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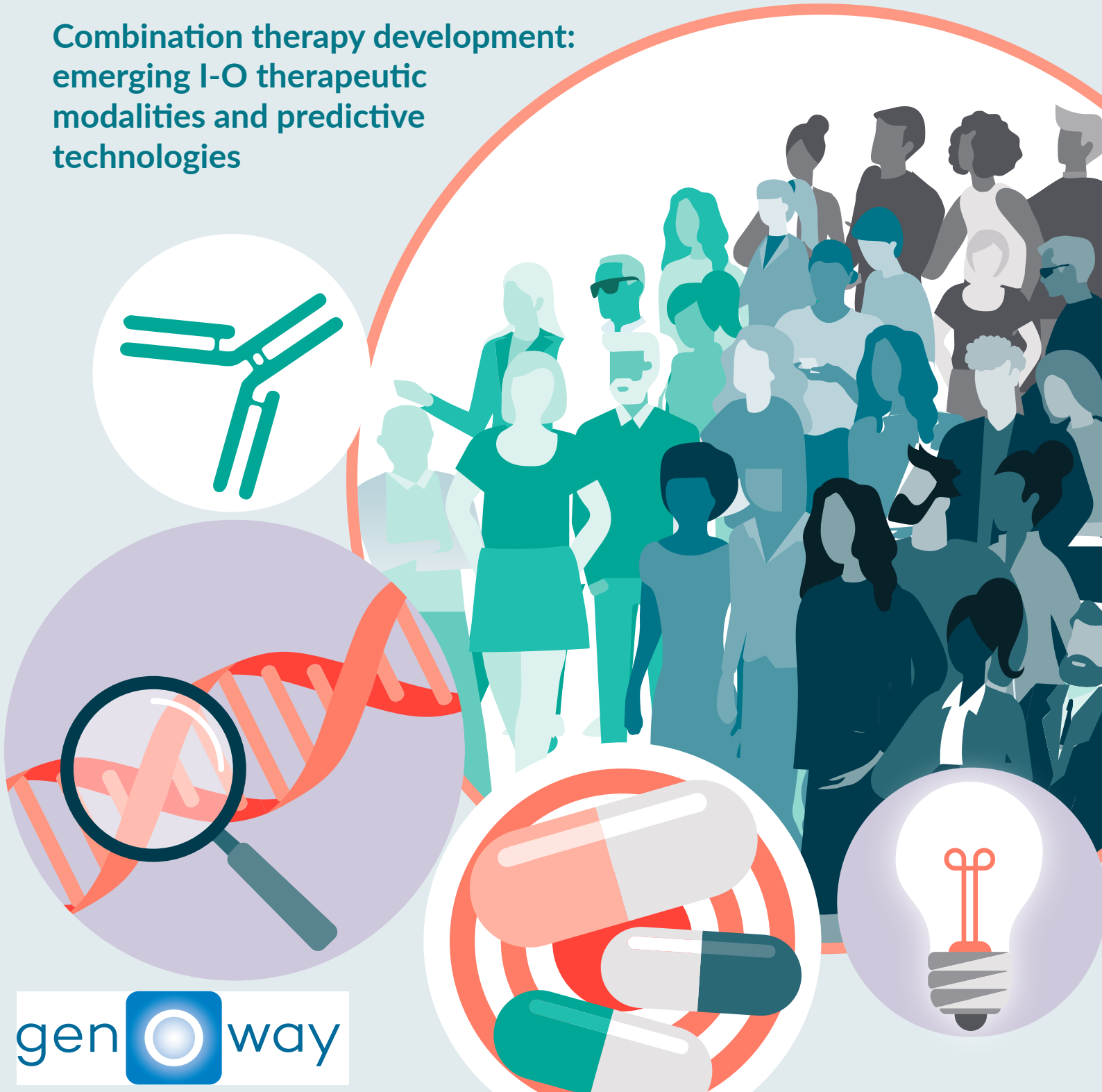
SPOTLIGHT ON:

Combination therapy development: emerging I-O therapeutic modalities and predictive technologies



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IMMUNO-ONCOLOGY INSIGHTS

COMBINATION THERAPY DEVELOPMENT: EMERGING I-O THERAPEUTIC MODALITIES & PREDICTIVE TECHNOLOGIES

SPOTLIGHT

EXPERT INSIGHT

Powering antitumor immunity: challenges & promise at the intersection of immunometabolism & cancer immunotherapy

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Since their approval, immune checkpoint inhibitors have drastically altered the landscape of cancer care. However, despite this substantial progress, many agents demonstrating considerable preclinical potential have failed to be successfully translated to the clinic. Therefore, an understanding of the processes underlying T cell responses will be crucial for the rational design of the next generation of immune checkpoint inhibitors. In parallel with these advances in tumor immunology, the field of immunometabolism has identified the importance of metabolic reprogramming during the initial steps of T cell activation in modulating clonal expansion, effector activity, and fate determination. Here, we will highlight the interplay between these processes, their suppression in the tumor-bearing state, and the putative mechanisms by which checkpoint blockade may restore metabolic reprogramming. Finally, we will discuss novel checkpoints under investigation and discovery-driven approaches to identify modulators of immune cell metabolism to synergize with new approaches to immune checkpoint inhibitors .

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Immune checkpoint blockade has demonstrated increasing success in many solid tumors [1]. By releasing the breaks on Cytotoxic T cell Antigen 4 (CTLA-4) and Programmed Death Receptor 1 (PD-1), the co-stimulatory receptors enforcing peripheral tolerance, these agents enable T cells to overcome the maladaptive appropriation of these physiologic processes in the tumor-bearing state. This in turn restores effective T cell activation and potentiates antitumor immune responses. However, complete responses remain rare, and most of the remaining partial responses are transient. These suboptimal responses are in part attributable to the immunosuppressive effects of the tumor microenvironment (TME), which pose a challenge to tumor infiltrating cytotoxic T lymphocytes (TILs) [2]. Highly metabolically active T cells must compete with tumor cells for the nutrients and oxygen required to sustain effective anti-tumor immune responses in the TME [3]. Therefore, identifying the coordinated signaling and metabolic cues driving productive and dysfunctional T cell responses will be critical to improve treatment outcomes. The burgeoning field of immunometabolism has shed light on processes underlying productive and dysfunctional immune responses, providing critical clues and strategies that can be leveraged in the rational design of the next generation immunotherapies. Here, we will review

1. The mechanisms underlying metabolic reprogramming during a functional T cell response;
2. The dysfunction in T cell metabolism in the TME and in periphery of cancer patients;
3. The impact of existing immunotherapies on T cell metabolism;
4. Novel approaches/the future of immunometabolism for cancer treatment.

GLYCOLYSIS FUELS EFFECTOR T CELLS

In order to augment the efficacy of immune checkpoint blockade, it is crucial to understand the metabolic transitions underlying productive effector T cell responses. Naïve T cells are present in circulation and in lymphoid structures, quiescent yet poised to respond to pathogens or cancer neoantigens. Upon recognition of their cognate antigen, effector T cells become activated, rapidly proliferate, and differentiate into effector cells with the capability to lyse their targets. These processes are not only associated with but are dependent on metabolic reprogramming to support the demands of cell growth, multiple rounds of division, and cytokine production. To become fully activated, T cells must receive signal 1 from the T cell receptors (TCR) interacting with cognate antigen, signal 2 from costimulatory molecules on antigen presenting cells (APCs) interacting with coreceptors like CD28 on T cells, and signal 3 from cytokines such as IL-2 [4]. TCR engagement and CD28 co-stimulation also synergize to induce metabolic remodeling, in which T cells undergo a 'switch' from primarily using oxidative phosphorylation (OXPHOS) to employing glycolysis as their main energy source in the presence of high oxygen, termed Warburg metabolism or aerobic glycolysis [3,5–11] (Figure 1A). Engaging in this process enables cycling through the pentose phosphate pathway (PPP), through which T cells generate crucial intermediates utilized in macromolecule production, which in turn sustain clonal expansion. Understanding the interplay between these early signaling events and glycolytic pathways is therefore necessary to modulate and enhance antitumor responses.

TCR signaling and co-stimulation are the critical inputs for T cell activation. These same signals also directly increase glycolysis by promoting lactic acid fermentation, increasing expression of surface nutrient transporters, and activating glycolytic enzymes. TCR signaling increases glycolysis by inducing tyrosine phosphorylation of a glycolytic gatekeeper, pyruvate dehydrogenase kinase 1 (PDHK1) [11] (Figure 1A). PDHK1 inhibits pyruvate dehydrogenase, thereby redirecting pyruvate from entering

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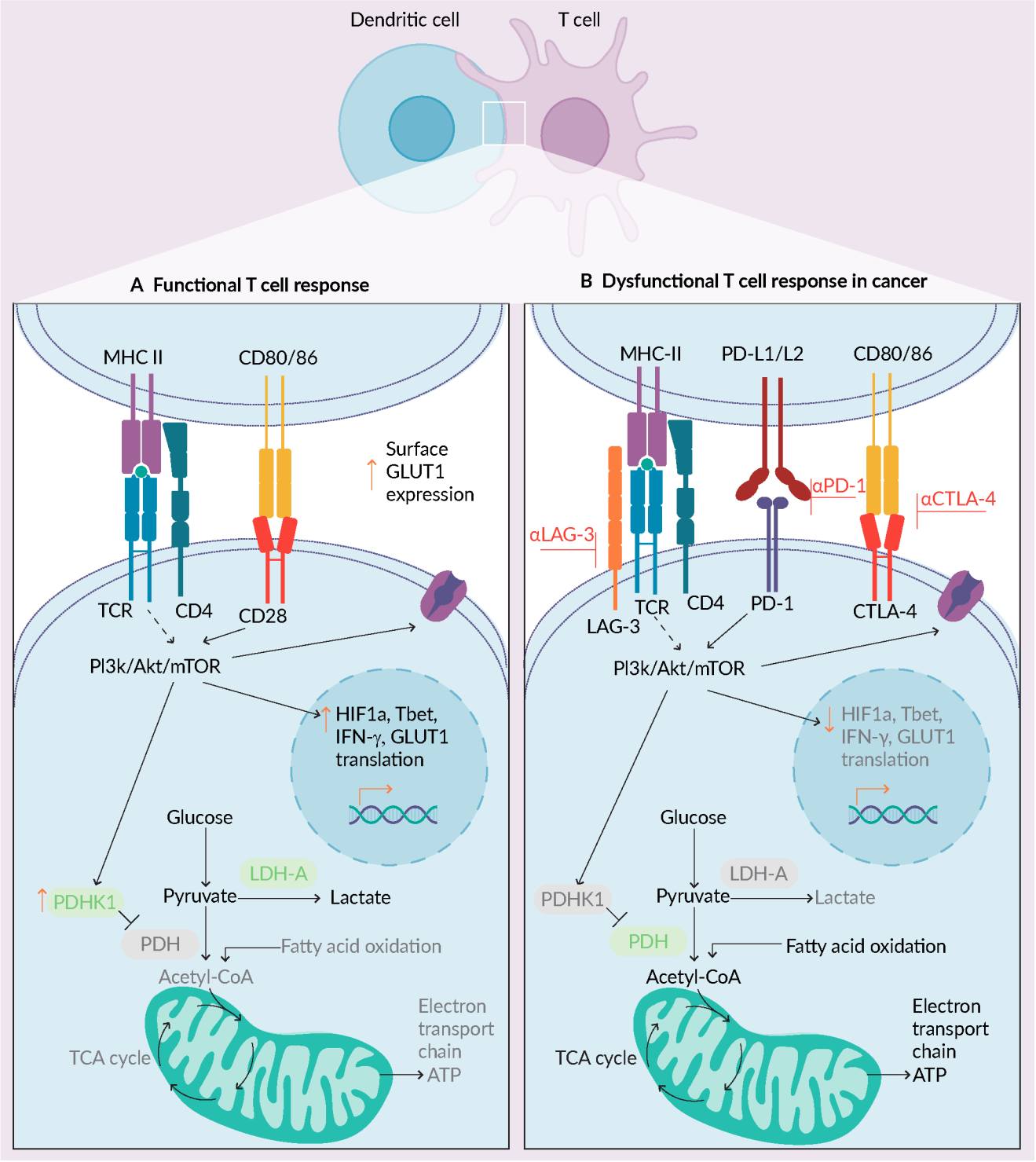
FIGURE 1

Panel schematic depicting metabolic reprogramming during A) a functional T cell response and B) a dysfunctional T cell response in the context of cancer.



A) During a functional T cell response, TCR stimulation and co-stimulation synergize to increase PI3k/Akt/mTOR signaling. This increases surface expression of the glucose transporter, GLUT1, the translation of key transcription factors, cytokines and nutrient transporters, and PDHK1 activity, which supports aerobic glycolysis by redirecting pyruvate from entering the TCA cycle to instead undergo lactic acid fermentation. **B)** In the context of cancer, reduced TCR stimulation and costimulation along with inhibitory receptor expression result in decreased PI3k/Akt/mTOR signaling. Cells fail to increase surface expression of GLUT1, expression of key transcription factors, cytokines, and nutrient transporters, or PDHK1 activity. Pyruvate primarily enters the TCA cycle in the mitochondria, supporting oxidative phosphorylation and fatty acid oxidation. Red blunt headed arrows denote the binding sites for anti-PD1, anti-CTLA4, and anti-Lag3 checkpoint blockade therapy.

the mitochondria for the tricarboxylic acid (TCA) cycle to instead undergo lactic acid fermentation. NAD^+ reduction to NADH is a feature of glycolysis, so in highly glycolytic cells lactic acid fermentation is necessary to regenerate NAD^+. Costimulatory signals such as CD28 can amplify TCR-mediated signals and maintain glycolytic function by engaging the phosphatidylinositol-3-kinase (PI3K), Akt and mammalian target of rapamycin (mTOR) kinase signaling cascades (Figure 1A). CD28-induced PI3K dependent Akt activation promotes an increase in cell surface expression of nutrient transporters, such as the glucose transporter GLUT1, and facilitates phosphorylation and increased activity of glycolytic enzymes, including hexokinase 2 (HK2) and phosphofructokinase 2 [8–10]. CD28 engagement also increases mammalian target of rapamycin complex 1 (mTORC1) activation, which signals through its downstream substrates 4EBP1 and S6 kinase to increase protein synthesis and hypoxia-inducible transcription factor (HIF1α) [3,8–10,12,13]. HIF1α acts as a switch between glycolysis and OXPHOS by inhibiting mitochondrial function and promoting the transcription of key glycolytic enzymes [14,15] (Figure 1A).

Following TCR engagement, T cells undergo massive clonal expansion, doubling every 4–5 hrs and generating over 1012 cells within a week [16]. This rapid proliferation necessitates the synthesis of macromolecules; glycolysis supports this demand by shuttling glycolytic intermediates into biosynthetic pathways to produce nucleic acids, amino acids, phospholipids, and fatty acids [3]. During the first step of glycolysis, glucose is phosphorylated by HK2 to glucose-6-phosphate (G6P), an intermediate that can enter the PPP to generate ribose-5-phosphate, which is the backbone of nucleic acids and reduced nicotinamide adenine nucleotide phosphate (NADPH)[3]. NADPH provides the reducing power for the synthesis of nucleotides, fatty acids, and non-essential amino acids in addition to playing a critical role in regenerating antioxidants to prevent oxidative stress [17]. Another glycolytic intermediate, glycerol-3-phosphate, can be converted to phosphatidate (PA), which is an intermediate for phospholipid synthesis, and storage lipid triacylglycerol (TAG) synthesis [3,18–20]. In addition, the glycolytic intermediate 3-phosphoglycerate can be converted into serine, an amino acid that feeds into the folate cycle by donating one carbon to folate. The folate cycle generates one-carbon units for biosynthetic processes and is another source of NADPH [3,21]. Pyruvate is the final product of glycolysis and is a building block for several non-essential amino acids, including alanine and arginine. Subsequent peptide synthesis supports the production of cytokines and effector molecules that underlies effective cytotoxic responses.

OXIDATIVE METABOLISM IS ESSENTIAL FOR T CELL ACTIVATION

While aerobic glycolysis is critical for the exit from quiescence and the development of functional effector T cell responses, an increasing appreciation for the role of oxidative phosphorylation has emerged. The critical inputs for T cell activation, TCR engagement and co-stimulation, are known to synergize to increase the glycolytic rate. However, co-stimulatory receptor signaling through CD28 or tumor necrosis factor ligand superfamily member 9 (4-1BB), have also been implicated in the induction of OXPHOS [12,22–24]. CD28 signaling leads to optic atrophy one (OPA-1)- mediated mitochondrial cristae tightening and mitochondrial fusion, which result in increased respiratory function [12,22]. Signaling through the 4-1BB receptor is even more efficient at promoting mitochondrial respiration by enhancing mitochondrial biogenesis and mitochondrial fusion via peroxisome proliferator-activated receptor-gamma coactivator (PGC1a) and OPA-1 modulation [12,23,24]. The 4-1BB receptor has been found to enhance the

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persistence and efficacy of chimeric antigen receptor (CAR) T cells for cancer therapy compared to the CD28 receptor, suggesting that increased mitochondrial respiration supports effector T cell function [23–27].

Prior work indicates that increased OXPHOS early during T cell activation is necessary for T cell proliferation and effector cell differentiation. While effector T cells are thought to primarily use aerobic glycolysis, the metabolic adaptations that ensue from the time that naïve T cells are initially primed until they differentiate into effector cells has remained unclear [5–7,28–31]. Recent advances in single cell metabolic analyses by mass cytometry by time-of-flight (CyTOF) have enabled the observation of single cell metabolic transitions during effector cell differentiation. These studies have revealed that T cells simultaneously exhibit peak expression of proteins involved in glycolytic and oxidative metabolism early after activation [32,33]. Shortly after entering this unique metabolic state, T cells rapidly proliferate to produce large numbers of highly activated effector cells, and subsequently downregulate metabolic markers expression upon differentiation [32]. Although little is known about the function of this intermediate increase in OXPHOS, studies have suggested that both glycolysis and OXPHOS are necessary for the effector T cell response [16,34,35]. In one study, authors demonstrated that inhibiting glycolysis, the TCA cycle, or the electron transport chain (ETC) can each disrupt effector cell differentiation, proliferation, or both [34]. Specifically, exposure to the glycolysis inhibitor 2-deoxy-D-glucose (2DG) and the TCA cycle inhibitor sodium fluoroacetate reduced T cell proliferation and Th1 effector cell differentiation, as measured by the production of the cytokine IFN-γ, in a dose dependent manner [34]. Ultimately, subsequent mechanistic studies determined that complex II of the ETC promotes Th1 differentiation but suppresses proliferation, while mitochondrial transport systems that support complex I activity (malate–aspartate shuttle and mitochondrial citrate export), are required for both T cell proliferation and transcriptional remodeling [34]. Together, these studies support a model in which co-stimulation increases mitochondrial respiration, and that this increase in OXPHOS is necessary for T cell proliferation and effector cell differentiation.

METABOLIC DYSFUNCTION UNDERLIES T CELL EXHAUSTION IN MALIGNANCY

Although T cells increase glycolysis and OXPHOS during a functional immune response, this metabolic remodeling is thwarted in the tumor-bearing state. Tumor cells alter T cell metabolism by directly competing for nutrients, by secreting lactate as an aerobic glycolysis biproduct, and by increasing the expression of inhibitory receptors, which act through various mechanisms to antagonize glycolytic and mitochondrial metabolic remodeling. Together, these maladaptive metabolic dynamics underlie T cell exhaustion and dysfunction in the TME.

In parallel with effector T cells, tumor cells utilize Warburg metabolism, characterized by high glucose and amino acid uptake, high rates of glycolysis, and high lactate secretion [3,36–38]. T cells in the tumor microenvironment (TME) therefore face unique challenges, including low glucose and glutamine availability and an acidic environment with high lactate, all of which blunt the effector T cell response [36–39]. It has been observed that T cells co-cultured with mouse sarcoma tumor cells had reduced glycolysis and IFN-γ production [36]. Glucose supplementation or pre-treating tumor cells with a glycolysis inhibitor, rapamycin, both rescued IFN-γ production [36]. Conversely, overexpression of glycolysis-related enzymes and transporters by tumor cells, including GLUT1, HK2, or PDHK1, resulted in reduced glucose uptake by TILs *in vivo*, and lower IFN-γ production *in vitro* [36]. Similarly, characterization of tumor cells isolated from renal cell carcinoma patients revealed high expression of GLUT1, which corresponded with poor $CD8^+$ T cell

infiltration and lower expression of the cytotoxicity markers granzyme B and perforin [38]. High levels of aerobic glycolysis in tumors not only restrict available nutrients, but result in high levels of lactate export and lower pH, which increase T cell anergy and promote polarization of $CD4^+$ T cells towards an immunosuppressive regulatory T cell (Treg) phenotype [40–43]. Glycolytic effector T cells require constant recycling of NAD^+ through the activity of lactic acid dehydrogenase (LDH) during lactic acid fermentation. High lactate reverses the LDH reaction and thus inhibits NAD^+ regeneration and subsequently effector T cell function [41]. Unlike effector T cells, Treg are less reliant on glycolysis and can metabolize lactate and convert it to pyruvate, which can fuel the TCA cycle [43]. Additionally, lactate metabolism is required for intratumoral Treg function such that inhibiting lactate transport reduces the suppressive functions and proliferation of intratumoral Treg [43].

The mechanisms by which effector T cell function is impaired by nutrient competition in the tumor microenvironment have been increasingly well understood. While glycolytic enzymes support effector cell function through their canonical activity, when not occupied by their substrates, they may also blunt cytokine production, a process known as moonlighting [29,34]. Activated T cells demonstrate impaired IFN-γ production in low glucose environments when cultured in glucose-free galactose media or when glucose uptake is prevented in the presence of the competitive inhibitor 2DG [29,34]. Further mechanistic interrogation reveals that glycolysis directly impacts cytokine production via glycolytic enzymes binding the mRNA of cytokines and preventing translation. Therefore, in low glucose environments, the glycolytic enzyme GAPDH is free to bind the AU-rich 3' UTR of IFN-γ and IL-2 mRNA, inhibiting their translation. When glucose levels and glycolytic flux are high, GAPDH is engaged in glycolysis and releases its inhibition of cytokine translation. Similarly, the enzyme LDH-A, which converts pyruvate to lactate, binds IFN-γ and IL-2 mRNA when flux is low. Increased aerobic glycolysis and lactic acid fermentation engage LDH-A thus releasing its inhibition of IFN-γ and IL-2 translation. When confronted with the low glucose, high lactate conditions of the tumor microenvironment, activated T cells that have undergone glycolytic reprogramming with attendant expression of GAPDH and LDHA may be vulnerable to moonlighting induced repression of effector function. Moreover, Treg and suppressive myeloid populations residing in the tumor microenvironment rely on oxidative metabolism and are less susceptible to these repressive mechanisms [41].

In addition to altering T cell function via nutrient competition, tumor cells modulate T cell metabolism within the TME by misappropriating mechanisms of peripheral tolerance. Immune checkpoint receptors serve as the 'brakes' to maintain immune homeostasis and prevent autoreactive T cell activation in the periphery. However, persistent exposure to tumor antigen dramatically increases T cell checkpoint receptor expression, ultimately resulting in T cell dysfunction [44]. In addition to their well characterized role in T cell exhaustion, checkpoint receptors also decrease T cell effector capabilities by altering their metabolic program.

CTLA-4 is a coinhibitory receptor expressed on the surface of activated T cell that shares its ligands, CD80 (B7-1) and CD86 (B7-2), with the costimulatory receptor CD28. In this way, CTLA-4 antagonizes CD28 co-stimulation, which abrogates CD28-mediated glycolytic and mitochondrial metabolic reprogramming [37,45,46]. By dampening CD28 signaling, CTLA-4 reduces GLUT1, HK2, and glutamine transporters (SNAT1, SNAT2), and inhibits glycolysis [37,45,47] (Figure 1B).

Similarly, programmed death-1 (PD-1) is a coinhibitory receptor expressed on activated T cells, NK cells, NKT cells and B cells. PD-1 binds the ligands programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (B7-DC) expressed on the surface of APCs and other hematopoietic

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cells, and attenuates signaling downstream of the TCR [37,45,48]. PD-1 engagement decreases PI3k/AKT/mTOR signaling, and subsequently alters the metabolic state and effector function of T cells [37,45,48]. PD-1 receptors translocate to TCR microclusters and accumulates at the signaling central supramolecular activation cluster (c-SMAC) [49]. Upon PD-1 engagement with its ligand PD-L1, the immunotyrosine inhibitory motif (ITIM) and immunotyrosine switch motif (ITSM) of the intracellular PD-1 tail recruit phosphatases like SHP-2, which dephosphorylate key signal transducers [45,48,50]. Like CTLA-4, PD-1 reduces SNAT1, SNAT2 and GLUT1 and reduces the rate of glycolysis [37] (Figure 1B).

Another checkpoint inhibitor, LAG3, is a CD4 homolog that binds its canonical ligand major histocompatibility complex-II (MHC-II) with higher affinity than CD4. LAG3 is expressed on the surface of activated T cells, NK cells, NKT cells, B cells and plasmacytoid dendritic cells and can bind other ligands found on the surface of tumor cells, including lymph node sinusoidal endothelial cell C-type lectin (LSECtin), fibrinogen-like protein 1 (FGL1), galectin-3, and α-synuclein [51–53]. LAG3 moves to the immune synapse where it localizes with the TCR/CD3 complex and disrupts CD4 or CD8 co-receptor-TCR signaling, thereby inhibiting downstream calcium influx, cytokine production and proliferation [37,53–58] (Figure 1B). Mechanistically, the LAG3 cytoplasmic tail contains a tandem glutamic acid–proline repeat that lowers the pH at the immune synapse and dissociates the tyrosine kinase Lck from the CD4 or CD8 co-receptor [51,58]. This disruption in CD4 or CD8 co-receptor-TCR signaling abolishes downstream metabolic reprogramming and increases glycolysis.

Additionally, the inhibitory checkpoint receptor T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), is a member of the CD28 family and binds the ligands CD155 and CD112, which are expressed on the surface of APCs and tumor cells. TIGIT is expressed by a subset of early activated T cells and NK cells [59], and shares the ligand CD155 in common with CD266. The ligation of CD155 with CD266 enhances T cell activation [60], whereas TIGIT binding suppresses activation by attenuating TCR and CD28 signaling [61,62]. Engagement of TIGIT results in phosphorylation of the tyrosine residue of the ITIM motif and the immunoglobulin tail tyrosine (ITT)-like motif on the TIGIT cytoplasmic tail. SHIP1 is recruited to the TIGIT tail, which blocks PI3K signal transduction and activates the NF-kB pathway, reducing glycolysis [47,53,63,64]. By directly competing for nutrients and increasing the expression of inhibitory receptors, tumor cells alter T cell metabolism both in the TME and in peripheral tissues. Taken together, these mechanisms represent important processes to be targeted by immune checkpoint blockade.

IMMUNE CHECKPOINT BLOCKADE RESTORES T CELL METABOLIC REPROGRAMMING

As checkpoint inhibitors have gained a growing foothold in clinical practice, greater understanding and appreciation of the pleotropic impacts of these agents on various aspects of T cell function has emerged. The field of immunometabolism has revealed the maladaptive metabolic reprogramming induced by the tumor-bearing state, and the potential ability of these agents to reverse them. While these processes have been well-characterized *in vitro* and in the context of *in vivo* tumor models, our understanding of the metabolic modulations induced by current FDA-approved and investigational checkpoint inhibitors remains incomplete. However, insights gained from chronic infection and tumor models have shed light of putative metabolic reprogramming induced by these agents.

While the metabolic adaptations underlying response to PD-1 blockade in human malignancy are incompletely understood, preclinical data implicate the early steps of de

TABLE 1

Developmental therapeutics targeting LAG-3 currently in clinical trials.

Drug	Class	Type of cancer	Additional agents	Target(s)/ mechanism	Phase	NCT	Status	Title
Relatlimab	Human IgG4 monoclonal Ab (mAb)	Unresectable stage III/IV melanoma	Nivolumab (PD-1 inhibitor)	LAG-3 blockade	2/3	NCT03470922	Completed	Relatlimab and Nivolumab versus Nivolumab in untreated advanced melanoma [71]
		Neoadjuvant stage II melanoma	Nivolumab		2	NCT05418972	Not yet recruiting	A phase 2 clinical trial of neoadjuvant Relatlimab and Nivolumab in high risk, clinical stage II cutaneous melanoma (Neo ReNi II)
		Adjuvant stage III or IV melanoma	Nivolumab		3	NCT05002569	Recruiting	A study to assess adjuvant immunotherapy With Nivolumab plus Relatlimab versus Nivolumab alone after complete resection of stage III-IV melanoma (RELATIVITY-098)
		Metastatic uveal melanoma	Nivolumab		2	NCT04552223	Recruiting	Nivolumab plus Relatlimab in patients With metastatic uveal melanoma
		Unresectable stage III/IV melanoma	Nivolumab, Ipilimumab (CTLA-4 inhibitor), Sarilumab (sIL-6R inhibitor)		2	NCT05428007	Not yet recruiting	Interleukin-6 receptor inhibitor Sarilumab in combination With Ipilimumab, Nivolumab and Relatlimab in patients With unresectable stage III or stage IV melanoma
		Head and neck squamous cell carcinoma (HNSCC)	Nivolumab, Ipilimumab		2	NCT04080804	Recruiting	Study of safety and tolerability of Nivolumab treatment alone or in combination With Relatlimab or Ipilimumab in head and neck cancer
		Gastric cancer, Gastresophageal junction cancer (GEJ)	XELOX, FOLFOX, SOX		2	NCT03662659	Active	An investigational study of immunotherapy combinations With chemotherapy in patients with gastric or gastroesophageal junction (GEJ) cancers
		Perioperative esophageal, Perioperative GEJ, Peroperative gastric	Nivolumab, Chemoradiation: Carboplatin + Paclitaxel		1	NCT03044613	Active	Phase IB trial of induction Nivolumab or Nivolumab/Relatlimab prior to concurrent chemoradiation in patients with operable stage II/III esophageal/ gastroesophageal junction cancer
		Locally advanced chordoma Unresectable chordomoa Metastatic chordoma	Nivolumab		2	NCT03623854	Recruiting	Nivolumab and Relatlimab in treating participants with advanced chordoma
		Acute myeloid leukemia AML	Nivolumab + Azacitadine		2	NCT04913922	Recruiting	Relatlimab With Nivolumab and 5-Azacytidine for the treatment of AML (AARON)
		Advanced MSS colorectal cancer (CRC)	Nivolumab		2	NCT03642067	Recruiting	Study of Nivolumab and Relatlimab in patients with microsatellite stable (MSS) advanced colorectal cancer
		Pre-treated advanced CRC	Nivolumab-FDC		3	NCT05328908	Recruiting	A Study of Nivolumab-relatlimab fixed-dose combination versus Regorafenib or TAS-102 in participants with later-lines of metastatic colorectal cancer (RELATIVITY-123)
		Perioperative hepatocellular carcinoma (HCC)	Nivolumab		1	NCT04658147	Recruiting	Feasibility and efficacy of perioperative Nivolumab with or without Relatlimab for patients with potentially resectable hepatocellular carcinoma (HCC)
		Advanced HCC	Nivolumab, Bevacizumab		1/2	NCT05337137	Recruiting	A study of Nivolumab and Relatlimab in combination with Bevacizumab in advanced liver cancer (RELATIVITY-106)
		TKI-refractory immunotherapy naïve HCC	Nivolumab		2	NCT04567615	Recruiting	A study of Relatlimab in combination with Nivolumab in participants with advanced liver cancer who have never been treated with Immuno-oncology therapy after prior treatment with tyrosine kinase inhibitors
		Advanced renal cell carcinoma (RCC)	Nivolumab		2	NCT02996110	Active	A phase 2, real-time assessment of combination therapies in immuno-oncology study in participants with advanced renal cell carcinoma (FRACTION-RCC)
		Neoadjuvant RCC	Nivolumab, Ipilimumab		2	NCT05148546	Recruiting	Neoadjuvant study with combination immuno-oncology for primary clear cell renal cell cancer (NESCIO)
		Advanced MSI-H tumors	Nivolumab		2	NCT03607890	Recruiting	Study of Nivolumab and Relatlimab in advanced mismatch repair deficient cancers resistant to prior PD-(L)1 Inhibitor
		Non-hodgkin lymphoma, Hodgkin lymphoma	Nivolumab		1/2	NCT05255601	Recruiting	A study to evaluate the safety, tolerability, drug levels, and preliminary efficacy of Relatlimab plus Nivolumab in pediatric and young adults with hodgkin and non-hodgkin lymphoma (RELATIVITY-069)
		Advanced lymphoma	Nivolumab		1/2	NCT02061761	Completed	A study to evaluate the safety, tolerability, and efficacy of Relatlimab in relapsed or refractory B- Cell malignancies
		Ovarian cancer, Fallopian tube cancer, Peritoneal cancer	Adoptive cell therapy (TIL, Nivolumab, Ipilimumab, Fludarabine		1/2	NCT04611126	Recruiting	T-cell therapy in combination with Nivolumab, Relatlimab and Ipilimumab for patients with metastatic ovarian cancer
		Advanced solid tumors	Ipilimumab + Nivolumab or Nivolumab + Linrodostat (IDO1 inhibitor)		1/2	NCT03459222	Recruiting	An investigational study of immunotherapy combinations in participants with solid cancers that are advanced or have spread
		Multiple myeloma	Pomalidimide, Dexamethasone		1/2	NCT04150965	Recruiting	A phase I/II assessment of combination immuno-oncology drugs Elotuzumab, Anti-LAG-3 (BMS-986016) and Anti-TIGIT (BMS-986207)

TABLE 1 (CONT.)

Drug	Class	Type of cancer	Additional agents	Target(s)/ mechanism	Phase	NCT	Status	Title
Sym022	monoclonal Ab	Biliary tract carcinoma, Esophageal SCC	Sym021 (Anti-PD-1 mAb)	LAG-3 blockade	1	NCT04641871	Active	An exploratory, open-label, multicenter phase 1b trial to evaluate safety and efficacy of Sym021 (Anti-PD 1) in combination with either Sym022 (Anti-LAG-3) or Sym023 (Anti-TIM-3) or Sym023 and Irinotecan in patients with recurrent advanced selected solid tumor malignancies
EMB-02	Bispecific antibody	Advanced solid tumors, lymphomas		PD-1 × LAG-3 blockade	1	NCT03489369	Completed	A phase 1, open-label, multicenter trial investigating the safety, tolerability, and preliminary antineoplastic activity of Sym022 (Anti-LAG-3) in patients with advanced solid tumor malignancies or ymphomas
		Advanced solid tumors			1/2	NCT04618393	Recruiting	A phase I/II trial of EMB-02, a bi-specific antibody against PD-1 and LAG-3, in patients with advanced solid tumors
TSR-033	Humanized IgG4 mAb	Advanced solid tumors, mCRC	Dostarlimab +/- mFOLFOX6 + Bevacizumab or FOLFIRI + Bevacizumab	LAG-3 blockade	1	NCT03250832	Active	A phase 1 dose escalation and cohort expansion study of TSR-033, an Anti-LAG-3 monoclonal antibody, alone and in combination with an Anti-PD-1 in patients with advanced solid tumors
Fianlimab	Human IgG4 mAb	Unresectable or metastatic melanoma	Cemiplimab (Anti-PD-1 mAb)	LAG-3 blockade	3	NCT05352672	Recruiting	A phase 3 trial of Fianlimab (REGN3767, Anti-LAG-3) + Cemiplimab versus Pembrolizumab in patients with previously untreated unresectable locally advanced or metastatic melanoma
		Advanced solid tumors	Cemiplimab		1	NCT03005782	Active	A phase 1, open-label, dose-escalation and cohort expansion first-in-human study of the safety, tolerability, activity and pharmacokinetics of REGN3767 (Anti-LAG-3 mAb) administered alone or in combination with REGN2810 (Anti-PD-1 mAb) in patients with advanced malignancies
ABL501	Bispecific Ab	Advanced solid tumors		PD-L1 x LAG-3 blockade	1	NCT05101109	Recruiting	A phase 1 dose escalation and expansion study of ABL501, a bispecific antibody of PD-L1 and LAG-3 as a single agent in subjects with any progressive, locally advanced (Unresectable) or metastatic solid tumors
HLX26	Recombinant humanized mAb	mCRC	HLX10 (Anti-PD-1 mAb)	LAG-3 blockade	2	NCT05584137	Active	A phase II study to evaluate the efficacy, safety and tolerability of HLX26 (Anti-LAG-3 monoclonal antibody injection) in combination with HLX10 (recombinant humanized Anti-PD-1 monoclonal antibody injection) in patients with metastatic colorectal carcinoma that have received 3 prior lines of therapy
RO7247669	Bispecific Ab	Metastatic melanoma, NSCLC Esophageal SCC, Advanced solid tumors		PD-1 × LAG-3 Blockade	1	NCT04140500	Recruiting	An open label, multicenter, dose escalation, phase 1 study to evaluate safety/tolerability, pharmacokinetics, pharmacodynamics and preliminary anti tumor activity of RO7247669, a PD1- LAG3 bispecific antibody, in patients with advanced and/or metastatic solid tumors
FS118	Bispecific Ab	Advanced solid tumors, HNSCC		PD-L1 × LAG-3 blockade	1/2	NCT03440437	Recruiting	A phase 1/2, open-label, first-in-human study to evaluate the safety and anti-tumor activity of FS118, a LAG-3/PD-L1 bispecific antibody, in patients with advanced malignancies
LAG525	Humanized IgG4 mAb	Metastatic triple-negative breast cancer (TNBC)	spartalizumab (Anti-PD-1 mAb), NIR178 capmatinib (MET-R inhibitor) Lacnotuzumab (anti-CSF1 antibody) canakinumab (IL-1 inhibitor)	LAG-3 blockade	1	NCT03742349	Active	A phase Ib, multicenter, open-label dose escalation and expansion platform study of select immunotherapy combinations in adult patients with triple-negative breast cancer
					2	NCT03484923	Active	A randomized, open-label, phase II open platform study evaluating the efficacy and safety of novel spartalizumab (PDR001) combinations in previously treated unresectable or metastatic melanoma
		Unresectable or metastatic melanoma	Spartalizumab					
INCA32459-101	Bispecific antibody	Melanoma HNSCC		LAG-3 × PD-1 blockade	1	NCT05577182	Active	A phase 1, open-label, multicenter study of INCA32459 in participants with select advanced malignancies
LBL-007	Human IgG4 monoclonal Ab	Advanced solid tumors	Tislelizumab (Anti-PD-1 mAb), BGB-A425 (anti-TIM-3 Ab)	LAG-3 blockade	2	NCT03744468	Recruiting	Phase 1-2 study investigating safety, tolerability, pharmacokinetics and preliminary antitumor activity of various combinations of BGB-A425 and LBL-007 with Tislelizumab in patients with advanced colid tumors
IN-CAGN02385	Fc-engineered IgG1k mAb	HNSCC	Retifanlimab (Anti-PD-1 mAb), INCAGN02390 (Anti-TIM-3 mAb)	LAG-3 blockade	2	NCT05287113	Recruiting	A randomized, double-blind, multicenter, phase 2 study of Retifanlimab in combination with INCAGN02385 (Anti-LAG-3) and INCAGN02390 (Anti-TIM-3) as first-line treatment in participants with PD-L1-positive (CPS ≥ 1) recurrent/metastatic squamous cell carcinoma of the head and neck
		Advanced solid tumors, Unresectable or metastatic melanoma	Retifanlimab (Anti-PD-1 mAb) INCAGN02390 (Anti-TIM-3 mAb)		1/2	NCT04370704	Recruiting	A phase 1-2 study of combination therapy with INCMGA00012 (Anti-PD-1), INCAGN02385 (Anti- LAG-3), and INCAGN02390 (Anti-TIM-3) in participants with select advanced malignancies
Tebotelimab	DART protein	HER2+ gastric cancer, HER2+ GEJ cancer	margetuximab (Anti-HER2 mAb), XELOX or mFOLFOX6	PD-1 × LAG-3 blockade	2/3	NCT04082364	Active	A phase 2/3 trial to evaluate Margetuximab in combination with INCMGA00012 and chemotherapy or MGD013 and chemotherapy in patients with metastatic or locally advanced, treatment-naïve, HER2-positive gastric or gastroesophageal junction cancer
		Advanced solid tumors	margetuximab (Anti-HER2 mAb)		1	NCT03219268	Active	A phase 1, first-in-human, open-label, dose escalation study of MGD013, a bispecific DART® Protein Binding PD-1 and LAG-3 in patients with unresectable or metastatic neoplasms
XmAb22841	Bispecific	Advanced solid tumors	Pembrolizumab	CTLA-4 x LAG-3 blockade	1	NCT03849469	Active	A phase 1 multiple-dose study to evaluate the safety and tolerability of XmAb®22841 monotherapy and in combination with pembrolizumab in subjects with selected advanced solid tumors (DUET-4)

novo T cell activation in this process. Blockade of PD-1/PD-L1 ligation disengages the inhibitory effects of SHP2, c-SMAC, and microcluster formation, and therefore enables productive metabolic reprogramming in recruited stem-like T cells [48]. For example, anti-PD-1 and anti-PD-L1 antibodies interrupted inhibitory microcluster formation and c-SMAC accumulation, prevented anergy, and restored IL-2 secretion in OT-1 T cells that were chronically stimulated with the OVA peptide [50]. Moreover, Pdcd1-/- p14 cells adoptively transferred into WT mice followed by LCMV clone 13 infection demonstrated enhanced proliferation and glycolytic metabolism, along with reduction in fused mitochondria and greater polyfunctionality suggestive of enhanced metabolic fitness early in the course of infection prior to the development of exhaustion [48]. This observation is consistent with the observed immutable epigenetic stability of terminally exhausted $CD8^+$ T cells in the setting of clone 13 infection, which are refractory to PD-L1 blockade in conditions of high antigen burden [65] Instead, $CXCR5^+CD8^+$ stem-like memory pools earlier in the course of the T cell differentiation trajectory provide the reinvigorated proliferative burst observed in response to PD-L1 blockade in this context [66]. Similarly, paired single-cell RNA and TCR sequencing of site-matched tumors of patients with basal cell or squamous cell carcinoma before and after PD-1 blockade with pembrolizumab or cemiplumab indicate that clonal replacement by recruiting novel T pools to the tumor accounts for increased $CD8^+$ T cell infiltration in response to therapy rather than the expansion of exhausted cell present at baseline within the tumor [67]. Taken together these findings suggest that blockade of PD-1/PD-L1 ligation disengages the inhibitory effects of SHP2 and c-SMAC, preventing inhibitory microcluster formation early during $CD8^+$ T cell activation, enabling productive metabolic reprogramming to proceed (Figure 1B). This intervention would enhance T cell metabolic fitness and prevent the epigenetic scarring and maladaptive metabolic adaptations that would otherwise ensue in the setting of chronic antigen exposure. The proximity of these steps to initial T cell priming implicates the preservation of a metabolically enhanced early activated state rather than the reinvigoration of these processes in terminally exhausted T cells.

Similarly, CTLA-4 blockade with ipilimumab may prevent maladaptive metabolic programming by retaining the ability of CD28 to engage with B7-1 and B7-2 as demonstrated by Xray crystallography [68]. Downstream signaling through the P13K/Akt/mTOR cascade is thereby restored, enabling the early steps of glycolytic reprogramming including receptor translocation, enzyme phosphorylation, and protein synthesis to proceed (Figure 1B). This may then further potentiate the priming of new metabolically active T cell clonotypes, furthering the process of clonal replacement. In addition to this potential metabolic offloading of effector subsets, CTLA-4 has also been found to promote glycolytic reprogramming of regulatory T cells leading to a loss of suppressive function in the tumor microenvironment [69]. Thus, the timing, localization and specificity of CTLA-4 and PD-1-directed therapies should be carefully considered in order to maximize their efficacy by enhancing the activation and metabolic fitness of early activated T cell subsets.

The recent FDA approval of the anti-LAG3 antibody relatlimab in combination with nivolumab for advanced melanoma (based on the results of the RELATIVITY-047 study) has generated even further interest in LAG3 as an immunotherapeutic target [51]. As the precise mechanism by which LAG3 mediates its inhibitory effect on $CD4^+$ and $CD8^+$ T cell TCR signaling has yet to be comprehensively defined, the immunomodulatory effects of relatlimab in cancer patients remains incompletely understood. However, one may hypothesize that LAG3 blockade may somehow permit resumption of TCR signaling, thereby promoting aerobic glycolytic reprogramming through PDHK1 phosphorylation (Figure 1B). However, these

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potential impacts on T cell metabolism have yet to be investigated. Interestingly, despite the absence of single-agent activity of this agent [70], when administered in combination with nivolumab, relatlimab exhibited benefit in patients with PD-L1 negative tumors independent of baseline LAG3 expression [51]. Therefore, while relatlimab was initially posited to reinvigorate exhausted T cells co-expressing these markers in the TME [71], this observation may implicate a yet to be elucidated upstream mechanism of action during the early phase of antigen engagement and T cell activation impacting both signals and their downstream effect on functional and metabolic remodeling. Therefore, further mechanistic and correlative investigation of both local and systemic T cell responses elicited by combining relatlimab and nivolumab are necessary to further enhance the efficacy of this novel regimen. At least 15 other LAG3-targeting agents are currently in development and data from these ongoing studies may further elucidate the mechanism of action underlying responses to these agents (Table 1) [72].

INVESTIGATIONAL CHECKPOINT INHIBITORS & METABOLIC MODULATORS MAY ENHANCE THE EFFICACY OF CURRENT APPROVED APPROACHES

In addition to CTLA-4, PD-1 and LAG3, additional immune checkpoints have been the focus of considerable attention and enthusiasm in the field. TIGIT has generated particular interest, as it represents an important checkpoint in T cell activation. CD155 expression has been identified across a range of tumors including melanoma [62,73,74], and gastric cancer [63]. Moreover, TIL and tumor antigen-specific T cells demonstrate increased TIGIT expression in melanoma patients [74]. Engagement of CD155 expressed by tumors with TIGIT on TIL has been implicated in the suppression of effector responses in the context of malignancy [62,73]. Combined TIGIT and PD-1 blockade enhanced the *ex vivo* effector function of $CD8^+$ T cells derived from melanoma patients [62] and has been demonstrated to act synergistically with PD-1 inhibition to enhance $CD8^+$ T cell effector function and mediate tumor regression in the CT26 murine model of colorectal carcinoma [62]. The metabolic impact of TIGIT ligation remains incompletely understood, with initial studies performed in T cell gastric cancer co-culture system suggesting that TIGIT may exacerbate the effects of metabolic competition between $CD8^+$ T cells and tumor cells in the TME. In this context, $CD8^+$ T cells demonstrated decreased glucose uptake and impaired effector function, which was reversed by either the addition of glucose or TIGIT blockade. Furthermore, CD155 silencing in gastric tumor cells enhanced T cell metabolism and cytokine production while CD155 overexpression further impaired these functions. However the functional impairments associated with CD155 overexpression were reversed by TIGIT blockade [63]. Yet despite these intriguing mechanistic studies generating considerable initial enthusiasm, TIGIT-directed therapies have yet to demonstrate efficacy in ongoing trials. However, over 14 TIGIT-targeted antibodies remain in development (Table 2) [75] and the outcomes of these studies have yet to be reported.

The continued challenges in translating novel approaches to checkpoint blockade to the clinic underscore the importance of target selection, study design, and rational combinatorial approaches to the successful clinical implementation of these investigational agents. Indeed, current approaches to further augment the efficacy of checkpoint blockade have centered on potent immunometabolic modulators such as 4-1BB agonists which stimulate T cell oxidative metabolism and augment antitumor immune responses [23]. Two monoclonal antibodies with 4-1BB agonist activity, urelumab and utomilumab have proceeded through early phase trials with little success, having been

TABLE 2

Immunotherapeutics targeting TIGIT currently under investigation in clinical trials.

Drug	Class	Type of cancer	Additional agents	Target(s)/ mechanism	Phase	NCT	Status	Title
HLX301	Bispecific Ab	NSCLC Urothelial carcinoma (UC) Gastric cancer GEJ carcinoma HNSCC		PD-L1 × TIGIT	1/2	NCT05102214	Recruiting	A phase 1/2 study of HLX301, a recombinant humanized Anti-PDL1 and Anti-TIGIT bispecific antibody, in patients with locally advanced or metastatic solid tumors
AZD2936	Bispecific Ab	Advanced NSCLC		PD-1 × TIGIT	1/2	NCT04995523	Recruiting	Phase I/II, open-label, dose escalation and dose expansion study to evaluate safety, pharmacokinetics, pharmacodynamics and efficacy of AZD2936 Anti-TIGIT/Anti-PD-1 antibody in participants with advanced or metastatic non-small cell lung cancer
HB0036	Bispecific Ab	Advanced solid tumors NSCLC		PD-L1 × TIGIT	1/2	NCT05417321	Recruiting	A phase I/II, open-label, multicenter study to evaluate the safety, pharmacokinetics, pharmacodynamics, and efficacy of HB0036 in subjects with advanced solid tumors
Tiragolumab	Human IgG1 monoclonal Ab	Neoadjuvant cisplatin ineligible UC	Atezolizumab (Anti-PD-L1 mAb)	TIGIT blockade	1	NCT05394337	Not yet recruiting	Neoadjuvant PD-1 plus TIGIT blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma
		Adjuvant esophageal SCC	Atezolizumab		3	NCT04543617	Recruiting	A phase III, randomized, double-blind, placebo-controlled study of Atezolizumab with or without Tiragolumab (Anti-TIGIT Antibody) in patients with unresectable esophageal squamous cell carcinoma whose cancers have not progressed following definitive concurrent chemoradiotherapy
		Extensive-stage small cell lung cancer (SCLC)	Atezolizumab Carboplatin Etoposide		3	NCT04256421	Active	A study of Atezolizumab plus Carboplatin and Etoposide with or without Tiragolumab in patients with untreated extensive-stage small cell lung cancer (SKYSCRAPER-02)
		Neoadjuvant HNSCC	Atezolizumab Tocilizumab (Anti-IL-6 mAb)		2	NCT03708224	Recruiting	A phase II study of preoperative immunotherapy in patients with squamous cell carcinoma of the head and neck
		Advanced NSCLC	Atezolizumab		2	NCT03563716	Active	A phase III, open-label, randomized study of Atezolizumab and Tiragolumab compared with durvalumab in patients with locally advanced, unresectable stage III non-small cell lung cancer who have not progressed after concurrent platinum-based chemoradiation [99]
		Advanced NSCLC	Atezolizumab		3		Active	Genentech reports interim results for phase III SKYSCRAPER-01 study in PD-L1-high metastatic non–small cell lung cancer
PM1021	monoclonal Ab	Advanced solid tumors	PM8001 (Anti-PD-L1/TGF-β)	TIGIT blockade		NCT05537051	Not yet recruiting	A phase I clinical trial to evaluate the safety, tolerability, pharmacokinetics, and preliminary efficacy of PM1021 (Anti-TIGIT) monotherapy and PM1021 in combination with PM8001 (Anti-PD- L1/TGF-β) in patients with advanced solid tumours
Domvanalimab (AB154)	Humanized IgG1 monoclonal Ab	Advanced gastric	Zimberelimab (Anti-PD-1 mAb)	TIGIT blockade	3	NCT05568095	Not yet recruiting	A randomized, open-label, multicenter phase 3 trial of Domvanalimab, Zimberelimab, and chemotherapy versus Nivolumab and chemotherapy in participants with previously untreated locally advanced unresectable or metastatic gastric, gastroesophageal junction, and esophageal adenocarcinoma
		Advanced GEJ cancer advanced esophageal eancer	+ FOLFOX or CAPOX					
		Unresectable or metastatic melanoma	Zimberelimab		2	NCT05130177	Recruiting	Phase II study of PD-1 inhibitor Zimberelimab (AB122) with TIGIT inhibitor Domvanalimab (AB154) in PD-1 relapsed/refractory melanoma
Ociperlimab	Humanized IgG1 monoclonal Ab	Limited-stage SCLC	Tislelizumab (Anti-PD-1 mAb)	TIGIT blockade	2	NCT04952597	Active	A phase 2, multicenter, randomized, 3-arm, open-label study to investigate the preliminary efficacy and safety of the Anti-TIGIT monoclonal antibody Ociperlimab (BGB-A1217) plus Tislelizumab plus concurrent chemoradiotherapy in patients with untreated limited-stage small cell lung cancer
(BGB-A1217)			+Concurrent Chemoradiotherapy: Carboplatin/Cisplatin + Etoposide					
		PD-L1+ Advanced Esophageal SCC	Tislelizumab		2	NCT04732494	Recruiting	A phase 2, multicenter, randomized, placebo-controlled study to compare the efficacy of Anti-PD- 1 monoclonal antibody Tislelizumab (BGB-A317) plus Anti-TIGIT monoclonal antibody Ociperlimab (BGB-A1217) versus Tislelizumab plus placebo as second-line treatment in patients with PD-L1 tumor area positivity (TAP) ≥ 10% unresectable, locally advanced, recurrent or metastatic esophageal squamous cell carcinoma
		Advanced cervical C cancer	Tislelizumab		2	NCT04693234	Active	Phase 2 study investigating efficacy and safety of Anti-PD-1 monoclonal antibody Tislelizumab (BGB-A317) combined with or without Anti-TIGIT monoclonal antibody BGB-A1217 in patients with previously treated recurrent or metastatic cervical cancer
		NSCLC	Tislelizumab		1	NCT04047862	Recruiting	Phase 1/1b study investigating safety, tolerability, PK and antitumor activity of Anti-TIGIT monoclonal antibody BGB-A1217 in combination with Anti-PD-1 monoclonal antibody Tislelizumab in patients with advanced solid tumors [100]
		NSCLC	Tislelizumab		3	NCT04746924	Recruiting	A phase 3, randomized, double-blind study of BGB-A1217, an Anti-TIGIT antibody, in combination with tislelizumab compared to Pembrolizumab in patients with previously untreated, PD-L1- selected, and locally advanced, unresectable, or metastatic non-small cell lung cancer
HLX53	Fc fusion protein	Advanced solid tumors lymphoma		TIGIT blockade	1	NCT05394168	Not yet recruiting	A phase I clinical study to evaluate the safety, tolerability, kinetic characteristics and preliminary efficacy of HLX53 in advanced/metastatic solid tumors or lymphoma

TABLE 2 (CONT.)

Drug	Class	Type of cancer	Additional agents	Target(s)/ mechanism	Phase	NCT	Status	Title
M6223	Human IgG1 monoclonal Ab	Advanced UC	Avelumab (Anti-PD-1 mAb)	TIGIT blockade	2	NCT05327530	Recruiting	A phase II, multicenter, randomized, open label, parallel-Arm, umbrella study of Avelumab (MSB0010718C) in combination with other anti-tumor agents as a maintenance treatment in participants with locally advanced or metastatic urothelial carcinoma whose disease did not progress with first line platinum-containing chemotherapy (JAVELIN Bladder Medley)
		Metastatic solid tumors	Bintrafusp alfa (Anti-PDL1/ TGFß Trap)		1	NCT04457778	Recruiting	Phase I, first-in-human, open-label, multiple ascending dose study to investigate the safety, tolerability, pharmacokinetics, pharmacodynamics and clinical activity of M6223, an Inhibitor of TIGIT, as single agent and in combination with Bintrafusp Alfa (Anti-PDL1/ TGFß Trap) in participants with metastatic or locally advanced solid unresectable tumors
JS006	Humanized IgG4k monoclonal Ab	Advanced solid tumors	Toripalimab (Anti-PD-1 mAb)	TIGIT blockade	1	NCT05061628	Recruiting	A phase I study to evaluate the safety, tolerability, pharmacokinetics and initial efficacy of recombinant humanized Anti-TIGIT monoclonal antibody (JS006) monotherapy and in combination with toripalimab in patients with advanced tumors
EOS-448	Human IgG1 monoclonal Ab	NSCLC HNSCC melanoma	Pembrolizumab Inupadenant (adenosine A2A receptor antagonist) Dostarlimab (Anti-PD-1 mAb)	TIGIT blockade	1/2	NCT05060432	Recruiting	A multicenter, open-label, phase I/II study of EOS884448 (EOS-448) in combination with standard of care and/or investigational therapies in participants with advanced solid tumors
Domvanalimab (AB154)	Humanized IgG1 monoclonal Ab	Glioblastoma	Zimberelimab (Anti-PD-1 mAb)	TIGIT blockade	0/1	NCT04656535	Recruiting	A multi-center phase 0/I trial of Anti-TIGIT antibody AB154 in combination with Anti-PD-1 antibody AB122 for recurrent glioblastoma
		Advanced solid tumors	Zimberelimab		1	NCT03628677	Active	A phase 1 study to evaluate the safety and tolerability of AB154 monotherapy and combination therapy in participants with advanced malignancies
		Metastatic NSCLC	Zimberelimab Etrumadenant (A2aR and A2bR antagonist)		2	NCT04262856	Recruiting	A phase 2 study to evaluate the safety and efficacy of AB122 monotherapy, AB154 in combination with AB122, and AB154 in combination with AB122 and AB928 in front-line, non-small cell lung cancer
BMS-986207	monoclonal Ab	Advanced endometrial neoplasms Advanced Ovarian Cancer Advanced Solid Tumors Advanced HNSCC	COM701 (poliovirus receptor related immunoglobulin domain (PVRIG)) Nivolumab	TIGIT blockade	1/2	NCT04570839	Recruiting	A phase 1/2 study evaluating the safety, tolerability and preliminary antitumor activity of COM701 in combination with BMS-986207 (Anti-TIGIT Antibody) and Nivolumab in subjects with advanced solid tumors [101]
		Multiple Myeloma	Pomalidomide Dexamethasone		2	NCT04150965	Recruiting	A phase I/II assessment of combination immuno-oncology drugs Elotuzumab, Anti-LAG-3 (BMS- 986016) and Anti-TIGIT (BMS-986207)
COM902	Human IgG4 monoclonal Ab	Advanced Solid Tumors Ovarian Cancer NSCLC	COM701	TIGIT blockade	1	NCT04354246	Recruiting	A phase 1 study of the safety and tolerability of COM902 in subjects with advanced malignancies [102]

TABLE 3

Investigational agents targeting 4-1BB currently in clinical trials.

Drug	Class	TypeofCancer	Additionalagents	Target(s)/ Mechanism	Phase	NCT	Status	Title
YH004	Humanized IgG1 mAb	Advanced solid tumors Non-hodgkin lymphoma		4-1BB agonist	1	NCT05040932	Recruiting	A first-in-human, multicenter, open-label, phase I dose escalation study to evaluate the safety, tolerability and pharmacokinetics of YH004 as a single agent and combination with Toripalimab in subjects with advanced solid tumors and relapsed or refractory non-hodgkin lymphoma
ADG106	Human IgG4 mAb	Metastatic NSCLC	Nivolumab	4-1BB agonist	1/2	NCT05236608	Recruiting	A phase 1b/2 study to evaluate the combination of Nivolumab (Anti PD1 Monoclonal Ab) with-ADG106 (4-1-BB Agonist Monoclonal Ab) in metastatic NSCLC after progression with anti PD1 therapy and platinum-based chemotherapy (ADIVO lung study)
NM21-1480	Trispecific Fv fusionprotein	Advanced solid tumors Advanced NSCLC		PD-L1 × 4-1BB × Human serum albumin (HSA)	1/2	NCT04442126	Recruiting	A phase 1/2 study of NM21-1480 (Anti-PDL-1/Anti-4-1BB/Anti-HSATri-Specific Antibody) in adult-patients with advanced solid tumors
RO7227166	Bispecific Ab	Relapsed/refractory Non-hodgkin lymphoma	Obinutuzumab (anti-CD20 mAb) Glofitamab (CD20 × CD3 bispecific Ab)	CD-19 × 4-1BB (CD19 targeted 4-1BB ligand)	1	NCT04077723	Recruiting	An open-label, phase I study to evaluate the safety, pharmacokinetics and preliminary antitumor activity ofRO7227166 (aCD19 Targeted 4-1BB ligand) in combination with Obinutuzumab and in combination with glofitamab following a Pre-treatment dose of Obinutuzumab administered in par-ticipants with relapsed/refractory B-Cell non-hodgkin's lymphoma
GEN1046(Duo-Body®-PD-L1×4-1BB)	Bispecific Ab	Metastatic NSCLC	Pembrolizumab	PD-L1 × 4-1BB	2	NCT05117242	Recruiting	A phase 2, multicenter, randomized, open-label trial of GEN1046 as monotherapy and in combi-nation Pembrolizumab therapy in subjects with relapsed/refractory metastatic non-small cell lung cancer after treatment with standard of care therapy with an immune checkpoint inhibitor
PRS-344/ S095012	Bispecific Ab	Advanced solid tumors		PD-L1 × 4-1BB	1/2	NCT05159388	Recruiting	A first in human phase 1-2 open-label, multicenter, dose escalation and expansion study of PRS-344/S095012 in patients with solid tumors
RO7122290	Bispecific Ab	Metastatic MSS CEACAM5-hiCRC	Cibisatamab (CD3 x CEAbispecific Ab) Obinutuzumab	4-1BB × FAP	1/2	NCT04826003	Recruiting	An open-label, multicenter, first-in-human, dose-escalation, phase 1 study of INBRX-105 and IN-BRX-105 in combination with Pembrolizumab in patients with locally advanced or metastatic solid tumors
INBRX-105	Bispecific Ab	Advanced solid tumors	Pembrolizumab	PD-L1 × 4-1BB	1	NCT03809624	Recruiting	An open-label, multicenter, first-in-human, dose-escalation, phase 1 study of INBRX-105 and IN-BRX-105 in combination with pembrolizumab in patients with locally advanced or metastatic solid tumors
EU101	Humanized IgG1 mAb	Advanced solid tumors Advanced CRC advanced NSCLC		4-1BB agonist	1/2	NCT04903873	Recruiting	An open-label, phase 1/2 study to evaluate safety, efficacy, and pharmacokinetics of EU 101, an agonistic Anti-CD137 (4-1BB) monoclonal antibody in patients with advanced solid tumors
ABL503	BispecificAb	Advanced solid tumors		PD-L1 × 4-1BB	1	NCT04762641	Recruiting	A phase 1 dose escalation and expansion study of ABL503, a bispecific antibody of 4-1BB and PD-L1, as a single agent in subjects with any progressive locally advanced (unresectable) or metastatic solid tumors [103]
GEN1042(Duo-Body®-CD40×4-1BB)	IgG1Bispecifi-cAb	Advanced solid tumors		CD40 × 4-1BB	1/2	NCT05491317	Not yet recruiting	A phase 1 dose escalation and expansion study of ABL 503, a bispecific antibody of 4-1BB and PD-L1, as a single agent in subjects with any progressive locally advanced (unresectable) or metastatic solid tumors [103]
Cinrebafuspal-fa(PRS-343)	BispecificAb	HER2+ gastric HER2+ GEJ carcinoma HER2- gastric or GEJ carcinoma	Ramucirumab (An-ti-VEGFR2 mAb) Paclitaxelor Tucatinib (HER2 inhibitor)	HER2 × 4-1BB	2	NCT05190445	Active	A phase 2, multi-center, open-label study of Cinrebafusp Alfa (PRS-343) in combination with Ra-mucirumab and Paclitaxelin patients with HER2-positive gastricor gastroesophageal junction (GEJ) adenocarcinoma and in combination with Tucatinib in patients with HER2 low gastric or gastro-esophageal junction (GEJ) adenocarcinoma
		HER2+ breast cancer, HER2+ gastric, HER2+ bladder can-cer,, HER2+solid tumors	Atezolizumab		1	NCT03650348	Active	A phase 1b, open-label, dose escalation study of PRS-343 in combination with Atezolizumab in pa-tients with HER2-positive advanced or metastatic solid tumors [104]
ATOR-1017	Human IgG4 mAb	Advanced solid tumors		4-1BB agonist	1	NCT04144842	Active	A first-in-human, multicenter, open-label, phase 1 study in patients with advanced solid malignancies to evaluate the safety of intravenously administered ATOR-1017 [105]
Utomilumab (PF-05082566)	Human IgG2 mAb	Relapsed-refractory Non-hodgkin lymphoma	Rituximab	4-1BB agonist	1	NCT01307267	Completed	A phase 1 study of PF-05082566 as a single agent in patients with advanced cancer, and in combina-tion with RITUXIMAB in patients with non-hodgkin's lymphoma (NHL) [106]
		Advanced solid tumors	Mogamulizumab (Anti-CCR4mAb)		1	NCT02444793	Completed	A phase 1B study of PF-05082566 in combination with MOGAMULIZUMAB (KW-0761) in patients with advanced solid tumors [107]
		Inoperable HPV + Oropha-ryngeal cancer	ISA101b (E6/E7 pep-tide vaccination)		2	NCT03258008	Completed	Phase II trial of Utomilumab and ISA101b vaccination in patients With HPV-16-positive incurable oropharyngeal cancer
		Recurrent ovarian cancer	CD8+ T cells Aldesleukin(recom-binanthumanIL-2) Cyclophosphamide		1	NCT03318900	Active	Phase I/Ib study of adoptive cellular therapy using autologous IL-21-primed CD8+ tumor anti-gen-specific T cells in combination with utomilumab (PF-05082566) in patients with platinum resis-tant ovarian cancer

TABLE 3 (CONT.)

Drug	Class	TypeofCancer	Additionalagents	Target(s)/ Mechanism	Phase	NCT	Status	Title
YH32367	Bispecific Ab	Advanced HER2 + solid tumors		HER2 x 4-1BB	1/2	NCT05523947	Recruiting	A phase 1/2, non-randomized, open-label, multicenter, first-in-human study to evaluate the safety, tolerability, pharmacokinetics and anti-tumor activity of YH32367 in patients With HER2-positive locally advanced or metastatic solid tumors
LVGN6051	Humanized mAb-AG	Soft tissue sarcoma	Anlotinib (RTKinhibitor)	4-1BB agonist	1/2	NCT05301764	Recruiting	An open label, phase Ib/II trial of LVGN6051 combined with Anlotinib in the treatment of locally advanced, metastatic or recurrent refractory soft tissue sarcoma
		Advanced solid Tumors	Pembrolizumab		1	NCT04130542	Recruiting	An open label, first in human (FIH), phase 1 trial of LVGN6051 as single agent and in combination-with Pembrolizumab (MK-3475-A31/KEYNOTE-A31) in advanced or metastatic malignancy
		Advanced solid tumors	Pembrolizumab			NCT04694781	Recruiting	An open label, phase 1 trial of LVGN6051 as single agent and in combination with embrolizumab in advanced or metastatic malignancy
		Metastatic esophageal	Nab-Paclitaxel			NCT05075993	Recruiting	study of LVGN3616 and LVGN6051 ± LVGN7409 in combination with Nab-Paclitaxel or Bevacizum-ab and Cyclophosphamide in metastatic solid tumors
		Gastric cancer, metastatic HNSCC, Metastatic HPV re-lated solid tumor, Metastatic ovariancarcinoma, Metastatic soft tissue Sarcoma, Meta-static uveal melanoma	Cyclophosphamide LVGN7409 (CD40 Agonist mAb) Bevacizumab					
CB307	Trispecifican-tibody	PSMA+ Advancedsolid tumors		4-1BB x PSMA x HAS agonist	1	NCT04839991	Recruiting	A phase 1 open-label, dose escalation and expansion trial to investigate the safety, pharmacokinetics and pharmacodynamics of CB307, a Trispecific Humabody® T-cell enhancer, in patients With PSMA+ advanced and/or metastatic solid tumours
		PSMA+ metastaticcastra-tion-resistantprostate cancer						
FS222	BispecificAb	Advanced solid tumors		PD-L1 x 4-1BB	1	NCT04740424	Recruiting	A phase 1, open-label, first-in-human study to evaluate the safety and anti-tumour activity of FS222, a CD137/PD-L1 bispecific antibody, in subjects with advanced malignancies
AGEN2373	CRD-IV binding, IgG1 mAb	Advanced solid tumors	Botensilimab (An-ti-PD-1 mAb)	4-1BB agonist (Conditionally active uponFc_{γ}R-cross-linkage)	1	NCT04121676	Recruiting	A phase 1 study of AGEN2373, an Anti-CD137 monoclonal antibody, as monotherapy and in com-bination With AGEN1181, an Fc-Engineered Anti-CTLA-4 monoclonal antibody, in patients with advanced cancer [108]
Urelumab	Human IgG4 mAb	Advanced solid tumors	Nivolumab	4-1BB agonist	1/2	NCT02253992	Terminated	A phase 1/2 dose escalation and cohort expansion study of the safety and tolerability of Urelum-ab administered in combination with Nivolumabin advanced/metastatic solid tumors and B-cell-non-hodgkins lymphoma [109]
		Non-hodgkin lymphoma , Advanced solid tumors			1	NCT01471210	Completed	A phase 1 study of the safety, tolerability, pharmacokinetics and immunoregulatory activity of Ure-lumab (BMS-663513) in subjects With advanced and/or metastatic solid tumors and relapsed/refrac-tory B-cell non-hodgkin's lymphoma (B-NHL)
		Non-hodgkin lymphoma , Metastatic CRC	Cetuximab (anti-EG-FR mAb)		1	NCT02110082	Completed	A phase 1b, open-label, multicenter study of Urelumab (BMS-663513) in combination with Cetux-imab in subjects with advanced/metastatic colorectal cancer or advanced/metastatic squamous cell carcinoma of the head and neck
		Advanced HNSCC, Advanced Solid Tumors	Nivolumab	Intratumoral Urelumab	1/2	NCT03792724	Not yet recruiting	Phase I-II study of intratumoral Urelumab combined with Nivolumab in patients with solid tumors
FS120	Bispecific Ab	Advanced solid tumors	Pembrolizumab	OX40 × 4-1BB	1	NCT04648202	Recruiting	A phase 1 open-label study to evaluate the safety and antitumor activity of FS120, an OX40/CD137 bispecific antibody, alone and in combination with Pembrolizumab, in subjects with advanced malignancies

beset with poor efficacy [76] and hepatotoxicity [77,78] respectively. However, the next generation of therapies leverages bispecific antibodies directed against 4-1BB in conjunction with either tumor associated antigens such as HER2 [79,80], FAP [81], or VEGF [82], or immune receptors such as PD-L1 (NCT03809624) (Table 3). This combinatorial approach has shown promise in preclinical studies and has been well-tolerated to date in a phase I clinical trial with early signs of clinical activity [80]. In addition to modulating metabolism through receptor ligation, combinatorial strategies to directly address the hostile environment and metabolic competition for nutrients in the TME may hold the key to further success. Recent studies suggesting synergistic effect of modulating tumor cell LDHA activity in conjunction with CTLA-4 blockade [69] or efficacy of reducing reactive oxygen species to enhance TIL mitochondrial fitness [83] raise the prospect of translating these approaches to enhance clinical outcomes.

Indeed, agents targeting diverse aspects of metabolic function have entered clinical trials in combination with immune checkpoint inhibitors. Among these approaches, efforts to target glycolysis, TCA Cycle, OXPHOS, amino acid metabolism and nucleic acid synthesis all aim to inhibit tumor cell metabolism and shift the balance of metabolic competition to favor immune cell function [84]. Among these putative targets, Indoleamine-2,3-dioxygenase (IDO) has been the focus of extensive clinical investigation. Inhibitors of this rate-limiting enzyme expressed and secreted by tumor cells and immune cells present in the TME, which metabolizes tryptophan giving off the toxic byproduct kynurenine, have been studied in several completed and ongoing clinical trials. Most prominent among these is epacadostat, a selective IDO1 inhibitor that demonstrated initial promise when administered to advanced melanoma patients in combination with pembrolizumab in the phase 1/2 ECHO-202/ KEYNOTE-037 study [85] but failed to meet progression free survival and overall survival endpoints in the phase 3 ECHO-301/KEYNOTE-252 trial [86] However, trials of both epocadostat and other IDO1 or IDO2/3 inhibitors remain ongoing in a variety of malignancies. Notably, BMS-986205, an IDO1 inhibitor currently under study in a Phase 1/2a clinical trial (NCT02658890) has demonstrated initial favorable safety and efficacy signal in metastatic bladder cancer [87]. Other agents demonstrating initial efficacy signal in early phase trials in combination with immune checkpoint inhibitors (ICB) include modulators of adenosine metabolism such as the CD73-receptor antagonist etrumadenant (NCT04381832 [88]), the adenosine A2a receptor antagonist ciforadenant (NCT02655822 [89,90]), and anti-CD73 antibody BMS986179 (NCT02754141 [91]). Additionally, therapeutics targeting arginine depletion to enhance T cell function include the arginase inhibitors INCB001158 (NCT02903914 [92]), and CB-1158 (NCT02903914 [93]). However, strategies to enhance arginine depletion to inhibit tumor cell metabolism with pegylated arginine deiminase (ADI-PEG 20) have also demonstrated initial success (NCT03254732, [94]).

Yet, while these approaches towards enhancing T cell function have focused on inhibiting tumor cell metabolism, direct metabolic modulation of T cell function remains incompletely explored, especially in the non-adoptive setting. Moreover, while these tumor cell-directed therapies may preserve T cell function in the TME, they do not enable more productive, durable metabolic remodeling during the steps of early activation. Thus, identifying and optimizing the appropriate cues to induce more potent T cell responses during activation at the immune synapse which may then be used in conjunction with these tumor-directed strategies remains crucial. However, to successfully implement these approaches, a more complete characterization of the metabolic dynamics of productive and dysfunctional immune responses will be critical.

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TRANSLATION INSIGHT: NOVEL APPROACHES TO METABOLIC PROFILING FOR FUTURE TARGET IDENTIFICATION & NEW COMBINATIONS

To develop rationally designed next generation therapeutics combining immune checkpoint blockade with approaches to enhance T cell metabolic fitness, it will be crucial to gain greater insight into the mechanisms by which currently approved and investigational checkpoint inhibitors mediate effective T cell reprogramming and identify novel metabolic and signaling targets with synergistic activity. These immunomonitoring studies will require a comprehensive characterization of the timing and localization of integrated programs of T cell metabolic transitions occurring both locally within the tumor microenvironment and systemically within the blood and tumor draining lymph node. Until recently, such translational efforts to profile rare antigen-specific cells in this fashion have been limited due to the large cell input and replicates required for standard metabolomics assays. However, advances in both transcriptional and proteomic single-cell analysis have now rendered such objectives within reach [95].

Leveraging highly multiplexed cytometric metabolic profiling techniques such as mass cytometry by time-of-flight (CyTOF) [32,33], Met-Flow [96], or SCENITH [97], serial immunomonitoring of the integrated functional program of rare, antigen specific cells in the blood will enable the identification of crucial transition states underlying treatment response and disease progression, while linking marker expression with metabolic phenotype [32,33,96] and single-cell bioenergetic profile [97]. These studies may correlate immune checkpoint co-expression with metabolic profile over the course of therapy, thereby permitting the design of more effective bispecific antibodies that may engage particular T cell subsets of interest at the appropriate timepoint. This approach would selectively expand metabolically robust populations for more effective antitumor activity. Furthermore, these populations of interest may be sorted for further exploratory analysis by single-cell transcriptomic metabolic profiling [98], thereby identifying novel targets to be combined with immune checkpoint blockade as next generation therapies.

Finally, it will be critical to understand the dysfunctional metabolic adaptations undertaken by T cells in the TME over the course of tumorigenesis along with the specific tumor cell and APC interactions that underlie these transitions towards exhaustion and promote immunosuppressive environments. Metabolic profiling by multiplexed ion beam imaging (MIBI) of FFPE specimens [33] enables investigators to query both archival specimens and those collected on prospective trials of both novel and FDA-approved immunotherapies, providing novel cross-sectional insights into the local and systemic metabolic processes underlying response and progression on treatment across multiple tissues in the neoadjuvant setting. Taken together, these strategies will inform the development of novel antibodies and adoptive approaches integrating proximal signals at the immunologic synapse with functional modulations of immune cell metabolism.

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COMBINATION THERAPY DEVELOPMENT: EMERGING I-O THERAPEUTIC MODALITIES & PREDICTIVE TECHNOLOGIES

SPOTLIGHT

INTERVIEW

A career in cancer vaccines: exploring the promise of B cell epitopes

Roisin McGuigan, Editor, *Immuno-Oncology Insights*, speaks to **Pravin TP Kaumaya,** Professor and Director of Vaccine Research at The Ohio State University

PRAVIN KAUMAYA is Professor of Medicine in the Department of Ob/Gyn at the OSU Wexner Medical Center and the James Comprehensive Cancer Center. Dr Kaumaya is internationally recognized as an expert in the fields of vaccine research with emphasis on peptide vaccines for cancer. His work over three decades in developing B cell epitope-based cancer vaccines is a paradigm shift in the immune-oncology landscape. Dr Kaumaya is an elected fellow of the American Association for the Advancement of Science (AAAS), and he was elected as the treasurer of the American Peptide Society since 2009. He has lectured worldwide and has published over 130 peer-reviewed articles in major scientific journals. He conducts research in the areas of immune-oncology, tumor immunology, peptide design and immune mechanisms supported largely by NIH, Pelotonia and more recently by Imugene, Ltd. He is an inventor on several issued and pending patents for peptide cancer vaccines and immune-therapeutic technologies. Vaccines developed for HER-1, HER-3, IGF-1R and VEGF at the university has been licensed to IMUGENE Ltd. Dr Kaumaya has conducted two first man/woman NCI funded and FDA approved Phase 1 Trial in Cancer Patients (Stage four) with solid tumors in several indications (Breast, Ovarian, GIST) at the OSU James Cancer Hospital has recently been completed successfully demonstrating the safety and efficacy of the vaccine. Dr Kaumaya's laboratory has recently developed a PD-1-Vaxx (programmed cell death) B cell peptide cancer vaccine that induces the body to produce polyclonal antibodies that block PD-1 signaling and produce an anticancer effect similar to the marketed immunotherapy drugs Keytruda® and Opdivo®.

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Cancer vaccines hold potential in the immuno-oncology space as an alternative to monoclonal antibodies or other approaches – but why have they not yet gained more traction in the immuno-oncology space? Professor and director of vaccine research at The Ohio State University, Pravin Kaumaya, once described B cell epitope cancer vaccines as a "new paradigm for combination immunotherapies". Here, he speaks about their untapped potential.

Q Can you tell us a bit about your own background and your current role?

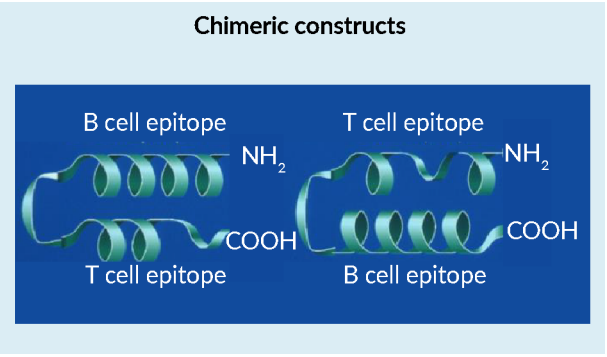
PK: I was born in Mauritius and completed my primary schooling there, and I then obtained my bachelor's degree in London, then spent four years at the University of Portsmouth to obtain my PhD in 1980. As soon as I graduated, I traveled to the US to do a post-doc at the University of Texas, Austin, School of Pharmacy. I then did a second post-doc at Northwestern University in Evanston, Illinois, where I became a research associate professor in 1987.

I was recruited at Ohio State University (OSU) in 1989, under a specialized program of protein engineering funded in perpetuity by the board of trustees. I was appointed assistant professor, became an associate professor with tenure in 1993, and a full professor in 1998. In 1995, I chaired the 14th American Peptide Symposium which attracted 1650 delegates from 33 different countries. I also served as Chairman, 1st International Symposium Peptide, Protein and Nucleic Acid Vaccine, held at Oxford University, England in 1998. I served as the treasurer of the American Peptide Society from 2009–2018. In 2006 I was elected Fellow of the American Association for the Advancement of Science (AAAS). I have 108 patents (issued and submitted) and I have served continuously on several National Institutes of Health Study Sections from 1997 to date, and am a permanent member of the National Cancer Institute (NCI) institutional training and education study section (T32, R25 and K12), July 1 2022–June 30 2026.

At Northwestern University, I started developing the idea of engineering secondary and tertiary B cell epitopes to be used as vaccines. I further developed chimeric B and T cell epitopes (Figure 1) incorporating 'promiscuous' T cell epitopes as a universal vaccine using human T-lymphotropic virus 1 (HTLV-I), the distant cousin of HIV, as the model antigen. In 1995, I began looking at cancer vaccines with the human epidermal growth factor receptor 2 (HER-2) oncogene

FIGURE 1

Chimeric B and T cell epitopes.



which is overexpressed in breast cancer (30%) as well as other cancers such gastrointestinal cancers, including colon cancer.

Q In your opinion, why have B cell epitope vaccines, and cancer vaccines in general, not yet gained more traction in the immuno-oncology space?

PK: There are a few cancer vaccines in existence, such as the range of available human papillomavirus (HPV) vaccines to treat cervical and anal cancers. There is also Provenge, a dendritic cell vaccine developed by Dendreon. Most cancer vaccines target the T cell component of the immune system, specifically cytotoxic T lymphocytes (CTLs). I would say 95 % of all research done to date has been on the activation of T cells, which is very important.

In the 1980s, crystallography showed the binding of peptides to major histocompatibility complex (MHC) class I and class II, and how they activate the T cell receptor. Most of those peptides are 8–10 amino acids in length. This discovery led to an explosion of CTL vaccines. Viral infection exposes multiple epitopes. We are unlikely to get a vaccine with just 8–10 amino acid peptides that bind MHC class I. We do not currently have a CTL vaccine approved by the FDA.

Our basic immunological knowledge has made great strides over the years, and we know that helper T cells are important, with helper T cell epitopes of between 10–30 amino acids that bind MHC class II. Vaccinologists and immunologists have started using those together. Even with this improvement, we do not yet have T cell vaccines.

We now know that checkpoint inhibitors are important. Scientists are using cytotoxic T cells together with the PD-L1 checkpoint inhibitor to put the brakes on T cell activation. Hopefully, that together with additional new discoveries might lead to a CTL vaccine.

In terms of B cell vaccines, there are several monoclonal antibodies (mAbs) that have been FDA-approved, such as Herceptin for HER-2 and Cetuximab for HER-1/EGFR. Cancer immunotherapy has recently been energized by the discovery of checkpoint inhibitor proteins. James P. Allison and Tasuku Honjo won the 2018 Nobel Prize in Physiology or Medicine for discovering immune checkpoints programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), demonstrated that they acted as a 'brake' role in immune function. More recently, we have seen mAbs to PD-1, PD-L1, and CTLA-4, which have all been FDA-approved.

If you can treat with mAbs, and they can prolong life or be effective for at least 20–30% of the adult population with cancer, why not have the immune system make those antibodies? That has been my driving force over the last 20–25 years. I started developing contraceptive vaccines first for LDH-C4, then for HTLV. HTLV is a viral oncogene – some cancers that are of viral origin have been caught early on with Provenge and HPV vaccines. The oncogenes that are over-expressed in tumors are considered self-protein. It is more difficult to develop a vaccine to a self-protein.

We have mapped B cell epitopes on protein antigens. The crystal structure (Figure 2) of the complex of lysozyme with its mAb was published in 1987. The large conformational interaction over 900 amino acids showed that short linear synthetic peptides of 10 amino acids would not mimic the surface-oriented secondary or tertiary structure. That is one of the major barriers to developing efficacious antibodies to B cell epitopes.

I embarked on engineering epitopes on protein antigens by mimicking the pertinent secondary attributes of the epitope by using our knowledge of protein folding and structure. In the 1990s, we published our findings that antibodies raised to those various secondary structures elicited high-affinity antibodies to the native protein and thus provide a potential strategy for developing an effective peptide vaccine. That was the first step to solving one of the problems with B cell vaccines, by designing peptides that are conformational in nature.

The second problem was that most vaccinologists used B cell epitopes and coupled them to a carrier protein, for example bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). When you vaccinate with these small peptides coupled to a carrier protein, you have no control over the immunogenic epitope, because the resulting vaccine requires processing by the immune system in a way that cannot be predicted, thereby resulting in a less effective immunogen [1].

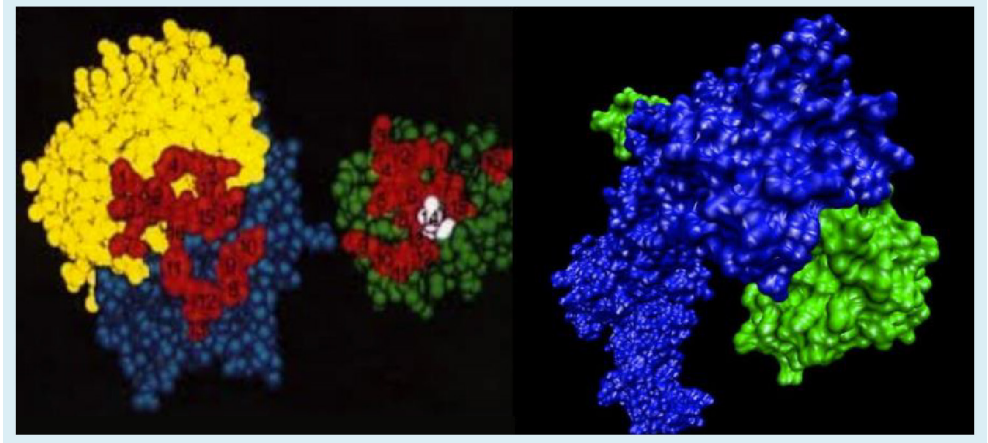
In the 1990s, the idea of 'promiscuous' T cell epitopes was published by a group in Switzerland and a group in Australia. They identified a number of 'promiscuous' T cell epitopes from measles virus or tetanus toxoid, that bind MHC molecules in a universal fashion.

I proposed the idea of using those T cell epitopes in combination with our B cell epitopes. At that time, we did not know whether they were going to be processed or not. Afterwards, we found out that those around 50 amino acid chimeric constructs are not processed *in vivo*, and therefore the immune response generally generates antibodies of high affinity to the native protein.

This was the start of my work at Northwestern and then at OSU in 1995, I started looking at HTLV as our model antigen to develop a vaccine for HTLV-I. I also started looking at cancer vaccines, using the HER-2 oncogene, which is overexpressed in breast cancer. We

FIGURE 2

Lysozyme (green) and mAb to lysozyme (blue/yellow) are shown to have a conformational interaction.



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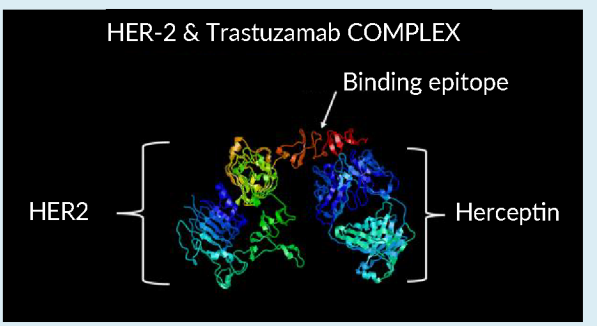
developed epitopes for HER-2 and translated those into a first-generation HER-2 vaccine. Then in 2000, the structure of trastuzumab (Herceptin®) in complex with HER-2 was published (Figure 3). Similarly, the complex of HER-2 with pertuzumab (Perjeta®) was published.

FIGURE 3

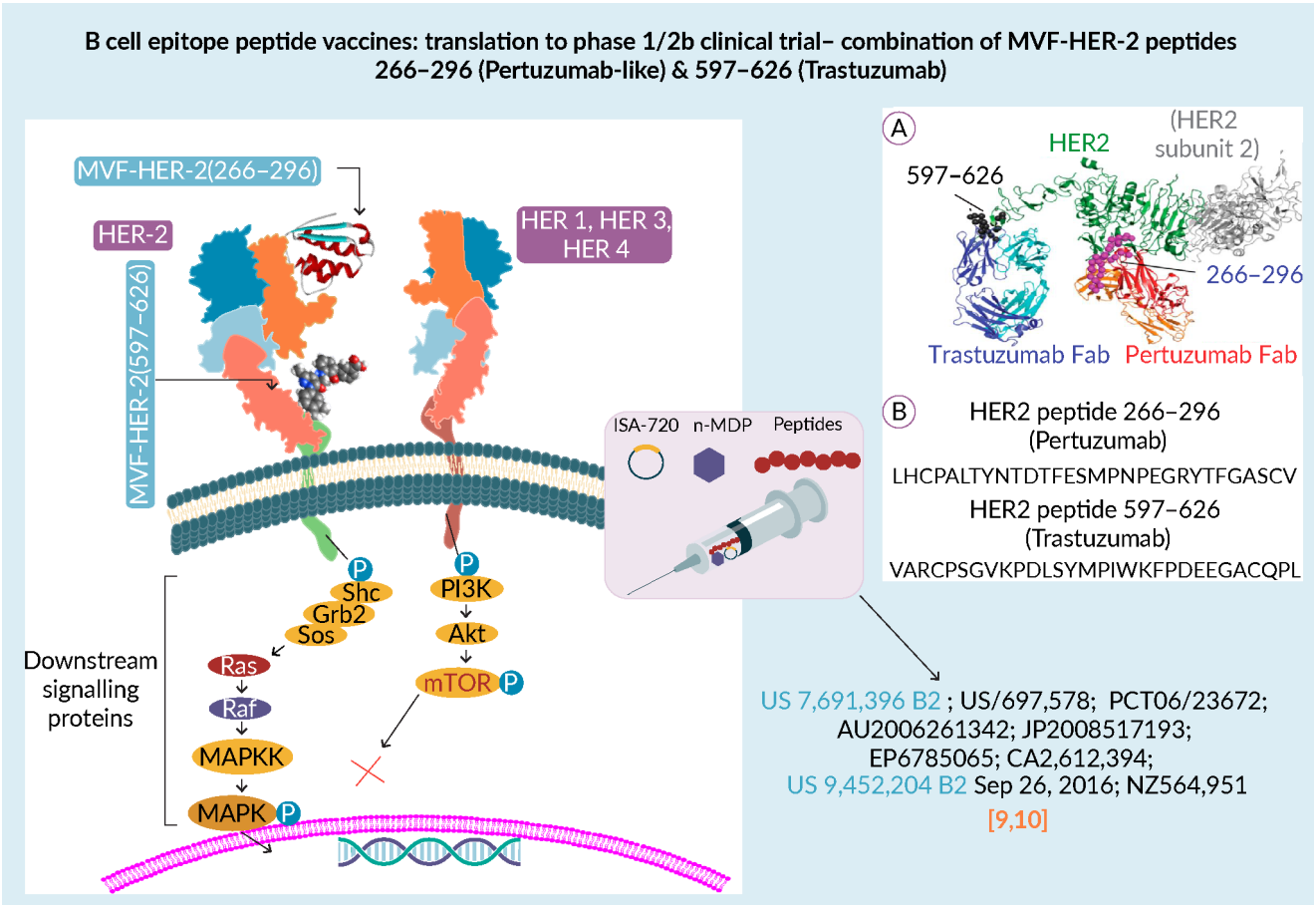
HER-2 and Herceptin binding molecular structure.



We developed several epitopes to mimic the binding region and discovered two novel epitopes that mimic Herceptin and Perjeta. We completed a combination immunotherapy vaccine of two epitopes MVF-HER-2 (266–296) (Perjeta-like) and MVF-HER-2 (597–626) (Herceptin-like) in animal models, then we translated the combined vaccines (B-Vaxx) to a Phase 1 clinical trial conducted at the James Cancer Hospital at OSU. We published the completion of a dose escalation Phase 1 trial in 2019. The combination vaccine was emulsified with ISA720 vehicle (water-oil-emulsion, Seppic INC, Fr) and

FIGURE 4

Combination HER-2 vaccine (B-Vaxx) in Phase 1 clinical trial.



[9,10]

amuramyl-dipeptide (nor-MDP) adjuvant to deliver our vaccine (Figure 4) intramuscularly [1].

Presently, we are conducting a Phase 1b trial, and have attained FDA approval to target HER-2-positive cancer patients. It has taken us 15 years to get here, but we are happy with where we are going with the HER-2 vaccine. The landscape for breast cancer has evolved from FDA-approved Herceptin, Perjeta, Kadcyla and now Enhertu and our HER-2 B cell vaccine should be competitive in the treatment of breast and other cancers overexpressing the HER-2 gene. We have also proposed how the vaccine works (Figure 5).

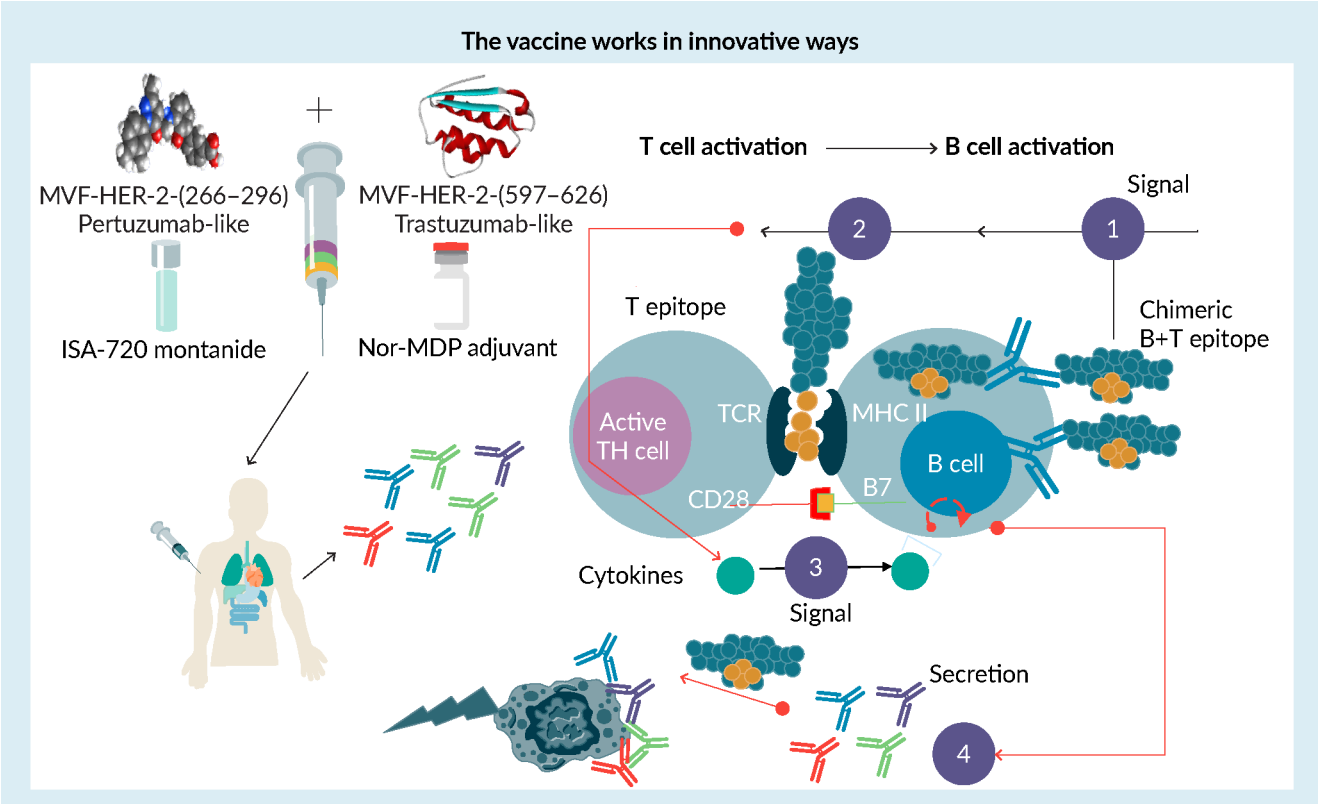
Q What are the key advantages that vaccine-based approaches offer, versus mAbs or other approaches?

PK: In general, mAbs are effective in about 20–30% of adults. The mAbs that have been used to treat breast cancer are Herceptin and Perjeta. As soon as patients were treated with these, they become refractive to treatment and stop responding. These antibodies are US $ 120000 per treatment, and they are not a cure.

Another set of FDA-approved mAbs such nivolumab (Opdivo) or pembrolizumab (Keytruda®) are monoclonal targeting PD-1 a protein on the surface of T and B cells that plays an important role in regulating the immune system's response. Ipililumab (Yervoy®) is a mAb targeting another checkpoint CTLA-4.There are also mAbs (e.g. Avelumab, Atezolizumab)

FIGURE 5

Proposed mechanism of how the vaccine works.



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to the PD-L1 located on tumor cells that play an important role in various malignancies through the PD-1/PD-L1 axis.

Nivolumab and pembrolizumab are also given together with a chemotherapeutic agent. However, many patients do not want chemotherapy. mAbs do have a place in our armamentarium of treatment for cancer, but they are also toxic. Our approach of using the immune system to generate vaccines is a paradigm shift. We are still having difficulty getting it generally accepted.

There are many advantages to peptide vaccines. They are safe, non-toxic, highly stable, can break tolerance, can elicit B and T cell memory response, and have no oncogenic material included. A multi-epitope approach could lead to broad antigen recognition and universal coverage.

Humanized mAbs have many disadvantages, such as full penetration, ineffective tumor targeting, a half-life of 12 days, and the requirement of weekly infusions of large quantities of humanized antibodies. Treatment is expensive and cardiotoxicity and gastrointestinal perforation can occur. The most important thing is that there is no immunological memory. This is a treatment, not a cure. A peptide vaccine could be used in both a prophylactic fashion, as well as in a therapeutic mode. There are many advantages to peptide vaccines over mAbs.

Q You mentioned you returned to cancer research in 1995. What inspired your work and made you pursue this particular area?

PK: My dad was diagnosed with leukemia in 1981. He was treated with vincristine, which at the time was an experimental drug. They have since worked out the right doses for vincristine, and it is now used often in leukemia. Within a month of being treated with this experimental drug, he passed away.

There were toxic events happening when my dad was treated with that drug. We know now that some similar drugs, such as axitinib, are highly toxic. You can extend life by a couple of months, but with extraordinary toxicity. This galvanized my passion for developing peptide vaccines because I knew these would be safe and non-toxic. Biological materials, including peptides, are well known to be very safe.

I also spent a lot of time studying how to make B cell vaccines immunogenic or antigenic, to provide high efficacy. In so doing, we developed our HER-2 vaccine. Signal transduction pathways involving the dimerization of HER-2 drive cancer metastasis. If this can be blocked, like with Herceptin, cetuximab, or pertuzumab, then you block cancer.

When patients are treated with mAbs, they develop resistance and stop responding. Over a span of 10 years, we hypothesized that one of the reasons for the resistance mechanism in those targeted therapies was the upregulation of the other oncogenes, such as HER-1, HER-3, HER-4, IGF-1R and VEGF. [1]. We developed a plethora of vaccines for all of those molecules, to use in combination. In 2010, with our combination of HER-2 with VEGF in animal models, we showed that we could increase the efficacy of those vaccines when used in combination. Cancer immunologists started to see the potential of using the immune system to try and conquer cancer.

The crystal structure of the checkpoint inhibitors enabled us to design a vaccine for PD-1 and PD-L1. We looked at the entire structure of PD-1 and PD-L1 and developed all those various antibodies. We studied them in multiple animal models. The epitope 92–110, which we now call PD1-Vaxx, is a chimeric construct with a measles virus promiscuous epitope linked to the B cell epitope. We published this in 2020 in *OncoImmunology* [2]; showing in syngeneic models that this particular epitope was quite effective in preventing tumors. We used a syngeneic model of colon cancer where the mice were treated with CT26, a carcinoma cell line, and we showed that we could duplicate the efficacy together with the mouse mAb to PD-1. Then, when we used those in combination with our HER-2 vaccine, we obliterated cancer growth in that CT26/ HER-2 model.

We have developed PD1-Vaxx (Figure 6). Imugene contacted me to enquire about our PD-1 vaccine, and within a few months we licensed not only the PD1-Vaxx but my entire portfolio to Imugene. We then developed protocols to conduct a Phase 1 trial in humans to apply for an IND and get FDA approvals. A study in dogs was published this year, where we defined how to deliver those peptides. Imugene contracted Charles River to conduct a non-human primate study in Ashland, Ohio, and raised money to do a clinical trial with three cohorts both in the US and in Australia [3].

The Phase 1 trial in advanced/metastatic non-small cell lung cancer (NSCLC) completed a dose escalation to determine the safety and Optimal Biological Dose (OBD) monotherapy of the vaccine. The results show the vaccine was safe and one patient had no observable recurrence for 20 months [4–7].

Now, Imugene and Roche have formed a collaboration in which Roche are going to provide their mAb to PD-L1, atezolizumab, and Imugene will conduct a combined treatment with PD1-Vaxx and atezolizumab. This Phase 1b trial in advanced/metastatic NSCLC dose escalation: NSCLC checkpoint inhibitor naïve or have progressed on/after checkpoint inhibitors will start in the next few months, and we are looking forward to what this study will teach us [7].

We have developed a PD-L1 B cell epitope vaccine (Figure 7). One of the epitopes, 130–147 (PDL1-Vaxx), has turned out to be one of the most effective epitope in several different syngeneic (BALB/c; C57BL/6)J models and carcinoma cell lines (CT26WT, CT26/

FIGURE 6

Molecular structure of human PD1-Vaxx.

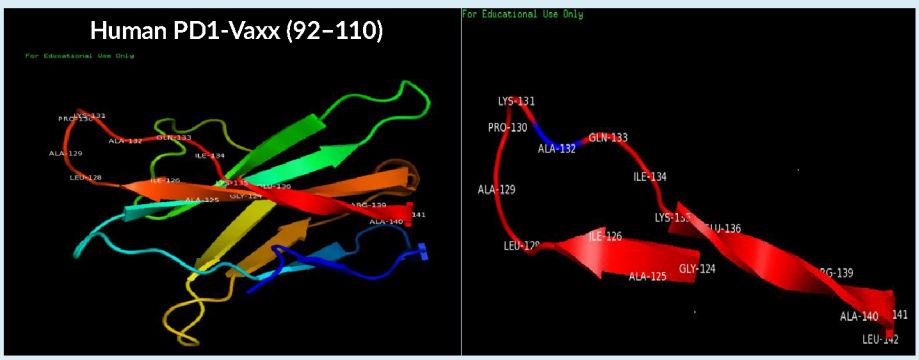

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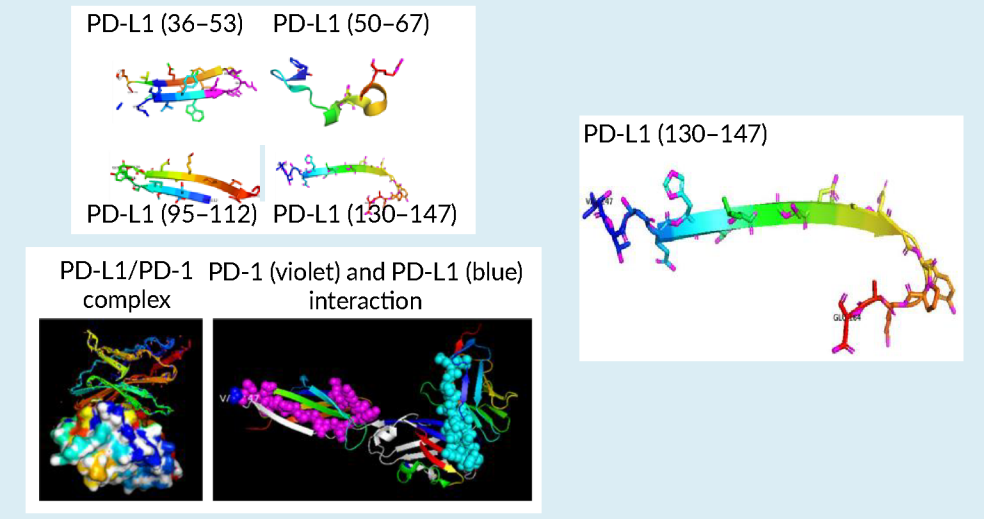
FIGURE 7

PD-L1 epitopes.



HER-2, 4T1, D2F2,D2F2/E2,MC38, MC38/HER-2 and B16.F10 in colon, breast cancers, triple-negative breast cancer, and melanoma). This work was recently published in *OncoImmunology* [8].

We have already completed PD-1 and PD-L1 combination immunotherapy in animal models which showed synergistic inhibition in several different cancer models. We have shown the efficacy of using both vaccines together, and in a triple version together with our HER-2 vaccine. These ongoing studies will expand to Phase 1 clinical trial in the near future.

Q What have been the most significant milestones of your work to date, and what's next?

PK: We have established the template of how to design B cell vaccines by using chimeric constructs, delivering them, and studying them in multiple syngeneic models. Next, we can translate this to human clinical trials.

However, one of the main important things going forward is regarding our CTLA-4 peptide vaccine (CTLA-4-Vaxx), which is similar to ipilimumab. We have completed a CTLA-4 and PD-L1 combination immunotherapy in a syngeneic mouse model, which is not yet published. This combination is going to be important.

We have also designed peptide B cell epitope vaccines to all the various checkpoint inhibitors. We have vaccines for PD-1, PD-L1, LAG-3, and TIGIT. Now, we are using all those in combination to explore how we are going to design combination immunotherapy. I think the PD-1 and PD-L1, or the PD-1 and CTLA-4 may gain FDA approval. I believe this is the future of cancer vaccines using peptides.

Q Could you tell us a bit more about the CTLA-4, LAG-3 and TIGIT vaccines that you are developing?

PK: Some of our work has not yet been published, but we have a large cancer vaccine project pipeline (Figure 8).

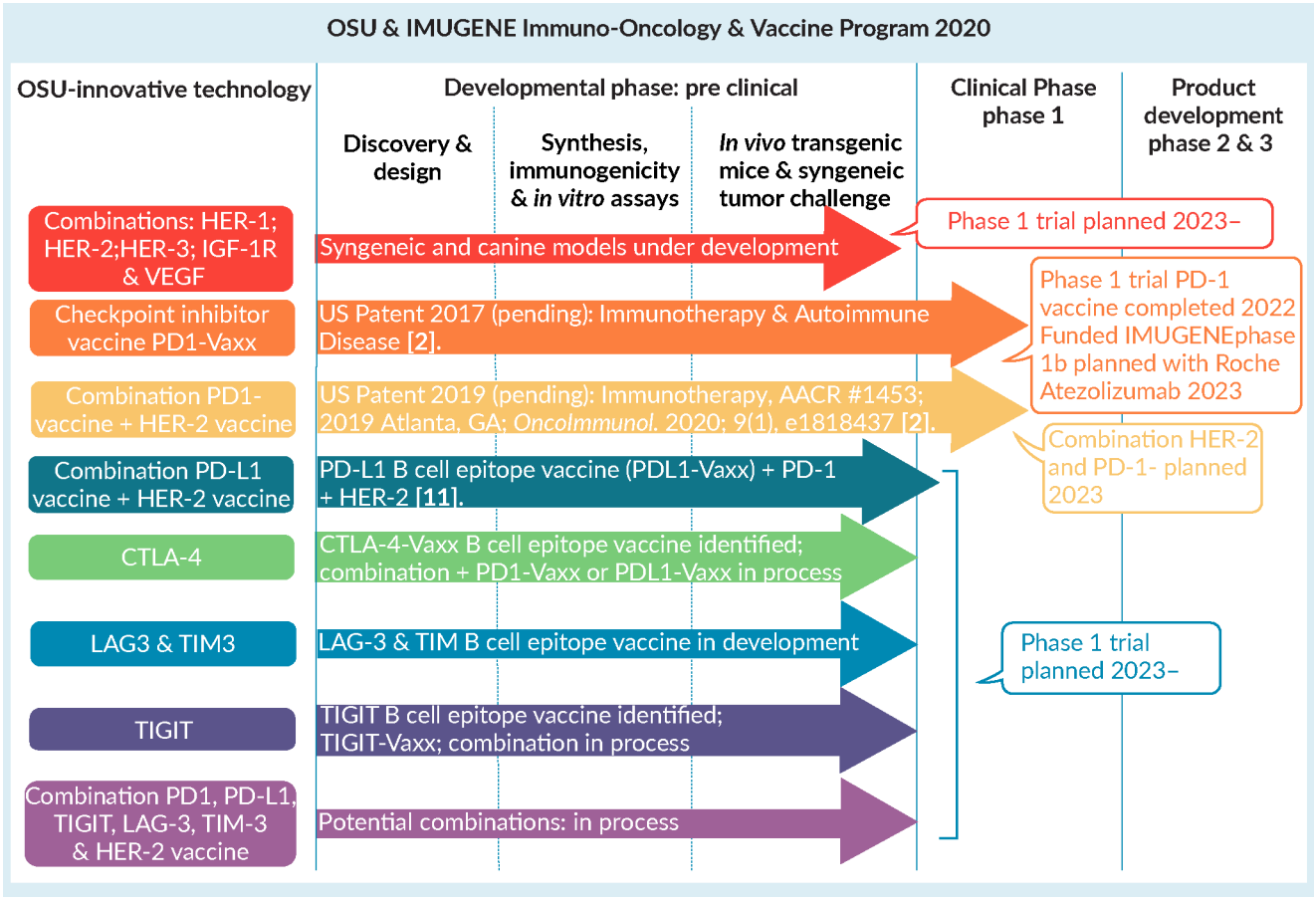
We looked at immunogenicity and antigenicity of the CTLA-4 peptides, first in rabbits, then in mice, and then at all four epitopes. Now, we are looking at several syngeneic models. They are highly immunogenic and recognize a native protein. However, we have also identified CTLA-4(130) as a good epitope to be used for vaccination.

Our second model, 4T1 breast cancer, showed that the results for the mAb were not very good. Our vaccine was much better. We saw similar results in the D2F2 model, a mammary tumor model. Based on that, we know now that the CTLA-4 epitope is good.

Now, we are looking at peptide mimics instead of vaccination. We are currently conducting a duplicate experiment to see if we can use only a peptide to prevent mammary tumors. We have identified 2 LAG-3 peptides and completed the study in a tumor model. We have an epitope that is acceptable. We also have 8 TIGIT peptides, and we are conducting studies in rabbits and C57BL/6 mice.

FIGURE 8

OSU and Imungene cancer vaccine project pipeline 2019–2022.



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Q You have previously described B cell epitope peptide cancer vaccines as "a new paradigm for combination immunotherapies". What unmet needs can novel combinations incorporating cancer vaccines potentially address?

PK: There is a multitude of checkpoint inhibitors and mAbs. All the big companies are now looking at combining those mAbs together. The problem there is that each mAbs has a toxicity profile, and when you add them together the toxicity is going to be elevated.

We can treat those and play with the amount of mAb or combine them with radiotherapy or chemotherapy. The goal now is to reduce toxicity. We know which checkpoint inhibitor can be used, and how you can reduce toxicity by decreasing the number of antibodies infused in the patient.

Biomarkers are going to be an important factor in finding which cancer to target, and in doing so, developing methods to reduce toxicity. Yervoy and Atezolizumab are now being used in combination.

But although these are good ways of treating cancer, we will prove that our vaccine platform is also a great method to treat cancer patients, with very little toxicity. We want to figure out how to deliver the peptides in combination. It is well known that combination immunotherapies with mAbs exhibit toxicity, and both Roche and Imugene were interested in finding out how the PD-1 vaccine when combined with Roche's PD-L1 mAb (atezolizumab) could have less toxicity. This could move the field forward. If we can have patients being treated by the proposed combination that could expand our platform of vaccines to checkpoint inhibitors together with targeted mAb therapy.

In the meantime, we still nee to figure out vaccines to CTLA-4, to TIGIT, to LAG-3, and how to combine those together. Once the scientists and the doctors that treat cancer patients find out that those vaccines are a plausible approach, then we will get recognition for the work that we have done on how to move our science forward.

Q What will be your own chief goals and priorities in the coming years?

PK: The chief goal is to see which combinations will be effective by looking at multiple syngeneic models.

We are in academia, not in big pharma where they have much more funding. Imugene is funding all the research for those combinations. From starting the combination to figuring out the efficacy, it takes between 6–9 months. Thus, Imugene must take the first step by developing those vaccines in GMP conditions and be ready off-the-shelf to go into clinical trials within 18 months.

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IMMUNO-ONCOLOGY INSIGHTS

COMBINATION THERAPY DEVELOPMENT: EMERGING I-O THERAPEUTIC MODALITIES & PREDICTIVE TECHNOLOGIES

SPOTLIGHT

VIEWPOINT

Customizing T cell biology: armoring approaches to optimize allogeneic CAR-T therapies

Jakob Dupont

"Within the past couple of decades, significant progress has been made in the landscape of immunotherapy, altering how certain cancers are treated."

VIEWPOINT

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Harnessing the power of a patient's immune system to target and eliminate disease-specific cells is a proven approach in the treatment of diseases that previously relied on highly toxic systemic therapies. Over the last decade, autologous chimeric antigen receptor (CAR)-T cell therapies have demonstrated groundbreaking clinical results in hematological cancers, yet they also present significant challenges. Now, leveraging new cell types and alternative approaches may bring further innovation to oncology and other therapeutic areas, potentially overcoming barriers associated with patient derived, also known as autologous, CAR-T cell products. Here, we discuss the cutting-edge science in T-cell platform approaches that have the potential to broaden applications in hematological and solid tumor settings and beyond.

Cell therapies fall into two main categories – autologous and allogeneic. Autologous, or patient-derived, cell therapies have shown remarkable efficacy, particularly in blood cancers, but have often been limited by complex manufacturing and challenging logistics [1]. In contrast, allogeneic, or donor-derived, cell therapies have the potential to be produced in large quantities from a single donor and stored in advance of patient need ready for delivery within days as an off-the-shelf treatment [2,3]. Despite these advantages, allogeneic cell therapies come with their own set of challenges that includes risk of host-versus-graft or graft versus-host disease which can be life-threatening. As such, manufacturers are utilizing innovative approaches to help minimize the potential for unwanted immunogenicity both from and to allogeneic T cells [4].

Ongoing research into developing next-generation allogeneic CAR-T cells is focused on trying to improve expansion, persistence, as well as the number of potential targets that may help pave the way for further therapeutic advances for patients [5]. At Atara Biotherapeutics, we are currently using allogeneic Epstein-Barr virus (EBV) T cells as a platform to develop investigational therapies for cancer and autoimmune diseases. Our EBV T cells are manufactured to specifically target EBV infected cells without the need for gene editing of the T-cell receptor. Leaving the endogenous T-cell receptor intact allows our EBV T cells to retain their natural T-cell functions which may help reduce the likelihood of unwanted immunogenicity. Natural attributes of EBV T cells offer other potential benefits, including a low likelihood of harming normal tissues, which may enhance tolerability and the ability to persist in the body long enough to fight the disease, offering the potential for durable response. As such, we believe allogeneic EBV T cells have the potential to transform the treatment of EBV-associated diseases including lymphoproliferative diseases and multiple sclerosis.

We are also investigating allogeneic EBV T cells to target diseases not associated with EBV by expressing modified CARs on the surface of the cells. These cells are designed to attack specific antigens, such as those associated with certain blood cancers and solid tumors while potentially retaining the natural abilities of T cells because the endogenous T cell receptor remains intact. Building on nature's αβ T cells, which are responsible for recognizing foreign or non-self antigens, we are applying new technologies aimed to enhance certain capabilities [5]. In particular, we're investigating novel co-stimulatory activity domains including Mut06 and a 1928ζ mutant (1XX) in investigational, off-the-shelf T cell candidates [6,7]. These cell products are designed to exhibit more physiologic levels of T cell signaling to help avoid activation-induced cell death and exhaustion, potentially improving persistence and tolerability.

Many cell therapy developers are also investigating ways to augment CAR-T cells to prevent cell death and improve allogeneic cell persistence, especially in tumor microenvironments. Due to inclusion of these advanced technologies, these cells have been dubbed 'armored CAR-Ts' based on their potential to help resist the immunosuppressive tumor microenvironment, typically mediated through PD-1 ligand, which may

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help improve efficacy in solid tumors. Current approaches include combining CAR-Ts with programmed cell death protein 1 (PD-1) inhibitors or generating new CAR-T cells with the ability to resist PD-1 inhibition.

We are investigating the expression of a PD-1 dominant negative receptor (PD-1 DNR) in allogeneic CAR-T cells with the aim to maintain endogenous PD-1 expression while still providing intrinsic checkpoint inhibition. Use of PD-1 DNR may help improve allogeneic CAR-T cell persistence and expansion by avoiding endogenous PD-1 ablation that in preclinical models was associated with premature CAR-T cell exhaustion [8]. Expression of PD-1 DNR, a PD-1 decoy receptor, may help protect against the immunosuppressive effects of PD-L1 within the tumor microenvironment as well as T cell exhaustion driven by the ablation of endogenous PD-1 both potentially leading to enhanced CAR-T proliferation, cytotoxicity and cytokine secretion.

Engineered T cells incorporating PD-1 DNR have several potential advantages over other approaches. Preclinical data has shown that PD-1 DNR provides a sustainable blockade of PD-1 signal that is tumor-limited [9]. The combination of novel co-stimulatory domains and PD-1 DNR may be particularly useful when targeting solid malignancies, as EBV T cells have unique immunological features including the ability to traffic to and embed within solid tumors to target cancer cells [10].

Within the past couple of decades, significant progress has been made in the landscape of immunotherapy, altering how certain cancers are treated. As allogeneic, off-the-shelf CAR-T cell therapies start to enter the clinic for hematological and solid tumor indications, we will better understand the potential benefit allogeneic cell-based immunotherapies may offer those diagnosed with cancer. By leveraging next-generation CAR-T cell designs or armored CAR-Ts, or a combination of both, we and other researchers seek to harness the immune system to make deeper inroads into our fight against cancers, autoimmune disorders, and other diseases.

BIOGRAPHY

JAKOB DUPONT is a renowned expert in the fields of cell therapy and oncology, with long-standing and deep experience in developing therapies and programs dedicated to addressing high unmet medical needs. He serves as Global Head of Research & Development including Medical and Regulatory Affairs. Dr Dupont has been involved in tumor immunology research and clinical investigations for more than 25 years, ranging from cell therapy to tumor vaccine therapy and immune checkpoints. Dr Dupont has received numerous grants and awards, and has co-authored 47 peer-reviewed publications, has 30 patents, and has served as a faculty member and laboratory researcher at Memorial Sloan Kettering Cancer Center and adjunct clinical faculty in medical oncology at Stanford University. Dr Dupont received his MD from the Joan & Sanford I. Weill Medical College of Cornell University, MA in philosophy from New York University, and undergraduate degree in philosophy from Vassar College.

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AUTHORSHIP & CONFLICT OF INTEREST

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