusion is mainly chemically driven by cytokines and certain suppressive chemical factors. However, Dr. Provenzano alluded that in additional to chemical barriers, physical barriers also likely have an important role in contributing to immune exclusion.

> "There are physical barriers and physical architectures and mechanical events in a tumor that also very much influence T cells, I would say, on an equal footing or sometimes the T cells have physical barriers that even prevent them to getting to the next level where they would see that level of [chemical] suppression."

In addition to physical barriers playing a role, Dr. Provenzano mentioned his interest in the renewed understanding of the role of metabolism within the tumor as relates to immune suppression.

> "The idea that metabolism affects immune suppression, T cell function, exhaustion, motility, raises some of these questions about metabolism, I think is a big part of the future as well. I think we're going to see a lot of information locally from imaging modalities where you can look at metabolic signatures. And I think that's going to give us another level of information. So that chemical realm of it, there are cytokines and growth factors that influence things, but there's a lot going on with just general metabolism that plays a very big role and I'm really excited to see how that plays out over the next five or ten years."

By using multiphoton microscopy, Dr. Provenzano can take a holistic approach to investigating poor prognosis cancers, including the barriers to anti-tumor immunity and engineering cell-based therapies. A deeper understanding of what limits the efficacy of immunity and cell-based therapies will contribute to the development of effective cancer therapies in the future.

## Learn More

To learn more about multiphoton microscopy and Bruker's Ultima multiphoton microscopes, visit www.bruker.com/multiphoton

### Author

Savana Lipps
Bruker, Life Science Writer
Savana.lipps@bruker.com