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IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT ON
Assessing the evolving I-O landscape: key challenges and opportunities for 2024

Guest Editor
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IMMUNO-ONCOLOGY INSIGHTS Volume 5, Issue 1

Assessing the evolving I-O landscape: key challenges and opportunities for 2024

ASSESSING THE EVOLVING I-O LANDSCAPE: KEY CHALLENGES AND OPPORTUNITIES FOR 2024

SPOTLIGHT

New horizons for I-O in 2024: what the experts have to say

Samik Upadhaya

"In this issue you'll find an array of themes and topics, but one thing all of our contributing authors would certainly agree upon is that an exciting year lies ahead for I-O."

FOREWORD

Immuno-Oncology Insights 2024; 5(1), 31–33

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Welcome to the February issue of *Immuno-Oncology Insights*: 'Assessing the evolving I-O landscape: key challenges and opportunities for 2024'. This issue focuses on emerging trends in I-O, their potential for overcoming the current challenges facing the field, and taking stock of where future priorities should lie.

First up, we spoke with Roy Baynes (Eikon Therapeutics) who shares his perspective on the evolving picture in cancer combination therapies. His insights touch upon the challenges within the I-O space, specifically that of I-O/I-O combinations and within the capital market, and the progress in innovative therapeutics entering the clinical stage,

particularly those dedicated to discovering novel treatment combinations.

Claire Palles and Ik Shin Chin (University of Birmingham) provide insights on the immune-related adverse effects (irAEs) and toxicity profiles of immune checkpoint inhibitors. They also highlight progress in searching for predictive genetic markers to determine treatment response and predict the likelihood of irAEs.

Roy de Souza and David Hawke (BreakBio) join an interesting discussion on the development of cancer vaccines, with an emphasis on targeting multiple cancer branches simultaneously. They also discuss their work at BreakBio in target selection and manufacturing, adjuvant vaccine development, and the design of clinical trials for early-stage cancers.

Last but by no means least, we spoke to Rising Star Katie Campbell (University of California). Katie discusses the successful implementation of genomics and transcriptomics in the I-O space. She addresses the challenges associated with successful integration into the clinical setting, and the role spatial profiling plays in understanding molecular drivers and cellular interactions.

In this issue you'll find an array of themes and topics, but one thing all of our contributing authors would certainly agree upon is that an exciting year lies ahead for I-O. We hope you enjoy reading (and listening) along!

BIOGRAPHY

SAMIK UPADHAYA is the Associate Director of Scientific Affairs at the Cancer Research Institute (CRI). He is passionate about harnessing the full potential of scientific advances in immunology and cancer immunotherapy to help patients live better, longer lives. In his current role at CRI, Upadhaya oversees scientific diligence initiatives, which involve the meticulous monitoring and assessment of the outcomes of CRI-funded research endeavors. He also contributes to the conception and implementation of novel programs aimed at bolstering fundamental, translational, and clinical research in immunology and cancer immunotherapy. Additionally, Upadhaya assists in CRI's clinical trial activities and in tracking of the emerging trends and challenges in the global cancer immunotherapy landscape.

Prior to joining CRI, Upadhaya completed his doctoral studies in pathology and molecular medicine at Columbia University where he focused on investigating the spatiotemporal dynamics of blood and immune cell production. Following his PhD, he pursued a postdoctoral research fellowship at New York University School of Medicine where he developed new techniques to visualize and analyze *in-vivo* behaviors of stem cells of the immune system.

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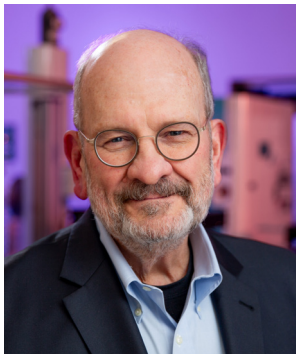
IMMUNO-ONCOLOGY INSIGHTS

ASSESSING THE EVOLVING I-O LANDSCAPE: KEY CHALLENGES AND OPPORTUNITIES FOR 2024

SPOTLIGHT

INTERVIEW

The future of combination therapies in I-O: insights, challenges, & future endeavors



Lauren Coyle, Editor of *Immuno-Oncology Insights,* interviews **Roy Baynes,** Executive Vice President and Chief Medical Officer of Eikon Therapeutics, about the development strategy of combination therapies, specifically in early-stage disease, in the I-O space.

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Q Could you describe the evolving landscape of combination therapies and what might we expect to see from research and development in 2024?

RB: Understanding the requirements for successful combinations has been largely led by the extramural collaborations group at Merck. They sit at the nexus of a remarkable amount of clinical trial data. To sum up a great deal of data, combinations are most likely to yield significant activity if the combination partners have firstly, independent action, and secondly, orthogonal or anti-correlated mechanisms of action.

"Importantly, combinations and monotherapies have advanced into earlier treatment lines. Adjuvant treatment... establishes the benefit of checkpoint inhibitors post-surgery, either as monotherapy or in combination."

Combinations of tyrosine kinase inhibitors have been most effective where the tyrosine kinase inhibitor has strong independent action such as in renal cell and endometrial cancer. While directional trends are observed in other tumor types, the independent action seems not to have been sufficient to offset tolerability issues with the combination.

Combinations with Receptor tyrosine-protein kinase erbB-2 (HER2)-targeted agents, like trastuzumab in HER2-positive gastric cancer, and HER2-targeted antibody-drug conjugates (ADCs) in HER2-positive breast cancer are great examples.

The data from combinations of chemotherapy have been clear, and this approach has redefined the treatment paradigm in many different advanced cancer types.

Emerging, we see combinations with ADCs which are considered as chemotherapy 2.0. ADCs rely on targeted chemotherapeutic agent delivery, thereby opening a therapeutic window. Certain ADCs have shown significant activity as monotherapy, and in some circumstances, have redefined the treatment paradigm in combination with checkpoint inhibitors. For example, enfortumab vedotin combined with Keytruda in advanced urothelial cancer has redefined the therapeutic landscape as was presented at the recent European Society for Medical Oncology meeting in advanced urothelial cancer (KEYNOTE-A39) .

Importantly, combinations and monotherapies have advanced into earlier treatment lines. Adjuvant treatment, supported by several studies, establishes the benefit of checkpoint inhibitors post-surgery, either as monotherapy or in combination. This is evident in Keytruda with non-small cell lung cancer (NSCLC) (KEYNOTE-091), and kidney cancer (KEYNOTE-564). Notably, this is the only adjuvant approach in renal cell cancer that has shown survival benefits. Melanoma, highlighted by KEYNOTE-53, -54, and -716, and recent combinations with chemotherapy in endometrial cancer (KEYNOTE-A18), further exemplify these advances.

The next focus area is perioperative treatment, where a paradigm of treatment involves neoadjuvant combination therapy inducing optimal pathological response, followed by continued immunotherapy. This approach, demonstrated in triple-negative breast cancer (KEYNOTE-522), has now become the standard of care, similarly observed in Non-small-cell lung cancer (NSCLC) (KEYNOTE-671), the only study in the perioperative space of NSCLC to show survival benefits. Potential translation into hormone receptor-positive breast cancer is suggested by promising initial data from KEYNOTE-756, indicating improved pathologic complete response.

Several novel mechanisms should be considered when looking at combinations that are likely to evolve. Firstly, evolving combinations in the ADC space are prolific, many of which will seek to explore combinations with checkpoint inhibitors. Secondly, there has been exciting data from the Moderna and Merck study of personalized cancer vaccines in combination with checkpoint inhibitors entering Phase III trials.

At my new company, Eikon Therapeutics, we have been working on systemically administered toll-like receptor agonists, that could have a promising future. Additionally, CatalYm GmbH, another company I collaborate with, is studying growth/differentiation factor 15 antagonist monoclonal antibodies and has shown some quite provocative data.

In summary, the field remains rich for exploration, however, challenges remain in the combinations of I-O/I-O drugs.

Q Are there any trends across the market or with investors in the IO space, and do you think the focus of this is accurate?

RB: Capital markets are currently challenging, driven by multifactorial influences such as industry trends, geopolitical forces, and various financial drivers. Investors face pressures, compounded by high global interest rates, providing de-risked investment opportunities for those willing to commit capital. This has created stress within the biotechnology ecosystem.

In addition, we have observed over the years, particularly in the I-O space, but in oncology and drug development generally, once a target is identified as validated, intense competition ensues, leading to numerous fast followers. Innovators must strategically build walls of data, incurring significant expenses, which plays to the strengths of larger pharmaceutical companies.

Significant overinvestment in areas has contributed to some of the challenges in the capital markets, particularly the I-O/I-O field. Currently, there is a notable influx of capital into ADCs, raising concerns about a potential repetition of past patterns.

Q Further to this, what problems do you think the industry might face in the upcoming year with combination therapies and how could these be addressed?

RB: Focusing on I-O/I-O, after many different IO/IO combination studies, the combination of LAG-3 plus a programmed cell death protein 1 (PD-1) was approved in the US. It should be noted that this approval was based upon a modest progression-free survival benefit in advanced melanoma, with no demonstrated survival benefit in the final analysis. Although updates may show minimal separation in survival curves over time, the trial has used up all of its alpha and can never demonstrate an overall survival benefit.

Some argue that this combination is safer than a cytotoxic T-lymphocyte associated protein 4 (CTLA-4) antibody combination. However, the CTLA-4/PD-1 combination has been complex, with a paucity of well-conducted trials and interpretable data. While the CTLA-4 antibody has single-agent activity in melanoma, its use in combination remains uncertain.

Approvals from US regulators for the CTLA-4/PD-1 combination include melanoma (CheckMate 067), intermediate and high-risk renal cell cancer (CheckMate 214), NSCLC (CheckMate 227 and 9LA), mesothelioma (CheckMate 743), and esophageal cancer (CheckMate 648). However, many questions persist, as many of these trials weren't designed to directly assess the contribution of individual components.

By contrast, five randomized controlled studies were appropriately designed to address the value of adding a CTLA-4 antibody including KEYNOTE-598 in NSCLC, the Lung MAP study in NSCLC, CheckMate 714 in squamous cell cancer of the head and neck, and

CheckMate 915, an enormous study in adjuvant melanoma. All of these have failed to show benefit. Generally, CTLA-4-containing arms were more toxic and overall tended to do worse. There is an ongoing study in renal cell cancer, a European Medicines Agency post-marketing commitment known as 8Y8, that is scheduled to readout in April 2024. Notably, this study, although long-enrolled, has progression-free survival and objective response rate as primary endpoints and lacks overall survival as a primary endpoint. If negative, it would mean all five appropriately designed trials failed. Other I-O/I-O combinations continue to release data, but none so far have demonstrated positive results in pivotal combination studies. It's crucial to highlight that, at present, no agent in the I-O space appears to surpass a good PD-1 antibody due to a lack of independent action among most agents.

Q Have you seen any progress being made in biosimilars, and is this something that we should shift our focus towards?

RB: The loss of exclusivity for the leading PD-1 antibodies is approximately 5 years away, and biosimilars are not yet a significant part of the landscape. In addition, the development of coformulations with hyaluronidase for subcutaneous use and combinations with other active agents are likely to extend the exclusivity periods.

In-licensing PD-1 antibodies developed and studied outside the US have faced approval challenges due to the need for multiregional studies that accurately reflect the US population; many such programs have been discontinued in the US. PD-1 innovators have established substantial data walls, enjoying a significant first-mover advantage. As a result, fast followers have struggled to gain a foothold. In my opinion, the PD-1 agents are the superior checkpoint inhibitors, and the programmed death-ligand 1s have appropriately struggled to gain share.

Q Could you discuss any recent milestones or achievements that Eikon has reached in its research toward solid tumor treatments?

RB: Eikon, a start-up company celebrating its fourth anniversary, is strategically and purposefully integrating science and engineering to pioneer innovative therapeutics. Grounded in super-resolution microscopy inspired by Eric Betzig's Nobel Prize-winning work, the company utilizes various assay tools, robust data analytics, including machine learning and AI, and clinical research and development.

Organic assets, visible on our website, include a novel androgen receptor modulator in lead optimization, a Werner syndrome helicase-targeted agent, a valosin-containing protein-targeted agent, and additional undisclosed targeted agents approaching lead optimization, androgen receptor splice variant 7-targeted agents in hit identification, undisclosed oncology targeting agents in hit to lead stage, and undisclosed programs in immunology and neuroscience.

Impressive progress in the past 4 years has propelled Eikon to become a clinical-stage company. We have strategically in-licensed promising assets to complement our portfolio, ensuring readiness as our internal agents advance.

Inorganic assets in the form of a systemically administered toll-like receptor 7 and 8-coagonist are entering Phase II and a poly [ADP-ribose] polymerase 1 (PARP-1) selective antagonist is in Phase I in collaboration with IMPACT Therapeutics. Collaborating with IMPACT Therapeutics, we are conducting IND-enabling studies for a brain-penetrant PARP-1 selective agent. The PARP-1 work is a testament to our commitment to advancing cutting-edge therapies.

Q Finally, could you summarize one or two key goals you wish to achieve in the upcoming year?

RB: My major goal is unchanged, and that is to continue to discover and advance novel therapeutics to address grievous diseases and make a meaningful difference in the lives of patients.

BIOGRAPHY

ROY BAYNES currently serves as Executive Vice President and Chief Medical Officer of Eikon Therapeutics, Inc., a privately-held biotechnology company. Prior to Eikon Therapeutics, Baynes served as Chief Medical Officer, as well as Senior Vice President and Head of Global Clinical Development, at Merck and Co, Inc., where he helped Merck become a leading oncology and global healthcare company. Baynes also previously served as Senior Vice President of Oncology, Inflammation and Respiratory Therapeutics at Gilead Sciences, Inc., which was preceded by his service at Amgen, Inc. as Vice President of Global Clinical Development, and Therapeutic Area Head for Hematology/Oncology. Before joining Amgen, Baynes was the Charles Martin Professor of Cancer Research at the Barbara Ann Karmanos Cancer Institute, a National Cancer Institute-designated Comprehensive Cancer Center, at Wayne State University. He received his medical degree and PhD from the University of the Witwatersrand in South Africa, and completed his medical training in the Department of Hematology and Oncology at Johannesburg Hospital. Baynes has received innumerable awards for his activities as a physician/scientist, and currently serves on the board of directors at Natera, Inc. and Travere Therapeutics, Inc. and is a paid advisor to Decheng Capital, CatalYm GmbH and Nurix Therapeutics Inc.

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ASSESSING THE EVOLVING I-O LANDSCAPE: KEY CHALLENGES AND OPPORTUNITIES FOR 2024

SPOTLIGHT

INTERVIEW

Navigating genomics and transcriptomics in the immuno-oncology space

This article is part of our 'Rising Stars' series, giving a platform to the emerging leaders of the sector. In this series, we share the perspectives of fledgling thought leaders, chosen by our Editorial Advisory Board members and Guest Editors as future stars in their field. Samik Upadhaya, Guest Editor of our February issue, had this to share on his Rising Star nomination:

> "Katie has a deep appreciation for and embodies the spirit of interdisciplinary collaboration. Among her many scientific pursuits, Katie is leading translational studies for clinical trials conducted by SWOG, an NCI-supported cooperative group. I am genuinely impressed by her ethos to support young scientists. Katie is currently the Chair of the AACR Associate Member Council and responsible for organizing the professional development events for the associate members."

RISING STARS

Lauren Coyle, Commissioning Editor, *Immuno-Oncology Insights*, interviews **Katie Campbell**, Postdoctoral Fellow, Cancer Research Institute, about her current research on genomics, transcriptomics, and spatial profiling to address challenges in the I-O field.

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Q Can you tell us a bit about your career in the immuno-oncology space so far and what you're currently working on?

KC: I first decided I wanted to be a scientist in my fourth-grade class when learning about microorganisms for the first time. It was then that I told my dad I was going to be a scientist, without fully understanding what that entailed or what that career trajectory would look or feel like.

My graduate studies at Washington University started in 2014 where I was initially introduced to cancer genomics. The genomic space quickly evolved into cancer informatics, and this shift expanded my focus to encompass both. From the start of my studies until 2018, I explored checkpoint inhibitors and gained insights into cancer and the immune system through a clinical trial focused on treating head and neck cancer with pembrolizumab in the neoadjuvant space.

Upon pursuing my postdoc studies, I further developed my research, delving into larger Phase 2 clinical trials involving various I-O agents. This led to the implementation of cutting-edge technologies in genomics, transcriptomics, and spatial profiling. This brings me to my current status as a junior faculty member at UCLA. Now, I integrate all of these technologies to continue understanding how we can best leverage cancer-immune interactions for therapeutic approaches, by studying clinical samples and large data sets.

Q Looking at genomics and transcriptomics data, how has this successfully been implemented in I-O so far and what challenges remain to be solved?

KC: Beginning with genomics and transcriptomics data, the successful sequencing of the human genome marked a significant milestone, providing a reference and foundational point for subsequent research. Additionally, the successful sequencing of the first cancer genome led to extensive data generation and technological advancements in the field. Consequently, genomics and transcriptomics have emerged as one of the most cost-effective means to comprehensively profile tumors, facilitating their successful implementation in the establishment of various companion diagnostics.

In the genomics space, known genetic drivers of cancer itself, as well as known genetic implications in driving resistance or sensitivity to drugs, have been identified. Having this companion information alongside clinical data allows an understanding of what works and what doesn't. Transcriptomics takes it another step further because it not only addresses the genetic status but also the behavioral aspects, thus several gene panels and predictors of response have been generated.

There have also been accomplishments in leveraging transcriptomics data to understand signaling events that occur upon the successful recognition and elimination of cancer cells by the immune system. Regarding challenges, one of the most significant obstacles is effectively implementing these findings into the clinical setting. Since there are various preferred gene panels and technologies, continued efforts are needed for the establishment and real-time testing of these predictors or companion diagnostics. Advancing companion diagnostic information has been somewhat limited, and addressing this limitation is crucial for further progress.

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"...genomics and transcriptomics have emerged as one of the most cost-effective means to comprehensively profile tumors, facilitating their successful implementation in the establishment of various companion diagnostics."

Q How can these challenges be addressed going forward?

KC: Tackling these challenges is crucial for adopting a forward-looking perspective. Drawing from my observations at UCLA and previous successful practices, a proactive approach incorporating insights from clinical trial planning—especially those involving newer agents, particularly in the earlier Phase 2 setting—would be effective when working with a smaller cohort of patients, allowing for comprehensive examination.

The utilization of knowledge acquired during these deep dives could be used to suggest secondary endpoints or additional translational objectives in planning subsequent clinical trials. While there may be challenges, such as the potential inaccuracy of the diagnostics themselves, we need to creatively integrate this technology into the trial planning process. By doing so, we can harness the benefits of technology and ensure that it becomes an integral part of strategic planning, ultimately contributing to more informed and successful outcomes.

Q How does the integration of spatial profiling data contribute to a better understanding of molecular drivers and cellular interactions? What considerations do you take when integrating these into your research?

KC: Spatial profiling, while somewhat lagging in standardization and effective implementation compared to genomics and transcriptomics, plays a pivotal role in enhancing understanding. In genomics and transcriptomics, established protocols, benchmarks, and testing exist, addressing the complexities of data analysis. In contrast, spatial profiling faces limitations due to the novelty of cutting-edge technologies, their cost, and the evolving understanding of the best methodologies.

Spatial profiling offers clarity to the information inferred from genomics or transcriptomics data. It moves beyond stating the presence of cells and their gene expression to elucidate how these cells interact and behave collectively. The rapid progression of spatial profiling technologies, especially in protein analysis, has evolved from single-stain IHC to multiplex immunofluorescence reaching complexities of hundreds or thousands of transcripts. When executed effectively, this high-resolution information provides a visible representation of what was initially interpreted from transcriptomic data.

Integrating this technology effectively into research, whether it's experimental, clinical, or translational, demands careful consideration. Cost is a primary concern, with varying degrees associated with resolution. Resource availability is another factor, relying on collaborations or

relationships to access specific platforms. The pivotal consideration is aligning the technology with the research question. The era of vast data informatics emphasizes moving beyond the excitement of new technologies to a hypothesis-driven approach. This shift involves questioning the scientific interest, and ensuring that the chosen technology aligns with the research goals.

One of the major mistakes with this consideration is the allure of using these advanced technologies without a clear scientific need. The selection of costs, resources, and technology should align with the research questions depth and focus. The field is seeing an intriguing juncture where informed decision-making, grounded in scientific inquiry, guides the progress of both science and technology. Overcoming this challenge could be achieved by incorporating this type of technology into research, particularly in the context of a grant, where the focus should lie on the underlying motivation for generating data.

Q In your current research on somatic alterations and antigen-presenting machinery, how do you approach the analysis of human leukocyte antigen (HLA) genes?

KC: HLA genes encode elements of the antigen presentation machinery on all of our cells, serving the mechanism through which the immune system distinguishes between normal and diseased cells. Diseased cells can range from an infectious agent to cancer where the antigens exhibit variation. The complexity arises from the diversity of the HLA genes, both within individuals and across the population as a whole.

While this diversity is beneficial from an evolutionary standpoint in terms of our immune system, allowing it to combat and eradicate diseases effectively, it poses a significant challenge for research due to its polymorphic nature. With thousands of different HLA alleles or gene versions, studying them with a single human reference becomes impractical.

To navigate this complexity, the HLA gene should be analyzed at an individual level. In our research, this is generally done with genomic and transcriptomic technologies as sequencing reads can effectively differentiate between various alleles. Utilizing this information, we can map the reads back to the HLA genes, enabling a more comprehensive and specific characterization of a patient's immune system in the context of their cancer.

A crucial element of our research involves leveraging prior work in the genomic space to account for variability across the HLA gene locus. By discerning and sorting differences between HLA alleles, we can sort and study them independently. This strategy enables us to examine HLA alleles in the context of an individual's cancer, offering valuable insights into the interactions between immune response and cancer progression.

Q Following that, what strategies have you employed to identify and characterize copy number alterations in the antigen-presenting machinery?

KC: We study this at the genomic level using whole exome sequencing data, and that allows us to perform HLA haplotyping and count the number of reads

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"Collaboration across diverse fields is fundamental in advancing scientific research and a strong emphasis should be placed on interdisciplinary collaboration to address multifaceted challenges."

associated with each allele. We can also study it at the transcriptome levels and separate the reads based upon the polymorphisms, and assign each read to each allele to compare and quantify the expression of each allele.

Finally, at the protein level, we can use various approaches. We can consider it with patient tissues, with cell line data, and we can stain those samples with allele-specific antibodies. Essentially, it comes down to how can you tell the difference between two HLA alleles in order to say one is from the maternal allele versus the paternal allele. Then we can either sort them at the sequencing level, or we can stain for them differentially at the protein level.

Q How do you collaborate with experts from different fields, and how has this contributed to the progress of your research?

KC: Collaboration across diverse fields is fundamental in advancing scientific research and a strong emphasis should be placed on interdisciplinary collaboration to address multifaceted challenges. The substantial burden on trainees who must rapidly acquire and apply knowledge should be recognized. My approach involves considering not just what they need to know, but also who they can collaborate with to ensure it is being addressed from all angles.

That being said, none of my work throughout my academic journey until now has been done alone—collaboration has been integrated at all points. Starting with my undergraduate years in biochemistry, to my molecular cell biology PhD in a genomics lab studying clinical trial samples, I was consistently collaborating with clinicians. For instance, during one of the immunotherapy clinical trials, I began attending the lab meetings of a cancer immunology lab. That collaborative experience was significant in my progress and I attribute much of my success to the 'team science' attitude.

Productive discourse, dialogue, and even disagreement have been essential in interpreting and validating our findings. The diversity in expertise, spanning technology, biology, and medicine, has integrated these varying perspectives to examine research questions.

Without incorporating diverse considerations and perspectives into a project, there is a high chance of overlooking crucial aspects. I not only collaborate with these individuals, but I consider my entire training a product of that mentorship across those disciplines. My hope, especially for individuals in bioinformatics and biological data science, is to be able to consider that coding alone might lack significance without integrating biological knowledge.

Q Finally, what are your main goals and aspirations for the future?

KC: In graduate school, I had incredible mentorship, leadership, and guidance and during this time, I realized I wanted to do good science, and so that has been

and always is my aspiration. I am fortunate to have had supportive academic training that aligned with that aspiration. The goal is to continue to do good science and progress through this faculty transition.

As a junior faculty member, my immediate goal is to continue pursuing that trajectory as long as I'm doing good science. One thing that I would like to mention is the collaboration with industry, nonprofit sectors, and the government. Incredible science and progress are happening in these settings, and as long as I get to contribute to the forward progress, I am continuing to achieve my goals.

BIOGRAPHY

KATIE CAMPBELL is a junior faculty member at the University of California, Los Angeles, where she previously completed her postdoctoral research in the laboratory of Antoni Ribas MD PhD. Her research focuses on integrating multiplexed spatial profiling with other high-dimensional 'omics data to understand the complex molecular drivers and cellular interactions responsible for immunotherapeutic response in melanoma clinical specimens. Campbell is particularly interested in understanding how the somatic alterations in the antigen presentation machinery modulate tumor-T cell interactions, particularly through copy number alterations that result in imbalance or loss of HLA genes.

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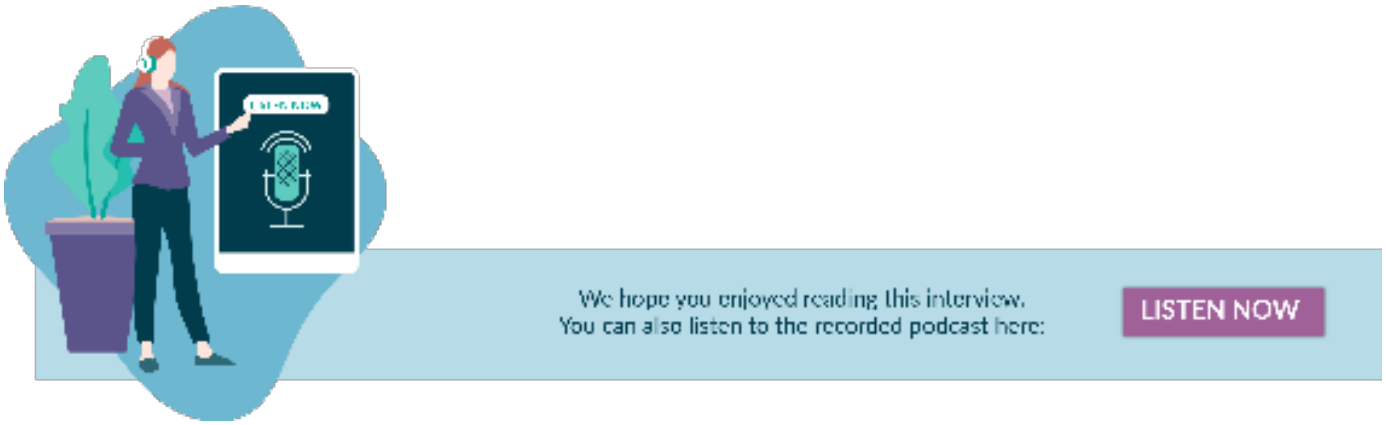

IMMUNO-ONCOLOGY INSIGHTS

ASSESSING THE EVOLVING I-O LANDSCAPE:KEY CHALLENGES AND OPPORTUNITIES FOR 2024

SPOTLIGHT

COMMENTARY

Will it be possible to find predictive genetic markers of immune checkpoint inhibitor toxicity that are not also predictive of survival?

Claire Palles and Ik Shin Chin

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A durable response to immune checkpoint inhibitor therapy is observed in 20–30% of patients, however, approximately 10–55% experience one or more grade 3+ immune related adverse events, depending upon whether they were treated with single-agent or combination checkpoint blockade therapy. In 2022, the first genome-wide association study of immune checkpoint inhibitor-induced immune related adverse events was published. We could now begin to predict which patients will experience serious immune-related adverse events requiring urgent treatment with immunosuppressive agents. There is a growing body of evidence that those who experience immune related adverse events have a better treatment response and survival outcome. This Commentary article reviews the evidence for the link between immune related adverse events induced by immune checkpoint inhibitors and efficacy. It summarizes the evidence for the interleukin-7 single nucleotide polymorphism, the first genome-wide significant biomarker of immune-related adverse effects.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) targeting programmed cell death 1 (PD-1), programmed cell death 1 ligand (PD-L1), or cytotoxic T cell associated protein 4 (CTLA-4) were first approved as anti-cancer treatments in 2011 [1] and their use has increased rapidly since. In the US alone there were 462,049 prescriptions for six ICI agents in 2021 [2]. ICIs are used in the first or second lines of treatment for approximately 50 cancer types because of their ability to reactivate immune T cells to mount strong anti-tumor responses [3]. One of the major problems of ICI treatments, however, are the unwanted auto-immune-like immune-related adverse effects (irAEs) that can affect multiple organs. Whilst the majority of irAEs are mild, 10–55% of patients can experience severe events requiring steroid or immune modulatory treatment [4–9]. The toxicity profile of the ICI therapies can also vary depending on the ICI agent used. Egeler *et al* reported hypophysitis and fatigue as the most common serious irAE for anti-CTLA-4 agents, asthenia in anti-PD1 agents, and diarrhea and rash in combined anti-PD1 and anti-CTLA4 regimes. Deranged liver function tests were also commonly reported from all three ICI regimes [10]. Rates of irAEs are similar for patients with auto-immune conditions but the majority of patients included in these studies did not have active or uncontrolled autoimmune disease symptoms [11–14]. For safety reasons, it has been suggested that autoimmune conditions be effectively treated before commencing treatment [15].

The severity of irAEs is evaluated using Common Terminology Criteria for Adverse Events with grade 1 representing mild, grade 2 moderate, and grades 3 and 4 severe events. ICI treatment normally continues following grade 1 irAEs, is interrupted for grade 2 until resolution to grade 1 or below and discontinued for grade 3 or higher. Systemic oral and intravenous high-dose steroids or immuno-modulatory agents are often required for grade 2 or higher irAE [16,17]. The toxicity-induced fatality rates for ICIs have been estimated at 0.36–1.23% [18]. Certain immune-related endocrinopathies can also result in chronic morbidity and the requirement for long-term hormone replacement therapies [19,20]. Despite this, no biomarkers are currently in use clinically to predict the patients at risk of these severe events. The ICI regimes currently in use are often combined with other anti-cancer therapies including chemotherapy or targeted therapies. Predictive biomarkers of risk of toxicity will need to be tested for their clinical utility in patients being considered for increasingly complex regimens. There is also a growing need for clinically useful biomarkers of treatment response and resistance.

Limited tumor biomarkers such as microsatellite stability status and PD-L1 expression are used to select patients for treatment with ICIs [21,22]. High tumor expression of PD-L1 and high tumor mutation load have both been shown to be associated with favorable responses in multiple settings, but efficacy has also been seen in patients whose tumors do not display these features [23–26]. Despite only using ICI in tumor types where a response is predicted e.g., due to the presentation of a large number of neo-epitopes on the surface of tumor cells, only 20–30% of patients experience a durable response to treatment [27,28]. Biomarkers are urgently required to identify this subset of patients. A potential challenge to overcome before implementing biomarkers is the potential overlap between markers that predict tumors of toxicity and markers of durable response. As shown in **Figure 1** multiple tumor and host factors are likely to be important in explaining the risk of irAEs and the likelihood of efficacy in response to ICIs.

WHAT EVIDENCE IS THERE FOR A LINK BETWEEN SURVIVAL AND TOXICITY?

It has been suggested that skin toxicities such as hand-foot syndrome are associated with

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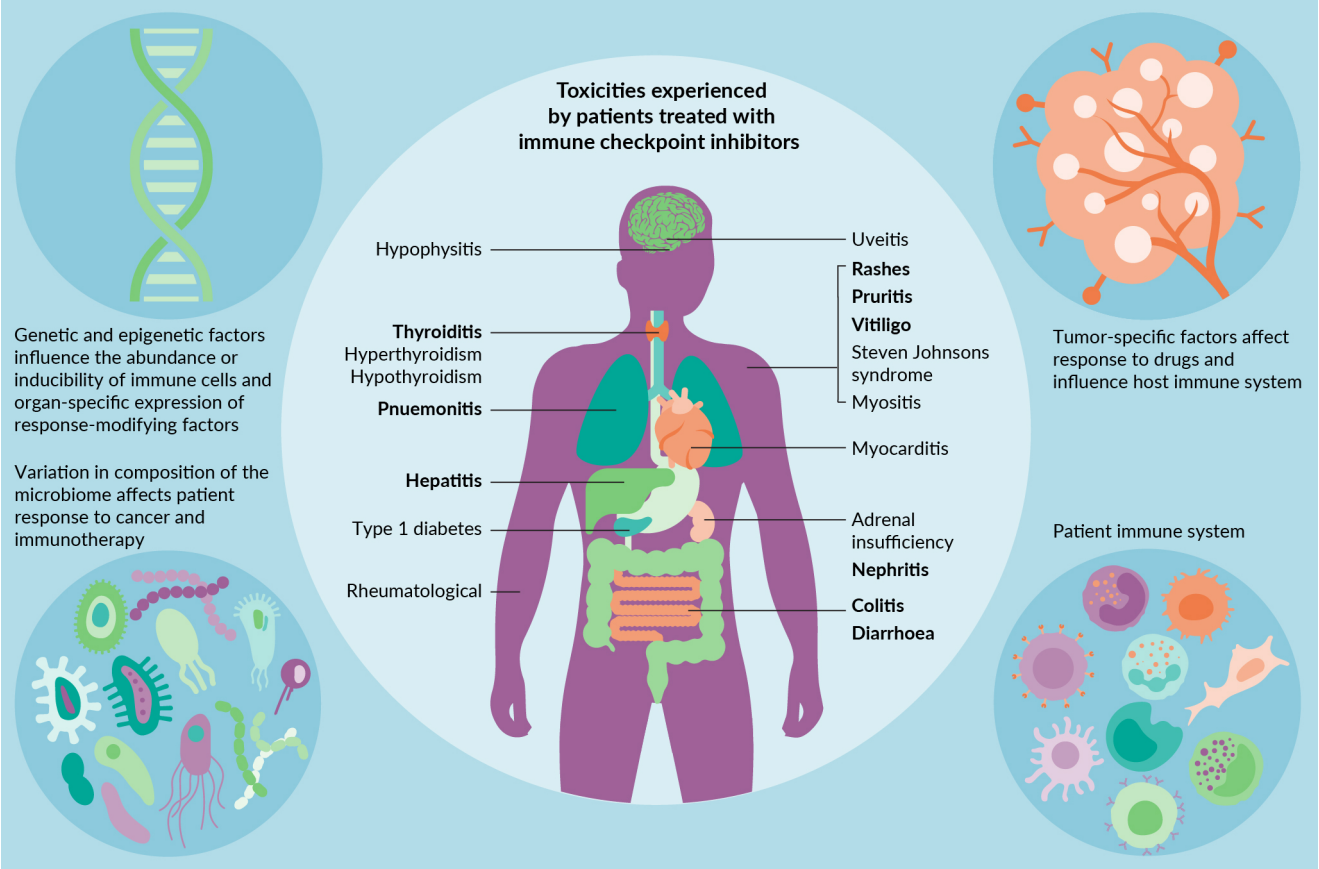
an improved response in patients treated with conventional chemotherapies [30,31]. There is now a considerable body of work suggesting that irAEs are a biomarker of response and survival [17, 18, 40, 41, 32–39] in patients treated with ICIs. Most of the studies investigating this coded the presence of irAEs as a binary explanatory stratification variable and did not account for the timing/onset of the irAE. In addition, the majority of studies analyzed the relationship between any grade irAE and outcome rather than investigating how this association changes with increasing grade of irAE. This may be due to the small number of high-grade irAEs observed in modestly sized studies [18,34,35,37,38,40].

In 2021, two meta-analyses were published which examined the relationship between irAEs and treatment efficacy [42,43]. Both studies stratified analyses by the outcome and methodology used in the source papers and the meta-analysis by Hussani *et al* [43] was also stratified by cancer type. Hussani *et al* included patients on single-agent Nivolumab (anti- PD-1), Pembrolizumab (anti-PD-1) or Ipilimumab (anti-CTLA4) or combination ICI therapies (anti-PD1 and anti-CTLA4) and reported significant increases in progression free survival (PFS), objective response rate (ORR) and overall survival (OS) in the group of patients who experienced any grade irAEs [43]. This effect was seen in both melanoma and

FIGURE 1

irAEs experienced at grade 3 by 1% or more of patients treated with single agent or combination checkpoint blockade are shown in bold.



For a breakdown in the frequency of individual toxicities please see Khoja *et al.* and Haanen *et al.* [9,29]. The complex interplay between host (genetics, epigenetics, modifiers, microbiome) cancer and drug affects both toxicity and response to checkpoint blockade. irAE: immune-related adverse effects.

NSCLC patients. The second meta-analysis, by Zhong *et al*, combined data from patients with multiple cancer types, stratifying by the outcome measure analyzed. The authors reported results from a random-effects meta-analysis of ORR, OS, and PFS because of the significant heterogeneity in effects seen across the included studies in each analysis (I2=55–73%, P=<0.0003). ORR, OS, and PFS were statistically significantly improved in patients who experienced any grade irAEs, however, in the sub-analyses restricted by the type of ICI agent used, the presence of irAE was only significantly associated with OS and PFS in the anti PD-1/PD-L1 group and not in the anti-CTLA4 group. This must be caveated by highlighting that the CTLA-4 evidence was from a small number of studies, consistent with the fact that single-agent Ipilimumab is not so commonly used.

Zhong *et al* were also able to perform meta-analyses to investigate which of the common irAEs were associated with OS and PFS (three to seven studies included in each analysis). Skin, endocrine, and gastrointestinal irAEs were associated with significantly increased OS and PFS but pneumonitis and hepatobiliary irAEs were not. Zhong *et al* also identified 3 studies where the grade of irAE had been investigated in relation to OS. No significant association between grade of event and OS was observed. Das *et al* [44] discussed in their review of ICI-induced irAE and efficacy that it is possible that patients experiencing high-grade events might not experience a survival benefit due to the immune suppression associated with treatment for irAEs.

There is a complex relationship between irAE onset and outcome measures which may not be fully captured in simple Cox regression models. Some of the patients who experience irAEs are likely to be those that stay on treatment longer and we know that despite treatment discontinuing following a grade 3+ irAE, the effects of ICIs can be long-lasting and durable. To assess if the occurrence of irAEs serves as a biomarker for PFS, Eggermont *et al* [33] incorporated irAE as a time-varying covariate. In their model, the irAE variable was coded as 0 until the time of onset of the irAE and then 1 thereafter, while also accounting for the time to irAE. 1019 patients with stage III melanoma, treated with pembrolizumab, were included in this study, and consistent with studies not considering the time dependency of both irAEs and PFS, the authors reported improved PFS (hazard ratio [HR] 0.61) in the group of patients who experienced irAEs. Another approach that tries to control for the time patients were at risk of experiencing irAEs, is to perform a landmark analysis. This involves defining a time point at which to perform an analysis of outcome e.g., once all patients have received the same number of cycles of treatment or at a timepoint where 90% irAEs seen across a cohort have already occurred [45]. Authors reporting the results of landmark analyses also identified associations between irAEs (either skin or any irAE) and improved measures of survival and response (PFS, ORR, or OS) [32,35,37,46]. Despite the different methods used for analysis, there is strong evidence that irAEs are truly biomarkers of PFS and OS, accepting that the magnitude of the association reported may have been mis-estimated in some studies due to the failure to consider competing risks. PFS is considered to be a good surrogate endpoint for OS in studies investigating evaluating the effectiveness of ICI therapy [47–49]. However, as the indications for ICI therapies move earlier in the treatment pathway such as in the peri-operative setting, OS may be a more appropriate endpoint to establish the efficacy of treatment used for curative intent.

ROLE OF GENETIC BIOMARKERS

As reviewed by us and others [50–52], germline genetic variation may explain the variable outcomes from ICI in terms of both toxicity and response to treatment. Genetic variation is likely to contribute to cellular responses which have been shown to differ between

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those that experience irAEs and those that do not, such as the ability of suppressive B cells to be induced and limit the self-reactive response of T cells [53]. Hundreds of genetic regions have also been found to explain risk of multiple autoimmune diseases and some of these have been shown to also be associated with response to ICIs [54]. Polygenic risk scores (PRS) can be calculated by summing the effects of multiple genetic markers to generate a per-person score. Scores can then be compared between groups such as those that experience irAEs and those that do not. PRS for autoimmune disorders have been shown to be associated with increased risk (odds) of irAEs. Khan *et al* reported a significant association between a PRS for psoriasis and increased risk of skin irAEs in patients treated with anti-PD-L1 inhibitor Atezolizumab [46]. The same group also showed significant associations between PRS for psoriasis, atopic dermatitis, and vitiligo, and OS in patients treated with Atezolizumab. Increased risk scores for psoriasis and vitiligo were associated with longer OS whereas decreased risk scores for atopic dermatitis were associated with longer OS. A PRS for hypothyroidism developed using UK Biobank data consisting of 1,502 single nucleic polymorphisms were also found to predict thyroid irAEs in non-small cell lung cancer patients [55].

Rarely, heterozygous (monoallelic) germline loss of function mutations in CTLA-4, leading to loss of protein expression, have been identified [56]. Patients present with autoantibody-mediated cytopenia, lymphadenopathy/splenomegaly, hypogammaglobulinemia, organ-specific autoimmunity, and lymphocytic infiltration of nonlymphoid organs in late childhood or early adulthood [57]. The penetrance of these mutations is incomplete, with 40% of carriers having no clinical signs of disease [58]. Screening for rare variants in genes like CTLA-4 and 54 other genes implicated in inborn errors of immunity [59] are likely to also be important to avoid severe reactions to ICIs.

PROGRESS SO FAR IN IDENTIFYING GENETIC BIOMARKERS OF IRAES

The first genome-wide association study of immune checkpoint blockade-induced irAEs was published last year [60] which identified a marker, rs16906115, associated with any grade irAEs ($P<5\times10^{-8}$). The discovery phase of the study was conducted in 339 cases and 1,412 controls. Patients were diagnosed with one of 12 different cancer types and predominantly received single agent therapy. Cases were defined as those who experienced an irAE as deduced from autoimmune-like electronic health record diagnoses codes. Importantly the marker, rs16906115, was also associated with irAEs in three independent cohorts (two multi-cancer cohorts and one melanoma cohort). The HR in the combined analysis was 2.1, p-value of 3.6×10^{-11}, with stronger effects seen in the discovery Dana Faber Cancer Institute dataset (HR 2) and Massachusetts General Hospital validation cohort (HR 2.5) and a weaker effect in the meta-analysis of 12 clinical trials (validation cohort 2) (HR 1.2). It was not clear which type of irAEs was driving the observed association as nominal significance was observed across multiple irAE subtypes, but it was noted that this single nucleic polymorphism has not previously been implicated in predisposition to autoimmune diseases and it is not part of existing PRS for autoimmune diseases. A second independent study of 214 melanoma patients receiving ICI [61] also found that rs16906115 was associated with an odds ratio (OR) of 2.24, p-value 0.046, and as also described in [60], the marker was associated with a much stronger effect in patients receiving single-agent ICI (OR = 6.0 [95% confidence interval: 1.5–23.0, P = 0.0084]).

Groha *et al* [60] investigated whether rs16906115 is associated with survival in patients treated with ICI and did not find a significant association with either PFS or OS across the cohorts studied. Interestingly they did find a significant association between the

irAE risk allele and improved survival in 433 primary and metastatic melanoma patients from the The Cancer Genome Atlas cohort. These patients received chemotherapy rather than immunotherapy suggesting that the irAE-associated allele exerts an anti-tumor effect independent of the presence of an ICI agent and that carriers of rs16906115, who are at increased risk of irAEs, may respond well to conventional chemotherapy.

POTENTIAL FOR IMPLEMENTATION OF BIOMARKER TESTING

Globally many groups are collecting samples; irAE and survival data from patients treated with ICIs and as this data is combined, it is likely that additional markers of ICI-induced irAEs will be identified. As more markers are identified that are associated with irAEs but not with survival, these could be combined into PRS to predict risk of irAEs. Similarly, if markers of survival and efficacy are identified PRS predicting likelihood of efficacy following treatment can also be generated.

The clinical utility of rs16906115 remains to be determined, particularly in patients receiving combination immunotherapy or chemo-immunotherapy regimens, which are increasingly being used, but, for the first time, we have a robustly identified biomarker of ICI-induced irAEs that deserves clinical evaluation. Determining the correct strategy for testing clinical benefit is challenging, as is implementing routine clinical testing of pharmacogenetic markers [62,63]. Possible study designs for the evaluation of predictive markers of irAEs include randomized controlled trials and comparison of incidence of irAEs before and after implementing testing and enhanced management of marker-positive patients. The lack of association between rs16906115 and PFS and OS in patients treated with ICIs demonstrates the possibility of identifying genetic variants associated with increased irAE risk but not improved ICI treatment efficacy. Careful evaluation of this variant and those that follow will be essential in informing how genetic biomarkers enhance clinical practice. Being able to predict both the risk of toxicity and the likelihood of favorable response prior to ICI treatment will enable clinicians to deliver personalized safer treatments and allow patients to make informed decisions.

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IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT

INTERVIEW

Revolutionizing cancer treatment? A conversation on the potential of personalized cancer vaccines

Lauren Coyle, Commissioning Editor, *Immuno-Oncology Insights*, speaks with **Roy de Souza** and **David Hawke**, co-founders of BreakBio, about innovative strategies for personalized cancer vaccines and the transformative potential of early intervention in cancer.

In the version of this Interview initially published, **Roy de Souza** expressed his opinion on the challenges companies have faced in the development of personalized cancer vaccines (pp 68–69); however, this has since altered. To reflect this change, we have amended the HTML and PDF versions of this article as of May 30, 2024.

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Q Could you tell me a bit about your career and what you are working on?

RdS: I studied engineering and initially worked in strategy consulting. Later, I ran a tech company that was eventually sold to Warner Bros Discovery. My career is predominantly in the technology space, but it took a turn in 2017 when my late wife was diagnosed with colon cancer. Navigating the challenges of cancer care became a personal mission.

The main problem is that many solid cancers cannot be cured. Life can only be prolonged, as the doctor told my wife and me. The realization that finding new drugs was the way to find a potential cure, fueled my exploration into the field. Initially, I sought existing solutions, but over time, I recognized the wealth of researchers and academics and the great work they have conducted often funded by the government via the National Cancer Institute.

Acknowledging the diversity of research ideas, I decided to leverage this. I started analyzing promising concepts and talking to researchers or doctors to see if we could initiate clinical trials and establish a pharmaceutical biotech company that could bring about great change.

Over time, I have come to understand that cancer is a complex tree of problems, unique to each patient. Though one branch may be dominant, attacking it alone proves insufficient. When a dominant branch of the cancer is killed, other branches grow and take its place. Developing an effective therapy that eliminates all the branches means acknowledging that different patients require different drugs. Further, within each patient, multiple drugs are required to kill multiple branches of the cancer.

It is a seemingly simple concept, yet its application is intricate. Eliminating all branches of cancer requires us to take an innovative approach to identifying suitable targets. That is essentially where the company vision started, and how we envision a future of potential curative treatments.

DH: My career started with a more traditional path. I initially studied chemistry with a focus on organic chemistry, and I had a keen interest in instrumentation. This led me to work at the City of Hope Cancer Center in Southern California where I joined an immunology group with a significant focus on cancer, particularly the cancer antigen, carcinoembryonic antigen.

Subsequently, I ventured into the instrumentation industry specializing in mass spectrometry. This prompted my return to research at the MD Anderson Cancer Center in Houston. I ran the proteomics facility for many years and became involved in major histocompatibility complex (MHC) peptide analysis. This then led to a greater involvement in immunology, potential vaccines, and T cell therapies.

My connection to cancer research also goes beyond a professional interest. I lost both my aunt and grandfather to lung cancer and my wife has faced two bouts of breast cancer. It is often these personal connections that drive us to tackle meaningful problems and invest time in addressing them.

Q What sparked your interest in the I-O space, and what key progress has been made so far?

RdS: The driving force behind my interest in I-O stems from the unique challenges posed by the diverse nature of cancer. As we discussed, unlike transmissible

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diseases, where a single protein or set of targets remains consistent across individuals, cancer manifests as a complex tree of problems with various branches or clones. These clones, termed polyclonal or heterogeneous, exhibit significant differences even among patients sharing a similar cancer type. While there may be some overlap in mutations at onset, the evolving nature of cancer leads to distinct challenges.

As mentioned, to work towards a cure, we must target multiple aspects simultaneously. This perspective led me to I-O, where the power lies in leveraging T cells. Instead of administering numerous drugs at the same time which could be toxic, where we do not have a sufficient breadth of existing drugs, various types of T cells can be deployed, each programmed to attack a specific target. By targeting 30 different antigens, for instance, we increase the likelihood of impacting various branches of cancerous growth.

T cells are particularly appealing due to their specificity—they seek out a precise peptide target. This specificity ensures that they only engage and destroy cells bearing that target, saving normal cells, and minimizing side effects.

DH: I worked in the immunology space at the start of my career, before the advancements of checkpoint inhibitors, when the field lacked clarity in cancer immunology. However, even then, there was the hope of being able to understand the immune system and harness it to combat cancer effectively.

While working at MD Anderson, my focus started to shift toward the I-O space. Although most of my research was primarily focused on general oncology and deep research, a project analyzing MHC peptides led to my involvement and participation in clinical trials.

The turning point was witnessing the significant potential of I-O during these trials. Patients treated with checkpoint inhibitors exhibited extended survival compared to traditional chemotherapy. Achieving lasting cures through the immune system became an exciting prospect—the holy grail of cancer treatments and potential vaccines.

In principle, vaccines offer a safety advantage. Activated T cells not derived from the patient, while possessing potent capabilities, can cause harm by targeting the wrong elements. As the body has the ability to control whether to create a T cell against a target or not, the vaccine route continues to emerge as a promising avenue.

Unlike chemotherapy, which often induces systemic toxicity, the immune system's selective targeting allows for more compartmentalized treatments. This radical shift towards personalized cancer vaccines has significant implications. T cells serve as reservoirs, ready to recognize and combat re-emerging antigens, contributing to the potential for long-term cures.

The contrast with chemotherapy is simple—having to continually find new drugs as cancer mutates creates challenges, often leading to toxic treatments. The immunotherapeutic approach, particularly through vaccines, opens avenues for improved survival rates beyond the standard 5-year benchmark. It offers hope for more widespread and long-term success in cancer treatment.

Q What are the biggest challenges that companies have faced in the development of personalized cancer vaccines, and what approaches are being used to overcome them?

RdS: My background is in software and AI/machine learning (ML), while David's expertise lies in immunopeptidomics and target detection through mass spectrometry per patient. Let me explain three challenges that other companies have faced and how BreakBio has overcome them.

The first is AI training data. mRNA companies like BioNTech and Moderna have proven that personalized cancer vaccines have some efficacy. That has created renewed serious interest in the space. However, Moderna and BioNTech focus on and excel in large-scale manufacturing. They use this manufacturing for all types of vaccines: mostly transmissible diseases. BreakBio is focused on curing cancer. Vaccines for transmissible diseases are the same for all patients so don't need personalization and AI/ML software. Therefore, mRNA companies are not deep into AI target detection in each patient which is the problem in cancer. BreakBio has amazing software and methodologies for AI/ML target detection per patient. For example, let's look at AI training. All other companies train their AI from past patients' data. We uniquely do AI training for each patient from scratch: the same AI platform, but new training data per patient. Why? Every patient's immune system is different, and then it evolves further in the tumor cells. We are therefore the first company to analyse each patient's tumor cells using mass spectrometry (proteomics). David was the Head of MD Anderson's Mass Spectrometry Facility before co-founding BreakBio and is a world leader in mass spectrometry of the immuno-peptidome. Mass spec finds thousands of peptides presented by the tumor cells at that time (after immune evasion/evolution). This mass spec data lays bare the result of the unique antigen-presenting machinery in that patient's tumor cells, from proteasomal cleavage to TAP molecules to MHC binding.

The second challenge is peptides at the right doses. mRNA platforms like Moderna and BioNTech make proteins but cannot make peptides (but again the good news is that they still get efficacy). For example, mRNA effectively made the spike protein for COVID-19 which is roughly 1200 amino acids long. These proteins are difficult to make, yet with an mRNA platform, it can be done at an incredible scale by making the mRNA instead of the protein. They probably manufactured a billion doses in a year. However, for cancer, the requirement is not to create proteins but to create small proteins: 8–24 amino acid long peptides. mRNA cannot create peptides. So, what do Moderna and BioNTech do? They join all the small peptides (say, 20) into one long protein and use mRNA to get the body to make that strange protein. This approach is not good for cancer. Their treatment trains the body to fight this one protein, not the 20 peptides. Their hope is that the patient's antigen-presenting machinery will break down the protein into exactly the right pieces by cleaving at the right locations often enough to create the right dose of each peptide. That works just a little- some peptides are probably created at sufficient doses, but most are not. Illustrating this, the BioNTech MSKCC paper on the pancreatic cancer personalized vaccine showed that of the 20 antigens they chose per patient, T cells were created against only 11%. This is a very low percentage. It means that only two out of the 20 antigens primed T-cells in each patient. Peptide vaccines expect to prime T-cells against 75% or more of the antigens. So, of our 30 antigens, we would expect over 20 new types of T-cells versus their 2. Based on this, we choose the obvious solution—manufacture synthetic peptides.

A third challenge is low mutational burden cancers. Moderna, BioNTech and Gritstone all target only neoantigens. Many solid cancers have a low number of mutations in the cancer or

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low Tumor Mutational Burden (TMB). They target mutations in cancers without many mutations! Strange? BreakBio targets mostly Tumor Associated Antigens (TAAs) and a few neoantigens associated with mutations. These TAA targets are not associated with mutations and are highly expressed in low TMB cancers. This strategy of targeting TAAs has proven to get better results in low TMB cancers like MSS metastatic colorectal cancer (see Mayo Clinic Trial by Dr Jolene Hubbard, 2022). Because our platform targets more TAAs than neoantigens, it should work for both low TMB and high TMB solid cancers, i.e. all solid cancers.

DH: Adding to that, historically, earlier papers on peptide vaccines often targeted a single antigen, which has not generally been highly effective, in part because many of these lacked sufficient adjuvants and limited targets. The evolution of this field has been marked by a shift towards better target selection.

Even if our current trial has 30 targets per patient as opposed to 20, the multiplicity increases the likelihood of a more effective response. Additionally, for peptide vaccines comprised of multiple peptides, responses typically exceed an immune response against 50% of the targets, often reaching 75–80%. The variability in response rates can be influenced by the nuance of target selection and adjuvants, highlighting an area where cancer vaccines can improve.

In the context of peptides, we have a significant advantage. Our comprehensive analysis of individual patients lets us identify antigenic pieces likely to be recognized by the patient's immune system. This personalized approach enhances the probability of selecting peptides that are highly effective for a particular patient.

If the immune response is effective only against a few peptides, there is a higher risk of the tumor evading the treatment. This highlights the argument for combination with chemotherapy, where using a combination increases the likelihood of eliminating more of the tumor. Chemotherapies alone may have immediate effectiveness, but their durability can be limited.

On the other hand, the immune system has a remarkable ability to remember. If we can successfully recall a few potent responses, it significantly contributes to our overall efficacy. This memory aspect of the immune system enhances our approach, offering a potential advantage in achieving sustained effectiveness against the tumor over time.

RdS: We are greatly confident in the efficacy of peptide vaccines. As David mentioned, we expect T cells primed against at least 75% of antigens in peptide vaccines which is seven times greater than the mRNA vaccine approach. This improvement is significant, especially in generating an immune response. Importantly, if we have selected the right peptides, the T cells they create could effectively target and eliminate the tumor. However, selecting the wrong peptides would result in T cells that fail to kill the tumor because the T cells primed are not targeting anything presented on the tumor cells.

"I hold an optimistic outlook on personalized treatments, particularly emphasizing that among all diseases in which personalized treatments can be used, cancer is the most logical and important disease."

David mentioned a few noteworthy aspects. Firstly, the significance of combinations in achieving robust response and a few complete cures. We believe the key is in combining established drugs with new immunotherapies for improved outcomes. While some companies opt for a universal approach with a single combination for all cancer types, we stress the importance of tailoring combinations to specific cancer indications. Different drugs have always been required for different cancer indications.

Another critical aspect is early intervention: treating patients at an early stage is necessary for successful immunotherapy. Waiting until patients have become refractory may hinder efficacy as a strong immune system is crucial for success. Our initial indication is colorectal cancer. We are aiming for a Breakthrough Therapy Designation and then Accelerated Approval due to the unmet medical need in this indication. The IND for this trial has been approved by the US FDA. We are now working on the IND for our next indication.

In light of Project FrontRunner announced by the FDA, our clinical trial, with an approved IND, is designed as a randomized controlled trial early in the treatment journey. It is for patients who have started chemotherapy but have not progressed, allowing a broader pool of patients who may benefit. This early intervention capitalizes on stronger immune systems and healthier patients. We are grateful that the FDA has understood this and launched Project FrontRunner. We may be one of the first companies to leverage this initiative.

Looking ahead, scalability is a key consideration. In the past, there have been investors dissatisfied by the limitations of scalability for CAR-T in terms of revenue growth. Peptide vaccines are proving to be cost-effective, easier to manufacture and administer, and present a more scalable alternative. While the manufacturing process is intricate and requires personalization, the widespread availability of software for personalization makes this very feasible and highly scalable, and it is much easier to make synthetic peptides than to manufacture cells like T cells.

Q Can you discuss any innovative approaches in adjuvant development for these vaccines? How are these selected, and what considerations being important in the formulation process?

RdS: Protein vaccines and peptide vaccines that have been developed previously have shown that adjuvants play a crucial role in their development. A good example is Dr Nina Bhardwaj, one of our advisors and head of immunotherapy at Mount Sinai. Her remarkable research includes adjuvants for peptide vaccines. We incorporate three adjuvants that we consider highly effective, with two of them being widely utilized and proven.

We do not overly emphasize innovation in this adjuvant aspect, so we use what works. The use of three adjuvants however is an innovative approach, including incorporating dendritic cell growth factor, a novel addition that we believe has demonstrated efficacy in generating

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T cells in humans. Adjuvants hold significant importance, and our use of three adjuvants aims to enhance the percentage of antigens that result in new T cells. When selecting 30 targets, our goal is to stimulate T cells against the vast majority (75% or more) of these targets, and the adjuvants play a vital role in achieving this. It is worth remembering that we directly inject the target, so all the T cells we prime aim to go after the correct targets.

Q What are some of the key components of designing a clinical trial for personalized cancer vaccines? How do you address regulatory challenges?

RdS: This has been a significant focus for us in recent years, as our IND application has been approved by the FDA for human use. Our randomized controlled trial has gained popularity with hospitals, as evident by its positive reception at an excellent conference organized by Michael Sapienza and the Colorectal Cancer Alliance in Miami.

The FDA is and will be closely monitoring our software, recognizing the individualized treatments we create for each patient. The FDA's understanding of our software intricacies was somewhat unexpected but entirely logical given the personalized nature of our approach. They allowed us to initiate the process after extensive discussions where we faced challenging questions, particularly regarding safety. To address these concerns, we provided a detailed explanation to the FDA, outlining why our approach is likely to be safe. In terms of the regulatory environment, I believe we are in a promising position and the FDA has moved fast and is up to speed on these new technologies.

DH: The primary concern for the FDA is and has always been safety, and while efficacy is crucial, safety will take precedence. The more measures that can be taken to address and mitigate any safety issues, the more secure our position becomes. It is worth noting that we have a committee overseeing our operations to ensure everything is running smoothly and in accordance with the guidelines.

There may be a prevailing impression that the FDA is excessively harsh in regulating these matters, however, as a consumer of medication, I prefer strictness. Ensuring the safety of the products is paramount and by adhering to the necessary protocols, we can navigate the regulatory landscape successfully. In the event of a problem, the principle is to act swiftly, assess the situation, and implement corrective measures or find alternative solutions. Overall, our treatment approach is designed to err on the side of caution, aligning with the safety standards prevalent in the industry.

Q How do you envision the future of personalized cancer vaccines and the role they will play within the larger cancer landscape?

RdS: I hold an optimistic outlook on personalized treatments, particularly emphasizing that among all diseases in which personalized treatments can be used, cancer is the most logical and important disease. The versatility lies in having various types of T cells circulating the body, actively seeking cancer cells without introducing potentially

> "Vaccines could play a pivotal role in prevention efforts rather than a cure, similar to the successful anti-HPV and antiviral vaccines."

toxic molecules. I believe personalized cancer vaccines will become increasingly important for all solid cancers as people recognize the need for individualized approaches. Creating multiple new CAR-Ts for each patient appears impractical while also lacking in terms of safety. But with cancer vaccines, we can achieve something similar: 30 new types of T cells per patient.

Cancer vaccines offer a high level of safety due to our comprehensive analysis of the injected components. The body's natural safeguards also come into play, selectively generating T cells only against targets that are not widely expressed as self-antigens. Personalized cancer vaccines will play a significant role in cancer treatment when administered early and in combination with existing therapies such as chemotherapy. It holds promise even for challenging cases like metastatic cancers.

Traditionally, different drugs were used for specific cancer indications: lung vs breast cancer for example. In our case, we go further: we craft 30 drugs for each patient's cancer cells, eliminating the need for generalized guesses based on cancer indications. The focus is on the individual patient's cancer cells, and I believe this strategy is the key to achieving real cures and effective treatments for all solid cancers. Early administration, coupled with a robust immune system, will be essential, and I anticipate that personalized cancer vaccines could be a game-changer for a significant percentage of solid cancer patients.

For metastatic diseases where patients already have existing tumors, the combination therapy approach holds the greatest potential. However, caution is necessary when selecting combinations to ensure they do not damage T cells. In contrast, for Stage 2 and 3, to prevent recurrence, monotherapy cancer vaccines are more feasible.

Our primary focus is on the significant challenge of metastatic disease in all solid cancers, as this is where achieving overall response rate and complete responses becomes particularly challenging. We are encouraged by the potential for accelerated approvals and breakthrough therapy designation from the FDA for our first indication: colorectal cancer. We are also actively working on INDs for other indications. Addressing less advanced stages of cancer presents comparatively simpler problems.

DH: Even in these cases, there might be some room for certain combinations, but it is much less of a concern. Vaccines could play a pivotal role in prevention efforts rather than a cure, similar to the successful anti-HPV and antiviral vaccines. However, it is crucial to acknowledge that prevention trials generally pose significant challenges due to their large size.

Looking into the long-term future, I believe this approach may gradually emerge as the tip of the iceberg. Vaccines, in general, have demonstrated remarkable tolerability among billions of people, making them considerably safer, possibly a thousand times safer than most over-the-counter medications that we commonly use. I anticipate that, in the long-term, preventive approaches through vaccines will play a substantial and transformative role even for cancer.

RdS: Prevention is an interesting aspect, as David rightly points out, as it is inherently more logical to prevent a problem rather than waiting for it to manifest.

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Cancer progresses through stages before reaching full metastatic cancer. If we can intervene and target the cells or DNA alterations that have gone awry before reaching the stage of fully developed metastatic cancer, there is a great potential for prevention.

Q Finally, what are your key goals and priorities for the upcoming year?

RdS: Our primary goals for the upcoming year include initiating the injecting of our first patients in our first approved randomized controlled trial and observing a reduction in tumors. The immediate goal is to witness a decline in tumors, a process that is expected to unfold over approximately 12–20 weeks in each patient. Also, we will be working on the INDs and clinical trials for six other solid cancer indications.

BIOGRAPHIES

ROY DE SOUZA is a tech and biotech entrepreneur. As CEO of BreakBio Corp, Roy is leading a new era of AI drug discovery per patient and personalized medicine manufacturing, where the overarching goal is to solve the problems surrounding cancer. It uses AI to analyze, design, and manufacture new targeted multi-peptide immunotherapy for each patient. Roy is also an investment advisor and investment committee member of Outside the Box VC. Previously, Roy was the founder of ZEDO, Inc., a software-as-a-service company. He started his career in strategy consulting at the COBA Group and at Zip2, Elon Musk's first company.

Roy recently received an award from the American Cancer Society for his work on curing cancer and is a Director of the Save Groundwater Foundation, which is working to recharge depleted groundwater across India, at scale, by digging hundreds of simple recharge pits.

DAVID HAWKE joined BreakBio Corp in 2021 and now serves as its Chief Technology Officer. His most recent experience prior to BreakBio was as the Director of the mass-spectrometry-based proteomics facility at the MD Anderson Cancer Center in Houston TX. His work there on the analysis of MHC class I peptides and their use in treating patients in both clinical trials and for compassionate cases led to his connection with BreakBio. He has co-authored over 130 peer-reviewed scientific articles and holds six issued US patents.

David has a BSc in Chemistry from the Massachusetts Institute of Technology, a MSc in Organic Chemistry from CalTech, and a PhD in Bioanalytical Chemistry from the University of the Pacific.

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