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Review

Novel Combination Strategies to Enhance Immune Checkpoint Inhibition in Cancer Immunotherapy: A Narrative Review

Jonathan A. Hermel,[1] Cassi M. Bruni,[2] Darren S. Sigal.[3]

Abstract

Programmed cell death protein-1 (PD-1) is an immune checkpoint receptor that induces and maintains tolerance of T cells, invariant natural killer T (iNKT) cells, and natural killer (NK) cells, among other lymphocytes. Immune checkpoint inhibition by PD-1 blockade restores the lymphocytic immunostimulatory phenotype and has been successful in the treatment of various malignancies. However, while immune checkpoint blockade has been shown to provide robust antitumor treatment outcomes, its overall response rate remains low in a significant portion of cancer patients. An essential unmet need in cancer therapy is the development of novel pharmacologic strategies designed to lower rates of resistance associated with immune checkpoint blockade. Therefore, efforts that seek to enhance the efficacy of PD-1 inhibition possess profound immunotherapeutic potential. Here, three promising combination strategies that harness the antitumor effects of immune checkpoint inhibitors (ICIs) together with non-ICI antitumor therapeutic agents are reviewed. These agents include (1) ABX196, a potent inducer of iNKT cells, (2) chimeric antigen receptor (CAR)-T cell therapy, and (3) NK cell therapy. A comprehensive literature search was conducted using the PubMed and ClinicalTrials.gov databases for scientific articles and active trials, respectively, pertaining to immune checkpoint inhibition, iNKT cells, CAR-T cells, and NK cell immunotherapy. Preliminary clinical and preclinical data suggest that these combination treatment regimens greatly suppress tumor growth and may serve as innovative methods to enhance and optimize anticancer immunotherapy.

Key Words: Immunotherapy; Immune checkpoint molecules; Invariant Natural Killer T Cell; Chimeric Antigen Receptor; Natural Killer T-Cells (Source: MeSH-NLM).

Introduction

Immunotherapy represents the newest pillar in anticancer therapy. The first fifty years of anticancer therapy consisted solely of cytotoxic chemotherapy, but this began to change in the 1990s with the advent of monoclonal antibodies and again in the early 2000s with the development of small molecule tyrosine kinase inhibitors. Each successive new approach has increased therapeutic efficacy and resulted in improved patient outcomes. United States Food and Drug Administration (FDA)-approved immunotherapies, in the form of immune checkpoint inhibitors (ICIs) to the cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1) checkpoint receptors, may represent the most profound advances in anticancer therapy in modern history. Cancers notoriously difficult to manage, including non-small cell lung cancer (NSCLC) and melanoma, have responded well to ICI immunotherapy, whether administered solely as monotherapy or in combination with chemotherapy in the neoadjuvant, adjuvant, and advanced late-stage settings.[1,2,3] However, despite their proven antitumor treatment outcomes, ICIs have modest overall response rates, not only between varying cancer types, but also among patients who share the same malignancy.[4] The identification of novel tumor biomarkers is an ongoing area of research aimed at predicting resistance to ICI immunotherapy and developing targeted approaches that seek to overcome this resistance.[5] It remains that a key unmet need in cancer therapy is improving the consistency and functional efficacy of ICIs in a majority of cancer patients. A variety of novel immune therapies and targeting approaches are in clinical development that may mark another important step forward towards this goal. This review will examine the preclinical and clinical data of ABX196, a non-checkpoint inducer of invariant natural killer T (iNKT) cells; chimeric antigen receptor (CAR)-T cell therapy; and natural killer (NK) cell therapy, each in combination with ICIs for improved anticancer immunotherapy.

Methods

An extensive scientific literature search was performed using the PubMed database for peer-reviewed articles published in academic journals related to immune checkpoint inhibition, iNKT cells, CAR-T cells, and NK cells. The literature search was conducted between the months of May and July 2020. Search parameters included combinations of the keywords "ABX196," "α-GalCer," "invariant natural killer T cell," "iNKT cell," "chimeric antigen receptor T cell," "CAR-T cell," "natural killer cell," or "NK cell," with the terms "PD-1," "immune checkpoint inhibitor," "immune checkpoint inhibition," or "immunotherapy." Only studies published in English with full available text were screened for use. Because of the intended comprehensive nature of this review, a small portion of the manuscript pertaining to the background and history of α-GalCer mechanistic studies includes original articles published from 1994 to 2018. For the remainder of the paper, only articles published within the past 10 years were considered for use. Article abstracts were closely reviewed for applicability to the research question and those without clear relevance were removed from consideration. For information regarding active clinical trials, a detailed search of ClinicalTrials.gov was conducted. Initial search parameters included the keyword phrases, "ABX196," "CAR-T cell," "NK cell" or "natural killer cell." Trials located in all countries were included for

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review; however, trials that were not in the recruiting or pre-recruiting stages were filtered out. Listed trial titles and descriptions were then manually screened for protocols that included additional ICI immunotherapies. Trials that did not meet the aforementioned criteria were excluded.

Results and Discussion

ABX196 in Combination with PD-1 Blockade

Background and rationale: iNKT cell anergy is associated with PD-1 upregulation

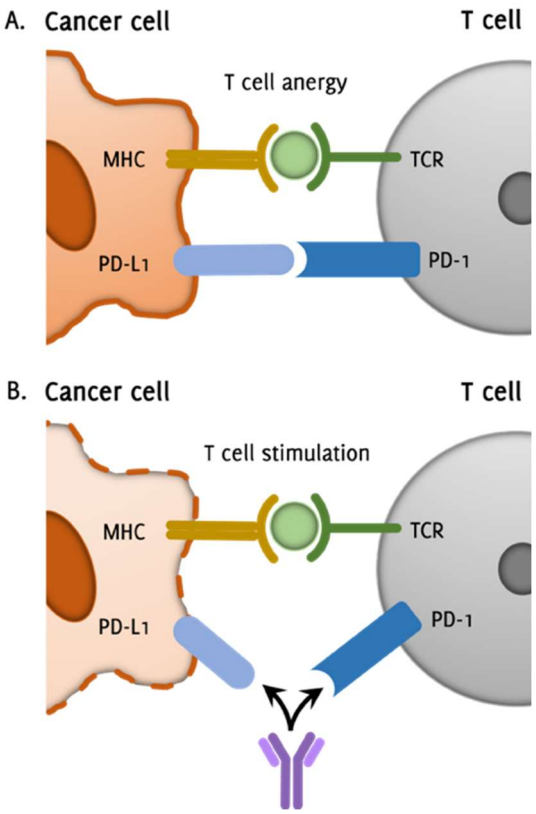
ABX196 is a synthetic glycolipid analogue of α-galactosylceramide (α-GalCer), a strong agonist of invariant natural killer T (iNKT) cells. In contrast to conventional T and NK lymphocytes, iNKT cells induce both innate and adaptive antitumor immune responses.[6] iNKT cells express an invariant T cell receptor (TCR) composed of Vα14-Jα18/Vβ8.2 gene chain rearrangements in mice and Vα24-Jα18/Vβ11 gene chain rearrangements in humans.[7] These invariant TCRs recognize endogenous and exogenous lipid moieties bound to and presented on non-classical, major histocompatibility complex (MHC) class I-like CD1d molecules.[8] CD1d is a membrane-bound cell surface glycoprotein expressed primarily by B lymphocytes, macrophages, and dendritic cells (DCs).[9] When lipid moieties are presented within the hydrophobic binding-groove of CD1d, they interact with the iNKT TCR; this association stimulates iNKT cells, which in turn modulate the immune system.[10] Specifically, *in vivo* administration of α-GalCer in mice triggers iNKT cells to i) rapidly proliferate, ii) release a variety of cytokines, including IFN-γ and IL-4, iii) cross-prime dendritic cells to release IL-12, and iv) slow tumor growth and prevent metastasis.[11,12] Although α-GalCer is a powerful iNKT cell-stimulating antigen, its antitumor efficacy is restricted by the fact that α-GalCer-stimulated iNKT cells release cytokines with opposing immune system actions, specifically IFN-γ, which initiates an immunostimulatory T helper 1 (TH1) response, and IL-4, which initiates an immunoregulatory T helper 2 (TH2) response.[13] The favorable effect of iNKT cells in antitumor immunity is due, in large part, to IFN-γ production.[14] ABX196 consists of an acetamide group linked to the galactosyl C6 of α-GalCer, and this structural modification has been shown to enhance the secretion of TH1 cytokines.[15] As compared to α-GalCer, ABX196 produces high levels of systemic IFN-γ but significantly lower levels of IL-4, confirming a pro-inflammatory TH1 skew in cytokine production.[15]

While ABX196 induces a more potent immunostimulatory response beneficial for antitumor immunity, iNKT cell activation quickly leads to a long term unresponsive, anergic state for two primary reasons: one, the iNKT TCR becomes downregulated;[16,17] and two, PD-1 receptors become upregulated at the surface of iNKT cells.[18,19] PD-1 is a T cell checkpoint receptor that, when bound to its ligand PD-L1, functions to induce and maintain T cell tolerance (***Figure 1***).[20] The FDA has approved a number of monoclonal antibodies that target and interfere with the PD-1/PD-L1 signaling pathway for use in cancer therapy.[21] These include anti-PD-1 antibodies nivolumab, pembrolizumab, and cemiplimab, as well as anti-PD-L1 antibodies atezolizumab, avelumab, and durvalumab.[21] The aforementioned ICIs have emerged in recent years as effective therapeutics for a variety of cancers due to their unique ability to block and reverse T cell anergy.[22] These findings have led to the pharmaceutical approach of simultaneously administering both an iNKT cell agonist and an anti-PD-1 antibody to limit iNKT cell anergy and thus enhance antitumor immunity.

Preclinical Data: iNKT Agonist with PD-1 Blockade

Several preclinical studies have explored the immunotherapeutic potential of α-GalCer-mediated iNKT induction in combination with PD-1 blockade. Parekh et al.[19] demonstrated that antibody-mediated inhibition of PD-1/PD-L1 interactions at the time of α-GalCer treatment prevented the induction of iNKT cell anergy and enhanced the anti-metastatic activity of α-GalCer in wild-type mouse models. The same study showed that PD-1 deficient mice were resistant to α-GalCer-induced iNKT cell anergy. Durgan et al. [23] performed similar experiments with murine melanoma models. In their study, PD-L1 deficient mice were administered DCs loaded with antigen and α-GalCer; these mice subsequently had a significant reduction in tumor size associated with increased trafficking of antigen-presenting cells (APCs) and $CD8^+$ cytotoxic T cells to the sites of tumors.[23] The importance of α-GalCer and PD-1 blockade on $CD8^+$ T cell cytotoxic activity has been demonstrated by Bae et al.,[24] who found that administration of an iNKT agonist in an anti-PD-1-resistant tumor model re-stimulated exhausted $CD8^+$ T cells through the enhanced secretion of IL-2 and IL-12. They also observed a synergistic increase in the antitumor effect between α-GalCer-loaded APCs and PD-1 blockade. Moreover, *in vitro* assays with human peripheral blood mononuclear cells (PBMCs) showed that the simultaneous co-administration of an anti-PD-L1 antibody and α-GalCer-pulsed APCs enhanced both the direct cytotoxic and indirect TH1 cytokine release functions of iNKT cells, enhancing their antitumor immunostimulatory functions.[25] Lastly, preclinical studies in mice have confirmed the similarities between ABX196 and α-GalCer concerning *in vitro* and *in vivo* stimulation of iNKT cells.[15] Toxicity reports from these same experiments suggested no considerable adverse effects of ABX196 in mice and monkeys at the doses necessary for immune activation, although hepatic toxicity in the form of elevated transaminases was observed in mice at doses higher than that required for immune activation.[15]

Figure 1. Mechanism of Checkpoint Inhibition in Promoting Anti-Tumor Immune Stimulation



Legend: Simplified illustration demonstrating the mechanism of immune checkpoint inhibition in cancer immunotherapy. ***Figure 1.A.*** shows the T cell programmed cell death protein-1 (PD-1) receptor interacting with the corresponding tumor programmed cell death protein-1 ligand (PD-L1) receptor, leading to T cell anergy and inactivity. This occurs despite appropriate antigen presentation on the major histocompatibility complex (MHC) and proper recognition by the T cell receptor (TCR). ***Figure 1.B.*** demonstrates the targeted interruption of the PD-1/PD-L1 interaction by a monoclonal antibody immune checkpoint inhibitor (ICI). This inhibition of PD-1/PD-L1 signaling promotes continued stimulation of the T cell to induce tumor cell death.

Clinical Data: ABX196 and Nivolumab in the Treatment of Hepatocellular Carcinoma

Clinical studies assessing the immunotherapeutic rationale of administering iNKT agonists in combination with ICIs are sparse and have only recently been initiated for the treatment of hepatocellular carcinoma (HCC). HCC nearly always develops secondary to chronic liver inflammation, as this produces an immunosuppressive microenvironment that accommodates immune cell exhaustion.[26] Exhausted immune cells exist in an inactive anergic state, expressing high levels of inhibitory co-receptors, such as PD-1 and CTLA-4, and low levels of effector cytokines. As a result, anti-PD-1 antibodies have been studied in HCC and found to be an effective treatment.[27] Nivolumab received FDA approval in September 2017 for patients with advanced HCC previously treated with the multi-kinase inhibitor sorafenib during the phase I/II dose-escalation and dose-expansion CheckMate-040 study.[28] Nivolumab resulted in significant tumor diminution compared to first-line sorafenib therapy; however, its objective response rate (ORR) remained low at 15% (95% CI 6-28) in the dose-escalation phase and 20% (95% CI 15-26) in the dose-expansion phase. This has prompted additional efforts to improve nivolumab response rates in HCC treatment, and the first open label, uncontrolled phase I/II clinical trial to assess combination therapy of ABX196 with nivolumab is now underway at the Scripps M.D. Anderson Cancer Center [NCT03897543]. Importantly, the trial addresses the question of whether the immunostimulatory effects of ABX196 may help bolster the efficacy of nivolumab immunotherapy in HCC by specifically targeting and reversing iNKT cell anergy.

Since Hepatitis B infection is associated with increased risk of HCC, the adjuvant activity of ABX196 may play a critical role in HCC immunotherapeutic control. In a phase I first-in-human dose-escalation study, ABX196 induced a strong anti-HepB antibody response when used as an adjuvant for a prophylactic hepatitis B vaccine.[15] In all forty-four healthy male subjects treated, ABX196 elicited a stimulation of NKT cells *in vivo* as demonstrated by the downregulation of NKT TCR and pronounced antibody response. Adverse side effects were mild to moderate and associated with elevated IFN-γ levels, consistent with acute activation of hepatic iNKT cells by ABX196. Similarly, many clinical trials (***Table 1***) have tested the anticancer therapeutic potential of α-GalCer in humans, and it has been shown to be a safe and well-tolerated treatment plan, although its effectiveness in these trials was limited due to iNKT cell anergy and the development of immunosuppressive tumor microenvironments.[42]

CAR-T Cells in Combination with PD-1 Blockade

Background: The Therapeutic Evolution of CAR-T Cells

Chimeric antigen receptor (CAR)-T cells are genetically modified T cells designed to express a synthetic TCR for use in anticancer immunotherapy. T cells are isolated from human blood and engineered to express a unique CAR. CAR-T cells are then stimulated to expand *ex vivo* and are infused back into the patient to kill tumor cells expressing the corresponding CAR-T cell antigen.[43] CAR constructs are hybrid molecules consisting of three regions: i) the extracellular ectodomain, usually composed of a single chain variable fragment (scFv) obtained from a tumor antigen-reactive antibody, ii) the transmembrane domain to support CAR stability, and iii) the intracellular endodomain, composed of signaling peptides responsible for cell activation and co-stimulation following tumor antigen recognition (***Figure 2***).[44] CAR-T cells are separated into four generations based on the composition of their endodomains.[45] First generation CARs contain a scFv attached to a CD3ζ-derived intracellular signaling molecule, the primary transmitter of endogenous TCR stimulation. Second generation CARs contain an additional co-stimulatory molecule as part of the signal transduction region.[46] Third generation CARs combine multiple intracellular co-stimulatory domains to increase cytokine production. Fourth generation CARs, also referred to as T cell redirected for universal cytokine-mediated killing (TRUCKs), contain a transgenic load of immune modifiers, such as cytokines, co-stimulatory ligands and enzymes, that

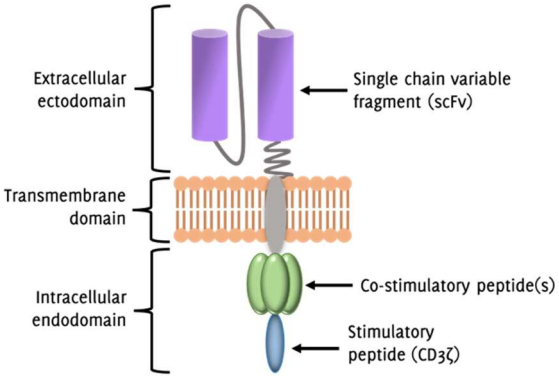
Table 1. Clinical Trials Evaluating α-GalCer-Mediated Stimulation of iNKT Cells

Clinical trial	No. of participants	Treatment	Result	Cancer type(s)
Giaccone et al.[29]	24	α-GalCer; intravenous	7 SD	Solid tumors
Nieda et al.[30]	12	α-GalCer-pulsed CD1d-expressing DCs; intravenous	3 reductions in tumor markers/mass	Solid tumors
Ishikawa et al.[31]	11 enrolled, 9 completed	α-GalCer-pulsed DCs; intravenous	5 SD	NSCLC
Chang et al.[32]	6 enrolled, 5 completed	α-GalCer loaded onto monocyte-derived mature DCs; intravenous	4 reductions in tumor markers or SD	MM, RCC, Anal SCC
Motohashi et al.[33]	6	α-GalCer-activated Vα24 iNKT cells; intravenous	4 SD	NSCLC
Uchida et al.[34]	9	α-GalCer-pulsed APCs; administered in nasal submucosa	1 PR, 5 SD	HNSCC
Motohashi et al.[35]	23 enrolled, 17 completed	α-GalCer-pulsed PBMC cultured with IL-2 and GM-CSF; intravenous	5 SD	NSCLC
Kunii et al.[36]	8	Intra-arterial infusions of α-GalCer- activated Vα24 iNKT cells + submucosal injections of α-GalCer-pulsed APCs	3 PR, 4 SD	HNSCC
Kurosaki et al.[37]	17	α-GalCer-pulsed APC injections into nasal or oral floor submucosa	Increased levels of iNKT cells/IFN-γ	HNSCC
Yamasaki et al.[38]	10	Nasal submucosal administration of α-GalCer-pulsed APCs + intra-arterial infusion of α-GalCer-activated iNKT cells via tumor-feeding arteries	5 PR, 5 SD	HNSCC
Nicol et al.[39]	12	α-GalCer-pulsed DCs; 2 treatments intravenous, 2 treatments intradermal	3 PR, 3 SD	Solid tumors
Nagato et al.[40]	4	α-GalCer-pulsed APCs; intravenous	Increased levels of iNKT cells in TILs, increased IFN-γ levels	NSCLC
Richter et al.[41]	6	α-GalCer-loaded monocyte-derived DCs + low-dose lenalidomide; intravenous	3 reductions in tumor-associated monoclonal immunoglobulin	Asymptomatic myeloma

Abbreviations: SD, stable disease; PR, partial remission; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; MM, multiple myeloma; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; DC, dendritic cell; APC, antigen presenting cell; TIL, tumor infiltrating lymphocyte.

upon release help activate and recruit innate immune cells to eliminate antigen-negative tumor cells.[47] TRUCKs enhance antitumoral activity through inducible IL-12, creating an immunostimulatory tumor microenvironment and favorably redirecting host lymphocytes toward the tumor site.[48]

Figure 2. Structure of the Chimeric Antigen Receptor (CAR)-T cell



Legend: Simplified diagram of the chimeric antigen receptor (CAR)-T cell structure. The extracellular ectodomain communicates with a specific tumor cell antigen via the single chain variable fragment (scFv), which is derived from an antibody that reacts with a given tumor antigen. The transmembrane domain provides stability to the CAR structure. The endodomain is responsible for communicating intracellular signals that promote T cell activation.

Rationale for CAR-T Cell Combination Therapy with ICIs

While conventional CAR-T cell therapy has demonstrated clinical success against B cell hematologic malignancies,[49,50] its efficacy is limited by several important obstacles, including high toxicity, immunosuppressive tumor milieu, and CAR-T cell dysfunction.[51] One of the primary reasons for poor treatment response and relapse after CAR-T cell therapy is inefficient T cell expansion and a lack of persistent T cell activation following infusion of CAR-T cells into patients.[44] It is thought that CAR-T cell dysfunction and non-persistence is driven by co-inhibitory pathways induced by checkpoint blockade that lead to T cell anergy.[52] CAR-T cells were shown to upregulate immune checkpoint receptors, such as PD-1, CTLA-4 and lymphocyte activating gene-3 (LAG-3), in patients with chronic lymphocytic leukemia (CLL) unresponsive to anti-CD19 CAR-T cell therapy.[53] CLL is a hematologic malignancy with particularly poor response rates to CAR-T cell therapy and is known to facilitate an immunosuppressive, pro-tumor microenvironment.[54] PD-L1 expression was found to be significantly higher in 112 CLL patients than in non-CLL controls.[55] Similarly, mesothelin-specific CAR TILs (tumor-infiltrating lymphocytes) administered to mice bearing human mesothelin-expressing flank tumors underwent rapid and spontaneous loss of functional activity associated with increased expression of the surface inhibitory receptors PD-1, LAG3, T cell immunoglobulin- and mucin-domain-containing molecule 3 (TIM3) and 2B4 (CD244).[56] The aberrant expression of inhibitory molecules has been demonstrated in CAR-T cell clinical trials as well.[57] Infusion of anti-CD19 CAR-T cells to patients with advanced B cell lymphomas resulted in at least three-fold increase in expression of PD-1 at the surfaces of $CD4^+$ CAR-positive cells in 8 out of 11 patients.[57] These studies suggest that checkpoint-based immunosuppression is an important mechanism mediating tumor resistance to CAR-T cell therapy. Therefore, strategies that block inhibitory immune checkpoint pathways in combination with CAR-T cell therapy possess powerful immunotherapeutic potential.

Preclinical Data: CAR-T Cells with PD-1 Blockade

CAR-T cell combination therapy with PD-1 blockade has demonstrated improved antitumor effects in multiple preclinical models. In adoptive transfer studies of mice bearing human epidermal growth factor receptor-2 $(HER\text{-}2)^+$ tumors, anti-PD-1 antibodies enhanced HER-2-specific CAR-T cell functionality, significantly increased markers of activation and proliferation, improved tumor growth inhibition, and reduced the percentage of myeloid-derived suppressor cells, which – when produced in excess – are known to aid in tumor metastasis and immune evasion.[58] Similarly, PD-L1 inhibition in mouse CLL models reactivated immune effector functions and restored cytotoxic $CD8^+$ T cell activity as well as immune synapse formation *ex vivo* and *in vivo* by preventing exhaustion-like T cell phenotypes.[59] Experiments conducted with an orthotopic mouse model of pleural mesothelioma showed that PD-1 pathway interference restored the effector function of exhausted CD28-specific CAR-T cells.[60] Gargett et al.[61] demonstrated that third generation GD2-specific CAR-T cells would undergo significant activation-induced cell death (AICD) after repeated antigen stimulation *in vitro*; however, PD-1 blockade enhanced both CAR-T cell survival and promoted killing of $PD\text{-}L1^+$ tumor cell lines. CRISPR-Cas9-mediated editing of CAR-T cells, which rendered them non-responsive to PD-1 signaling, improved antitumor CAR-T cell activity both *in vitro* and *in vivo*.[62] Finally, Hui et al. [63] showed that PD-1/PD-L1 interactions suppressed CAR-T cell activity by blocking CD28 signaling, suggesting that upregulation of costimulatory pathways is an important mechanistic response of CAR-T cells to anti-PD-1/PD-L1 therapy.

Clinical Data: CAR-T Cells with PD-1 Blockade

Clinical trials employing CAR-T cell combination therapy with PD-1 blockade have already shown promising results. In a single-institution study at the Children's Hospital of Philadelphia, fourteen pediatric patients with heavily treated, relapse B cell acute lymphoblastic leukemia (B-ALL) and poor responses to CAR-T cell therapy were treated with CD19-specific CAR-T cell therapy in combination with an anti-PD-1 monoclonal antibody.[64] Encouraging results were particularly observed in patients with early B-cell recovery and bulky extramedullary disease. Three of 6 patients treated with PD-1 inhibitor and CAR-T cells for early B cell recovery reestablished B cell aplasia, an indication of persistent CAR-T cell activation. In a cohort of four patients treated with pembrolizumab and CAR-T cells for extramedullary disease, 2 partial remissions (PRs) and 2 complete remissions (CRs) were seen. However, in the 4 remaining patients who were unsuccessful in achieving remission with initial CAR-T cell therapy, only PRs were observed with CAR-T cell and pembrolizumab combination therapy. Adverse effects of combination therapy included fever, acute pancreatitis, hypothyroidism, joint pains, as well as moderate to severe pancytopenia. The study supports the hypotheses that upregulation of the PD-1/PD-L1 signaling axis may be a driving force in the development of resistance to CAR-T cell immunotherapy. The study also suggests that ICI combination therapy at the time of CAR-T cell administration may be a safe and durable strategy for preventing subsequent AICD in the treatment of B-ALL.

A similar single-institution trial at the Abramson Cancer Center of the University of Pennsylvania attempted to evaluate the role of pembrolizumab as salvage therapy for patients who experienced worsening disease following initial CAR-T cell infusion.[65] The study enrolled 12 patients with progressive or relapse B cell non-Hodgkin lymphomas with partial or no response to CD19-specific CAR-T cell therapy. Pembrolizumab was administered to these patients every 3 weeks until disease progression or adverse toxic side effects were observed. The addition of PD-1 blockade after prior ineffective anti-CD19 CAR-T cell therapy produced an ORR of 27% (1 CR and 2 PRs), including 1 patient with stable disease and 7 patients with progressive disease. Nine of 12 patients demonstrated a re-expansion of peripheral blood CAR-T cells after the first pembrolizumab dose, although this cellular re-expansion did not correlate with clinical outcome. Nevertheless, this study highlights the key theory that ICIs may reinvigorate exhausted CAR-T cells in patients with poor or failed responses to initial CAR-T cell therapy, and further studies should be explored to translate this CAR-T cell re-expansion into clinically efficacious use. At the moment, several clinical trials (***Table 2***) are attempting to address the optimal timing of administration, dosing, efficacy, and safety of CAR-T cell combination therapy with ICIs, particularly in patients who have failed first-line therapies with relapsed or refractory progression of their cancers. These

Table 2. Clinical Trials Evaluating CAR-T Cell Combination Therapy With PD-1 Blockade

Clinical trial identifier	Sponsor/study name	Patients enrolled	CAR-T	ICI	Cancer type(s)
NCT03310619	Celgene/PLATFORM	100	JCAR017	Durvalumab	NHL, DLBCL, FL
NCT03287817	Autolus Limited/ALEXANDER	120	AUT03	Pembrolizumab	DLBCL
NCT02706405	Fred Hutchinson Cancer Research Center	42	JCAR014	Durvalumab	NHLs + gene rearrangement(s), DLBCL, PMBL
NCT03630159	Novartis Pharmaceuticals/ PORTIA	32	CTL019	Pembrolizumab	DLBCL
NCT02926833	Kite, A Gilead Company/ZUMA-6	37	KTE-C19	Atezolizumab	DLBCL
NCT03726515	University of Pennsylvania	7	CART-EGFRvIII	Pembrolizumab	Glioblastoma
NCT04003649	City of Hope Medical Center	60	IL13Ralpha2-CRT T cells	Ipilimumab, nivolumab	Glioblastoma
NCT03525782	The First Affiliated Hospital of Guangdong Pharmaceutical University	60	Anti-MUC1 CAR-T cells	Nivolumab	NSCLC
NCT02414269	Memorial Sloan Kettering Cancer Center	66	iCasp9M28z, T cells	Pembrolizumab	Lung/breast cancers, mesotheliom

Legend: NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; PMBL, primary mediastinal B cell lymphoma; NSCLC, non-small cell lung cancer

trials address ICI and CAR-T cell combination therapy in two primary treatment scenarios: 1) when the agents are administered simultaneously, or 2) when the ICI is administered for a limited duration shortly after CAR-T cell infusion. Taken together, these studies attempt to assess both the potential of concurrent ICI and CAR-T cell therapy to prevent the development of future AICD as well as the salvage potential of ICIs to enhance prior ineffective CAR-T cell therapy. Investigating ICI and CAR-T cell combination therapy from both of these angles may provide a better understanding of the appropriate timeframe in which ICIs can most effectively enhance and prevent resistance to CAR-T cell therapy.

NK Cells in Combination with PD-1 Blockade

Applications, Advantages, and Challenges of NK Cell Therapy

Natural killer (NK) cells are cytotoxic innate lymphoid cells that play a vital role in antitumor immunity due to their unique ability to detect and eliminate malignant cells with downregulated surface expression of self-MHC-I molecules.[66] NK cell functions vary widely and include degranulation, cytokine secretion primarily in the form of IFN-γ, and direct cytotoxicity due to an elaborate interaction of inhibitory and activating signals.[67] Many antitumor therapeutic strategies have emerged in recent years that utilize the cytotoxic and immunoregulatory activities of NK cells. Adoptive transfer therapy, in which NK cells from a healthy donor are isolated, activated *ex vivo* in an IL-2 or IL-15 solution, then infused into cancer patients, has proven to be an effective and nontoxic antitumor treatment.[68] More recently, isolated NK cells have been genetically modified to express a unique tumor-antigen CAR prior to re-infusion. CD19- and CD20-specific CAR-NK cells have shown successful preclinical tumor growth inhibition in a variety of B cell malignancies.[69] CAR-NK cells targeting HER-2, epidermal growth factor receptor (EGFR), natural killer group 2D (NKG2D), and disialoganglioside GD2 receptors, all of which are overexpressed in tumor cells, have also shown preclinical antitumor efficacy against solid tumors.[69] CAR-NK cell therapy has advantages over CAR-T cell therapy in that it does not induce graft versus host disease (GVHD),[70,71] nor does the CAR modification prevent the NK cell from carrying out its non-specific innate functions, thus limiting the occurrence of antigen loss and tumor escape.[72] In addition, CAR-NK cell therapy eliminates the need for a personalized, autologous product typically required with CAR-T cell therapy. CAR-NK cell therapy, therefore, has the potential to be an affordable and readily available, "off-the-shelf" treatment option. The aforementioned therapeutic benefits are limited in part by the ability of injected NK cells to migrate to tumor sites, persist, and expand *in vivo*.[73] NK cells express a range of inhibitory receptors, including PD-1, PD-L1, CTLA-4, T cell immunoglobulin and mucin-containing protein 3 (TIM3), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT), LAG-3, interleukin-1 receptor 8 (IL-1R8), and CD96, in addition to more well-established inhibitory receptors, like killer-cell immunoglobulin-like receptor (KIR) and the C-type lectin inhibitory receptor CD94/natural killer group 2A (NKG2A).[74,75] Research into the blockade of these inhibitory NK cell immune checkpoint pathways is ongoing and early data reflect an encouraging possibility of immune checkpoint inhibition to overcome the immunosuppressive limitations currently associated with NK cell therapy.[76] For the purposes of this review, literature pertaining specifically to inhibition of the PD-1/PD-L1 axis was the focus.

Rationale for Combining NK Cell Therapy with PD-1 Blockade

PD-1 is highly expressed on a distinct subpopulation of NK cells with impaired immunostimulatory capabilities that are detectable in approximately 25% of healthy people.[77] NK cell populations with high PD-1 expression demonstrate significantly reduced function and are found in greater proportion in patients with ovarian carcinoma,[77] Kaposi sarcoma,[78] multiple myeloma,[79] and head and neck cancers.[80] *In vitro* studies have confirmed that PD-1 receptors become upregulated at the surface of healthy control NK cells upon extended contact with activating ligands,[78] suggesting that PD-1 helps induce, as it does for T lymphocytes, NK cell anergy. This increased expression of PD-1 at the surface of NK cells correlates with poorer survival prognosis in esophageal and liver cancers.[81] More recent studies have determined that upregulated PD-L1 expression at the surface of NK cells also mediates exhaustive NK phenotypes. Dong et al., [75] for instance, discovered that some tumors can induce PD-L1 expression on NK cells via protein kinase B (AKT) signaling. These discoveries support the idea that NK cells are a valuable target in immunotherapeutic approaches that inhibit PD-1/PD-L1 interactions, especially when these ICIs are used to treat tumors that are MHC-I deficient. Thus, utilizing PD-1 blockade may be an excellent additive strategy for immunotherapy regimens that harness the antitumor capabilities of NK cells.

Preclinical data: NK cells with PD-1 blockade

Preclinical studies have shown that inhibiting PD-1 and PD-L1 checkpoint receptors enhances the immunotherapeutic efficacy of NK cells. Benson et al.[79] demonstrated that a PD-1 blocking antibody improved human NK cell functionality against autologous, primary multiple myeloma cells *in vitro* through a mechanism involving NK cell trafficking, immune complex formation, and enhanced cytotoxicity directed toward PD-L1$^+$ tumor cells. Blocking PD-1/PD-L1 signaling notably enhanced cytokine secretion and inhibited NK cell apoptosis *in vitro*. Importantly, administration of an anti-PD-1 antibody significantly slowed tumor growth in HCC xenografts, and this beneficial antitumor response was diminished by NK cell depletion, indicating an NK-dependent antitumor mechanism in response to PD-1 blockade.[81] Hsu et al. [82] performed similar experiments on several mouse models of cancer, including lymphoma, melanoma, prostate adenocarcinoma, and colon carcinoma, and determined that the release of PD-1-imposed inhibition activated an NK response that was indispensable for the full

effect of ICI immunotherapy. Oyer et al. [83] observed a significant improvement in NK cell antitumor efficacy, persistence, and retention of cytotoxic activity in mouse ovarian cancer models when combined with anti-PD-L1 antibody. The group showed that expanded NK cells secreted large amounts of IFN-γ, which induced expression of PD-L1 on human ovarian cancer cells *in vivo*. These findings support NK cell combination therapy with anti-PD-L1 antibody, irrespective of initial tumor PD-L1 status. Lastly, Dong et al. [75] determined that various PD-L1$^-$ tumor cell lines still responded favorably to anti-PD-L1 monoclonal antibody therapy, because the anti-PD-L1 antibody directly targeted and activated PD-L1$^+$ NK cells in a PD-1 independent process.

Clinical Data: NK Cells with PD-1 Blockade

While preclinical data has confirmed the immunostimulatory advantage of PD-1 blockade on NK cell functionality, clinical trials are new and in their early stages. A phase II study assessing the effects of pembrolizumab on NK cell exhaustion in patients with malignant melanoma was recently terminated due to enrollment difficulties [NCT03241927]. Nevertheless, several clinical trials (***Table 3***) evaluating the therapeutic benefit of PD-1 blockade on NK cell antitumor activity are currently underway.

Conclusion

Immunotherapy continues to revolutionize cancer treatment in the twenty-first century. ABX196, CAR-T cell and NK cell immunotherapies, in particular, have shown compelling preclinical data and are in early phase studies to determine if this activity can be translated into patient care. These therapeutics have mechanisms of action that are distinct from approved ICIs, which may overcome some of the limitations that have plagued ICI immunotherapy. Modulating multiple levers of the immune system simultaneously by co-administering ICIs with either ABX196, CAR-T cells or NK cells may further the essential, as-yet unmet, goal of overcoming tumor resistance to ICI immunotherapy. While awaiting the results of these agents' early clinical trials, additional studies should be pursued to both enhance and optimize these promising new immunotherapy approaches.

Table 3. Clinical Trials Evaluating NK Cell Combination Therapy With PD-1 Blockade.

Clinical trial identifier	Sponsor	Patients enrolled	ICI + NK	Cancer type(s)
NCT03958097	First Hospital of Jilin University	20	Sintilimab (anti-PD-1) + NK cells (autologous NK cells; collected by apheresis)	NSCLC
NCT03815084	Allife Medical Science and Technology Co., Ltd.	100	Pembrolizumab + DC-NK	Solid tumors
NCT03937895	SMT bio Co., Ltd.	40	Pembrolizumab + SMT-NK (allogeneic NK cells)	BTC

Legend: NSCLC, non-small cell lung cancer; BTC, biliary tract cancer

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Conflict of Interest Statement & Funding

Darren S. Sigal is an advisor for Celularity, Molecular Stethoscope, Curematch, and DrugCendR, has a patent on a method of use of TRK inhibitors in neuroendocrine tumors, and is on the speaker bureau for Celgene and Bayer. Jonathan A. Hermel and Cassi M. Bruni have no conflicts to declare. This research received no external funding.

Author Contributions

Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Supervision, Validation: JAH, DSS. Visualization: JAH, DSS, CMB. Writing – Original Draft Preparation: JAH, DSS, Writing – Review & Editing: JAH, DSS, CMB.

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