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REVIEW ARTICLE

Immune checkpoint blockade response and resistance: Next generation therapeutic agents for solid tumors

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Abstract

Immune checkpoint blockade has evolved in the realm of cancer immunotherapies to become standard of care in front-line settings, as well as in adjuvant and even neoadjuvant settings, especially in immunogenic tumors such as advanced melanoma. However, while many patients respond to these therapies with long-term robust clinical outcomes, there exists a considerable degree of non-responders. Studies have attributed this clinical situation to a number of correlative and causative factors, and a new generation of therapies are being developed to be used alone or in combination with anti-CTLA-4 and anti-PD-1 therapies to improve survival and overall response rates. This paper discusses the next generation immune checkpoint inhibitors against LAG-3, TIGIT, and TIM-3 and highlights emerging insights into their mechanisms of action. Another approach is the use of tumor infiltrating lymphocytes, discussed in the context of feasibility and randomized trials. These two approaches outlined in this paper explain distinct avenues to address the issue of non-responders and provide ways to circumvent the difficulties they pose for patients and in the clinic. The paper concludes on future directions in the form of reverse translation methods and their use and application for addressing non-responders to immune checkpoint blockade.

keywords: *immune checkpoint blockade resistance, next generation inhibitors, tumor infiltrating lymphocytes, MHC restriction downregulation, reverse translation*

Introduction

Immune checkpoint inhibitors, since their first regulatory approvals in 2011, have become established forms of therapies for solid tumors. Their efficacy was first demonstrated in melanoma due to the tumor's high immunogenicity, with robust overall survival and objective response rates when compared to standard of care, or chemotherapy regimens. However, patients treated with immune checkpoint inhibitors have been shown to be non-responders. Ipilimumab and nivolumab, the anti-CTLA-4 and anti-PD-1 immune checkpoint inhibitors respectively, demonstrate lack of clinical efficacy and adverse safety profiles in advanced staged patients, and exhibit relapse and recurrence. Initial studies evaluating the combination of ipilumimab and nivolumab for melanoma reported at ORR of 53% with tumor reduction in a subset of patients [1]. Substantial basic, clinical and translational research into the foundational nature and applications of cancer immunotherapies have been conducted to understand the correlative and causative mechanisms whereby there exists responders and non-responders, and why some patients are more at risk than others for exhibiting poor objective response rates and "only a small percentage of patients respond to ICI treatment due to intrinsic or acquired resistance to therapy. Currently, there are few clinically useful biomarkers of response (e.g., PD-L1, tumor mutational burden, microsatellite instability) and limited approved therapies to augment response to ICI. As immunotherapies become more prominent in the treatment of cancer, there is an immense need for understanding the mechanisms of ICI resistance and for the development of new therapeutic strategies to induce long-term responses" [2].

Much of this research has attributed this clinical situation to a combination of irreversible T cell exhaustion and tumor heterogeneity, and intrinsic and acquired resistance, while other studies have also shown roles for MHC restriction downregulation, the complexity of the tumor and immune microenvironment and vulnerabilities in the cancer immunity cycle. The development of therapies is ongoing to overcome this resistance and they belong to immunotherapy classes such as cancer vaccines, cytokine therapies, bispecific antibodies, CAR T cells, natural killer cells, and combination constructs.

Resistance can take the form of primary or secondary, or acquired, resistance. A number of mechanisms are implicated. Immunoediting has suggested that despite high immunogenicity, resistance can develop from neoantigen loss due to the continuous interfacing of the tumor cell and the immune cells. Subclones that are not sensitive to the immune cells are selected for, leading to resistance and tumor evasion. Genomic factors are also at play since BRCA2 positive melanoma tumors have higher response rates and DNA damage genes such as ATM, POLE and FANCA have shown improved outcomes. Tumor-associated macrophages, particularly M2 macrophages, promote tumor progression through modifications of the TME.M2 macrophages are known to stimulate tumor cell motility, angiogenesis, growth, and immune evasion [3].

This paper describes several types of the novel treatments and approaches being developed for overcoming resistance to the traditional checkpoint blockades that lead to non-responders. These include inhibition of MHC-I downregulation, the next-generation immune checkpoint inhibitors, LAG-3, TIGIT and TIM-3, whose mechanisms of action have recently been elucidated and are elaborated in this article. Antibodies against these inhibitors have been produced that are in different phases of the translational process from phase 1 studies to regulatory approvals, and are used in combination with anti-PD-1 for improved response. Tumor infiltrating lymphocyte therapies and the latest clinical trial evaluating their use are also discussed, along with their rationale for clinical utilization and prior clinical trials. The approaches discussed in this review are meant to outline innovative avenues that could either be used alone or in combination with anti-CTLA-4 and anti-PD-1 inhibitors to achieve synergistic effects for treating solid tumors.

Methods

Assessment of recent data and material from the Society for Immunotherapy of Cancer 2022 Annual Meeting and Keywords on Pubmed and Google Scholar databases were conducted: "MHC Restriction Downregulation Inhibition" OR "Next-Generation Immune Checkpoint Blockade" OR "LAG-3" AND "TIGIT" AND "TIM-3" AND "LAG-3" and "RELATIVITY-47" OR "Autologous Tumor Infiltrating Lymphocytes" AND "Advanced Melanoma". Large Language Models were also employed with the Keyword "TIGIT."

MHC restriction in immune checkpoint blockade restriction

Tumor cells have the propensity to avoid immune recognition through downregulation of the Major Histocompatibility complex I or MHC I, a peptide complex that allows for tumor antigen presentation to CD8+ T cells that release cytotoxic molecules. There are number of mechanisms whereby this can take place: genetic defects in the antigen presentation pathway such as in Beta2M [4]; transcriptional silencing via suppression of the Nf-Kb [5] and downregulation of type I and II IFN pathways [6]; and post-transcriptional silencing mediated by non-coding RNAs such as microRNAs that can repress expression of MHC I [7,9] (Figure 1).

Nguyen et al. at Memorial Sloan Kettering Cancer Center have conducting a recent study revealing that targeting LSD1, or lysine specific demethylase -1, rescues MHC-I antigen presentation in small cell lung cancer (SCLC) [10]. Lysine-specific demethylase 1 (LSD1), is encoded by the gene KDM1A and regulates gene expression by removing methyl groups on monomethylated and dimethylated lysine 4 and 9 of histone H3 protein. KDM1a has been shown to have higher expression in SCLC. Investigators have hypothesized that there is a correlation between KDM1A and a variety of genes necessary for antigen presentation, leading to creating a set of experiments to determine if LSD1 overexpression is correlated to MHC-I mediated antigen presentation in this malignancy. These experiments include pharmacologic inhibition and RNA interference which derepress MHC-I cell surface expression in SCLC. They elucidated differentially expressed genes involved in the antigen-presentation machinery of the MHC-I complex that become activated upon LSD1 inhibition (through LSD1 inhibitors and shRNA knockdown), including NLRC5, a member of the regulators of inflammatory response gene family. They also found that "targeting LSD1 can sensitize SCLC tumors to PD-L1 blockade" [10].

SCLC has low or absent MHC-I expression and antigen presentation deficiency in SCLC is reversible through epigenetic de-repression. The epigenetic modifier LSD1 regulates gene expression through H3K4/K9 methylation. In an inducible shRNA knockdown model, LSD1 deficient mice restored MHC-I presentation since LSD1 affects the KDM1A enzyme that in turn affects MHC I levels through irreversible catalytic inhibition. Interferon-gamma stimulation and LSD1 inhibition induce transcription of HLA genes. LSD-1 inactivation overcomes SCLC resistance to immune checkpoint blockade. In the presence of both LSD-1 inhibitors and anti-PD-1 inhibitors tumor burden is overcome through synergistic effects and the anti-tumor response is T-cell dependent. As the study authors state, "targeted inhibition of LSD1 in SCLC restores MHC-I cell surface expression and transcriptionally activates genes encoding the antigen presentation pathway. LSD1 inhibition further activates interferon signaling, induces tumor-intrinsic immunogenicity, and sensitizes SCLC cells to MHC-I–restricted T cell cytolysis. Combination of LSD1 inhibitor with ICB augments the antitumor immune response in refractory SCLC models." They conclude that "[t]ogether, these data define a role for LSD1 as a potent regulator of MHC-I antigen presentation and provide rationale for combinatory use of LSD1 inhibitors with ICB to improve therapeutic response in SCLC. Epigenetic silencing of MHC-I in SCLC contributes to its poor response to ICB. Our study identifies a previously uncharacterized role for LSD1 as a regulator of MHC-I antigen presentation in SCLC. LSD1 inhibition enables MHC-I–restricted T cell cytolysis, induces immune activation, and augments the antitumor immune response to ICB in SCLC" [10].

Next Generation Immune Checkpoint Blockade: LAG-3, TIGIT, TIM-3

Recent investigations have uncovered novel therapeutic applications for a set of immune checkpoint inhibitors, also known as next-generation immune checkpoint blockade. Most notably, they include anti-LAG-3 and anti-TIGIT antibodies and the inhibitory receptor TIM-3. New insights into their mechanisms of action have been revealed demonstrating the distinctive roles of cytokines, chemokines and other types of molecules in the immune microenvironment that have also been shown to be relevant for the anti-PD-1/PD-L1 and anti-CTLA-4 mAbs accounting for their immunosuppressive functions in tumors. LAG-3 in particular is termed "the third checkpoint inhibitor", and clinical trials have led to the approval of anti-LAG3 antibodies; and these inhibitory receptors have heralded the "next wave of co-inhibitory receptor targets that are being explored in clinical trials," as stated in earlier reports [11]. Earlier reports confirm the use of antibodies directed against these checkpoints in preclinical and clinical models. A study by Woo et

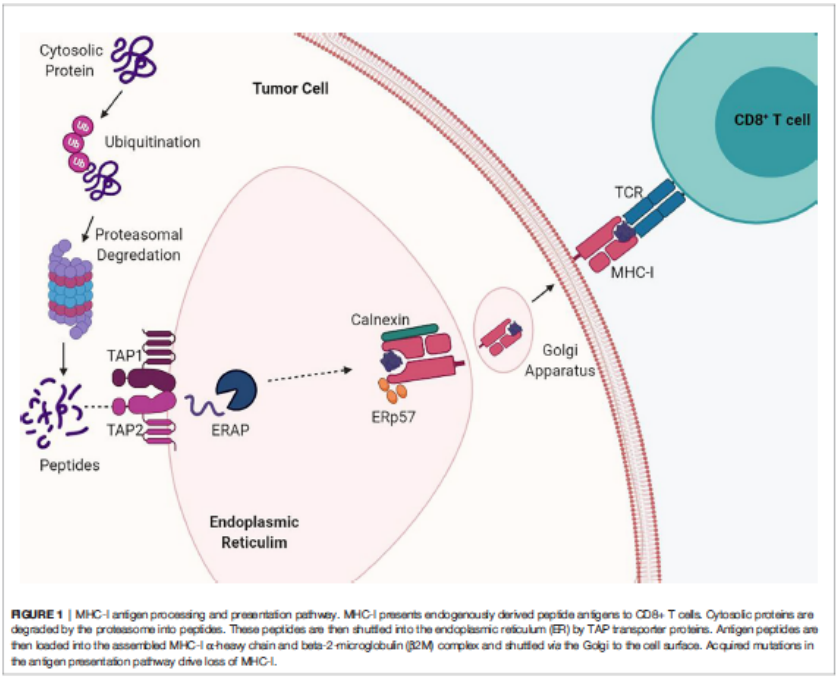

FIGURE 1 | MHC-I antigen processing and presentation pathway. MHC-I presents endogenously derived peptide antigens to CD8+ T cells. Cytosolic proteins are degraded by the proteasome into peptides. These peptides are then shuttled into the endoplasmic reticulum (ER) by TAP transporter proteins. Antigen peptides are then loaded into the assembled MHC-I α-heavy chain and beta-2-microglobulin (β2M) complex and shuttled *via* the Golgi to the cell surface. Acquired mutations in the antigen presentation pathway drive loss of MHC-I.

Figure 1: MHC-I Antigen processing and presentation pathway (adapted from Taylor and Balko 2022)

al. showed that anti-LAG3 and anti-PD-1 antibodies had antitumor effects in mice models that were resistant to single use agents [12]. Wooreveal extensive co-expression of PD-1 In "murine model of chronic lymphocytic leukemia dual PD-1/LAG-3 blockade was shown to limit tumor growth compared with either treatment as monotherapy." TIM-3 positivity was significantly correlated with duration of PD-1 blockade in genetically engineered mouse models. Earlier reviews explored the association between tumor resistance and the emerging roles of LAG-3, TIGIT, and TIM3 inhibition. A review published by Marin-Acevedo et al. in 2018 presented the latest clinical trials and the antibodies demonstrating efficacy in preclinical models [13].

Anti-LAG-3 antibodies in advanced melanoma: novel mechanisms and regulatory approvals

LAG-3 (CD223) is an inhibitory receptor that was discovered in in vitro-activated T cells. It has multiple biological inhibitory effects on T cell function and CD4 T cell activation [14-16]. LAG-3 plays a regulatory role in the immune system comparable to PD-1 and CTLA-4, generally consisting of inhibition of cell proliferation, immune function, cytokine secretion, and homeostasis. LAG3 is considered the MHC Class II canonical ligand. Vignali and colleagues have depicted how LAG3 works, how it impacts T cell function and how it mediates its inhibitory signals. They constructed PD-1, LAG3 and combination PD-1/LAG3 knockout mice to determine efficacy and found that exhausted T cell lineages are a result of substantial decrease in TOX expression drives T cell exhaustion. PD-1 in combination with LAG-3 decreases interferon-gamma production and while loss of PD-1 in combination with LAG-3 increases IFN-gamma production and IFN-gamma responsive genes. A primary loop is created whereby synergism of IFN-gamma feeds into an autocrine loop (unpublished data, 2022).

MHC Class II-independent LAG3 limiting T cell activation was observed by Guy et al. [16]. They found an evolutionary conserved glutamic acid-proline repeat in the cytoplasmic tail that caused dissociation of the tyrosine kinase Lck from the CD4 or CD8 co-receptor in the presence of acidic environments, that led to the loss of co-receptor TCR signaling and T cell effector functions through activation. The conclude that "these observations indicated that LAG3 functioned as a signal disruptor in a major histocompatibility complex class II-independent manner, and provide insight into the mechanism of action of LAG3-targeting immunotherapies" (Figure 2) [16].

Recently, a LAG-3 immune checkpoint inhibitor was approved by the FDA led by the Phase II/III RELATIVITY-047 clinical trial, which led to the FDA's approval of the LAG-3 antibody relatlimab for patients with advanced melanoma. Following the approval of relatllimab, the FDA approved an agent that combines anti-LAG3 mAB relatlimib with nivolumab for first-line treatment of metastatic melanoma in a phase 2–3, global, double-blind, randomized trial evaluating "fixed-dose combination as compared with nivolumab alone when administered intravenously every 4 weeks to patients with previously untreated metastatic or unresectable melanoma. The primary end point was progression-free survival as assessed by blinded independent central review" [17].

According to Tawbi et al. the mPFS "was 10.1 months (95% confidence interval [CI], 6.4 to 15.7) with relatlimab–nivolumab as compared with 4.6 months (95% CI, 3.4 to 5.6) with nivolumab (hazard ratio for progression or death, 0.75 [95% CI, 0.62 to 0.92]; P = 0.006 by the log-rank test). The percentage of patients with progression-free survival at 12 months was 47.7% (95% CI, 41.8 to 53.2) with relatlimab–nivolumab and 36.0% (95% CI, 30.5 to 41.6) with nivolumab. Progression-free survival, assessed by blinded independent review of 391 events, was significantly longer with relatlimab–nivolumab than with nivolumab." The Kaplan-Meier curve depicts the progression status of both cohorts as shown in (Figure 3) [17].

Anti-TIGIT antibodies

"TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an immune checkpoint protein that is expressed on T cells and natural killer (NK) cells. It functions as an inhibitory receptor and can bind to its ligands, such as PVR and PVRL2, on antigen-presenting cells (APCs) to suppress T cell and NK cell activation. TIGIT can also bind to CD155 on cancer cells, leading to suppression of the immune response against the cancer cells. Immune checkpoint inhibitors such as TIGIT antibodies are designed to block the interaction between TIGIT and its ligands on cancer cells, thereby restoring the ability of the immune system to recognize and attack cancer cells" [18].

TIGIT and PD-1 are mechanistically convergent checkpoints that regulate T cell differentiation. TIGIT and PD-1 blockade lead to T

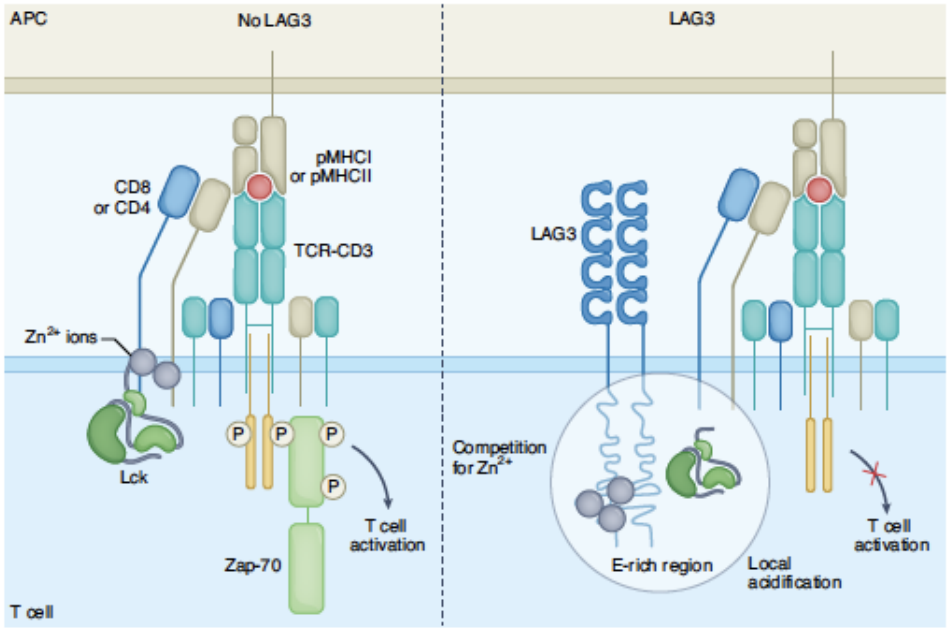

Figure 2: The inhibitory effect of LAG-3 through local acidification (adapted from Hlvroz 2022)

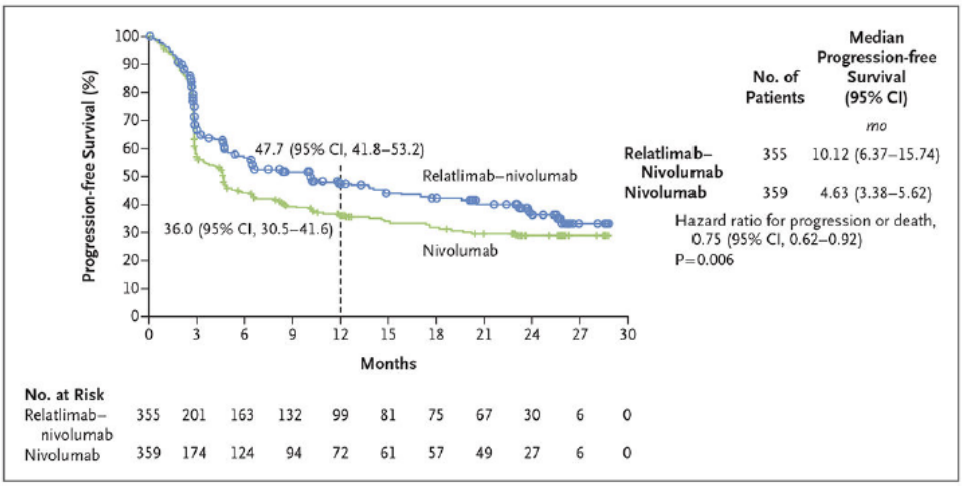

Figure 3: Anti-LAG-3 inhibitor Relatlimab and nivolumab versus nivolumab alone Progression Free Survival (adapted from Tiwab et al 2022)

cell exhaustion reversal, and expansion of the T cell compartment. This is relevant since myeloid cells express a ligand for CD28 cells, and in PD-L1 knockout mice, tumors do not grow. Macrophage specific dendritic cells play outsize role in process PD-L1/DC axis enhance T cell activation, differentiation, expansion, and attacking PD-1 inhibition prevents T cell exhaustion, which is not reversible. TIGIT's ITIM domain blocks phosphorylation of CD226; when removing the 2 phosphorylation sites, the same degree of inhibition and ligand competition is observed. PD-1 blockade enhances CD226 phosphorylation. TIGIT inhibition leads to similar CD226 activity but only through ligand competition. The intact Fc domain on anti-TIGIT antibodies are necessary for maximal tumor regression. Tiralogamab is an anti-TIGIT antibody; TIGIT blockade and PD-L1 combination lead to less of the transcription factor Tox, and decreased Tox expression depends on an intact Fc domain on anti-TIGIT. PD-L1/PD-1 and TIGIT blockade re-directs the differentiation of T effector cells compared to T memory cells than T cell exhaustion.

Banto et al. have recently shown that PD-1 and TIGIT act on the costimulatory CD226 receptor in concert through a number of mechanisms that involve biochemical and T cell activation pathways, and that CD28 was co-expressed by distinct populations in intact cells. They showed that WT mice that were administered anti-PD-1 or anti-TIGIT had less tumor volume compared to control antibody animals. However, they found remarkably that PD-1 played a role in controlling the anti-tumor effects of TIGIT inhibition that is mediated by PVR and PVRL2 regulation of CD226 signaling, indicating that CD226 expression associates with the combined TIGIT and PD-1 inhibitory responses. The biochemical mechanism of this effect is robust phosphorylation of CD226 residue Y322 within the intracellular domain of CD226 was "the major phosphorylation site for CD226 in activated T cells". The phosphorylation of CD226 is a result of the compounded effects of anti-PD-1 and anti-TIGIT, which was found by the investigators to be mediated by the ICD of PD-1 rather than the ICD of TIGIT despite its ITIM, which was unexpected [19,20]. "While TIGIT and PD-1 can independently regulate CD226, coordinate blockade of both inhibitory receptors was required to fully restore CD226 signaling…If PVR is expressed in excess of TIGIT, CD226 would become activated were it not for the ability of PD-1 to restrain CD226 phosphorylation via Shp2" [19].

The phase 2 trial CITYSCAPE enrolled stage IV PD-L1+ NSCLC patients comparing atezolizumab with atezolizumab and the anti-TIGIT drug tiragolumab. mPFS was 3.9 versus 5.6 in each arm, respectively. Based on this promising data, the FDA granted Breakthrough Therapy Designation in 2021 in these patients who harbored no targetable mutations. Follow-up data at EMSO showed a mOS of 23.2 months in the combination arm versus 14.5 months in the monotherapy arm. In TPS ≥ 50% cohorts, the clinical outcome is significantly more pronounced with mOS of over 30 months in the tiragolumab arm versus 12.8 months in the atezoluzimab arm [21].

Mechanisms of TIM-3 inhibition: The T cell exhaustion marker

T cell immunoglobulin-3 also known as Tim-3 is a cell surface marker expressed on interferon gamma producing CD4+ helper and CD8+ cytotoxic T cells. Tim-3 is encoded by Haver2, and has been discovered to play roles in autoimmunity, tuberculosis, and viral infections and cancer [22].

Anderson et al. reported that TIM-3 marks terminally dysfunctional CD8+ T cells with data showing the partitioning of TILs into TIM-3 and PD-1 areas that produce IL-2, TNF-alpha, IFN-gamma. There is loss of IL- 2, TNF-alpha and IFN-gamma in the presence of both TIM-3 and PD-1, leading to the designating of TIM-3 as the marker for terminally exhausted TILs. Anti-TIM-3 strongly synergizes with PD-1 blockade in established CT26 lines with 2/5 mice showing tumor regression (and is recapitulated in preclinical models of acute myeloid leukemia, now being translated into a Phase 1b clinical trial). Anti-TIM-3 antibodies for AML and MDS are also showing promising data for advanced melanoma and NSCLC.

The multiple ligands for Tim-3 are CEACAM-1, Galectin-9 and PtdSer; Fyn binds to Tim-3's cytoplasmic tail along with Bat3. However TIM-3 lacks the canonical inhibitor ITIM/ITSM signaling motif in its cytoplasmic tail, leading to a need for further understanding of its inhibitory mechanism. It was confirmed in reports published in 2012 that Bat3 (HLA-B associated 3) binds to TIM-3 through a Yeast 2-hybrid screen tail. On the TIM-3 cytoplasmic tail, tyrosine residues that were mutated to alanine lost the ability of Bat-3 to bind to Tim-3 that was not replicated when these tyrosines were mutated to phenylalanine, which shows structural dependence rather than the implication of a biochemical pathway. Galectin-9 binding to TIM-3 triggers Bat-3 release, and when Bat-3 is depleted, IFN-gamma decreases such that Bat-3 binding to the TIM-3 tail acts as a repressor of TIM-3 inhibitory function. (unpublished data, 2022)

However, the questioned remained as to what was mediating the inhibitory function of TIM-3. RNA-Seq experiments found genes co-expressed with TIM-3 in CD8+ TILs. The Cbl-b E3 ligase highly correlated with TIM-3 expression in mouse and human CD8+ TILS (melanoma). Cbl-b dampens T cell responses by interfering with signaling downstream of CD28. Cbl-b protein is also upregulated in Tim3+ TILs. In co-immunoprecipitation experiments, Cbl-b precipitates with Tim-3. Cbl-b binds to the TIM-3 cytoplasmic tail that maps to a similar region where Bat3 binds, the Y256 and Y263 residues, in particular. Cbl-b was also found to bind to both Tim-3 and CD28. T cell co-localization of TIM-3 with Cbl-b at the immunological synapse was also determined through microscopy

experiments. Aggregation of Cbl-b and TIM-3 occurs at the immunological synapse. Anderson and colleagues are now developing models whereby TIM-3 ligand binding triggers Bat3-Cbl-b exchange on the TIM-3 tail thus converting TIM-3 from being permissive to being inhibitory for T cell binding. (unpublished data, 2022)

A monoclonal anti-TIM3 antibody, sabatolimab (MBG453) blocks TIM-3 interaction with the phosphatidylserine ligand and partially blocks interaction with the Galectin-9 ligand. In NSCLC and melanoma patients, sabatolimab demonstrated clinical outcomes in combination with PD-1 directed antibody spartaliuzmab (PDR001) in a phase I/Ib part for determining safety and tolerability of sabatolimab with or without spartalizumab (11,12). "The most frequent AE (≥5% of patients) reported with sabatolimab was fatigue (n=12; 9%), and most frequent AEs for combination treatment were fatigue (n=13; 15%), decreased appetite (n=7; 8%), diarrhea (n=6; 7%), rash (n=6; 7%), elevated aspartate aminotransferase (n=5; 6%), and nausea (n=5; 6%)... Median follow-up for efficacy was 5.7 months (0.2–39.6 months). Per RECIST v1.1, no responses were observed in patients receiving single-agent sabatolimab" [23].

Tumor Infiltrating Lymphocytes (TIL) therapy

Melanoma has been established as the solid tumor with high degree of response to immune checkpoint inhibitors, augmented by the development of targeted therapies against BRAF mutations present in these tumors. However, despite the high tumor mutational burden and T cell reactivity, melanoma tumors develop resistance to immune checkpoint blockade. First line treatment includes anti-LAG-3 inhibitors such as relatlimab and nivolumab combination therapy and ipilimumab and nivolumab combination agents, and targeted agents such as BRAF + MEK inhibitors in BRAF V600 mutated disease, have led to less than optimal clinical outcomes.

An alternative therapy has been developed using autologous tumor infiltrating lymphocytes or TIL an ex vivo cellular therapy approach depicted in (Figure 5). In the process of administering TIL therapy, TIL populations are generated from freshly resected tumor samples from melanoma patients after IL-2 expansion. The cultured TIL population is grown to an enormous extent on the order of 1X10^10 to 1X10^11 in a 14 day rapid expansion protocol. After lymphodepletion chemotherapy, these cell products yielded from RIP are intravenously administered "high-dose (HD) bolus IL-2 infusions are given to support the growth and survival of the infused T cells. On average, TIL therapy has shown clinical responses in approximately 50% of treated individuals, mostly in anti-PD-1 treatment naïve patients, with durable complete remissions (CR) in 10%–15% of patients with treatment-refractory metastatic melanoma" [24]. The prognostic value of TIL therapy was demonstrated in earlier studies showing correlation with pCR rates and increases in survival. Investigators observed a positive correlation in TNBC patients between TIL levels and CD8+ T-cell density. Higher residual disease TILs were also associated with "improved RFS (HR: 0.86; 95% CI 0.79–0.92; P < 0.001), and improved OS (HR: 0.87; 95% CI 0.80–0.94; P < 0.001)" [25].

Given these initial observations, a phase I/II feasibility study for TIL therapy in metastatic melanoma by the Netherlands Cancer Institute which showed long-term survival and CR for a subset of patients was conducted. Ten patients received TIL therapy with 5 being PD-1 naïve that showed an ORR, with two CR for more than 7 years. Immune monitoring demonstrated detectable neoantigen-specific T cells in TIL infusion products to relate their expansion to tumor regression, however the responders had different neoantigens observed between them. ("Specifically, in patient 3, CD8+ T cell reactivity against five different neoantigens was observed, with CD8+

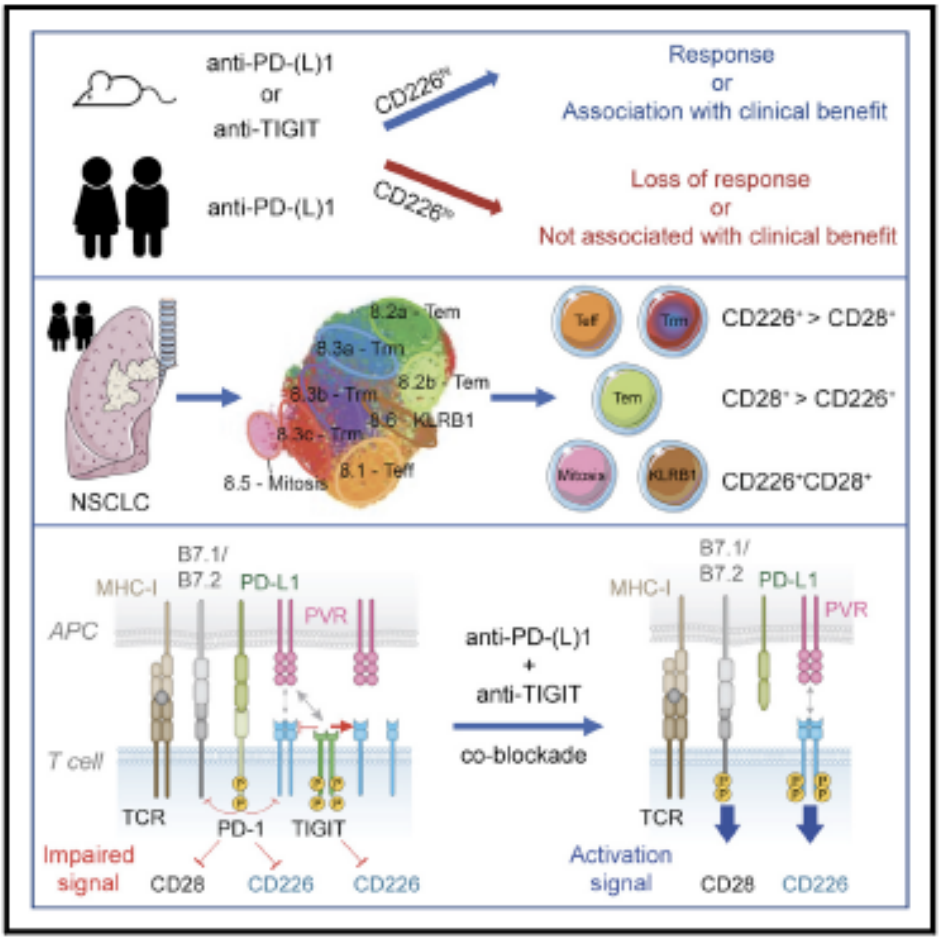

Figure 4: Anti-TIGIT and Anti-PD-1 antibody synergistically effect anti-tumor response through CD226 (adapted from Banta et al 2022)

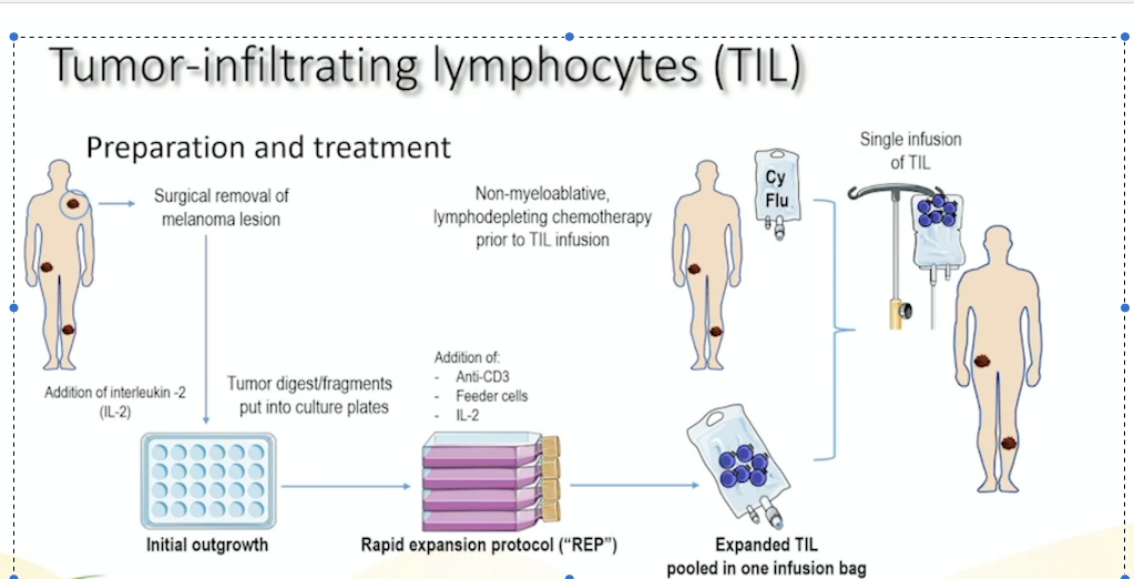

Figure 5: Tumor-infiltration lymphocytes protocol for preparation and treatment (adapted from Haanan 2022)

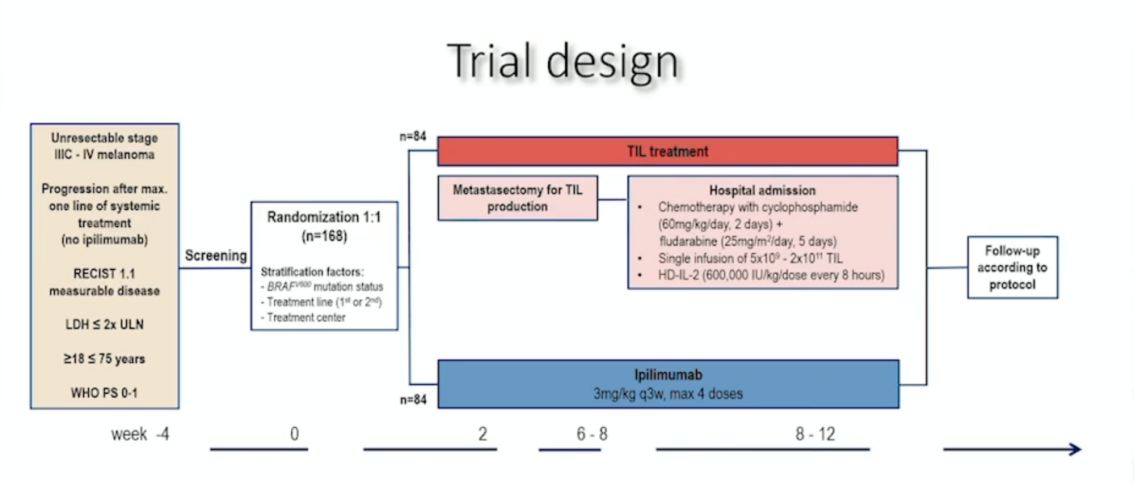

Figure 6: Randomized Phase III Trial evaluating TIL for advanced melanoma (adapted from Haanan 2022)

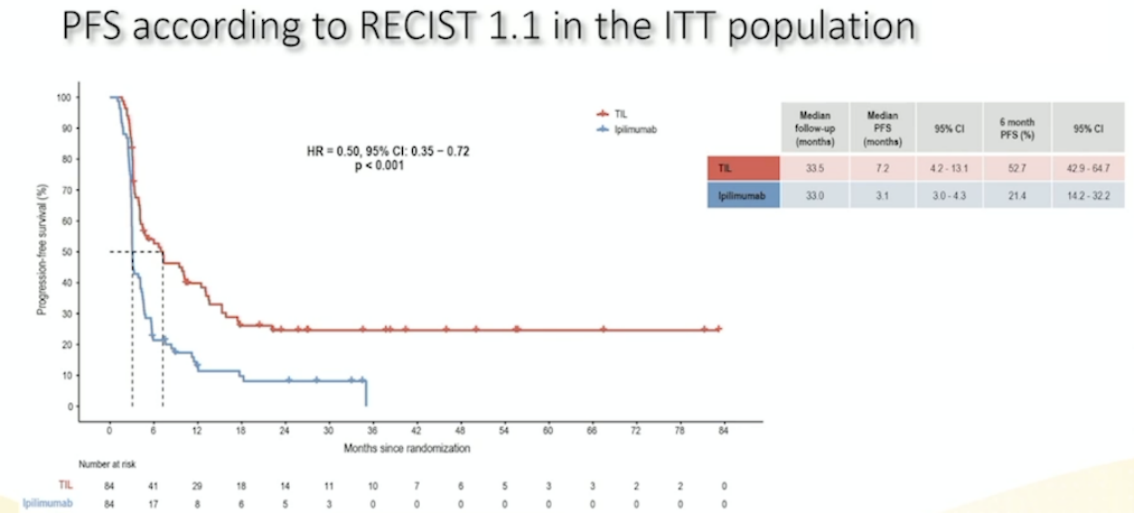

Figure 7: Kaplan Meier survival curves with mPFS comparison between TIL versus Ipilimumab (adapted from Haanan 2022)

	TIL (n=84)	Ipilimumab (n=84)
Best overall response	**n (%)**	**n (%)**
Complete response	17 (20.2)	6 (7.1)
Partial response	24 (28.6)	12 (14.3)
Stable disease	16 (19.1)	15 (17.9)
Progressive disease	24 (28.6)	40 (47.6)
Not evaluable/done[#]	3 (3.6)	11 (13.1)
Overall response[†]	41 (48.8)	18 (21.4)
Clinical benefit[‡]	57 (67.9)	33 (39.3)

Table 1: Overal responses to TIL therapy compared with Ipilimumab (adapted from Haanen 2022).

T cells directed against neoantigen RBM12 S>L forming a major Component") [24].

The phase I/II study as a result of its success was followed by a randomized phase III trial on 168 patients with unresectable stage IIIC – IV melanoma after meeting eligibility criteria. The trial design is shown in Figure with ECOG at 0,1 and LDH not more than two times the upper limit. Patients were randomized 1:1 administered either TIL treatment (n=84 (80 received infusion)) or ipilimumab (n=84 (82 received at least one cycle)) with BRAF mutation status and treatment line serving as stratification factors. Patients had unresectable stage IIIC-IV melanoma and LDH of less than or equal to 2, and were between 18-75 years of age. Follow up occurred after week 12. mPFS in the ITT population served as the primary endpoint and was 7.2 and 3.1 for TIL and ipilimumab, respectively and 52.75% and 21.4% of patients achieved 6-month progression free survival. Prior to TIL treatment, anti-PD-1 inhibitors has more than 50% increased risk of progression according to Forrest plot. (unpublished data, 2022, Figure 6)

Table 1 shows the best overall response rate for both treatment arms according to RECIST 1.1. CR was achieved for 17 patients and 6 patients in the treatment cohorts respectively, with 24 and 12 partial responders respectively. 16 and 15 patients achieved stable disease. 24 and 40 patients had progressive disease. According to the clinical outcomes analysis the patients do extremely well with the Kaplan-Meier survival curves plateauing. (Percentage of patients in parentheses). Toxicities and grade 3 treatment related events included neutropenia (n=80), lymphopenia (n=57), anemia (n=16) and elevated ALT (n=7)and AST (n=4), and fatigue (n=4) in the chemotherapy TIL arm; In the TIL + IL-2 arm febrile neutropenia (n=58), fever (n=36), dyspnea (n=15), hypertension (n=11), and rash (n=9) were among the adverse events. Adverse events in the ipilimumab included colitis, diarrhea, elevated ALT and AST usually associated with immune checkpoint inhibitor treatments (unpublished data, 2022).

Future directions for TIL therapy included careful selection and training of centers once it is approved in the refractory setting and the possibility of treatment administration in first line settings along with PD-1 combination therapies. Less toxicity from lymphodepletion chemotherapy and cytokine stimulation and better T cell product leading to greater tumor reactivity and better efficacy would constitute refinements of TIL therapy for clinical use. Other questions surround who should receive TIL therapy since good organ function, serum LDH less than 2X the upper limit of normal and the ability to withstand high doses of IL-2 and tumor without surgical resection are the criteria for eligibility; why they only work in a subset of patients; and if TIL could be used in first line settings which may be unclear if it is not more clinically robust than anti-PD-1. In this case, combination therapy with anti-PD-1 might be more efficacious as a result of potential synergy, as alluded to earlier. Side effects must be manageable, and this trial demonstrated that the side effects were resolved, and there may be less cytotoxicity if IL-2 is replaced.

Discussion

Many of the approaches described here are based on targeting the tumor based on immunogenicity. The situation of non-responders has lead researchers to try to find biomarkers to predict response and immune related adverse events. Reverse translation is a method developed by Sharma and colleagues at MD Anderson Cancer Center in immunological research that "takes" tumor from patients, generates a hypothesis to be tested in the lab and returns the relevant results to form the basis of a clinical trial. This reverse translation method has been pivotal in performing treatment in the neoadjuvant setting such that tumor samples could be obtained prior to surgery.

This method was experimented on prostate cancer, a tumor that has been shown to be resistant to immune checkpoint inhibitors. Two doses of ipilimumab prior to surgery were administered. There were very few infiltrating immune cells prior to treatment, but after the tumor became "hot", producing interferon gamma. Single cell RNA sequencing was performed to reveal additional targets such as CD73 and epigenetic pathways that inhibited Ezh2, which leads to regulatory T cells that are no longer capable of suppressing Teff cells, decreasing tumor volume and increasing survival. These sets of experiments set up a paradigm whereby the data from patients, leads to an hypothesis modeled in mice and then "go back" to patients in a clinical trial with pathologic complete response as the primary endpoint.

Conclusion

The eclectic nature of these approaches: inhibiting MHC-I downregulation, novel immune checkpoint blockade development, and tumor infiltrating lymphocytes show how the field has given due attention to the problem of non-responders, and paradigmatic methods such as reverse translation offer innovative workflows into how to make cold tumors respond. One of the main features of these therapies is that they can be used in combination with anti-CTLA-4 and anti-PD-1 inhibitors for more pronounced effects, as many have these studies have noted. Future directions would lead to the refinement of ex vivo approaches and the continuing clinical development of checkpoint blockade and their therapeutic applications for the attainment of more "success stories" in the field of cancer immunotherapies.

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Key Points

- *While immune checkpoint inhibitors have led to high degree of clinical success, there are a considerable number of non-responders to anti-CTLA-4 and anti-PD-1 treatment in patients with solid tumors, particularly advanced stage melanoma.*
- *Recent studies have attributed this to tumor immunogenicity, acquired resistance, T cell exhaustion and dysfunction and tumor heterogeneity.*
- *A number of clinical agents are being developed to address this issue of non-response, among them the development and therapeutic application of next-generation immune checkpoint inhibitors, tumor infiltrating lymphocytes, and the targeting of MHC-I downregulation.*
- *The mechanisms of action and new checkpoints are discussed along with the randomized clinical trial in advanced melanoma for TILs.*
- *Reverse translation has become a paradigmatic method to take pre-surgical tumor samples, evaluate tumor efficacy in mice models and return back to patients in clinical trials.*

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