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IMMUNO-ONCOLOGY INSIGHTS

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Safety: preclinical and clinical

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COMMENTARY

2024 and beyond: addressing ongoing safety and toxicity challenges for the future of the I-O field

Rakesh Dixit

On September 12, 2023 **Roisin McGuigan**, Editor, Immuno-Oncology Insights, spoke to **Rakesh Dixit** PhD, DABT Cofounder, President and CSO, Regio Biosciences, Bionavigen, LLC and ex-VP of AstraZeneca, about addressing the safety and toxicity hurdles in the I-O field today. As a key opinion leader in the oncology biologics and safety assessment space, Rakesh Dixit comments on the field's attempts to achieve the holy grail of I-O: targeting tumor cells with greater accuracy to improve safety and efficacy. This article has been written based on that discussion.

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ONGOING CHALLENGES IN THE I-O FIELD

The biggest challenges in I-O therapy are the rise of tumor resistance and long-term chronic or delayed toxicities. Although I-O therapeutics have revolutionized the treatment of cancers, these therapies are mostly effective when the patient's immune system is functioning reasonably well and is immunologically resilient. This has translated into varying benefits in about 30% of patients. Still, the benefits are marginal in patients with immunologically unresponsive tumors, and these

patients die rapidly. What to do with the remaining 70% of patients remains a fierce challenge in the I-O field.

Most I-O therapies are based on antagonizing PD-1, PD-L1, and CTLA-4 targets with a very strong ability to limit immune attacks. Over time, with constant hits of immune checkpoint-based immunotherapy, tumors develop alternate pathways for survival. They lose dependence on these immuno-suppressor pathways and induce other pathways to evade T cells, NK cells, neutrophils, and macrophages. Despite ongoing research, we have not yet been able to beat the resistance mechanism effectively. There are not many available treatments for the metastasis of cancers, which remains a crucial challenge in the space in terms of efficacy. Tumor-induced resistance against immune checkpoint inhibitors (ICIs) and the development of alternate pathways for tumor survival remains a fierce challenge in maintaining the long-term survival of cancer patients.

One of the most fierce challenges in the I-O space is the long-term safety that affects cancer patients' quality of life and long-term survival with ICIs. Over two thirds of ICI patients, including PD-1:PD-L1 axis antagonists-treated patients, are likely to experience acute or short-term adverse effects (AEs). As many as 10–15% of these immune-related severe adverse effects (IRAEs) affecting multiple organs, including the heart, lung, liver, GI tract, skin, and endocrine system, can be fatal or severe. Nearly 40% of ICI patients display chronic or long-term, often irreversible, adverse effects. Other agents, such as chemotherapy and targeted agents, including poly-ADP ribose polymerase (PARP) and tyrosine kinase inhibitors, have exacerbated many IRAEs. It is worth mentioning that severe pneumonitis, or interstitial lung disease, cases have been observed in cancer patients treated with a combination of ICIs and chemotherapeutics, such as taxanes.

The mechanism of IRAEs related to approved ICIs is related to the systemic activation of the immune system, following the release of the brakes on PD-1/PD-L1 -expressing T cells and other immune effector cells, including macrophages. In some cases, the preexisting autoantibodies (e.g., anti-CTLA-4 and anti-PD-L1) might also exaggerate these toxicities.

Other targeted I-O agents, such as CAR-T cells, often produce mostly CAR-T-specific IRAEs, including moderate to severe cytokine storm and neurotoxicity. Activated T cells produce many pro-inflammatory cytokines that fuel their growth and survival, often leading to systemic cytokine storm-associated IRAE affecting multiple organs. CAR-T cell toxicity is often chronic, and a small number of patients experience significant neurotoxicity, which is very difficult to manage.

Furthermore, the high cost of these new immunotherapies poses another great challenge. Much of the world cannot afford any of these I-O therapies. There is financial toxicity, even in the US, with these drugs. From a big pharma perspective, it costs a lot to innovate, make, and test these drugs. However, a balance is urgently needed between the high cost of innovation and the unaffordable cost of most ICI drugs to patients.

ADDRESSING SAFETY AND TOXICITY CHALLENGES

Moving beyond the classic targets, PD1, PD-L1, and CTLA-4, or focusing on other immune checkpoints and immunosuppressive mechanisms are ways to address these challenges. The promise for new therapies lies in looking for additional immunosuppressive targets. The question of managing the immunosuppressive environment and tumor stroma also needs work. Clearing up tumor microenvironment-based immune suppression is needed to bring the activated T cells and other tumor-killing immune cells inside the tumors.

IRAEs mainly involve inflammatory adverse events on the skin, endocrine glands, gastrointestinal system, and liver. Other organs,

such as cardiovascular, pulmonary, musculoskeletal, ocular, and central nervous systems are also affected less frequently. ICI-based IRAEs tend to differ from those caused by chemotherapy and radiation and often have delayed onset and prolonged duration. Most IRAEs are treated with appropriate immunosuppressive and immunomodulatory strategies; however, concerns remain with treating severe and fatal adverse events, such as pneumonitis and endocrine toxicities. While many IRAEs that affect the skin and gastrointestinal system can be treated well with standard immunosuppressive drugs, endocrine toxicities are often irreversible and may require chronic lifelong hormone replacement therapy.

Efforts are needed to identify patients who are most susceptible to IRAEs. There is some evidence that patients with preexisting autoimmune diseases tend to have a greater degree of IRAEs, and their autoimmune disease is often exacerbated by treatment with ICIs. As many as 10% of patients with a history of autoimmune rheumatoid disease show severe rheumatic IRAEs. Additional susceptibility factors include genetic susceptibility and the ICI-suppressed host microbiota with a clear role in immune homeostasis. IRAEs predicting safety biomarkers are urgently needed to inform patients and their physicians about the risks and benefits of ICI therapies.

Effective preventive and therapeutic strategies must be employed to improve the long-term effectiveness of I-O agents. Most grade 1 and grade 2 IRAEs are generally well managed with immunomodulatory therapies, including antihistamines, oxygen, fluids, opioids, corticosteroids, and bronchodilators. Often, when IRAEs resolve after immunosuppressive treatments, most patients can be retreated with ICIs. However, permanent discontinuation of the ICI should be considered when patients develop grade 3–4 IRAEs, including pituitary inflammation, thyroiditis, hepatitis, colitis, pneumonitis, neurological (e.g., Guillain-Barré Syndrome, encephalitis, myasthenia gravis, seizures, etc.) rheumatoid arthritis, type 1 diabetes, proteinuria, and uveitis.

So far, the most successful approaches utilized include proper management of IRAEs, dose reduction, or dosing-free holidays. Information regarding AEs, quality of life, and living cancer-free should also be appropriately communicated to patients. The quality of life issue requires greater discussion in the I-O field, especially about combination approaches. There are some drugs being combined that patients cannot tolerate.

Combination approaches do hold potential in treating tumors with 'cold' immunity. However, in an environment with around 5,000 I-O drugs, finding relevant and meaningful combinations can be challenging. This can be a difficult investment environment to navigate, especially with the existence of copycat drugs, though finding successful combinations remains paramount.

Managing IRAEs is a prerequisite to offering cancer patients a better quality of life and long-term survival. Unique ways to minimize the toxicities of the current agents with next-generation improved ICI therapies would be precious. New unique solid tumor target-based therapeutics with a role in transforming cold tumors into responsive hot ones whilst remaining synergistic to current targets of ICIs are badly needed. Novel unique combination approaches that increase efficacy without enhanced toxicities should also be developed. Furthermore, measures beyond the RECIST criteria, the current gold standard to monitor the efficacy assessment, are needed to determine which patients have increased cancer-free survival rather than simply measuring tumor size.

TARGETING TUMOR CELLS WITH GREATER ACCURACY

Targeting tumor cells more accurately to improve both safety and efficacy is the holy grail of oncology, though tumor-specific targeting has multiple challenges. The most significant hurdle is the expression of the targeted tumor antigen on normal healthy cells. Clean targets with minimal off-target expression for

biologics, including monoclonal antibodies and CAR-T cells, have been designed to target cancer cells and spare normal healthy cells. However, finding very clean targets has been challenging, and better efforts are needed to find these. All targeted therapies are distributed to normal tissues, irrespective of the target expression, through nonspecific uptake. The off-target nonspecific adverse effect profile remains a challenge in finding a target in solid tumors, and efforts are needed to reduce nonspecific systemic uptake of targeted biologics and target-based and off-target toxicities.

OPPORTUNITIES IN SOLID TUMOR APPROACHES

Unfortunately, it has been difficult thus far to find effective CAR-T cell therapies for solid tumors. Once T cells are activated, if they find a target, they kill it, whether it is a tumor or a healthy cell. Finding targets for solid tumor therapies poses many challenges, though many novel research areas are being pursued to reach solid tumors. A range of these approaches being explored by various companies are listed below:

- The utilization of mutated neo-antigen targets that are typically absent in healthy cells. This field has not yet seen much success, as no clean targets have been found.
- Bispecific and biparatopic antigen targeting can minimize resistance due to one target antigen loss. Bispecific/biparatopic targeting can increase tumor uptake and reduce normal tissue antigen binding.
- The high level of lactic acid in many tumors versus normal healthy cells lowers the pH on the surface of tumors. A method to activate antibody binding to tumor antigens only at the lower pH is actively pursued.
- Modulating the affinity and valency of the targeting moiety. Developing differentiated antigen-binding complementarity determining region (CDR) sequences on tumors versus CDRs on regular tissue targets, increasing valency, and avidity/valency-based target binding with innovative antibody engineering approaches are being considered.
- Reducing hydrophobicity, aggregation, and Fcγ receptor-based toxicities (mutating the Fc–FcγR receptor) are some approaches to minimizing off-target toxicities of the I-O agents.

2024 & BEYOND: FUTURE IMPROVEMENTS & REMAINING CHALLENGES IN I-O SAFETY

In addition to proper safety management, new, cleaner, target-based I-O therapies are urgently needed to reduce target- and off-target toxicities. Antidotes that can reduce toxicity should also be developed.

The number of bispecific I-O antibodies (e.g., CD3 T cells-based bispecifics) and bispecific CAR-T cell therapies are rising. The field has seen some success and approvals in CD3-based bispecifics, including BCMA CD3 T cells, although these pose some similar safety issues to those seen with CAR-T cell therapies. In the future, we may see trispecific or tetraspecific antibodies targeting multiple tumor antigens. We could also see new therapies targeting cancer-killing NK cells, macrophages, and neutrophils. Regulatory acceptability of new liquid biopsy-based biomarkers (e.g., circulating tumor DNA) to predict the efficacy before the invasive RECIST assessment will be likely pursued.

The biggest challenge in treating cancers with I-O therapies is beating resistance and increasing effectiveness in patients with highly immunosuppressed inactive immune systems. The mechanisms of immunoresistance are complicated and not fully understood. It is increasingly recognized that new

therapies targeting the TME immunosuppressive environment in addition to tumor cells are urgently needed to convert cold tumors to immunoactive hot tumors.

Allogenic CAR-T cells could be another area in which a critical transformation could ease the need for individualized therapies. There are only a few places in the world where one can receive CAR-T cell therapy treatment as it is so specialized, and few centers are offering the therapies globally. We need to find reasonably priced immunotherapy drugs to increase accessibility to patients in less developed countries world.

BIOGRAPHY

RAKESH DIXIT is an accomplished executive, inventor, and scientist with over 35 years of success with top biotechnology and pharmaceutical companies, including Merck, Johnson & Johnson, MedImmune and AstraZeneca. He is President and CSO of Regio Biosciences and Bionavigen, LLC. He is a board member of Regio Biosciences and a key member of multiple scientific advisory boards for ADC companies. Dr Dixit is also a chief adviser and consultant for over 20 companies worldwide. From 2006–2019, Dr Dixit was a Global Vice President of the Biologics R&D at MedImmune-AstraZeneca. He has unique expertise in developing biologics (e.g., monoclonal antibodies, bispecific biologics, antibody-drug conjugates, fusion proteins, peptides, gene and cell therapies, etc.) and small-molecule biopharmaceuticals. Dr Dixit conducted extensive graduate and post-graduate training in pharmacology/toxicology–biochemistry with both Indian and USA Institutions (e.g., Case Western Reserve University, Medical College of Ohio, University of Nebraska) and is a Diplomate, Board Certified in Toxicology from the American Board of Toxicology, Inc. since 1992.

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INTERVIEW

Balancing act: improving safety while retaining efficacy in I-O

Lauren Coyle, Editor, *Immuno-Oncology Insights*, speaks with **Paolo Ascierto**, Director of the Unit of Melanoma, Cancer Immunotherapy and Innovative Therapy, National Tumor Institute 'Fondazione G. Pascale', to discuss development of I-O treatments and emerging strategies, specifically in melanomas, improving the balance safety profiles and treatment-related toxicities.

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Q What progress has been made in balancing efficacy and safety in the I-O field?

PA: The immune checkpoint inhibitor marked the beginning of modern immunotherapy. Ipilimumab was the first approved immune checkpoint inhibitor in the field of melanoma, and produced impressive results. Previously, most metastatic melanoma patients did not survive; with ipilimumab, 20% of patients achieved a cure. However, the price to pay for this success was toxicity, in the form of immune-related adverse events (irAE). The powerful immune response following treatment with ipilimumab led to strong activity against the tumor but also triggered a potentially harmful autoimmune reaction. The percentage of severe irAEs from ipilimumab was relatively high, and mainly seen with high dosages.

Next, we saw the emergence of anti-PD-1 therapies, which brought significantly greater efficacy and fewer side effects. In melanoma, the overall survival (OS) rate increased from 20–40% and the incidence of grade III and IV adverse events dropped from 30% to 12–15% compared with ipilimumab. This represented a crucial advancement.

The introduction of combination therapies provided even more potency. Now, we see a remarkable 50% response rate in melanoma treatment; however, it results in more side effects and increased toxicity due to the inclusion of ipilimumab. This evolution primarily affected the field of melanoma and, in contrast with other cancers, a similar toxicity profile with anti-PD-1 monotherapy was found. However, when combined with chemotherapy, the side effects increased. Further, the combination of anti-CTLA-4 and anti-PD-1 therapies also led to a higher incidence of irAEs.

Q Are there any emerging strategies that could help moderate the risk of toxicities for I-O treatment?

PA: There is a common saying in the medical community, "No side effect, no efficacy." If you don't have side effects, it means that the drug is not efficacious. An example of this was epacadostat, an IDO1 inhibitor that, in phase I/II, showed no side effects, even when used in combination with pembrolizumab, but also had no efficacy. Naturally, we aim for treatment strategies with greater potency and fewer side effects.

An example was recently approved by the US FDA and EMA: the combination of nivolumab and relatlimab. Relatlimab is an anti-LAG-3 checkpoint, which follows CTLA-4 and PD-1 checkpoints. This combination provides increased efficacy compared to single-agent treatments, but it comes with a slight (10%) increase in side effects, especially in the field of melanoma. At this point, we cannot definitively say that this new combination matches the efficacy of ipilimumab and nivolumab, but it does promise fewer side effects. This is a promising development and likely the direction of travel in the future.

Combinational approaches will involve new targets that can enhance efficacy, although there may still be some additional toxicity. It is an idealistic notion to expect a combination strategy that significantly enhances efficacy without any side effects. The introduction of new targets, like LAG-3, exemplifies this trend of achieving increased efficacy with only slightly more toxicity.

Recently, we have been focusing more on biomarkers to predict why some patients respond better to I-O treatments, with limited attention to predicting side effects. Now, there are some interesting studies looking into genetic modification and genetic predisposition of patients toward adverse effects. Given the high rate of toxicity, this is an area where we must strive for improvement. While we are seeing some promising data in this field, there is still much more we could and should do.

Q Can you shed light on ongoing research focusing on improving safety profiles in melanoma?

PA: After obtaining important data in the field of metastatic disease, we shifted our focus to the adjuvant setting to prevent metastasis with the adjuvant treatment.

Anti-PD-1 therapy and BRAF/MEK inhibitors have now become a standard of care. However, even in this context, 50% of patients benefit from these treatments while the other 50% do not, highlighting the need for further research and new therapies.

We have recently seen some impressive data from a randomized phase II with an mRNA vaccination constructed of 38 new epitopes. Further, when this mRNA vaccine was combined with pembrolizumab, there was an absolute benefit at 2 years compared to pembrolizumab monotherapy, with a risk reduction of 44% for relapse. Additionally, at ASCO 2023, we saw significant data with this combination regarding distant metastasis-free survival, which is a surrogate for OS. Notably, the increase in toxicity was minimal, primarily limited to local injection reactions and fever.

This combination therapy aligns perfectly with what we've been discussing—more efficacy with only a slight increase in irAE. There is an ongoing randomized phase III trial with this vaccine which is showing great promise and could open a new avenue of research and not only for melanoma. This personalized vaccine works on the neoepitope in the tumor, which can be applied to other cancers. In my opinion, these developments represent the most significant news in the field right now.

A noteworthy topic here is adoptive cell therapy, which is an area of increasing interest in the I-O field. However, it still carries a degree of toxicity due to the use of IL-2, which can be particularly taxing in the short-term. I believe that we need to focus more on the long-term side effect as there is currently few data.

Q When patients show no response to I-O treatment but develop treatment-related toxicity, how this should be addressed?

PA: Generally, what we observe is that when there is toxicity, there is usually a response—but this isn't true for all patients. Sometimes we see activation of the immune system but it falls short of effectively curing the tumor. It is important to note that when patients are treated with immunotherapy, they actually welcome some degree of toxicity. Naturally, they prefer mild toxicity, but they understand that it comes hand-in-hand with the activation of the immune system.

In cases of significant disease with no efficacy despite immune system activation, the patients naturally question the effectiveness of the treatment. It's a challenging subject to explain because it seems that it should be straightforward. When situations like this arise, it's a clear sign that the treatment is not delivering the expected results.

Q What challenges do you expect in ensuring long-term safety of these treatments, especially in combination, and how can these be tackled?

PA: The challenge, of course, is to find a treatment that can increase the number of patients experiencing long-term benefits. The Society of Immunotherapy of Cancer (SITC) is doing important work in the field of safety in the survival to immune checkpoint

inhibitors, particularly in patients with long-term benefit. We urgently need more data, especially from all the pivotal trials, particularly those that were started 7 or 8 years ago.

By gaining access to the long-term safety data with long survival in these patients who have an activated immune system, we can then further determine if there are late irAEs. Organizations like SITC and regulatory bodies should pull together all this data in order to gain a larger dataset that will consider the increase in the number of patients with the long-term survival.

BIOGRAPHY

PAOLO ANTONIO ASCIERTO is the Director of the Department of Skin Tumors, Experimental Oncological Immunotherapy and Innovative Therapies, as well as Director of the Unit of Experimental Oncology Melanoma, Immunotherapy and Innovative Therapies of the National Cancer Institute IRCCS 'Fondazione G. Pascale' of Naples. Ranking first in the Expertscape ranking of the University of North Carolina as the leading melanoma expert in the world for the decade 2013–2023, he is a pioneering oncologist of immuno-oncology, as well as author of over 650 publications in the field. Ascierto is a member of the working group responsible for drawing up the ASCO (American Society of Clinical Oncology) guidelines, coordinator of ESMO (European Society of Clinical Oncolgy) and the AIOM (Italian Association of Medical Oncology) on melanoma. Considered one of the best Italian researchers, Ascierto has made his clinical and research activity a true vocation, basing all his work on the prevention and treatment of cancer, in particular, the management of patients with melanoma and on the use of immunotherapy. To date, he is the promotor of numerous training and awareness events and initiatives, aimed at specialists and the general public.

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Enhancing dynamic cell health insights with AI-driven analysis

Jasmine Trigg, Scientist, BioAnalytics group, Sartorius

Live-cell imaging enables the acquisition of phase contrast and fluorescent images in a non-perturbing manner. This poster introduces the Incucyte® AI Cell Health Analysis software, an artificial intelligence (AI)-driven approach to label-free segmentation and live/dead cell classification.

AN INTRODUCTION TO LIVE-CELL IMAGING

Alongside the incorporation of AI into image analysis workflows, live-cell imaging has empowered accurate quantification of a broad spectrum of cellular models, making it a powerful approach to aid data-driven decisions. These innovative technologies, based on neural network algorithms, are more complex than traditional image analysis and facilitate more accurate segmentation of heterogenous cell morphologies whilst minimizing user-introduced bias.

INCUCYTE AI CELL HEALTH ANALYSIS

The recently launched Incucyte® AI Cell Health Analysis software module is powered by pre-trained neural networks and is a robust solution for label-free segmentation and live/dead classification of individual cells. This AI-driven approach can be used in combination with optional fluorescent readouts to enhance insights into cell health and function, including phagocytosis, caspase-dependent apoptotic pathways, and cell cycle perturbance.

With the Incucyte® AI Cell Health Analysis **(Figure 1)**, a neural network (pre-trained with validated datasets) informs segmentation and classification algorithms for accurate processing and quantification of live or dead cells. This AI-driven analysis is applied to all wells and timepoints, providing robust data and visualization of live or dead masks. In cases where optional fluorescence images are acquired, this can be quantified within all cells and within live or dead populations.

Figure 1. Incucyte® AI Cell Health Analysis workflow.

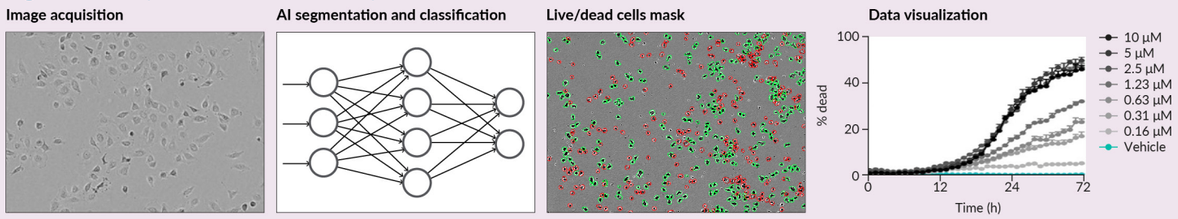
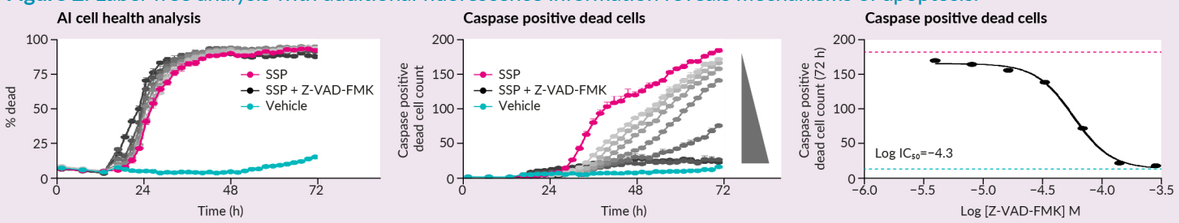


Figure 2. Label-free analysis with additional fluorescence information reveals mechanisms of apoptosis.



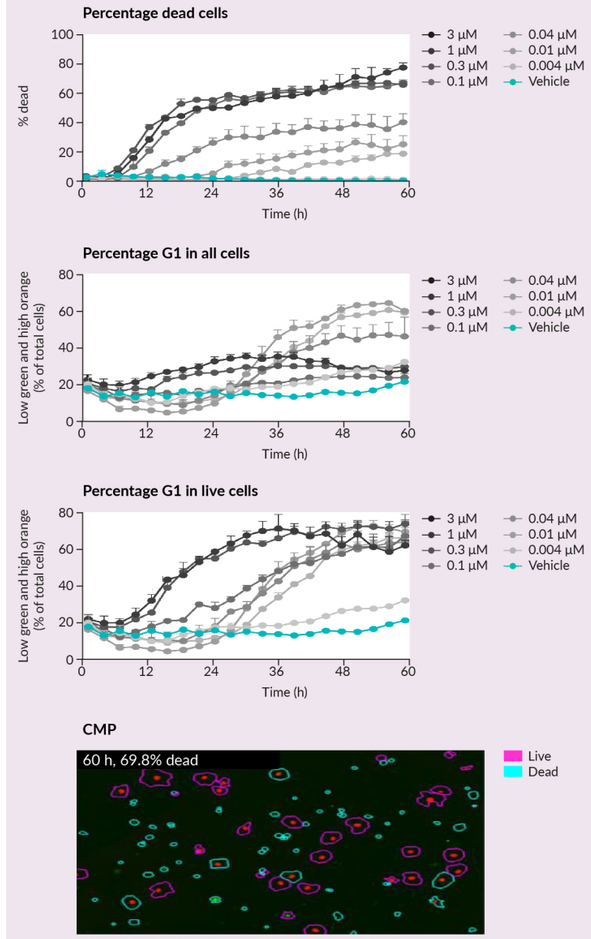
INSIGHTS FROM COMBINED LABEL-FREE AND FLUORESCENCE ANALYSES

The Incucyte® AI Cell Health Analysis Software Module provides label-free analysis of live and dead cells, although this enables deeper insight into cell behavior when combined with optional fluorescence measurements, as demonstrated in **Figure 2**.

Staurosporine is known to induce cell death via both caspase-dependent and caspase-independent mechanisms. To examine these pathways, MDA-MB-231 cells were treated with staurosporine (1 µM) in the presence of pan-caspase inhibitor Z-VAD-FMK (3–250 µM). To measure caspase activation, Incucyte® Caspase 3/7 Apoptosis Dye was included. Total cell death was quantified using Incucyte® AI Cell Health Analysis and indicated that staurosporine induced rapid cell death in the presence of all concentrations of Z-VAD-FMK. Within the dead cell population, caspase activation was measured using fluorescence classification. The number of caspase-positive dead cells decreased as the concentration of Z-VAD-FMK increased with efficacy log IC_{50}=−4.3.

HT-1080 cells expressing Incucyte® Cell Cycle Green/Orange Lentivirus were treated with a concentration range of camptothecin, shown in **Figure 3**. Incucyte® AI Cell Health Analysis was performed to identify live versus dead cells and fluorescence classification within the live cell population. We observed better separation of populations when removing the dead cells, with fluorescence classification of the live population showing time- and concentration-dependent increases in cells in G1 (orange lentivirus).

Figure 3. Analysis of cell cycle markers within live cell population.



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In partnership with: SARTORIUS

IMMUNO-ONCOLOGY INSIGHTS

IN VIVO TOOLS FOR PRECLINICAL APPLICATIONS

CHANNEL CONTENT

INTERVIEW

Multiomics integration: advancing pediatric cancer immunotherapy

Lauren Coyle, Editor, Immuno-Oncology Insights, speaks with **Raoul Santiago**, Clinical Investigator and Associate Professor of Pediatric Hematology and Oncology, University Hospital Center, Laval University, to discuss the role of multiomics in the I-O setting and how these tools can be leveraged to improve pediatric cancer care.

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Q 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.

Q 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.

Q 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 it to leverage the development of personalized immunotherapy and to find targets for novel combinations of immunotherapy in pediatric oncology.

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