Could you start by telling me a bit about your current role and the work that you’re doing?

RS: I am a clinician investigator and an assistant professor at the University Hospital Center in Laval University, Quebec City, Canada. In the clinical space, I work in pediatric hematology and oncology. My research revolves around tumor environments, particularly the tumor immune microenvironments of pediatric tumors. My lab primarily operates as a dry lab, where we engage in computational analysis of big data, predominantly sequencing data.
How do you apply multiomics approaches in your work?

RS: In my work, I apply multiomics approaches for deciphering the complexities of tumor microenvironments. The main goal is to identify pediatric tumors that are more susceptible to be responsive to immunotherapy by immune checkpoint inhibitors. Additionally, I am looking at the correlation between the tumor environment and potential complications that can occur in pediatric cancer.

For the multiomics approach, we are mostly focusing on using gene expression, specifically transcriptomics. RNA is easily obtained and is a very powerful tool to analyze both the tumor bulk and its environment. It serves as a proxy of the cell function, as RNA is the precursor of proteins. So, through transcriptomic analysis, we can look at distinct signatures and phenotypes.

Another multiomics approach that we’re using is immuno-oncogenomics. With RNA sequencing data, you can deduce the composition of the immune environments. There are some deconvolution tools that quantify the immune cells present in the tumor. Furthermore, you can look at the specificity of immune cells (such as T cells and B cells) by looking at the TCR and BCR rearrangements.

For tumor immune environments we (and many others) are using the tumor mutation burden that is extracted by DNA sequencing, effectively integrating genomics with transcriptomics. We are also using DNA methylation data and we are now moving toward proteomics too.

You mentioned DNA methylation—is integrating this into multiomics models important?

RS: We initially started our research studying transcriptomic gene expression and subsequently integrated genome methylation. This has proven a great tool for studying the immune environment and allowed us to identify subsets of immune phenotype, based on DNA methylation, that are different from those identified by gene expression. When the two methods are integrated, we are able to find more subsets of immune environments to refine the classification.

There is also a substantial body of data indicating that DNA methylation is a major modulator of immune environments. It has the capacity to silence important genes and proteins that are decisive for antigen presentation and recognition of the tumor cells. Numerous preclinical studies have shown that combining immune checkpoint blockade with methylation modulators could improve the efficacy of immune checkpoint blockade.

I believe that by considering both gene expression and DNA methylation, we may be able to identify a subgroup of tumors with an immune environment altered by methylation. This subgroup could potentially represent an ideal population for exploring combinations of methylation modulators with immune checkpoint blockade.

There is also a substantial body of data indicating that DNA methylation is a major modulator of immune environments.
**Q** What impact are multiomics tools having in the immune-oncology space?

**RS:** Predicting the efficacy of immunotherapy involves the consideration of various biomarkers. At first, biomarkers were used with immunohistochemistry and the expression of the PD-L1 protein, which is predictive of the response to immune checkpoint blockade. Other biomarkers include the infiltration of T cells into the tumor and tumor mutation burden, which quantifies somatic mutations expressed by the tumor.

Later biomarker studies looked at high-throughput, deep omics-based information, mostly in adult tumors. The first notable approach involved a transcriptomic gene expression profile, which analyzed signatures, such as interferon-gamma signature, that are more likely to be sensitive to immune checkpoint blockade.

These biomarkers are decent predictors of the response to immune checkpoint blockade, but they cannot completely predict how the patient will respond. Even among patients with these biomarkers, not all show a favorable response.

It is important to note that these biomarkers are only partially correlated between each other but that combining multiple biomarkers increases accuracy in predicting the response. It shows that one single biomarker cannot completely depict the complexity of the tumor immune microenvironment.

Numerous adult studies have explored the different biological components coming from gene expression profiling, tumor mutation burden, DNA methylation, proteomics, single-cell analysis, spatial annotation of the tumor, and now, plasma-circulating proteins. Each of these independent omics is able to predict or find signatures associated with immune checkpoint blockade response. However, there are a limited number of studies that combine all of these omics’ approaches.

A more comprehensive multiomics integration should enable us to depict the intricate interaction that exists between the molecular levels to define the tumor microenvironment complexity. It is known that usually, multiomics enhances the accuracy and specificity of biomarker discovery. Thus, a multiomics approach could help to discover more robust and reliable biomarkers.

**Q** What about the ongoing search for novel biomarkers in the I-O space?

**RS:** I think that the next steps in research should focus on proteomics, which is the final effector of the cells and offers a more functional view of the tumor immune environment. Looking directly at proteins would offer a better way to understand which patients would best respond to immunotherapy. When you consider the biomarkers that are currently known, one of the strongest is still the protein expression of PD-L1 by immunohistochemistry.

I also believe that single-cell sequencing is a very powerful method for directly investigating the functional state of cells. Research shows some specific functional states associated with good response to immunotherapy, both for T cells and B cells, characterized by the presence of memory effector cells, which are highly important for the efficacy of immune checkpoint blockade.
We have a very good definition of the different functional states that can exist for tumor-infiltrated leukocytes and I believe we can use this atlas of functional states to better understand the immune composition that enables a good response to immunotherapy.

**Q** What should be the next specific targets for the oncology field?

**RS:** The next step will likely involve predicting the most effective combinations of immunotherapy. PD-L1 and CTLA-4 inhibition have both proven effective in melanoma, and the combination of the two increases the efficacy. New combinations have shown great promise, such as PD-1/PDL-1 inhibition in combination with LAG-3 inhibition.

I think the next logical step will be to re-evaluate which patients will likely respond to specific combinations. For that, we should look at deeper correlation expression of those biomarkers in the tumor and possibly even at the cellular level. This would help identify the cells that co-express the two different checkpoints that can be inhibited.

**Q** What would be at the top of your wish list for new innovations in this space?

**RS:** The first would be to achieve a comprehensive multiomics integration. Currently, there are various omics data sets being independently studied, resulting in missing biological layers. Pediatric research is still severely lacking compared to adult studies in this regard. To fully understand the complexity of the tumor immune environment, there is a need to integrate all these biological layers together.

Next, I believe the composition of the immune environment from circulating proteins in the plasma is something that needs to be explored more. The objective would be to develop a liquid biopsy method for inferring the immune environment and predicting patient responses to immunotherapy. Obtaining tumor samples can be difficult and invasive, so accessing this information from circulating blood would simplify the process significantly.

The immune environment is not stable over time but has plasticity so, a tumor sample analysis should occur at every relapse to better predict the response to immunotherapy. Changes in the immune environment could be the result of either the treatment that the patient received or the modification of the tumor cells, such as a selection or an increase of a tumor clone at relapse. A liquid biopsy would allow us to more easily track the dynamic nature of the tumor immune environment. If we can deduct the tumor immune environment from circulating blood by analyzing the protein in the plasma, it will be a huge gain for the patient.

**Q** What are your own key goals and priorities in the next few years?

**RS:** The primary goal for my research is to introduce immune checkpoint blockades and immunotherapy in the treatment of pediatric cancer. There have only been around 300 pediatric cancer patients treated with immunotherapy reported so far from clinical
Obtaining tumor samples can be difficult and invasive, so accessing this information from circulating blood would simplify the process significantly.

trials and the response rate sits at around 3%. However, when you look at progression-free survival and stabilization of disease, around 15% of tumors can be controlled with immunotherapy, sometimes for extended periods of several months or even years.

The challenge we face in pediatric cancer is the absence of specific histologies that respond well to immunotherapy, as seen in adults. For example, in adult melanoma studies, immunotherapy demonstrated dramatic sensitivity, making it easier to identify a responsive group. In contrast, there is no histologic group in pediatric tumors that consistently responds to immunotherapy, apart from Hodgkin’s lymphoma.

My goal is to harness big data multiomics analysis of the immune environment to offer children the same opportunities that are offered to adults. This would help to gain an understanding of tumor immune interaction and find the tumors that are responsive to immunotherapy in pediatric patients.

I firmly believe that we should conduct an in-depth analysis of the immune environment at different stages of disease, from diagnosis to relapse. This will help determine which patients are likely to respond to immunotherapy, the optimal timing for immunotherapy, and the most effective combination for pediatric tumors. It’s possible that we may need to consider administering immunotherapy earlier in disease evolution, before the host immune system has been exhausted or altered by successive treatments. Ultimately, this will enable personalized immunotherapy as a therapeutic weapon in pediatric cancer.

BIOGRAPHY

RAOUL SANTIAGO is a Clinical Investigator in Pediatric Hematology and Oncology and Assistant Professor at the University Hospital Center of Quebec, Laval University in Canada. He runs a translational research program of multiomic analyses for deciphering the complexity of tumor immune micro-environment of pediatric tumors. The aim of his research is to leverage the development of personalized immunotherapy and to find targets for novel combinations of immunotherapy in pediatric oncology.

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