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# TUMOR MAPPERS

The unique cooperative research model of the Ludwig Center at Harvard is being productively harnessed by the Tumor Atlas Project, an ambitious effort to create high-dimensional maps of any and all tumors.

When Peter Sorger set out to develop a method for mapping the different cells in tumors, he didn't expect actual mapmakers would be involved. So when members of Harvard's Department of Architecture approached him one day following a presentation, Sorger was surprised. "They said, 'That's really cool. Let's work together,'" recalls Sorger, an investigator at the Ludwig Center at Harvard and professor of systems pharmacology at Harvard Medical School. "Unknown to me, Harvard was the place where the initial GIS"—geographic information system—"was developed back in the 1950s."

The Harvard cartographers' expertise would prove useful for organizing and visualizing the flood of tumor data that the Ludwig Tumor Atlas Project (TAP), led by Sorger, was generating. Launched in January 2019 with funding from Ludwig Cancer Research, TAP aims to develop a multi-dimensional "map" that captures the locations and identities of not just cancer cells but also the noncancerous immune and supporting cells that contribute to tumor evolution,

progression and response to therapies. It is also a sort of technological avatar of an idea central to the structure of the Ludwig Center at Harvard: to bring together diverse biomedical disciplines and their associated technologies to tackle the most intractable problems of cancer research and care.

## It takes a village

It's no coincidence that TAP originated at Ludwig Harvard, which has a special focus on drug resistance in cancer. "The Tumor Atlas Project fits into every single project we have," Ludwig Harvard Co-director Joan Brugge observes. "The technology makes it feasible to follow many different proteins in real human tumors, which is key to an understanding of the state of individual cells in tumor tissue prior to and after drug treatment."

TAP, Sorger expects, will not only help transform our understanding of cancer biology but drive innovations in diagnostic pathology as well. The first phase of the project will map tumor cells, unraveling their

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information systems specialists.

"The foundational technology that underlies modern digital maps is conceptually applicable to our Atlas," Sorger says. "On our website, you can zoom in and out on millions of tumor cells from different diseases. The technology behind that is the same one used in Google Earth."

## Community building

Ludwig Harvard's model was forged in the earliest days of its establishment, when Brugge and Demetri were appointed its co-directors and had to decide how to distribute the annual interest of the $90 million endowment from Ludwig Cancer Research. "George and I were in sync from the very beginning," says Brugge, whose own thinking was influenced by her experience co-founding a biotech company. "I saw how well it can work when you have multiple people with different expertise coming together to help solve a problem."

With the new funding, the co-directors saw an opportunity to build a truly multidisciplinary model for cancer research. "What we wanted to do was to bring the other people who are really interested in a given problem from multiple areas of science, and then together develop the strategy to attack the problem, so that from the very beginning, we would be functioning as a unit."

In practice, this means that every research team that is part of Ludwig Harvard receives about $150,000 in seed funding annually to pursue its research. This has helped forge a community, says Demetri, who is also the associate director for clinical sciences at the Dana-Farber/Harvard Cancer Center.

"Our pitch to faculty was, 'If you join our community, we will have the ability to come up with new ideas, intersect in different ways, and provide seed money to get great multi-institutional, multi-investigator grants going forward,'" Demetri says. "Did we get

interactions with supporting noncancerous cells and immune cells, and pin down the cell signaling pathways involved in driving tumor growth and drug resistance. The second, Sorger says, will deploy machine vision, artificial intelligence and multi-dimensional visualization to combine data from many specimens, facilitate expert annotation by human pathologists and develop algorithms for predicting the responses of individual patients to specific therapies.

The technology benefits from a unique "cooperative research model" that Brugge and Co-director George Demetri have implemented at Ludwig Harvard. That model seeks to bring together researchers from multiple disciplines at the outset of every inquiry. The framework is vital to TAP, which relies on contributions from not just oncologists and pathologists but also software developers, computational biologists and, of course, geographic

Ludwig Harvard Co-directors George Demetri and Joan Brugge.

pushback? You bet we did. But in the end it worked. Remember, this was right around the time when team science was starting to catch fire. People were realizing that the translation from basic science to patients is too complicated for any one person, and we need to figure out how to work together."

Brugge and Demetri also implemented a weekly Monday meeting to which anyone associated with Ludwig Harvard research—from principal investigators to postdocs, graduate students, and clinicians—is invited. The "Ludwig Monday meetings," as they've come to be known, are a chance for researchers from different disciplines to come together to learn what their colleagues are working on and determine how their projects might intersect.

Jennifer Guerriero, who has been attending the weekly gathering since her postdoc days,

says the meeting had a strong influence on her as a young scientist. "I remember sitting there in awe and watching and listening to all of these senior and junior people just talking about science together," recalls Guerriero, who now directs the Breast Immunology Laboratory in the Women's Cancer Program at the Dana-Farber Cancer Institute. She too has become a key part of the TAP and tCyCIF team, with a special focus on the roles of macrophages in chemotherapy and immunotherapy.

The effect Guerriero described was by design. "In some ways, it felt like part of what we did was introduce people to other people," says Demetri. "It was like a junior high school dance, where the basic scientists were on that side of the room and the clinical scientists were on the other side, and the two groups were too shy or unable to talk to each other."
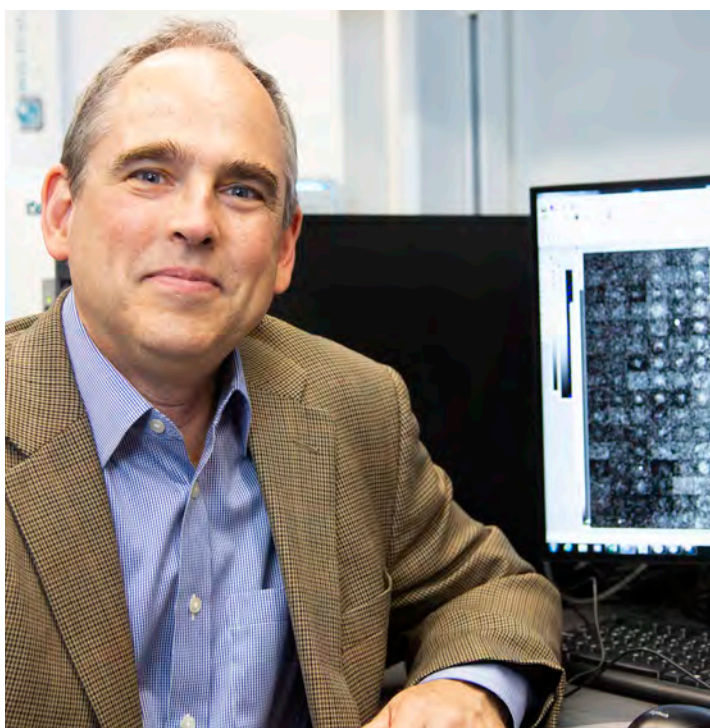
### E pluribus unum

At the heart of TAP is a method dubbed tissue-based cyclic immunofluorescence, or tCyCIF, which is being developed at Ludwig Harvard under Sorger's direction. tCyCIF allows researchers to obtain images containing multiple layers of protein information about tumors—including their cancer cells and their associated immune and other noncancerous cells—at subcellular resolution. It combines the output of multiple existing instruments and reagents into a workflow that can scan a tissue sample dozens of times without damaging its constituent cells. Each scan looks for three to five different protein markers. When compiled, the information generates a composite image of a tumor constructed from 40 to 60 "channels" of information.

Sorger likens the process to getting to know a stranger by asking many simple yes and no questions. "But instead of asking just one stranger, imagine asking these questions to a stadium full of people simultaneously. tCyCIF is very much like that," he explains.

The result is a remarkably rich and nuanced picture of tumors. "Before, we had a very unidimensional view of individual cells," says Sandro Santagata, an investigator at the Ludwig Center at Harvard, co-leader of the TAP and an associate professor of pathology at Brigham and Women's Hospital. "Now we can not only spot an immune cell, but determine specifically which type it is and define the functional state that it's in, and then compare it to a slightly different immune cell that occupies a different space. Not only do you get to really probe deeply the identity and the properties of individual cells, now you also get to see how they interface with each another."

### tCyCIF at work

tCyCIF is designed to use the kinds of standard biopsy samples that hospitals and researchers have been collecting from patients for nearly a century (it also works with mouse models of cancer). "Our goal was to hack directly into the standardized clinical workflow," says Sorger, who is also a principal investigator in the U.S. National Cancer Institute's Human Tumor Atlas Network, for which he is mapping premalignant tissues associated with certain skin and blood cancers. "We wanted to develop a method that allows us to get deep molecular insights from a sample that is collected from virtually every single cancer patient." This continuity means that tumor samples collected in completed clinical trials can still be analyzed, as can accumulated samples of rare cancers collected over the decades.

Sorger envisions tCyCIF as a complement rather than a competitor to other cell-screening technologies. For example, single cell RNA sequencing can provide a wealth of information on the gene expression profile of individual cells. "You get detailed information on individual cells, but you get no information about their locale," Sorger says. By contrast, while tCyCIF tracks only a few dozen proteins, it can interrogate many square centimeters of tissue—hundreds of thousands of individual cells—and determine the precise

morphologies of cells and their spatial relationships to each other. Combining the results of RNA sequencing and other large scale, or "omics", assays with maps generated by tCyCIF is a central goal of TAP.

To help people expand on TAP using research data from their own labs, Ludwig Harvard is placing details about tCyCIF and TAP in the public domain. "The patient data is anonymized, but all of the data and any insights we glean from it will be publicly accessible," Sorger says. "We want to make the data and the code freely available to the Ludwig community to demystify and democratize high dimensional histology."

Currently, TAP consists of dozens of images of six types of tumors—including triple-negative breast cancer, ovarian cancer, and acute myeloid leukemia—but Sorger envisions the project growing to encompass a greater variety of cancers as other centers and hospitals contribute samples. A key step will be combining image data from many different patient specimens into general-purpose maps. It is not yet clear how this will be accomplished, and Sorger and Santagata look forward to investigators from many Ludwig Centers becoming involved.

### A higher order experiment

The idea for TAP was partly inspired by Ludwig Harvard's cooperative research model, and Demetri thinks the project could be a vehicle for disseminating the model to other centers. "I see it as a social experiment," Demetri says. "Can we use it as a testing ground to see how we can all work better together? Everybody wants this kind of information, and there are whole companies being formed to do this, but if we can do it academically and openly distribute the tools and the data to people, we will engender trust and enable people to ask better questions and get answers faster."

All tissue samples for TAP have so far come from U.S. hospitals, but members of the

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Ludwig Harvard team have travelled to Ludwig Lausanne to initiate a collaboration on ovarian organoid cultures. As another step toward more open collaboration, the Ludwig Center at Harvard and the Lausanne Branch are experimenting with a workflow in which tumor samples scanned with tCyCIF at one center are analyzed with software at the other. Work on Barrett's esophagus with Ludwig Oxford is being planned as well, and Ludwig Harvard is working closely with Ludwig MIT to apply t-CyCIF to mouse models of cancer.

"We envision interactions with additional Ludwig Centers and Branches, either through direct collaboration or the transfer of technologies and methods," Sorger says. "We're comfortable with either approach. Within a few years we hope that this grows beyond a technical collaboration into a Ludwig-wide effort to efficiently ask and answer questions about shared data on drug resistance and the prospects for improving therapeutic responses." ◣