SPOT LIGHT

Cancer immunity, autoimmunity and COVID-19

YONGPAN YAN, PhD

FLUIDIGM®

Using Imaging Mass Cytometry to define the diversity within cell response

Yongpan Yan, PhD, co-founded Beijing Gencode Diagnostics Laboratory with the goal of developing a platform and tools to revolutionize the study of cancer immunity. With a background in cancer research and a focus on cell biology and bioinformatics, Yan directed an approach that combines molecular biology and immunology with cell biology tools that could uniquely contribute to our understanding of cancer and other diseases.

Building an effective pipeline

Yan believes that in order to answer difficult questions about cancer immunity and immune response to any disease, we must go back to fundamentals. He and his colleagues designed their research process to focus on events at the cellular level.

How can we better stratify immune cells across subtypes and between different diseases? What combination of cell behavior and cell type can help drive research and clinical decisions? Why start with biomarker studies and patient stratification when we have more to learn about cell function?

“For example, only a few patients can benefit from a PD-1 treatment. Why not more, and what can we do to benefit more?” Yan asked. “Instead of trying to solve this question from a molecular biology point of view, running NGS and trying to measure the tumor microenvironment with expensive single-cell RNA-seq, I think cell biology is the key to more successful immunotherapy and understanding autoimmunity.”

Following this approach, the company integrates three disciplines to gain comprehensive insight into cell function and interactions within an environment: a molecular biology lab, an immunology lab and a clinical pathology lab. Coordination among the labs makes it possible to generally focus on cell behavior in disease discovery and then conduct a deep dive into specific questions that arise.

“We integrate Imaging Mass Cytometry™ (IMC™) into our immunology technology platform, using IMC and CyTOF® systems along with cell culture and flow cytometry,” Yan adds. “We can then also use IMC for pathology studies quality controlled with H&E and IHC. Blending all of these technologies really helps validate the results.”

The team can take advantage of remote analysis with expert pathologists using IMC, providing services for analyzing pathology slides to various studies in the area. Yan notes that since pathologists offer a unique skill set, their inclusion is important to allow thorough examination and provide proper guidance.

Yan finds this organic combination across the teams has helped create a strong relationship that enables the group to better generate profound solutions.

A valuable resource on tumor microenvironment heterogeneity

One foundation for success is strong collaborations. Through collaborators within China, it became possible to collect the types of samples needed to complete studies across different diseases. When performed by Yan and team, these studies benefit from using the same platform, applying knowledge from each study to the next one in order to generate unique hypotheses that result in new discoveries.

Yan and colleagues recently published a study revealing a heterogeneous tumor microenvironment (TME) in oral squamous cell

carcinoma (OSCC) patients. Researchers from the Department of Oral and Maxillofacial Surgery at the Peking University School and Hospital of Stomatology in Beijing collaborated with Yan to analyze FFPE tumor tissue samples from four OSCC patients, stained using a panel of 26 immune or tumor-related antibodies and run on the Hyperion™ Imaging System.

'With the aim of stratifying patients, we used IMC, which is the perfect immunology tool above NGS, bulk RNA-seq and DNA-seq ...'

The clinical research team was looking for ways to assess early-stage tumors and determine patient prognosis, an area where there is a lack of information about how to improve treatment planning and predict prognosis. With no current optimal biomarkers, there was interest in biomarker identification for contributing factors of prognosis of early-stage OSCC. Imaging the four samples enabled the team to analyze the TME and correlate patient outcome.

"With the aim of stratifying patients, we used IMC, which is the perfect immunology tool above NGS, bulk RNA-seq and DNA-seq, to see the real story behind a cancer type and solve questions that have long plagued clinical scientists," Yan says.

High-dimensional proteomics analysis on the heterogeneity of tumor samples distinguished a variety and proportion of cell types including CD4+ T cells, CD8+ T cells, CD19+ B cells, CD11c+ dendritic cells, CD56+ natural killer cells, granulocytes and others, suggesting that ratios of cells within the tumor could contribute to different patients' prognosis in the future.

The study aligns with the goal of Yan's company, to learn more about the underlying cellular events occurring before, during and after cancer or immunotherapy treatments. However, the same obstacle arises across most diseases: a lack of robust biomarkers. As in this study, the identification of effective biomarkers to better predict disease outcome is critical to the development of immunotherapies or the assessment of combination therapies. The experiment is ongoing, with a plan to enroll 20 patients and obtain a better overall picture of what happens in the OSCC TME.

Moving to COVID-19

"Additional collaborations with the Jinyintan Hospital and Union Hospital in Wuhan led to a unique opportunity to ask questions about outcomes in some of the first severe cases of COVID-19. These hospitals specialize in infectious disease and were the frontline for patients at the early stages of the COVID-19 pandemic.

An aspect of this study that makes it stand out from others on COVID-19 was that the team had access to tissue samples of multiple organs from a group of patients. Most studies analyze samples from one organ, primarily the lung. In this one, researchers could associate immune changes across organs in the same patient. They obtained samples from three patients through autopsy and analyzed FFPE slides from the lung, heart, liver, kidney, brain, intestine and spleen of each, plus controls, for a total of 28 tissue samples.

Yan's lab was one of the few in the area capable of a large IMC study to investigate COVID-19 in severe or critical phase post-mortem patients. The samples were run over a two-week period using an IMC panel containing 26 antibodies to decipher different immune cell subtypes. Immunology and bioinformatics analysis were run in parallel.

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In addition to comparing immune response across the different organs, the group worked to understand infections in the lung and how resulting tissue damage arose and spread.

Based on earlier publications discussing cytokine storm and how it can affect tissues, they focused on cytokine activation and certain types of immune cells: T cells, macrophages and NK cells. Contrary to expectations that IL-6 would be most active, TNF-α and IL-10 had significant up-regulation in all 3 patients compared to the controls. These findings provided robust biomarkers for further study and suggest that TNF-α could be a potential therapeutic target.

Analyzing the data

Having already developed an efficient pipeline that produced robust data, the team could rely on it to study COVID-19. But with so many different tissue types, researchers had no comparisons for what they might find in the lung vs. the heart vs. the brain within a single patient, or between patients. Yan's collaborations came into play again, with specialists in the different tissue types to help optimize and analyze the data. They provided knowledge of the various tissue types in the different organ samples, something that was fundamental to processing the COVID-19 tissues.

The growing impact of IMC

"Originally, we did think about using fluorescence, but you can only achieve analysis with a handful of markers, generally not more than five. If it's more than five, it's a really difficult technology," Yan explains.

"COVID-19 and cancer immunology have complicated stories. The samples are complex, composed of many different cell types, and involve cytokines, not to mention what interactions occur between the cells. Fluorescence won't give you a whole picture of what happens in a tissue at the cell level. Fortunately, with IMC we can see into the tissue structure, especially at cellular or even subcellular definition."

Through Yan's approach using IMC, the team became the first to look into the tissue structure of COVID-19 patients not only in the lung, but in other tissue types as well. This is of significant value to the scientific community.

Yan hopes that patients will benefit from the study and that hospitals can start to think about using different treatments for this serious infectious disease.

In the future

The group is ramping up to use IMC in more studies this year. Between a follow-up to the OSCC preliminary study and other collaborations with a group working on immunotherapy for breast cancer, the labs are busy preparing for more preclinical research.

"This is a great direction for us, and we are interested to see benefits to both research and clinical decisions."

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References:

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