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SPOTLIGHT ON
Combination therapy

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INTERVIEW

Better together: assessing the evolving combination therapy picture in I–O

Roisin McGuigan, Editor, *Immuno-Oncology Insights,* speaks to **Yisrael Katz,** Senior Medical Director at Viracta Therapeutics, who shares his insights and predictions for the current and future progress of I–O combination therapy.

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Q Can you share your reflections upon the story of I–O combination therapy development to date?

YK: The introduction of ipilimumab in 2011 and the subsequent wave of programs demonstrating the therapeutic success of immune checkpoint inhibitors represents a watershed moment in oncology history. This long-overdue win not only fueled interest in exploring novel targets and treatment modalities, but also reminded us that answers leading to breakthroughs are frequently found in disciplines outside those being immediately studied. In this case, tapping into the discipline of immunology has drastically advanced our understanding of cancer and the central role of anti-tumor immunity. However, as

target populations and indications across these trials broadened, response rates have expectedly cooled, leading to the natural next questions around synergy and combination trials.

Combination immunotherapy studies have faced two major issues in their approach, with one being founded on strong scientific rationale while the other relies on recycling older and more toxic anticancer agents. The former uses innovative techniques like targeted therapy agents or immunomodulatory strategies to harness synergistic lethality or optimize anti-tumor response, which have largely been grounded in good foundational science. In contrast, the other common 'shotgun' approach leverages immuno-oncology (I–O) agents in combination with older or poor-performing therapies—I think it is a wasted opportunity to put all of your eggs in this basket, instead of focusing on creating something new or innovative. As an approach, it has not been founded in good scientific rationale, and ultimately costs the field in terms of stagnation, dollars, and patient-years.

Q What are the biggest challenges for the combination therapy space as you see them today? Can we say we've reached the stage of conducting rational development of I–O combinations?

YK: Certainly; everything from trial design to assessing endpoints, funding, patient involvement, companion diagnostics, and essentially every element of clinical development has grown in the past decade largely thanks to the success of I–O. That said, challenges of patient selection, predictive biomarkers, and streamlining of patient enrollment are some of the monumental tasks that all need to equally progress to allow for rationally-selected I–O combinations to have the best shot at success. And it's not just the science we need to look to, but also the buy-in from all stakeholders including regulators, investors, and individual clinicians, in order to progress further with rationally selected combinations.

A major feature that has been lagging behind is biomarker identification—including companion, preventative, prognostic, and so on. Reliably identifying predictive biomarkers will help to minimize toxicity, improve patient selection, and clear space for new therapies that may represent better modalities for certain populations. Resistance to immunotherapy remains another key challenge, and a better understanding of the underlying mechanisms is needed in order to develop strategies to overcome it. This includes investigating the role of the tumor microenvironment (TME) and identifying potential immune escape mechanisms.

Specific to combination therapies, while this is a promising and attractive tactic, objective tools to assess endpoints will need to evolve similarly to modification of immune-modified radiologic criteria (iRECIST, imRECIST, etc.). We may need to leverage machine learning to accelerate this process and reduce bottlenecks in clinical development to make this happen. Regulatory authorities must also evolve to allow more communication, feedback, and support of more 'unconventional' ideas. In the past we have seen that these are largely the success stories that have moved the field forward.

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"The field of immuno-oncology is in its renaissance, with each of the disciplines under this constellation of modalities potentially serving as a key to future success."

Q What would you place in the frame as the next modalities or combinations that might move the efficacy dial?

YK: The field of immuno-oncology is in its renaissance, with each of the disciplines under this constellation of modalities potentially serving as a key to future success. Checkpoint inhibitors reawaken anti-tumor immunity; cancer vaccines hone the anti-tumor response; cell therapies optimize the durability and degree of tumor killing; RNA-based therapies can target multiple genes or proteins involved in cancer development, and the list goes on—but it is unlikely any of these alone will be the solution to cancer as we know it. The highest likelihood of success comes from progressive approaches to combination therapies that harness the power of these tools, as well as our ability to target cancer earlier in its course. Focusing on prevention, diagnostics, and combination will yield the greatest advances in this field.

Q What are the key questions still to be answered in terms of better identifying and developing effective combinations? How does our approach need to change?

YK: Focusing on genomic targets as the sole type of 'targeted' therapy in I–O greatly limits the potential for success by neglecting the complexity and heterogeneity of the TME. Tumor clonal evasion and resistance mechanisms are diverse and often multifactorial, and may not be effectively targeted by a single genomic target. Multi-targeted approaches that aim to minimize the risk of resistance mechanisms from several different angles may have a higher chance of success by targeting multiple aspects of the tumor-immune interface. These approaches may include combination therapies, such as the use of checkpoint inhibitors with other immunomodulatory agents or targeted therapies, and personalized treatment strategies based on the patient's individual tumor characteristics. Ultimately, a comprehensive and integrated approach to I–O therapeutic development is necessary to maximize the potential for success in improving patient outcomes.

Q How could innovative approaches to combination trial design help address these issues?

YK: Currently, the thought process behind clinical trial design is evolving along with the trials themselves. The use of new and innovative designs, including adaptive trials, basket design, and others, in parallel with new tools that lower the barrier to patient recruitment, allow for more efficient use of resources and for streamlining of the drug development process. Additionally, these designs can increase the likelihood of success by enabling real-time modifications to the trial protocol, testing multiple treatments simultaneously, and creating more accurate control groups.

These improvements aren't groundbreaking in and of themselves, but the theme is taking a holistic approach and ensuring everything surrounding and supporting the trial process is helping to maximize success. Leveraging technology and remote patient monitoring can improve patient access and convenience, reduce costs, and minimize disruptions to patient care. And ultimately, by accelerating therapeutics development in I–O, patients with cancer will benefit from faster access to potentially life-saving treatments.

Q How will the combination therapy picture continue to develop in the immuno-oncology field over the near-to-mid-term?

YK: Regulatory requirements must accommodate the agility of growth in this field. In small molecule development, moving away from the maximum tolerated dose paradigm has its advantages, but new regulatory requirements such as Project Optimus may adversely impact the ability of smaller companies to move forward with dose-ranging studies.

CBER has done a great job of upscaling and expanding its scope, interaction with sponsors, and ability to provide feedback to enable continued development. That said, it must be explicitly stated that rigorous requirements, especially unnecessary or overly-specific requirements, may be all that stands between a therapy being approved and the patient who is waiting for it to save their life. We have to ensure the process isn't so difficult that the best, cutting-edge things end up getting stopped on the one-yard goal line.

A lot of the bottleneck, and even the way we even think about potential success, stems from how slow the regulatory process is—how much it costs and how many years it takes. That on its own is a big barrier to entry, even though you might be sitting on the next breakthrough. Regulatory agencies must act on this, and do all they can to lower the threshold for entry of new candidates and promising therapeutics.

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BIOGRAPHY

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AUTHORSHIP & CONFLICT OF INTEREST

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IMMUNO-ONCOLOGY INSIGHTS

COMBINATION THERAPY

SPOTLIGHT

EXPERT INSIGHT

Checkpoint on I–O resistance: lessons learnt & future perspectives

Ross Stewart, Martin L Miller, Ana Camelo & J Carl Barrett

Immune checkpoint blockade via anti-PD(L)1 has revolutionized anti-cancer treatment, with durable responses observed across multiple cancer types. However, some patients are resistant to treatment and many relapse, following initial response. Here we propose a conceptual framework aimed at promoting clearer discussion and understanding of I–O resistance. Within this framework, we define two critical factors that determine the success of anti-PD(L)1 therapy. The first is visibility of the tumor, as a foreign entity recognizable by the host's immune system. The second is T cell functionality, as an effective means of eliminating the tumor once recognized. These two core factors are subject to modification by several different tumor cell intrinsic and extrinsic aspects of biology, which are themselves interdependent. In this perspectives article, we take each of these modifiers in turn, summarizing the field's current state of knowledge, and considering how it can be leveraged to better direct the next generation of therapies.

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Since the first approval in 2014, the use of anti-PD-(L)1 based therapy has transformed the treatment of cancer. As of September 2022, anti-PD-(L)1, either alone or in combination with other therapies, had received more than 90 FDA approvals, in 20 different tumor types and in concert with three different biomarkers defined by companion diagnostics; PD-L1 expression, tumor mutational burden (TMB) and mis-match repair (MMR) deficiency. Despite this unprecedented impact on the field, the fact remains that the majority of patients still experience disease progression, and given such broad and rapid clinical success, it has been challenging for scientific understanding to keep pace. As a result, our knowledge of

when and how resistance emerges remains in its infancy, and such knowledge has the potential, in the future, to help drive informed drug development that addresses resistance through combination or alternative therapy.

A significant challenge in tackling the biology of anti-PD(L)1 resistance stems from the disconnect between target engagement and effect, that is inherent to checkpoint blockade. A typical targeted oncology therapy, such as a tyrosine kinase inhibitor (TKI) or antibody drug conjugate (ADC), acts in a very direct way, binding its target and as a result mediating a, typically, cytotoxic effect on the tumor. Drivers of response and resistance to such therapies tend to be tumor intrinsic and are frequently modifiers of the target itself, either via expression or mutation, or of pathways associated with the target or the downstream cytotoxic effect, such as proton pumps or alternative oncogenic pathways that enable escape. Anti-PD(L)1, and other T cell checkpoint inhibitors such as anti-CTLA-4, mediate their anti-tumor effects indirectly. Binding to their target has no direct effect on tumor cells, but rather modulates the immune system, increasing the probability of eliciting an anti-tumor immune response. Ultimately, the immune system is the drug, and resistance can be driven by anything that modulates its effective function. The result is a complex network of interdependent response and resistance drivers that can be both tumor intrinsic and/or tumor extrinsic.

Here, we propose a conceptual framework aimed at promoting clearer discussion and understanding of immuno–oncology (I–O) resistance, which can then drive more effective use of knowledge to improve therapies. Within this framework, we define two critical features that determine the success of anti-PD(L)1 therapy, with a complex network of modifiers impacting one or both these features. We take each of these modifiers in turn, summarizing the fields current state of knowledge, considering how this knowledge might be pursued to better direct the next generation of therapies, and speculating as to where the next generation of insights may come from.

A FRAMEWORK FOR EXPLORING ANTI-PD-(L)1 RESISTANCE

Anti-PD-(L)1 therapy relies upon the ability to mount an effective anti-tumor immune response. At a fundamental level, such a response depends on two components. The first is visibility of the tumor, since in order to mount a response the immune system must first recognize the tumor as foreign. The second is T cell functionality, since, in the context of anti-PD-(L)1 treatment, tumors are only effectively eliminated by an antigen specific cytotoxic T cell response that is allowed freedom to operate within the tumor microenvironment (Figure 1). By reducing the significant complexity of the anti-tumor immune response, downstream of anti-PD-(L)1, to these key components, we can formulate and test hypotheses around resistance drivers. Each component can initially be considered in isolation, but eventually together, as part of a network that shapes the two core drivers of tumor visibility and T cell functionality.

Presence & presentation of antigens

T cell killing of target cells is dependent upon a T cell receptor (TCR) recognizing a cognate antigen presented in the context of the class I major histocompatibility complex (MHC-I), which is canonically expressed on the surface of all mammalian cells. The majority of antigens presented by tumor cells are 'self' antigens, the recognition of which is prevented by the processes of central and peripheral tolerance, in order to avoid widespread tissue damage. However, the mutational processes that underpin oncogenesis have the potential to generate altered-self peptides that are recognizable by T cells; termed 'neoantigens'.

The presence of such neoantigens is a critical driver of tumor visibility, because in their

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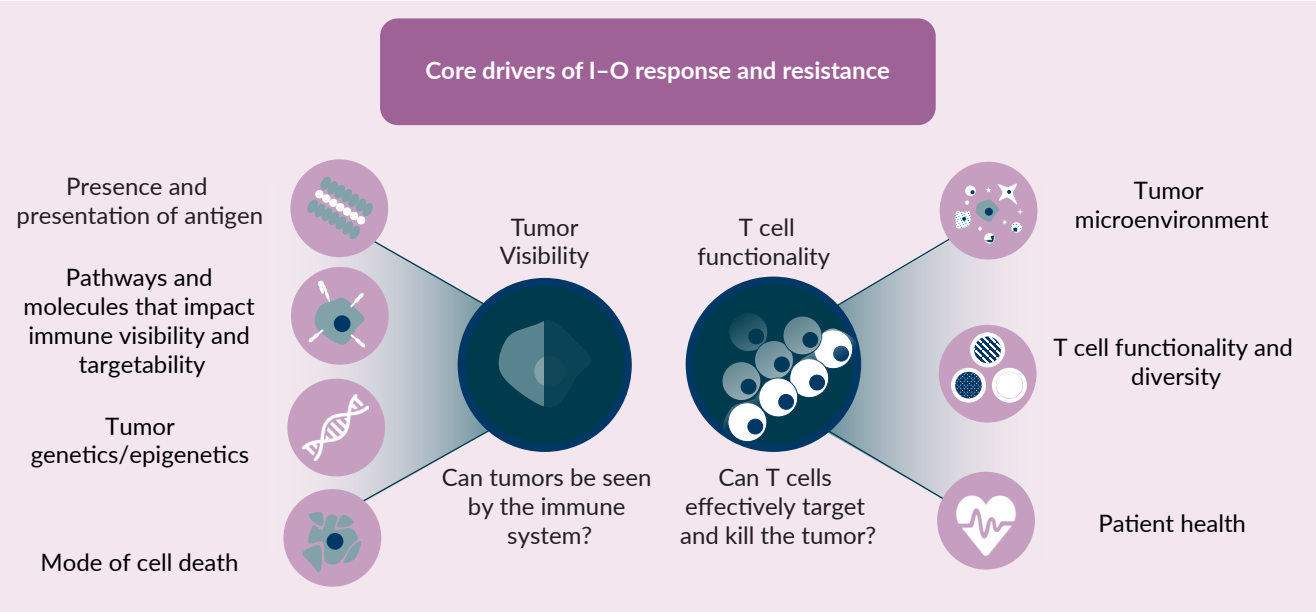
FIGURE 1

A framework for understanding the core drivers of I–O response and resistance.



I–O response and resistance is underpinned by two central drivers:
1) whether or not a tumor is visible to the immune system and;
2) whether or not T cells can effectively target and kill those tumor cells.
These two core components, tumor visibility and T cell functionality, are subject to modification by a number of different tumour cell intrinsic and extrinsic aspects of biology, which are themselves interdependent. With respect to tumor visibility, the existence of recognisable neoantigens and their effective presentation via the antigen presentation machinery, pathways and molecules that effect visibility at the tumor cell intrinsic level, such as PD-L1 expression, genetic or epigenetic mutations that can impact both of these features, and the mode of tumor or stromal cell death can all independently or together impact the ability to induce a productive and durable immune response. With respect to T cell functionality, the cells and associated cytokine and chemokine signals within the microenvironment are a major determinant of T cell function within that environment, additionally the overall diversity and functional state of the T cells as well as the patients' current health status, can have a major role on whether T cells can productively kill tumor cells.

absence the tumor cell is largely invisible to the T cell repertoire. The relationship between presence of antigen and T cell recognition of tumors is what underpins TMB as a predictive biomarker for response to anti-PD-(L)1. The greater the number of mutations present in a tumor, the higher the probability that a recognizable neoantigen is generated, and that an anti-PD-(L)1 driven T cell response can be stimulated. Neoantigen presence though, like any mutational event, is subject to tumor heterogeneity and clonal evolution in response to treatment. Evidence suggests that tumors with higher levels of clonal neoantigens are more likely to respond to treatment [1] and that the loss of neoantigens, in response to treatment, may represent a potential route to acquired resistance [2].

Simply having recognizable antigens is however not enough to guarantee tumor visibility, since recognition is dependent both on the presence of a cognate TCR, which will be touched on later, and also on the presentation of any antigen by MHC at the surface of the cell. The loss of MHC from the surface of tumor cells would represent a significant escape route from T cell mediated cytotoxicity, and mutations in key components of antigen presentation by MHC, such as B2M [3] and TAP [4] have been associated with resistance to anti-PD-(L)1. Complete loss of MHC is, however, a challenging state for a tumor to maintain, because MHC negative cells are rendered sensitive to killing by natural killer (NK) cells. As an alternative to losing MHC, tumors can also modulate the diversity of MHC present at the genetic or transcriptional level, and by doing so reduce the potential diversity of neoantigens available to the immune system.

Each person carries up to six different MHC I alleles, three inherited from each parent, and increased diversity of these alleles with respect to the peptides they bind, also called the HLA-I evolutionary divergence (HED), has been shown to link to benefit from anti-PD-(L)1 [5] while loss of heterozygosity (LOH) at the MHC locus has been shown to lead to lack of benefit to anti-PD-(L)1 [6]. It is important to note though, that modification of MHC is not only a potential route of immune escape [7], but also evidence of selective pressure being applied by an active immune response, and as such may not always associate with reduced benefit from anti-PD-(L)1 [8], and in some settings or lines of treatment could actually be a predictor of benefit [9]. A deeper understanding of the role of MHC in resistance and response may be gained by assessing its impact in concert with other markers, such as PD-L1 and CD8, combined with a productive interferon-γ (IFN-γ) response [10,11], but will more likely come from advances that improve our ability to measure the expression of individual MHC allotypes, and to link those allotypes to specific neoantigens and cognate TCRs.

Molecules/pathways impacting visibility

The fact that tumor-intrinsic transcriptional programmes play a key role in immune escape and mediating I–O resistance is underlined by several observations. For example, there is wide variability in the response rates to I–O across tumor types with distinct oncogenic signaling processes, and vice versa, the response rates are similar across different histologies when cancers are driven by similar processes such as microsatellite instability. Furthermore, in metastatic disease settings, different tumors from the same individual can have different activity of immunosuppressive pathways, such as Wnt, that track inversely with intra-epithelial infiltration of $CD8^+$ T cells [12–14]. This suggests that tumor-intrinsic processes, as well as systemic immune features, can drive the heterogenous tumor-immune microenvironments that are often observed clinically within the same patient. Supporting this, the response to immunotherapy can have clinically diverse temporal and spatial patterns in different sites of the same patient associated with distinct transcriptional programmes, as exemplified in a longitudinal study of an exceptional responder case in a patient will metastatic melanoma [15]. Thus, a clear understanding of tumor-intrinsic oncogenic programmes and how they shape the anti-tumor immune response in treatment-naïve patients and during treatment with immunotherapy is essential.

Although it remains to be systematically characterized and mechanistically assessed how tumor pathways drive I–O resistance across disease stages and cancer types, emerging evidence from both pre-clinical and clinical insights suggest that a range of cancer pathways can directly or indirectly contribute to modulating the tumor-immune interface. These include Wnt-β-catenin, MAPK, CDK4-6, LKB1 (STK11), PTEN and Myc signaling, the roles for which have been summarized recently [16,17].

While these pathways are associated with I–O resistance in specific settings and indications, a central pathway that is ubiquitously involved in both response and resistance is the IFN-γ response pathway; due to its important role in sensing IFN-γ secreted from T cells during a productive response against tumor antigen (reviewed in [17]). IFN-γ binds the IFN-γ receptor and triggers activation of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway which in turn activates interferon stimulated genes (ISGs) partially through interferon response factors (IRFs). The ISGs have pleotropic effects with important tumor-intrinsic effects including upregulation of the antigen presentation machinery as well as PD-L1, serving as positive feedback loop enhancing T cell recognition and leading to induction of cell cycle arrest and apoptosis [18]. Tumor-extrinsic effects of ISGs include enhancing the cytolytic activity of both

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innate and adaptive immune cells. In general, IFN-γ sensing by tumor cells leads to a stronger anti-tumorigenic response, particularly in the early stages of tumor development. In line with this, IFN-γ response signatures assessed through data analysis from bulk tumors have been associated with better responses to anti-PD1, across cancers [19]. Meanwhile alterations of the IFN-γ pathway that include JAK-STAT and the antigen presentation machinery have been associated with resistance to I–O (discussed in the following section). Recently, mounting evidence points to the IFN-γ response playing a reversed role in mediating pro-tumorigenic effects particularly in the late stages of tumor progression (reviewed in [20]). Similarly, pre-clinical evidence in immuno-competent syngeneic B16 mouse models where the cancer cell lines were pre-treated with sustained levels of IFN-γ prior to implantation, were found to subsequently develop acquired resistance to ICB *in vivo* [21].

Tumor genetics & epigenetics

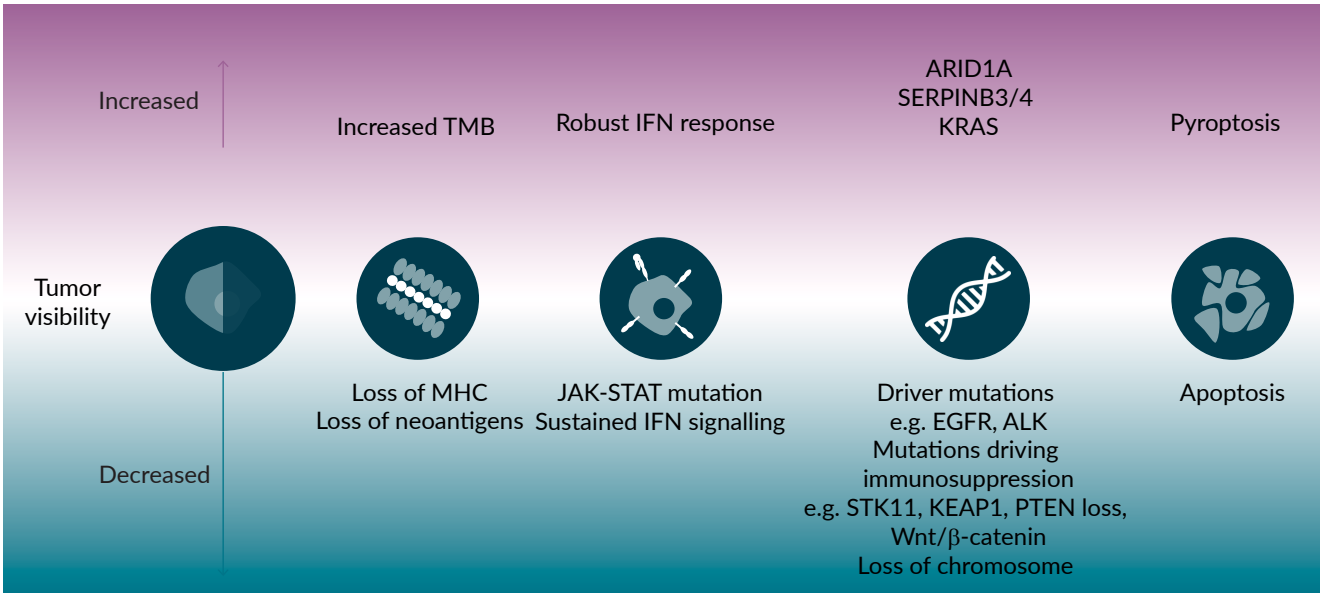
Historically, a rich source of understanding with respect to response and resistance in oncology, the presence of mutations in one or more genes have yielded less generalizable insights in the context of anti-PD-(L)1 treatment. In NSCLC, it is clear that tumors harboring mutations in dominant oncogenes, such as EGFR and ALK, have limited benefit from anti-PD-(L)1 [22] potentially because these tumors have lower TMB, and so limited opportunity for neoantigen generation, but also because it is challenging to control the growth of such tumors without addressing the central oncogenic drivers of that growth. Mutations in STK11 and KEAP1, while initially proposed as resistance drivers for anti-PD-(L)1, are prognostic in nature [23,24], identifying a group of patients that respond poorly to all current therapies. In Melanoma, mutations in the JAK-STAT pathway have been associated with both acquired [25] and primary [26] resistance to anti-PD-(L)1, presumably due to the critical nature of these genes with respect to the IFN-γ response; itself central to an effective T cell response. Other potential genomic drivers of resistance include PTEN loss and mutations in the WNT/β-Catenin pathway, both of which have been associated with reduced presence of T cells [27,28] and acquired resistance to anti-PD-(L)1 [29]. The majority of these genomic drivers have however not been validated as baseline predictors of response to anti-PD-(L)1 in large, randomized settings, and in a recent meta-analysis, were not seen to be consistent, or statistically significant, with respect to their impact on outcome across a range of studies [9]. More recently deletion of the chromosome 9p21.3 region, containing the genes CDKN2A, CDKN2B and MTAP, has been associated with reduced activity for anti-PD-(L)1 in multiple settings and with reduced immune infiltration [30,31], and may represent a promising genomic marker of resistance to anti-PD-(L)1 [30].

On the opposite side of the spectrum, a number of genomic features have been associated with increased benefit from anti-PD-(L)1, including APOBEC [32], smoking [33] and UV exposure [34] associated mutational signatures as well as mutations in SERPINB3/4 [35], ARID1A [36] and KRAS [37]. Interpreting the independent impact of such genomic features has however been challenged by the fact that many are also associated with increased TMB, a known predictor of improved outcome in many settings, and by the fact that several can have prognostic as well as predictive value in some settings (Figure 2).

While it seems clear that independent, genomic drivers of response and resistance to anti-PD-(L)1 are likely rare, it is inevitable that the genetic background of a tumor will impact the signals it can both receive and produce. Striving to better understand the interface and interlink between tumor genetics and the surrounding inflammatory microenvironment will be critical to deepening our understanding of how tumors and the immune system converse, and to grasping

FIGURE 2

Mechanisms impacting tumor visibility to the immune system.



Several tumor-intrinsic mechanisms can affect visibility to immune cells, and therefore susceptibility to immune mediated killing, including decreased tumor mutational burden, inability to present neoantigens or a deregulated antigen presentation machinery, an abnormal IFNγ response, mutations that drive immune suppression and the mode of cell death.

the complexity of response and resistance to anti-PD-(L)1.

Modes of cell death

Death of cancer cells and immune cells occurs spontaneously as well as in response to therapies and pathogens. Multiple modes of regulated cell death occur that may influence the immune response. The major modes of regulated cell death include apoptosis, pyroptosis, ferroptosis, necroptosis, NETosis, autophagic cell death, and cellular senescence (reviewed in [38]). Although each process has unique pathways of execution, activation of cysteine proteases of the caspase family is a common theme. Apoptosis activates caspases 3, 8 and 9 whereas caspases 1 and 4 are involved in the proinflammatory process of pyroptosis. Caspases are proposed to connect cell death processes to maintain homeostasis [39].

From the perspective of response to anti-PD-(L)1, cell death that leads to immunologic memory is key to controlling tumor growth and to therapeutic response. The hallmarks of immunogenic cell death (ICD) are antigenicity, adjuvanticity and environment [38]. Pyroptosis is an evolutionarily conserved mechanism that plays a critical role in innate immune defense to microbial infections. Unlike apoptosis and other cell death processes, pyroptosis results in an inflammatory response. The inflammatory mechanisms driven by viruses, bacteria and some toxicants include the activation of caspase-1 mediated by pattern recognition receptors (PPRs) and a multicomponent complex called the inflammasome. Following pathogen infection, an inflammatory signal is mediated by microbial associated molecular patterns (MAMPs) and toxicants activate damage-associated molecular patterns (DAMP) inflammasomes. MAMPs and DAMPs are sensed by PPRs on myeloid cells that can lead to ICD and a downstream immunologic memory response. A large number of chemotherapies can induce ICD. For example, oxaliplatin but not cis-platinum, can induce ICD in model systems [38]. Caspase-1 activation leads to cleavage of the gasdermin family (GSDMD and GSDME) that oligomerize and form

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pores in the plasma membrane resulting in release and cleavage of the precursors of the proinflammatory cytokines IL-1β and IL-18. GSDM-D cleavage is mediated by the inflammasome caspases 1, 4,5 and 11 whereas GSDM-E is cleaved by caspases 3 and 8 during apoptosis. This coverts noninflammatory apoptotic signals into pyroptotic death signals, further illustrating the interconnectivity of death processes [40]. GSDM-D is the immediate effector of pyroptosis after inflammatory stimulation, but GSDM-E can enhance IL-1beta release secondarily [41]. In addition to antigenicity and stimulation of inflammatory adjuvanticity, ICD requires a permissive environment, such as absence of immunosuppressive factors like adenosine, prostaglandin E2 and myeloid derived suppressor cells (MDSCs) [38].

Not all damage inducers or pathogens induce ICD. Features associated with ICD include inhibition of transcription and microtubular disruption, though these mechanisms are yet to be fully understood. A clear distinguishing feature between ICD inducers and cytotoxic agents that do not induce ICD is the activation of the integrated stress response (ISR) pathway which involves phosphorylation of the eukaryotic translation inhibitor factor eIF2a, currently proposed as a pathognomonic biomarker of ICD [38]. eIF2a phosphorylation also activates autophagy which can affect cell survival vs death. It is proposed that eIF2a activates a coordinated stress response that protects cells when stress levels are limited and possibly repairable, yet controls cell elimination when damage is extreme [42]. PDL-1 expression in the nucleus can switch tumor necrosis factor (TNF-α)-induced apoptosis to pyroptosis and tumor necrosis via the transcription of GSDM-C and its cleavage through caspase 8. High levels of GSDM-C correlate with poor prognosis in certain cancers and may represent a non-canonical pathway for pyroptosis and tumor necrosis in cancer cells [43]. GSDM-E is often downregulated in cancers with the exception of pancreatic cancers where it has been shown to play a novel function in mediating resistance to digestive enzymes that are produced by pancreatic ducts [44]. The balance between apoptotic and pyroptotic inflammatory cell death is important in cancers. Caspase 3 that mediates apoptotic death, inactivates cGAS and IRF3 that suppress IGFN type 1 production and keep apoptotic death immunologically silent [45]. Bcl2 is well known to inhibit apoptosis, but can also reduce GSDM-D activation by dropping caspase 1 cleavage and promoting cleavage at a site D87, a mechanism that inactivates pyroptosis [46]. Targeted therapies can also influence modes of cell death. In BRAF mutated melanoma cells, combination of BRAF and MEK inhibitors can be an effective therapy and induce markers of pyroptosis with concomitant T cell infiltration, a mechanisms that is not present therapy resistant tumors [47].

Ferroptosis is another form of non-apoptotic, regulated cell death. It is characterized by iron-dependent accumulation of oxidized polyunsaturated fatty acid containing phospholipids that can lead to membrane rupture and cell death. The pathway was identified by cysteine depletion shown originally to lead to death of cells in culture by Harry Eagle [48], and reviewed in [49]. Cysteine levels control the intracellular pool of reduced glutathione (GSH), essential for the activity of the enzyme glutathione peroxidase 4 (GPX4) that reduces peroxidized phospholipids and suppresses activation of the arachidonic acid metabolizing enzymes. Control of ferroptosis mediated death is a complex interplay between lipids, iron and cysteine metabolism. Ferroptosis has been shown to occur in certain cancer cells as well as in immune cells, including T cells, MDSCs, B cells, dendritic cells and NK cells. Ferroptosis susceptibility in cancer cells is influenced by mutations in oncogenes (like TP53 and RAS) and in genes involved in the stress response (NFE2L2), autophagy, hypoxia and epithelial-to-mesenchymal transition (EMT) [50]. Different cancers show differing levels of heterogeneity in their susceptibility to ferroptosis which can relate to states of inflammation. CD8 T cells

have been found to be able to regulate ferroptosis in tumors during immunotherapy [51], through IFN-γ, and along with arachidonic acid, can also induce tumor cell ferroptotic death [52]. Ferroptosis can also impact immune cells themselves, which can result in immune suppression in certain cancers, for example pathologically activated neutrophils, and PMN-MDSC cells die spontaneously by ferroptosis [53].

Through the knowledge we have now gained of the different mechanisms of ICD, and their potential to modulate the tumor microenvironment and tilt the needle to an immune-productive milieu and even sensitize tumors to PD-(L)1 blockade [54], conducting trials where combinations of ICB with either chemotherapy or antibody-drug conjugates (ADCs) are administered using different dosing schedules rather than simultaneously, or even sequentially, may lead to improved patients' outcomes, with the obvious considerations to take into account, such as toxicity and potential for adverse events.

Tumor microenvironment

It is now well understood that neither tumor progression nor response to IO therapies are solely driven by cancer cell-intrinsic genetic or epigenetic changes, but that these processes are tied to a large communication network of immune cells, stromal tissue, and molecular mediators both within, and at the boundaries of solid tumors. This ecosystem, or tumor microenvironment (TME), through its dynamic and multi-directional interactions with the tumor and the immune system can be both friend and foe with respect to response to anti-PD-(L)1.

Several studies have shown that a microenvironment with a coordinated, Th1 immune response is far more likely to respond to anti-PD-(L)1 therapy. Such an environment is characterized by increased CD8 infiltration [55], evidence of active IFN-γ signaling [19] and presence of tertiary lymphoid structures [56]. Through direct interactions and release of signaling molecules, cancer cells can co-opt the microenvironment, creating an immune-suppressive milieu, in which, the stromal compartment cooperates to promote tumor growth and metastases, and immune cells are modulated to a suppressive state. Evasion of the immune system and chronic inflammation, which can generate a forward feeding circle, are well recognized hallmarks of cancer [57], and have the potential to create a significant barrier to the activity of anti-PD-(L)1 [58]. In this section, we focus on the immune cell components of the TME, and their potential to positively and negatively impact such activity. Although we recognize there are some reports of stromal cells other than immune cells playing a role in resistance to I–O therapy [59], and strategies that target the stromal compartment are beginning to emerge [59], we do not consider them in depth here.

Innate immune cells in the TME

Tissue resident and circulating innate immune cells are key contributors to the inflammatory state of the TME, with major players having been identified in both the myeloid and lymphoid lineages. Innate immune cells, like macrophages, neutrophils, NK cells and innate lymphoid cells (ILCs) provide a bridge to adaptive immunity, and their interactions with T cells through either soluble mediators and/or cell-cell interactions warrant some exploration to understand the mechanisms by which they can impact resistance to I–O therapy. Tumor associated macrophages (TAMs) are one of the most abundant immune cell populations in the TME, and their density in the tumor tissue has been correlated to poor outcomes and resistance to immunotherapy [60]. Generally, macrophages in the TME are thought to exhibit one of two phenotypes, a classically activated or pro-inflammatory, antigen presenting, M1 phenotype, or the alternatively activated, anti-inflammatory, M2 phenotype, each defined by expression of different surface markers, and cytokine secretome. However, in reality, a degree of

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plasticity has been observed in TAM phenotypes and functions, depending on the tumor milieu, the stage of development of the tumor, and the cancer type [61]. They have been demonstrated, both pre-clinically and in human tumors, to contribute to a pro-tumorigenic TME through promotion of angiogenesis and tumor metastases [62], and while their biology within the TME is complex, some trends are emerging that suggest that they likely play a role in resistance to chemotherapy, radiotherapy and ICB [63]. As such combination therapies that aim at targeting macrophages, together with anti-PD-(L)1 may help with overcoming resistance. Multiple potential mechanisms of macrophage modulation could be considered including modifying survival or recruitment in or order to reduce their presence in the TME, re-polarizing M2 to M1 and re-educating TAMs to an anti-tumor, pro-inflammatory function, or through blockade of myeloid immune checkpoints that induce a pro-phagocytic phenotype [64].

The number of phase 1 and 2 clinical trials that include myeloid targeting agents in combination with I–O is starting to take off, shifting the landscape away from single I–O agents. The challenges with therapies that target myeloid cells, like macrophages and other myeloid-derived heterogenous cell types with a known T cell inhibition/ Treg inducing role in TME and impact on ICB resistance such as myeloid-derived suppressive cells (MDSCs) [65], will be to dissect the mechanisms by which they modulate resistance to I–O in different indications, and throughout tumor development, and to effectively address combinations in different tumor types with evidence-based dosing schedules.

Dendritic cells (DCs) represent another innate, tissue resident cell with a major role in the antitumor immune response. They are the professional antigen presenting cells (APCs) that detect environmental signals – tumor associated and tumor specific antigens (TAAs and TSAs) – and shape T cell mediated immunity, by transporting these antigens from tumor to lymph nodes, and inducing robust CD8 T cell priming, a process that has been shown to be dependent on type I IFN signaling [66] presence of conventional DCs in tumors correlated with improved response to PD-1 therapy and higher CD8 T cell infiltration and was generally associated with better prognosis in several indications [67]. Lack of tumor immunogenicity has been identified as a key factor contributing to anti-PD-(L)1 resistance, and effective DC-T cell crosstalk through the IFN-γ and IL-12 axis has been shown pre-clinically to be a critical requirement for both priming and function of cytotoxic T cells, and for the success of anti-PD-(L)1 therapy [68]. So far, there has been surprisingly little clinical evidence of effectiveness for therapies aimed at enhancing DC function in different indications, such as DC vaccine approaches using *ex vivo* generated autologous DCs from blood-derived monocytes pulsed with TAAs, with only one FDA approval for DC cell therapy, based on a 4.1 month survival improvement and delay in disease progression in metastatic prostate cancer [69]. This is likely due to the immunosuppressive environment of the TME, and thus future combinatorial strategies may focus on rendering the TME more favorable to effector cell infiltration and reveal a potential for TAA-loaded DC cell vaccines. There are challenges to DC targeting, however, including the lack of biomarkers for patient stratification, the cost of personalized therapy, the influence of the immune suppressive TME and lack of mechanistic understanding of how DCs can help overcome resistance to ICB through T cell mediated immunity, as thus far most of knowledge of DC biology comes primarily from animal models.

On the lymphoid arm of innate immunity, NK cells are well recognized as the frontline innate cytotoxic T cell counterparts for tumor cell killing. NK cells are tightly regulated through a balance of activating or inhibitory signals that are highly dependent on cellular and molecular cues from the microenvironment within the tissue where they circulate. In homeostasis, recognition of MHC-I by NK killer Ig-like inhibitory receptors (KIRs)

and NKG2A provides a signal that induces self-tolerance and the distinction between healthy 'self' or tumor or virus-infected 'missing-self' [70]. One of the known tumor evasion mechanisms is the downregulation of MHC-I to escape from CD8 T cell-mediated killing, an event that should activate NK cells to control tumor progression. However, other co-signals are necessary for a full activation cascade and a concomitant productive cytotoxic response by the NK cells, and these pathways are highly dysregulated within the tumor tissue, reducing NK cell effectiveness [71].

Increased numbers and fitness of NK cells in the tumor tissue have been associated with better survival outcomes in several different types of cancers [72]. However, in NSCLC, elevated NK cells have been associated with worse prognosis, and these cells were found to express high levels of inhibitory receptors, and present an immature, and pro-angiogenic phenotype [73]. Despite seemingly conflicting reports, the fact that NK cells harbor surface expression of CTLA-4, PD-1, TIM-3 and TIGIT, are equipped with Fc-mediated effector functions that can be triggered by therapeutic monoclonal antibodies (mAbs), and secrete a myriad of cytokines such as IFN-γ, TNF-α, amongst others, that contribute to adaptive immune cell infiltration and activation ([70], means that leveraging NK cell biology to augment response to anti-PD-(L)1 may become a successful therapeutic strategy. Indeed promising results have already been observed for the combination of pembrolizumab and allogeneic NK cells [74] and for durvalumab and, the NKG2A targeting antibody, Monalizumab in NSCLC [75].

Neutrophils typically account for 70% of total white blood cells in peripheral blood, playing a crucial role as a first responder in infection [76]. However, their role of in cancer has been debatable until now, with both pro- and anti-tumor properties having been assigned to this cell type, likely due to the fact that they can retain some functional plasticity and can respond differently to cues in their microenvironment [77]. Three populations of neutrophils have been identified in the circulation in cancer patients, mature high density neutrophils (HDN), mature low density neutrophils (LDN) and immature LDNs, with associated cytotoxic phenotypes for the first and immune suppressive for the latter two [78]. In a retrospective data analysis of single cell RNA-seq from 29 public datasets in NSCLC, a tumor resident neutrophil signature was found to be associated with atezolizumab treatment failure, and identified as a potential negative prognostic biomarker [79]. In line with these findings, a prospective study monitoring circulating LDNs in NSCLC has similarly found that elevated baseline LDNs could predict primary resistance to first line anti-PD-(L)1 therapy. The authors further their investigation and proposed that the mechanisms by which neutrophils might confer resistance are through soluble molecules within the HGF/c-MET pathway which were in an *ex vivo* setting able to dampen T cell cytotoxicity. Interestingly, in the same study, the cohort receiving ICB therapy in combination with chemotherapy, there was no association between high levels of LDN and resistance to treatment and authors suggest that this combination may favorably deplete neutrophils and hence potentiate the effect of immunotherapy [80]. In contrast to these findings in NSCLC, the neutrophil-to-lymphocyte ratio (NLR) in head and neck and salivary cancers was found to correlate with survival outcomes but not with response to pembrolizumab and vorinostat [81], highlighting a need to better define the functional role of neutrophils in the TME. Additionally, it is critical to define what is a true prognostic or predictive measure for this biomarker that can be standardized to be used across tumor indications, in order to truly understand its value and differences across cancer types for better patient stratification.

Adaptive immune cells in the TME

Given their critical nature with respect to the anti-tumor immune response, it is perhaps of no surprise that increased infiltration of

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tumors by CD8 T cells has been associated with both improved prognosis [82] and improved response to anti-PD-(L)1 [83] in a number of settings and studies. Despite this, patients with comparable levels of CD8 infiltrate can have disparate responses to treatment, this could be driven by differences in the accessibility of some tumoral regions [84], but also due to the fact that a measurable proportion of infiltrating CD8s are bystanders, with specificity for common pathogens such as EBV and CMV [85]. Recent studies have begun to try and unpick some of the complexity of CD8 populations within the tumor, utilizing techniques such as single cell Ranse to define signatures of neoantigen specific CD8s [86] and using flow cytometry to identify a stem like population, characterized by TCF1 expression [87]. This stem-like subset gave rise to more terminally differentiated effectors, and was resident within niches populated by antigen presenting cells, the lack of which was associated with more rapid progression in renal cancer [88].

In classical immunology, CD4 T cells are a central player, given their critical role providing support for both B cell and CD8 T cell activation [89]. Their role in the anti-tumor immune response, and particularly in the response to anti-PD-(L)1, is far less well defined. Recently a CXCL13$^+$ population of highly clonal intratumoral CD4 T cells has been identified in a range of tumor types [90]. This population was shown to associate with antigen presenting cells within the TME, and has been associated with response to anti-PD-(L)1 in breast cancer [91]. CXCL13 is a key chemokine involved in the recruitment and organization of B cells within the lymphoid follicles [92], which itself has been associated with improved response to anti-PD-(L)1 in bladder cancer [93], and it is tempting to posit that the role of these CD4, CXCL13$^+$ cells in the context of the tumor, may be to help drive assembly of TLS like structures that mimic these follicles to some degree, promoting antigen presentation and activation of downstream CD8 T cells.

As part of their inherent plasticity, CD4 T cells are known to come in a range of 'flavors', e.g. TH1, TH2, TH17, etc., each aligned largely to the cytokines they predominantly produce and each with differing primary functions. One CD4 sub-set of particular relevance to resistance is regulatory T cells, Tregs, typically defined by the expression of the transcription factor FOXP3. These cells have been proposed to play a suppressive role, inhibiting the activity of CD8 T cells, and have been associated with poor prognosis in a range of cancer types [94]. Interestingly there are very few studies in which the expression of FOXP3 has been explored with respect to anti-PD-(L)1 response, and in one such study in bladder cancer the presence of FOXP3$^+$ cells was actually associated with improved responses [95]. One confounding factor, mentioned by the authors, is that Treg infiltration tends to correlate to CD8 T cell infiltration, and so assessing FOXP3 as an independent marker is a challenge. It is possible that additional studies in other settings may reveal a more critical role for Tregs in resistance to anti-PD-(L)1, but to date clinical evidence of this has not been forthcoming.

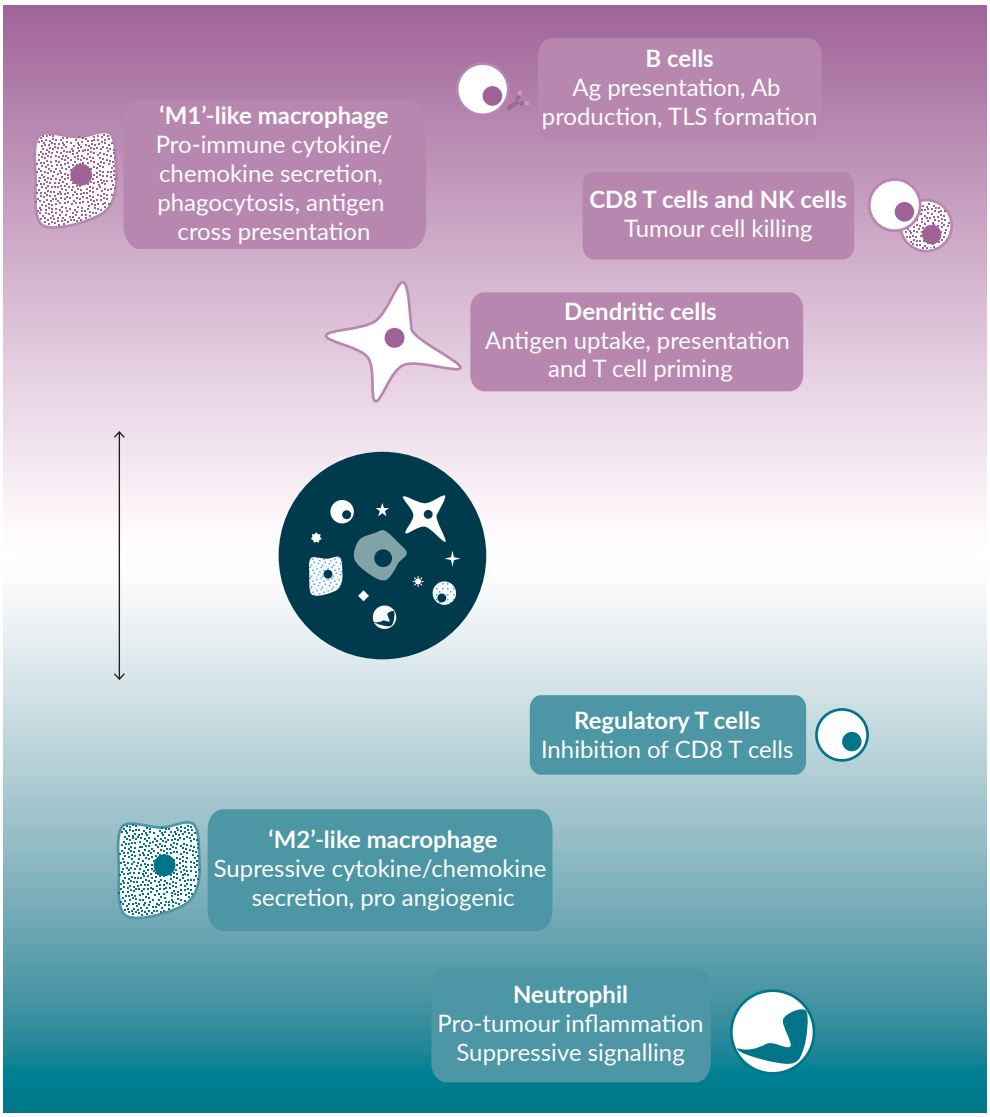
The other critical component of the adaptive immune response, the B cell, has for many years been largely unexplored with respect to its impact on response to anti-PD-(L)1 therapy. This lack of attention may have stemmed from the mixed results observed with respect to the prognostic impact of B cells in cancer [96], and the lack of clear evidence that the humoral response was key to tumor rejection. However, the other critical role for B cells, outside of antibody production, is driving antigen presentation and priming of the cellular response. More recently, a number of studies have highlighted the potential importance of this aspect of B cell biology with respect to anti-PD-(L)1 therapy. Studies in both melanoma [97–99] NSCLC [100] have illustrated the relationship between the presence of B cells, and the formation of TLS, and improved benefit following anti-PD-(L)1 treatment. In one of these studies [97], it was the patients with both TLS and high levels

of CD8 infiltrate that gleaned the highest benefit.

These data, together with those discussed above for innate immune cells, suggest that it is a coordinated and holistic response, in which all parts of the immune system are engaged and directionally aligned, that primes for response to anti-PD-(L)1 (Figure 3). The key questions that now present themselves are what are the factors that prevent that holistic response, are they common across patients or unique in every case and are there means by which we can intervene to overcome them and promote a more effective immune response. Delivering answers to these questions will be facilitated by advances in our ability to study and characterize the TME, such as single cell RNAseq, mass cytometry and multi-parameter immunofluorescence. It is only with these approaches that we can begin

FIGURE 3

The tumor microenvironment has a major role in shaping the T cell response, and the potential effectiveness of anti-PD-(L)1.



Multiple immune cell types populate the tumor microenvironment, and while most of these cell types can have diverse impact on the anti-tumor immune response, they can also be broadly classified into those, highlighted in green, that promote anti-tumor immunity and which therapeutic approaches would aim to enhance, and those, highlighted in red, that promote immunosuppression and which therapeutic approaches would aim to remove or reprogram.

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to dissect the many interactions and moving parts within the TME and understand how best to modify them.

T cell functionality & diversity

As described above, while increased CD8 infiltration into tumors has been shown to predict anti-PD-(L)1 activity, not all CD8 cells in the tumor will be relevant to the anti-tumor response. One way to better understand the nature of T cell populations is through profiling of the T cell receptor (TCR) repertoire. Such sequencing allows an assessment of the composition of the repertoire with respect to its diversity, i.e. how many different TCRs are present, and its evenness or clonality, i.e. does each TCR make up a comparable proportion of the population or is the population dominated by a small number of clones. Treatment with anti-PD-(L)1 therapy has been shown to alter the TCR repertoire within the tumor, leading to increased clonality [55,101]. These changes in clonality were accompanied by loss of neoantigens and changes in neoantigen clonality [101], supporting the concept that expansion of tumor-specific T cells is driving this increased clonality. Interestingly, increased intratumoral clonality prior to treatment has been associated with improved response to anti-PD-(L)1, while increased diversity has been associated with improved response to non-PD-(L)1 based therapy [102].

Increases in clonality, similar to those described for intratumoral T cells, can also be observed in peripheral blood following treatment with anti-PD-(L)1 [103]. Interestingly treatment with anti-CTLA-4 based therapy appears to have the opposite impact on the TCR repertoire, driving increased diversity in a number of studies [104,105]. In one study, exploring sequential treatment, clonality was not associated with outcome on anti-CTLA-4, but patients with increased clonality following CTLA-4 demonstrated increased benefit from subsequent anti-PD-(L)1. These data are in keeping with the concept that anti-PD-(L)1 functions, at least in part, to enhance activation of existing anti-tumor T cells while anti-CTLA-4 predominantly acts to drive activation of T cells not already participating in the anti-tumor immune response.

One potential advantage of TCR repertoire analyses is that they can be conducted on a blood sample, potentially allowing for less invasive biomarker monitoring. However, there have been very mixed results to date with respect to the predictive value of such peripheral measures [106]. This is perhaps unsurprising since only a fraction of the peripheral repertoire consists of anti-tumor T cells. In a recent study, increased diversity in flow cytometrically isolated PD-1$^+$, CD8$^+$ T cells from the peripheral blood was associated with improved response to anti-PD-(L)1 [107]. This suggests that one potential approach to improving the utility of peripheral measures could be to focus analysis on cells more likely to be tumor-reactive.

While the TCR sequence of a T cell defines its specificity, it does not say anything about its functional state, which is also a potentially important driver of response to anti-PD-(L)1. The concept of T cell exhaustion, through chronic stimulation, is one that has underpinned the development of PD-(L)1 targeting agents. Measuring exhaustion functionally in a clinical context remains very challenging, but attempts have been made to measure it phenotypically via expression of a number of surface receptors such as PD-1, TIM-3 and LAG-3 [108]. Expression of these markers has been associated with reduced activity for anti-PD-(L)1 in some studies [109,110], but the extent of impact and the role of one marker vs the other are not necessarily consistent. This lack of consistency may be driven by the fact that many exhaustion markers are also activation markers, which correlate to each other and other markers associated with response such as CD8 infiltration and PD-L1 expression. These complex relationships confound analysis relative to outcome and almost demand a move to technologies that allow analysis at the single cell level.

Patient health

A number of susceptibility factors related to patient heath influences the initial response to anti-PD-(L)1. These include high levels of inflammatory markers such as C reactive protein (CRP) or lactate dehydrogenase (LDH) [111.112], reduced body weight or cachexia [113,114], presence of liver metastasis [115,116] and age [117]. Many of these factors are prognostic in nature, and impact response to all cancer treatments, but given their close relationship to inflammation and hematological health, they may have a uniquely significant contribution with respect to response to immunotherapies such as anti-PD-(L)1. Large, randomized data sets combined with the power of machine learning approaches may enable us to begin to pull apart which of these factors are most important and in which settings.

TRANSLATIONAL INSIGHT

Here we proposed a framework to understand I–O resistance based on two fundamental questions: is the tumor visible to the immune system and are the T cells fit enough to kill the tumor? Each of these two aspects is subject to modification by a number of interlinked tumor cell intrinsic and extrinsic aspects of biology. By reducing the significant complexity of the anti-tumor immune response, downstream of anti-PD-(L)1, to these key components, we can formulate and test hypotheses around resistance drivers and appropriate combinations to overcome them. For example, in patients with negligible neoantigen burden, or total loss of antigen presentation machinery alternatives to anti-PD-(L)1 may be more appropriate, such as targeted T cell engagers or antibody drug conjugates. In patients where T cell exhaustion appears to be restraining effective responses, overcoming this exhaustion through blockade of additional checkpoint receptors, such as TIM-3, or through blockade of downstream signaling molecules such as HPK1 could provide a path forward. In tumors with molecular drivers of resistance, those drivers may bring with them alternative targetable susceptibilities, for example loss of chromosome 9 results in loss of MTAP, [118] which may sensitize to inhibitors of PRMT5 [118].

Breaking down these components of resistance in a framework such as this facilitates their independent investigation via available multi-omics and clinical data either from clinical trials or real-world practice. However, the more significant power comes from then reintegrating them together and validating findings in the lab and clinic. This integration of knowledge across an incredibly complex biological system, can provide the clues for which combination therapies will be more effective across different indications and can help identify markers for the optimal selection of patients for treatment according to their underlying biology. In an era where big data is booming and expanding rapidly to encompass immunological as well as molecular measures, this outcome for patients may not be in a too far distant future.

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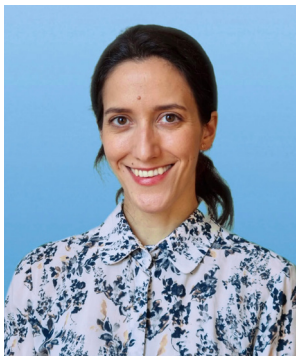
INTERVIEW

Supporting I–O combination therapy development with correlative assays & biomarker discovery



Abi Pinchbeck, Assistant Editor, *Immuno-Oncology Insights,* speaks to (pictured left to right) **Ana Rosa Saez-Ibanez** and **Samik Upadhaya** from the Cancer Research Institute, about their research in combination therapies across the I–O landscape and the importance of implementation of correlative assays in combination clinical trials.

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Q What are you working on right now?

AS: One focus of my work at the Cancer Research Institute (CRI) is on our immuno-oncology intelligence database. We are constantly monitoring the drug development

landscape in immuno-oncology (I–O), gathering and curating information about clinical trials in the space. We use this database to guide our clinical strategy and understand the gaps and opportunities. We also use this intelligence to inform the I–O community at large about trends in the field through regular publishing. In these publications, we dissect the space based on types of drug modalities, targeted proteins, indications, and the geography of clinical trials and sponsors. Last year, we published an update on the cell therapy landscape in oncology clinical trials, in addition to a dedicated piece on racial and ethnic diversity among patients in I–O trials. Last month, we published an update for the entire I–O clinical development landscape, and during the second half of this year, we are aiming to publish an update on the PD-1 and PD-L1 trials landscape, with an emphasis on understanding how resistance to prior I–O therapies is affecting trends in the usage of this type of treatment.

SU: CRI supports the research continuum from bench to bedside, driving significant discoveries in the field of immunology and I–O. We do so by funding scientists through the postdoctoral and early-mid career levels. We also have a new program to fund investigator-initiated clinical trials and provide additional support and coordination in those studies. We place a big focus on enabling translational studies and biomarker discoveries and have been doing so in a few different ways. For instance, over the past 6–7 years, we have been supporting multi-center, science-driven Phase 1b and Phase 2 clinical trials with deep correlative studies. We like to find niches in the field that the industry may not be prioritizing, and we bring in pharma, biotech, academic investigators, and other non-profits to support these studies.

One of the studies that we are currently supporting is called REVOLUTION. This is a platform trial to evaluate different immunotherapy combinations in first-line treatment of patients with metastatic pancreatic cancer. Pancreatic adenocarcinoma is a challenging cancer type for I–O, as it is poorly immunogenic and has shown limited responses to checkpoint inhibition. Through our support, investigators are working to address this unmet medical need. We currently have three ongoing cohorts in this trial, which all have the standard of care chemotherapy, gemcitabine, paclitaxel, and ipilimumab. In addition to this, Cohort A has nivolumab, Cohort B has hydroxychloroquine, and Cohort C has a novel compound, NG-350A, which is an oncolytic virus. Cohort B has now completed enrolment, and we are waiting for the translational studies to be completed in that study. We are hopeful and excited that this study will provide much needed learning to advance the pancreatic cancer field.

AS: Another trial that we are supporting now is titled Immunotherapy Platform Study in Platinum Resistant High-Grade Serous Ovarian Cancer (IPROC). This is a Phase 2 clinical trial carried out in collaboration with the Canadian Cancers Trial Group. This ovarian cancer setting is one in which there are very limited treatment options and is a highly unmet medical need in oncology. This is considered a 'cold' type of tumor, so it has been difficult to treat with immunotherapy. However, we think that with the right drug combination, we can make immunotherapy work in this type of indication. IPROC is a platform study in which we are testing different promising I–O drug combinations in separate cohorts. This is done under the guidance of our drug selection committee, which is

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a group of academic leaders in the field of immunotherapy and ovarian oncology who help us find drug combinations with the strongest scientific rationale. An important angle of this trial is that we design a strong plan for correlative analysis to hopefully identify biomarkers of response. In the two currently open cohorts, we are working in collaboration with two companies, AstraZeneca and BioAtla, with two different combinations of a PD-L1 inhibitory antibody and an antibody-drug conjugate. We hope to see results for these two cohorts later this year. In parallel, we are exploring new I–O combinations to test in future cohorts that we want to open in the near future.

SU: Yet another ongoing trial we have is to capture the tumor burden dynamics during immune checkpoint blockade using circulating tumor DNA (ctDNA). This study uses ctDNA to monitor response and potential resistance to immunotherapy, and could potentially allow patients with primary resistance to be quickly identified in real-time and redirected to alternative therapy.

The Clinical Innovator program was launched this year and is designed to investigate initiated trials that address areas of high unmet medical need in oncology. The aim is to seek mechanistic insights into patients using deeply translational correlative studies. We are targeting academic clinician scientists who have applied for this program and we have budgeted $3 million for three potential grants. CRI is aiming to coordinate these selected trials and focus on standardizing sample collection, correlative assays, and analysis. Our focus is on data sharing and the immunogenomic data that comes out of these trials is deposited into CRI's data platform, CRI iAtlas. The CRI iAtlas is an open-source platform with data curated from various immunotherapy trials. This harmonized data set allows people to compare clinical correlates to any immunogenomic features [1].

AS: We are also working on our company investment portfolio. Since 2021, CRI made the strategic decision to deploy part of our venture fund in early-stage private biotech companies. These must be biotech companies with a direct affiliation with CRI and have a focus on advancing immunotherapy. We have made several investments, mostly in seeds and series A companies that are working either with new I–O drug modalities or with platforms for target discovery that can be leveraged to find new immunotherapies. I am extremely excited about our growing portfolio and sharing this journey with entrepreneurs and other investing firms.

Q What evolution are you seeing in I–O combination therapy development and how is CRI contributing to this?

AS: Highlights of the I–O landscape publication we have recently released [2] include the insight that companies and sponsors are pivoting away from PD-1/PD-L1 combination clinical trials. These checkpoint inhibitors have revolutionized treatment in oncology and have become a standard of care in many indications and the preferred combination partner for new assets that advance in the clinical development pipeline. This

"...companies and sponsors are pivoting away from PD-1/PD-L1 combination clinical trials. These checkpoint inhibitors have revolutionized treatment in oncology and have become a standard of care in many indications..."

– **Ana Rosa Saez-Ibanez**

year is the first time we have seen a general decrease in the number of Phase 2 clinical trials in I–O, and this seems largely driven by a reduction in the number of trials using PD-1/PD-L1 inhibitors. This is a shift in a decade-long trend, possibly influenced by the fact that patent expiration is approaching for many of these inhibitors. This brings with it an exciting opportunity for the field to reinvent itself and keep innovating. In fact, we see an increase in Phase 1 trials, as well as more trials exploring new targets and modalities, with many drugs targeting immune cell types other than T cells. This is a fascinating time for the field.

SU: Before the REVOLUTION Platform Study came to fruition, there was the PRINCE trial, which also addressed pancreatic adenocarcinoma patients. This was another I–O combination trial using chemotherapy in combination with a CD40 agonist to activate and prime the antigen-presenting cells, and a PD-1 blockade to overcome immunosuppression and invigorate the T cells for effective tumor-killing. There were three arms in the study: the CD40 agonist plus chemotherapy arm, the nivolumab plus chemotherapy arm, and the triple combination arm. The baseline immune features in patients' blood were identified in each arm. The overall survival was close to 58% in the chemotherapy plus nivolumab arm. The impact of that trial cannot be understated – it demonstrated significant clinical benefits. More importantly, multiomic correlative analysis was performed on an unprecedented scale, and the outcome revealed some baseline immune characteristics that could predict response to each treatment arm. We are further exploring these results towards the design of a future clinical trial to guide precision treatment to stratify patients based on these identified immune signatures which will hopefully significantly increase clinical response and overall survival.

The REVOLUTION Platform Study is based on the learnings of the PRINCE Trial with the aim to push the field even further. We also leverage our internal I–O intelligence that we have been gathering for many years to inform our clinical strategy, and we work with our investigators to figure out the right immunotherapy combinations in indications of interest.

On the clinical innovation front, we are providing a mechanism to support investigator-initiated trials, focusing on biomarker discovery and correlative assays. We are learning from both the successes and from failures where clinical signals have not been seen. Leveraging learnings from every single patient is key in our clinical efforts.

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Q Why is the implementation of correlative assays in combination clinical trials important? What other emerging technologies can be used to speed up clinical trial development?

AS: The emerging technologies and novel trial designs that can help us bring the field forward include implementing correlative assays and creating complex translational assay plans for these trials. There is a lot of heterogeneity among patients in terms of response to immunotherapy that we do not fully understand. Correlative analysis will help us unveil what biomarkers are associated with response and what molecular pathways are involved.

Another aspect of our work that helps us move quickly in clinical trials so that we can reach more patients in a timely manner is that we design our trials as platform studies. This allows us to open a master investigational new drug (IND) and create sub-INDs to test new combination therapies in separate cohorts. This is a way to move faster in clinical trial development, as a fully new clinical trial does not need to be created each time we want to test a new combination. It also has an adaptive design, so that if we see that patients respond well in the initial enrolled cohort, we can expand that cohort and implement biomarker-driven strategies learned from the analysis of samples from the first set of patients.

In terms of other specific technologies that will help us move forward in the field, I think that ctDNA analysis holds great potential. As we see in the BR.36 trial that we are currently supporting, ctDNA can help identify, faster and more accurately than radiographic response, whether patients are responding to immunotherapy or not. Therefore, ctDNA can become a powerful decision-making tool for clinicians to decide whether to continue treatment or escalate/change therapy for patients.

Q Where is further improvement needed to fully realize these combination efforts?

SU: We need to think more about how these tools can be clinically implemented for a wide uptake in the field. How can the field prepare itself to incorporate these innovative correlative studies and technologies, in addition to the learnings from them, into future clinical trials? Should there be discussions about how to make these assays into companion diagnostics in the future? We also need to work to enable the clinical application of these technologies in varying kinds of settings, not just highly specialized medical centers and clinics but also in community settings. How can these diagnostics be rapidly implemented and utilized? For instance, investigators working on our ctDNA study are constantly thinking about pragmatic approaches aimed towards the broad applicability of the findings. I think the field needs to constantly question itself and think about clinical relevance, broad applicability, and accessibility.

Q As the field works to further rationalize combination therapy development, what are your predictions for how the space may evolve over the next decade?

> "Cancer is not one disease – every indication has unique biology and even within the same indication there is wide patient heterogeneity. We need to drill down further, for example, to understand the immune profile of patients, even before treatment."
>
> – **Samik Upadhaya**

SU: Going back to the learnings from biomarkers and correlative assays, I think there will be continued focus and advancement towards biomarker-directed patient stratification approaches. Cancer is not one disease – every indication has unique biology and even within the same indication there is wide patient heterogeneity. We need to drill down further, for example, to understand the immune profile of patients, even before treatment. This involves understanding what biomarkers, immune profiles, and immune cell states these patients have and whether they can be leveraged to stratify patients into specific treatment cohorts. I think we will see a push towards more biomarker-directed precision oncology, which is a trend we have seen over the past few years in our internal analysis. I believe we will continue to see that accelerate even further.

AS: I am excited about some of the new targets and approaches emerging in immunotherapy. My prediction is that the field will diversify the ways in which it harnesses the immune system. We have seen that checkpoint inhibitors have released the brakes of the immune system by interacting with T cells. There are so many other components of the immune tumor microenvironment that we can modulate to facilitate an immune attack on the tumor. I am excited about some of the drugs targeting the tumor microenvironment and which inhibit the immune suppressive cells that are impeding the function of T cells. That is an important mechanism by which some tumors do not respond to immunotherapy.

In general, it is important to see the immune system as a complex system with many different players, some of which need to be activated, and others inhibited. Every piece needs to fall into place to achieve the desired immune function.

We also see signs of increased efforts in making drug development more patient-centric, which is the way to go. We need immunotherapies that translate well to the real world and regular clinical practice, and which allow patients to have a life as normal as possible while on treatment. Another very important angle is diversity. How can we implement diversity in clinical trials to ensure that, once drugs are approved, they can be safely administered to heterogeneous patient populations? There are many types of diversity in patients (for example related to race and ethnicity, age, or presence of comorbidities) that are necessary to integrate

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in clinical trials to ensure that the drugs work for all patients once they are approved. Fortunately, we see the field making increasing efforts in this direction.

Q What are your own goals and priorities in the same timeframe?

SU: We will continue to monitor our I–O intelligence database that keeps track of the field in terms of global drug and clinical trial development. We will continue to expand that database and adapt it to the wider field. For example, we recently published an analysis on patient diversity in I–O trials. Given that checkpoint therapy has been approved for more than a decade, we felt it was necessary to investigate the diversity in the pivotal trials that have led to FDA approvals [3]. We are also continuing to assess the field to gain a more comprehensive view of the landscape in terms of biomarker usage. We have an additional focus on I–O resistance, as this is an area both driving innovation and limiting progress in the field. Our aim is to understand what is driving resistance to I–O therapies and what approaches are being undertaken to address this.

AS: Our goal is to keep expanding the support of innovative clinical concepts. We are approaching that in many ways at CRI. We have programs targeted toward academic investigators who want to pursue unique and bold ideas in investigator-initiated trials and we also have multi-centered platform studies, in which we bring several partners from academia, non-profits, big pharma, and big biotech to collaborate. We also support new small biotechs that are bringing forward fascinating new modalities in I–O. I want to keep supporting all these different strategies that work towards the same common goal.

The CRI has many patient-focused activities to increase the general understanding of immunotherapies. For example, we conduct a CRI virtual immunotherapy summit in both English and Spanish, which acts as a platform for patients to gain answers to questions about how immunotherapy works. We bring leaders in the field to explain this in layman's terms so patients can easily understand how to find the right clinical trials for them. This is just one example of the patient-centric activities that CRI is doing as a beautiful part of our bigger goal.

SU: I would like to also take this opportunity to highlight CICON, a Cancer Immunotherapy Conference that CRI has organized since 2015. This is an international conference that academic scientists and clinicians as well as colleagues in the industry have attended in the past, and many stakeholders in the field would find it valuable [4].

BIOGRAPHIES

ANA ROSA SAEZ-IBANEZ joined the Cancer Research Institute in 2022 as a Research Analyst at the CRI Clinical Accelerator and Venture Fund. In this role, she provides intelligence on immuno-oncology drug development trends and the competitive landscape, informing CRI's clinical trials strategy, business development activities and start-ups investment decisions. Prior to joining CRI, Ana Rosa earned her Doctorate in Cancer Cell Biology at Uppsala University (Sweden) where she explored novel roles of receptor tyrosine kinases in promoting cancer cell migration.

Following obtention of her PhD degree, Ana Rosa joined Albert Einstein College of Medicine and Icahn School of Medicine at Mount Sinai (New York) as a postdoctoral fellow, where she investigated the role of chaperone-mediated autophagy in cell fate determination and development of metastatic breast cancer. She received her BSc and MSc in Biology and Biomedicine from the University of Valencia (Spain). Over the years, Ana Rosa has cultivated an interest in the different steps of the drug development process. In 2020, she was selected for the FDA-AACR oncology educational fellowship, a competitive regulatory affairs program led by the FDA OCE. In addition, she has received training in biomedical entrepreneurship and has consulted for different investment banks as a biotech equity researcher. Finally, Ana Rosa holds a co-chair position in the fundraising committee of Women in Autophagy, a NY non-profit built to empower women and underrepresented minorities in the field of autophagy research.

SAMIK UPADHAYA is the assistant director of scientific affairs at CRI. He is passionate about harnessing the full potential of scientific advances in cancer immunotherapy to help patients live better, longer lives. Together with the Clinical Accelerator team, he leads the program's scientific diligence efforts, including analyses of emerging trends and challenges in the global cancer immunotherapy landscape. He assists in the team's collaborative ventures, clinical trial design, drug development plan, and maintenance of immuno-oncology landscape databases. He is also involved in all of CRI's research programs, and closely follows the research done by CRI grantees, evaluating their potential contributions to the field as a whole. Prior to joining CRI, Samik 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. He also received his MSc in Chemistry and a dual BSc, *summa cum laude*, in Biochemistry and Biomedical Sciences from Central Michigan University.

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INTERVIEW

Trialing reovirus in combination for I–O applications

Roisin McGuigan, Editor, *Immuno-Oncology Insights,* speaks to (pictured) **Houra Loghmani,** Senior Scientist, Oncolytics Biotech, about her role in the development of pelareorep, an intravenously-administered oncolytic virus currently in clinical trials.

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Q What are you currently working on?

HL: I am a Senior Translational Research Scientist at Oncolytics Biotech. We are developing an oncolytic respiratory enteric orphan virus (reovirus) – pelareorep – via several ongoing clinical Phase 2 trials. As a senior scientist, I help with developing protocols ranging from preclinical studies for new drug combination testing, to planning different exploratory experiments on the clinical samples we receive to look for biomarkers of response. We want to identify what kinds of biomarkers we can correlate with the responses that we are seeing in our clinical trials.

I also support the company's business development efforts, including evaluating new collaborators. We are constantly looking for collaborators to work with. In the checkpoint

blockade field, we are currently working with Roche, Pfizer, Incyte, and we also have collaborators for our CAR-T program.

Q Can you tell me more about pelareorep?

HL: It is a naturally occurring reovirus that has demonstrated oncolytic properties thanks to the double-stranded RNA introduced into cancer cells and its ability to induce an inflamed phenotype in certain tumors. There are two main differentiating factors that this oncolytic virus has compared to the rest of the oncolytic viruses in the field: first, it is naturally occurring, and has not been genetically modified, therefore it is categorized under the biosafety level two and can be administered in a typical chemo suite. Second, it is given intravenously, which is important to note as most other oncolytic viruses must be given intratumorally, a complex means of administration not favored by most oncology practices.

In addition, the virus is very safe, with the most commonly observed adverse events being under the category of flu-like symptoms. We have administered pelareorep to more than 1,100 patients so far and have observed severe reactions at much lower rates than those of the chemotherapies and/or PD-1/L1 agents that pelareorep is co-administered with.

By its nature as an oncolytic virus, pelareorep selectively replicates in and kills tumor cells and not healthy cells. Part of that is via direct lysis. That said, as we've understood more about the mechanism of action for pelareorep, we've come to realize that its true value is in its ability to activate the innate and adaptive immune response to weaken tumor defense mechanisms and make them more susceptible to a broad range of oncology treatments.

We ran a window of opportunity study, AWARE-1, in which we were able to collect breast cancer tumor samples from newly diagnosed HR+/HER2- breast cancer patients. We looked at the tumor microenvironment to understand how the virus is activating the T cells and bringing them to the tumor. We observed that pelareorep can highly activate the interferon gamma signaling pathway, which has been shown to help traffic the T cells to the tumor and increase PD-L1 expression, and therefore prime the tumor for checkpoint blockade therapy. Plus these T cells have more of an active memory phenotype, rather than an exhaustive type [1–4] .

Q Can you give an overview of the current clinical development strategy for pelareorep? What have been the most promising developments so far?

HL: We think of pelareorep as an enabling technology for both chemotherapies and I–O therapies, and we have published a number of papers demonstrating that the virus can turn a cold microenvironment into a hot one [5–7]. Besides chemotherapy agents, we have observed pelareorep synergies with chimeric antigen receptors (CAR-Ts) and checkpoint inhibitors, as well as bispecific antibodies. For any treatment that needs an immune component to it, we think that pelareorep could enhance that. We have already proven

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"We also know that by loading pelareorep on CAR-T cells we are able to make CAR-Ts expand more, be more persistent, and have more of a memory phenotype. We call these dual-specific CAR-Ts because they are specific to both the CAR target and also to pelareorep epitopes."

this in our clinical trials with checkpoint inhibitors, and we hope we can do so with other combinations.

Our near-term focus is on metastatic breast and pancreatic cancer, as we have observed very promising responses across multiple Phase 2 trials. We have observed a near doubling of overall survival in HR+/HER2- metastatic breast cancer when we compared pelareorep plus paclitaxel to standard of care paclitaxel. In first-line pancreatic cancer, we have observed a near tripling of the response rate for the the pelareorep and atezolizumab plus chemotherapy arm compared to the standard-of-care chemotherapy control arm [8]. Pelareorep's demonstrated potential to synergize with both chemotherapy and a range of immuno-oncology agents to treat a number of tumor targets has led to collaborations with Pfizer, Roche, Merck Serono, and Adlai Nortye.

Q What advantages can an oncolytic virus and CAR-T combination offer over other approaches in the solid tumor space?

HL: CAR-T therapy faces challenges in solid tumors. Firstly, it is difficult for CAR T cells to reach the tumor – and if they manage to get there, they encounter a harsh and suppressive microenvironment. With pelareorep, we are able to traffic the T cells to the tumor.

We also know that by loading pelareorep on CAR-T cells we are able to make CAR-Ts expand more, be more persistent, and have more of a memory phenotype. We call these dual-specific CAR-Ts because they are specific to both the CAR target and also to pelareorep epitopes. We have also observed that a subsequent, IV-delivered single pelareorep boosting dose can re-activate those CAR-T cells, which would otherwise become exhausted. This CAR-T work has been done at the Mayo Clinic by Professor Richard Vile's group [6].

Q What are your own, and your company's, key goals and priorities looking at the next few years?

HL: My focus will be mainly on advancing our translational understanding of how pelareorep could be leveraged to treat multiple tumor targets, especially when combined with PD-1/PD-L1 inhibitors, CAR-Ts, chemotherapy, and bispecific

antibodies. I want to deepen our understanding by looking at the translational data that we get from patients, as well as the preclinical models we are using.

BIOGRAPHY

HOURA LOGHMANI received her doctorate degree in Molecular Medicine from Hanover Medical School (Germany) and then went on to complete two postdoctoral fellowships at University Hospital Tubingen (Germany) and the Center of Blood Research (CBR) at the University of British Columbia. Throughout her academic career, Houra's research has focused on investigating the molecular mechanisms underlying hematopoiesis, blood diseases and malignancies, as well as small molecule drug testing and cell signaling, which the results of her work have been published in many peer-reviewed journals. Currently, Houra is a Senior Scientist at Oncolytics Biotech, where she is helping with the development of pelareorep, a proprietary isolate of the naturally occurring reovirus, a promising therapeutic oncolytic virus.

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