European Journal of Cancer 187 (2023) 147–160

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Review

Shining a LIGHT on myeloid cell targeted immunotherapy

Casey W. Shuptrine [a,c], Vincent M. Perez [b], Sara R. Selitsky [b], Taylor H. Schreiber [a,c], George Fromm [a,c,*]

[a] *Shattuck Labs Inc., Austin, TX, USA*
[b] *QuantBio, Part of Tempus Labs, Durham NC, USA*
[c] *Shattuck Labs Inc., Durham, NC, USA*

Received 29 November 2022; Received in revised form 31 March 2023; Accepted 31 March 2023
Available online 5 April 2023

KEYWORDS
LIGHT;
HVEM;
LTβR;
Acquired resistance;
Myeloid therapy;
Checkpoint inhibition;
Co-stimulation

Abstract Despite over a decade of clinical trials combining inhibition of emerging checkpoints with a PD-1/L1 inhibitor backbone, meaningful survival benefits have not been shown in PD-1/L1 inhibitor resistant or refractory solid tumours, particularly tumours dominated by a myelosuppressive microenvironment. Achieving durable anti-tumour immunity will therefore likely require combination of adaptive and innate immune stimulation, myeloid repolarisation, enhanced APC activation and antigen processing/presentation, lifting of the CD47/SIRPα (Cluster of Differentiation 47/*signal regulatory protein alpha*) 'do not eat me' signal, provision of an apoptotic 'pro-eat me' or 'find me' signal, and blockade of immune checkpoints. The importance of effectively targeting mLILRB2 and SIRPAyeloid cells to achieve improved response rates has recently been emphasised, given myeloid cells are abundant in the tumour microenvironment of most solid tumours. TNFSF14, or LIGHT, is a tumour necrosis superfamily ligand with a broad range of adaptive and innate immune activities, including (1) myeloid cell activation through Lymphotoxin Beta Receptor (LTβR), (2) T/NK (T cell and natural killer cell) induced anti-tumour immune activity through Herpes virus entry mediator (HVEM), (3) potentiation of proinflammatory cytokine/chemokine secretion through LTβR on tumour stromal cells, (4) direct induction of tumour cell apoptosis *in vitro*, and (5) the reorganisation of lymphatic tissue architecture, including within the tumour microenvironment (TME), by promoting high endothelial venule (HEV) formation and induction of tertiary lymphoid structures. LTBR (Lymphotoxin beta receptor) and HVEM rank highly amongst a range of costimulatory receptors in solid tumours, which raises interest in considering how LIGHT-mediated costimulation may be distinct from a growing list of immunotherapy targets which have failed to provide survival benefit as monotherapy or in combination with PD-1 inhibitors, particularly in the checkpoint acquired resistant setting.

* *Corresponding author.*
E-mail address: gfromm@shattucklabs.com (G. Fromm).

https://doi.org/10.1016/j.ejca.2023.03.040

1. Introduction

Over a decade has now passed since the landmark approvals of anti-PD1/L1 antibodies, and despite many attempts to combine other checkpoint targets with a PD-1/L1 backbone, most clinical studies examining checkpoint/checkpoint combinations have failed to improve upon the response to anti-PD1/L1 alone in any clinical setting [1]. This includes efforts that have focused on targeting compensatory checkpoint pathways like TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), TIGIT (T cell immunoreceptor with Ig and ITIM domains), IDO (Indoleamine-pyrrole 2,3-dioxygenase), NKG2A, CD73 (Cluster of Differentiation 73) and CD39 (Cluster of Differentiation 39), which can be up-regulated on T, NK, and other cell populations downstream of PD-1, and are associated with the acquisition of an exhausted phenotype [2–10]. An unfortunate and frustratingly common pattern in immunotherapy drug development follows the treatment paradigm of: identified lack of single-agent activity → subsequent encouraging activity in combination with a PD-1/L1 inhibitor in a single-arm study → ultimate lack of benefit of the combination compared to the PD-1/L1 control arm in a randomised and controlled clinical trial. So far, the only checkpoint combinations that have demonstrated additive benefit are combinations of PD-1/L1 and CTLA-4 inhibitors, or PD-1/L1 and LAG-3 inhibitors [1,6,7]. The recurring hypothesis tested the most over this decade of clinical experience has relied on the assumption that checkpoint/checkpoint combinations would yield profound response rates even in tumours which did not respond to PD-1/L1 monotherapy. Unfortunately, the data that has emerged thus far has proven this hypothesis to be invalid.

Usually, preclinical studies guide which combinations of checkpoint inhibitors and immune costimulators have the potential to improve anti-tumour responses, however with the exception of those targeting CD3 (Cluster of Differentiation 3) or CD28 (Cluster of Differentiation 28), most others have failed to translate into meaningful improvements in the clinic [11,12]. The largest class of costimulatory receptors fall within the tumour necrosis superfamily, which differs structurally from CD3 or CD28 and most require clustering as trimers or higher-order multimers of trimers for efficient downstream activation [13–15]. Some have proposed two 'categories' of TNF receptors, where category I TNFR (including BAFFR (B-cell activating factor receptor), DR3 (Death receptor 3), GITR (Glucocorticoid-induced TNFR-related protein), LTBR and TNFR1) signal following trimer formation, and category II TNFR (including 41BB, CD40, OX40, and others) which require hexamer formation for efficient signalling [16]. As a result, bivalent antibodies, and monovalent bispecific antibodies, have failed in a variety of clinical settings to efficiently activate trimeric or hexameric TNF receptors and multiple agents have been discontinued in early clinical development due to (1) safety concerns, (2) atypical bell-shaped dose/response profiles, and/or (3) lack of activity [17]. High doses of agonist antibodies can saturate target TNFRs and effector Fc (Fragment crystallizable) receptors independently, thereby reducing effective TNFR (tumor necrosis family receptor) clustering on target cells.

In the absence of ligand, a full-length TNFR exist in cell membranes as a mixture of monomers and dimers, whereas soluble TNFR exist primarily as monomers. Quantitative high-resolution microscopy studies of cells with physiological expression of TNFR1 demonstrated that 66% of TNFR1 molecules are present as monomers and 34% are present as dimers [18]. Following stimulation with ligand, the balance shifts to 13% TNFR1 monomers, 64% trimers, and 23% higher-order oligomers. Dimerisation of TNFR can occur primarily as a result of non-covalent, low-affinity, interactions between pre-ligand assembly domains (PLAD), which are typically in a low micromolar affinity range [19–21]. Ligand-induced trimerisation of TNFR is likely influenced by a variety of non-covalent interactions, including the PLAD domains, but the quantum of signalling transmitted by the cytoplasmic domains increases when ligand-induced avidity interactions lead to trimerisation, hexamerisation, and potentially higher-order network formation [22].

Fortunately, these learnings have increased our understanding of the immune microenvironment in the context of cancer, and we now know with increasing precision the types and effector potential of immune cells present in the tumour microenvironment (TME) of human tumours, the inhibitory and costimulatory receptors they express on their cell surface, and the extent to which certain cells can be awakened or repolarised under appropriate stimulation. Certain immune cells, such as gamma delta T cells, are associated with a highly favourable survival benefit, but are rare [23]. Myeloid cells are amongst the most common immune cells across tumour types but demonstrate a degree of plasticity which complicates defining specific subsets, which are generally associated with poor survival prognosis [15,24–28]. The development of combination strategies that target both the adaptive and innate immune systems will likely require thoughtful efforts to identify and validate synergistic activities. Here we present an analysis of the tumour and immune microenvironments using publicly available transcriptomic data and use it to identify abundantly expressed co-regulators in the TME. This analysis confirmed the high expression of many known checkpoint and costimulatory genes, and also identified less understood co-stimulatory pathways which could play key roles in T/NK/myeloid-mediated anti-tumour immunity in the setting of CPI (Check point inhibitor) resistance. We focus on members of the LIGHT (homologous to lymphotoxin, exhibits inducible expression and competes with HSV glycoprotein D for binding to herpesvirus entry mediator, a receptor expressed on T lymphocytes)/Herpes virus entry mediator (HVEM)/Lymphotoxin Beta Receptor (LTβR) signalling axis and why they may represent compelling therapeutic targets.

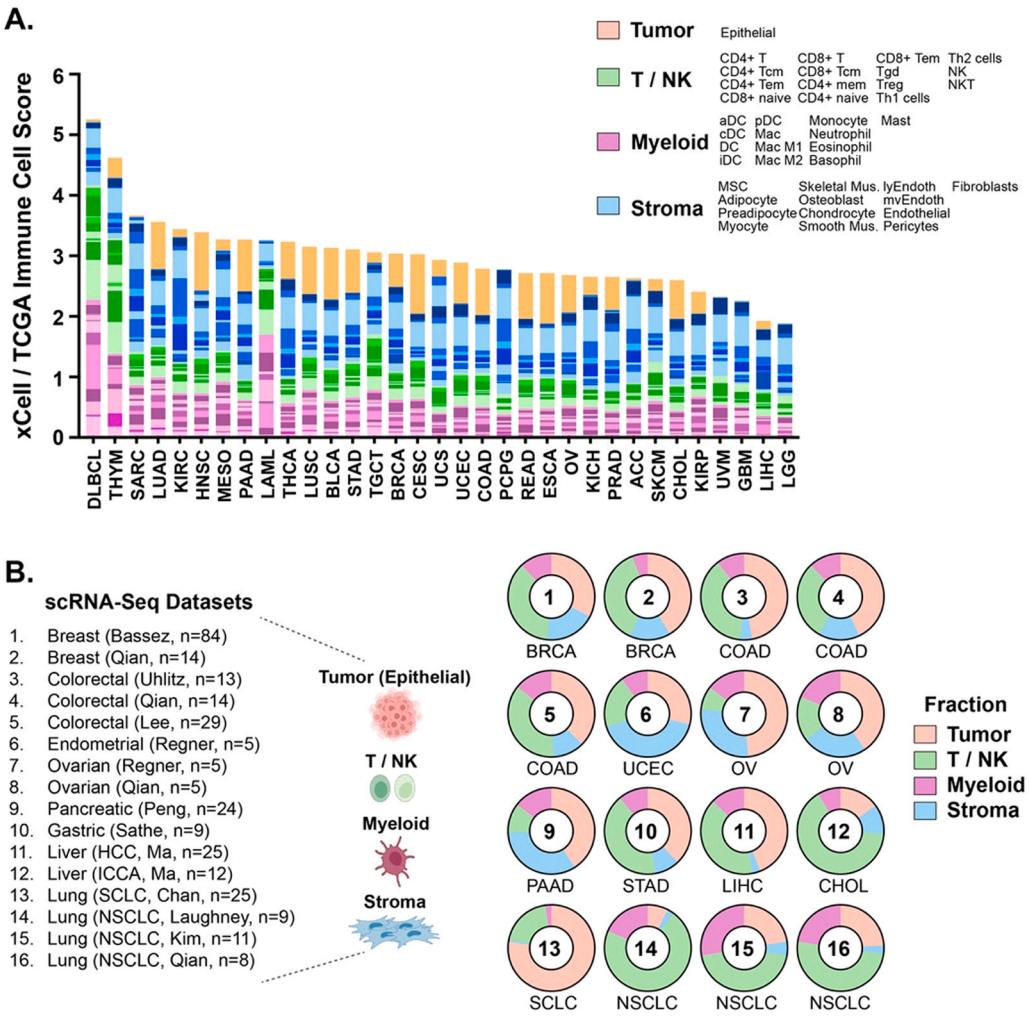

Fig. 1. Immune cell enrichment across tumour types. (A) Tumour, T/NK, myeloid, and stromal cell gene signatures defined by xCell were used on bulk RNA-seq from The Cancer Genome Atlas (TCGA) tumours to determine the enrichment scores of T, NK, and myeloid cells within tumours. (B) 16 publicly available single-cell RNA sequencing (scRNA-seq) datasets across a range of tumour types were evaluated for the fraction of tumour, T/NK, myeloid, and stomal cells based on the authors' defined cell type labels, grouped into the higher-level cell type categories.

2. Assessing the TME

We have entered an era where the dissection of the tumour/immune microenvironments using next-generation sequencing (NGS) is commonplace, and while much of this information has been generated at the transcriptomic level—which can differ from corresponding protein levels—RNA sequencing has nonetheless become a critical tool for identifying novel mechanisms underlying cancer therapy resistance to develop strategies to improve patient responses. The Cancer Genome Atlas (TCGA) is one such publicly available resource, and here we analysed bulk RNA-seq data from patients across 33 different TCGA tumour types and used xCell definitions for tumour, T/NK (natural killer), myeloid, and stromal cells (Fig. 1A) [29]. The overall enrichment scores for these four populations were rank ordered and clearly demonstrated that besides T and NK cells—which are known to be abundant in various solid tumours—that myeloid cells were also highly enriched (Fig. 1A). It should be noted that xCell is not intended to inform on cell type frequency and instead is simply a scoring method to estimate cell type enrichment. To extend this analysis, we assessed the relative frequency of tumour, T/NK, myeloid, and stromal cells across 16 distinct publicly available single-cell RNA sequencing (scRNA-seq) datasets generated from breast, colon, endometrial, ovarian, pancreatic, gastric, liver, and lung cancers [30–40]. Using author-defined immune gene signatures, the relative abundance of T/NK and myeloid cell populations was assessed and found to be consistently high with that of the TCGA analysis (Fig. 1B).

To better understand what other tumour/immune regulators are abundant in the TME that may be targetable, we performed transcriptomic analysis to assess the expression of 39 checkpoint (CP) and 57 co-stimulatory (co-stim) genes in cancer. CP (check point) gene expression was rank-ordered based on mean expression across all TCGA tumour types or the 16 scRNA-seq datasets presented in Fig. 1 (Fig. 2A–D). Interestingly, TCGA ranking identified enrichment of myeloid or

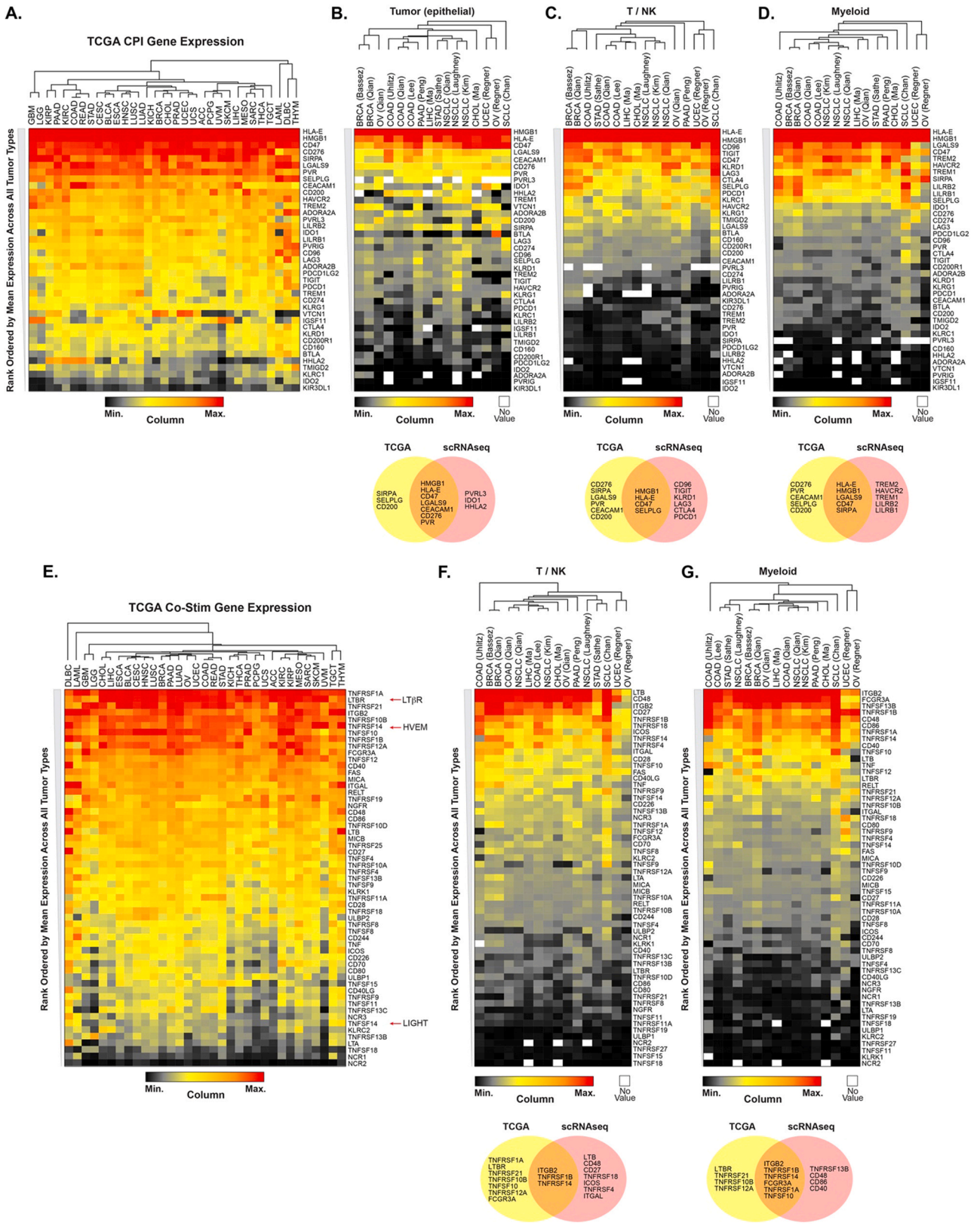
A.
TCGA CPI Gene Expression
Rank Ordered by Mean Expression Across All Tumor Types
Min.
Column
Max.
B.
Tumor (epithelial)
C.
T / NK
D.
Myeloid
No Value
TCGA
scRNAseq
HMGB1
HLA-E
CD47
LGALS9
CEACAM1
CD276
PVR
SIRPA
SELPLG
CD200
PVRL3
IDO1
HHLA2
CD96
TIGIT
KLRD1
CD160
TREM2
HAVCR2
LILRB2
LILRB1
E.
TCGA Co-Stim Gene Expression
LTβR
HVEM
LIGHT
F.
G.

Fig. 2. Checkpoint (CP) and co-stimulatory (co-stim) gene expression in cancer. The expression of CP genes was rank-ordered from (A) bulk The Cancer Genome Atlas (TCGA) RNA-seq and the single-cell RNA sequencing (scRNA-seq) datasets presented in Fig. 1, assessing CP expression in (B) tumour cells, (C) T/NK cells, or (D) myeloid cells. The expression of co-stim genes was rank-ordered from (E) bulk TCGA RNA-seq or the scRNA-seq datasets presented in Fig. 1, assessing co-stim expression on (F) T/NK cells or (G) myeloid cells. Genes are ranked according to the mean expression across all samples within a heatmap. The column min/max are depicted for each gene and hierarchical clustering (one minus Pearson correlation) was used to demonstrate the distances between tumour types. The top 10 expressed genes in each dataset are shown below the heatmaps in a Venn diagram that displays the overlap in the identified genes between TCGA and scRNA-seq. TCGA is based on the log2 average Transcripts per million (TPM) values of each gene across each patient within a tumour type. scRNA-seq represents the mean expression for each gene of interest and cell type, normalised by the observed UMIs in a cell for a particular gene divided by the total UMIs for that cell (across all genes), then multiplied by 10,000. Normalised counts were then transformed using log(x + 1) to put on a log scale, preserving zeros as zeros. For each gene and cell type combination in each study, the mean of the transformed values across cells was calculated.

tumour-specific checkpoints, including CD47, Cluster of Differentiation 276, B7 homolog 3 (CD276 (B7-H3)), SIRPA, and Selectin P Ligand, P-selectin glycoprotein ligand-1 (SELPLG (PSGL-1)), whereas T/NK checkpoint genes were ranked much lower, including PDCD1 (PD-1), TIGIT, LAG3 (Lymphocyte-activation gene 3), and CTLA4 (cytotoxic T-lymphocyte-associated protein 4) (Fig. 2A). Single-cell RNA sequencing allowed for the assessment of CP gene expression on isolated cell types, including tumour, T/NK, and myeloid cell fractions (Fig. 2B–D). There was a high overlap in the top expressed CP genes between TCGA and the tumour fraction of the single-cell datasets, however scRNA-seq more accurately defined CP genes that are specific to T/NK (Cluster of Differentiation 96, T cell activation, increased late expression (CD96 (TACTILE)), TIGIT, killer cell lectin-like receptor subfamily D, member 1 (KLRD1), LAG3, CTLA4, and PDCD1) and myeloid (TREM1 (Triggering receptor expressed on myeloid cells 1), HAVCR2 (TIM-3), TREM2 (Triggering receptor expressed on myeloid cells 2), LILRB2 (Leukocyte immunoglobulin-like receptor subfamily B member 2), and LILRB1 (Leukocyte immunoglobulin-like receptor subfamily B member 1)) cells. The analysis of bulk RNA-seq alone would not have resulted in the identification of these targets; however, the integration of bulk and scRNA-seq across tumours appeared to refine these results. The analysis of immune co-stimulatory genes rank ordered by mean expression across TCGA or scRNA-seq also demonstrated differences in the top hits identified between platforms (Fig. 2E–G). These hits included targets that have been pursued clinically, including CD27 (Cluster of Differentiation 27), TNFRSF18 (GITR), ICOS (Inducible T-cell costimulator), TNFRSF4 (OX40), CD86, and CD40.

Interestingly, this analysis framework also identified less appreciated tumour/immune co-regulators and may be useful as an initial triage for identifying targets of interest, particularly in patient populations where our mechanistic understanding is lacking, due to the availability of data (e.g. CPI-acquired resistance following anti-PD(L)1 therapy). For example, two of the most abundant co-stimulatory genes identified through both TCGA and scRNA-seq were TNFRSF14, also known as HVEM, and LTBR; which are both activated by the same TNF-ligand known as LIGHT (tumor necrosis factor superfamily (TNFSF14)). LTBR is highly expressed by both myeloid and stromal cells and is reported to participate in a wide range of stimulatory functions associated with anti-tumour activity. HVEM, the other major receptor for LIGHT, is most commonly associated with expression on T and NK cells, but can also be expressed on myeloid, B, endothelial, and some tumour cells. HVEM can provide direct costimulatory signals to lymphocytes and also competes with the co-inhibitory signals transmitted by BTLA (B- and T-lymphocyte attenuator). In the following sections we will provide an overview of what is known and highlight some of what is left to be learned, about the spectrum of costimulatory functions mediated by LIGHT.

3. TNF co-stimulatory ligand LIGHT

The LIGHT (homologous to Lymphotoxin, exhibits Inducible expression and competes with Herpes Simplex Virus glycoprotein D for Herpes Virus Entry Mediator, a receptor expressed by T cells) signalling axis is complex by design, as a mechanism to tightly control immune activities. The observation that expression of LIGHT in the thymus was associated with negative selection of potentially autoreactive T cells provided an important clue regarding the overall role of LIGHT in immunity [41]. LIGHT potentiates a range of immune activation signals through its receptors HVEM and LTβR. HVEM and LTβR can be co-expressed on the same cell and the ratio of HVEM to LTβR can result in differential signalling outcomes, which makes assessing the contribution of each receptor to the observed activity a challenge. HVEM is a TNF co-stimulatory receptor that is broadly expressed on immune cells, including effector T and NK cells. The importance of HVEM in modulating immune function is highlighted by the fact that herpes simplex virus evolved to bind HVEM as an entry point into immune cells that would otherwise be important for viral clearance, namely T and NK cells [42]. HVEM is stimulated by LIGHT, which is expressed by activated T cells and some antigen-presenting cells (APC) (Fig. 2E–F), resulting in NFκB/NIK mediated signalling, Th1 cytokine production, increased effector T cell function, and anti-tumour immune responses (Fig. 3A). Similar to other TNF-

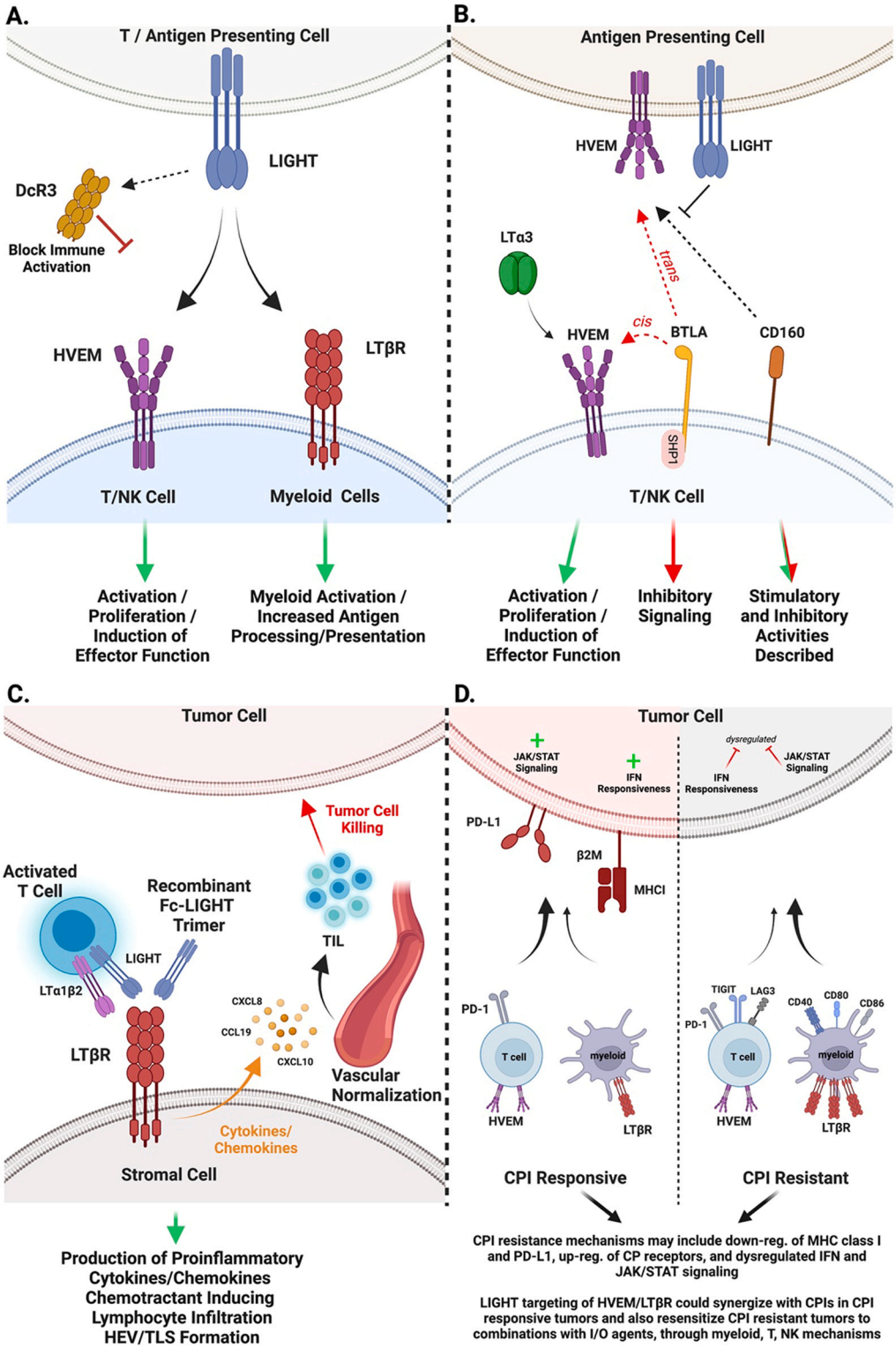
A.
T / Antigen Presenting Cell
LIGHT
DcR3
Block Immune Activation
HVEM
LTβR
T/NK Cell
Myeloid Cells
Activation / Proliferation / Induction of Effector Function
Myeloid Activation / Increased Antigen Processing/Presentation
B.
Antigen Presenting Cell
HVEM
LIGHT
LTα3
trans
cis
HVEM
BTLA
CD160
SHP1
T/NK Cell
Activation / Proliferation / Induction of Effector Function
Inhibitory Signaling
Stimulatory and Inhibitory Activities Described
C.
Tumor Cell
Tumor Cell Killing
Activated T Cell
Recombinant Fc-LIGHT Trimer
LIGHT
LTα1β2
LTβR
TIL
CXCL8
CCL19
CXCL10
Vascular Normalization
Cytokines/ Chemokines
Stromal Cell
Production of Proinflammatory Cytokines/Chemokines Chemotractant Inducing Lymphocyte Infiltration HEV/TLS Formation
D.
Tumor Cell
JAK/STAT Signaling
IFN Responsiveness
dysregulated
IFN Responsiveness
JAK/STAT Signaling
PD-L1
β2M
MHCI
PD-1
T cell
myeloid
HVEM
LTβR
TIGIT
LAG3
PD-1
CD40
CD80
CD86
T cell
myeloid
HVEM
LTβR
CPI Responsive
CPI Resistant
CPI resistance mechanisms may include down-reg. of MHC class I and PD-L1, up-reg. of CP receptors, and dysregulated IFN and JAK/STAT signaling
LIGHT targeting of HVEM/LTβR could synergize with CPIs in CPI responsive tumors and also resensitize CPI resistant tumors to combinations with I/O agents, through myeloid, T, NK mechanisms

Fig. 3. LIGHT (TNFSF14) Signalling Pathway. (A) Simplified view of LIGHT signalling, where binding to the Herpes virus entry mediator (HVEM) receptor expressed on T and NK cells can induce NFκB/NIK signalling, activation, proliferation, and enhanced effector function; and to Lymphotoxin Beta Receptor (LTβR) expressed on myeloid cells which activates target cells, enhances antigen processing/presentation and the secretion of proinflammatory cytokines. LIGHT can also bind to the soluble decoy receptor DcR3 which can competitively inhibit LIGHT binding to HVEM and LTβR. (B) HVEM can also interact with BTLA (inducing inhibitory signalling), CD160 (potential for both stimulatory and inhibitory activities), and LTα3 (inducing T/NK cell activation). These signalling events can further provide immune co-stimulation and/or inhibition and the remodelling of secondary lymphoid tissues. (C) Stromal cells in the tumour microenvironment express LTβR, which can be activated by LTα1β2 or LIGHT expressed on the surface of activated T cells or with therapeutics that present an active LIGHT trimer. This binding induces the production of proinflammatory cytokines and chemokines that can serve as chemoattractants that facilitate high endothelial venules (HEV)/tertiary lymphoid structures (TLS) formation, the normalisation of tumour vasculature, and the infiltration of tumour infiltrating lymphocytes (TIL) to induce anti-tumour responses. (D) CPI-responsive tumours can be associated with functional signalling, T cell infiltration, and tumour antigen-driven anti-tumour responses. CPI-resistant tumours evade the host immune system through various mechanisms. An increase in myeloid cells poised for activation in the TME may result in anti-tumour activity and/or the re-sensitisation of resistant tumours to CPI in combination with costimulation (figure created using BioRender).

receptors, LIGHT-dependent costimulation of lymphocytes can function through both T cell receptor (TCR)-dependent and -independent mechanisms [15]. LIGHT's interactions with LTβR can occur on a range of cell types, as LTβR is expressed on myeloid cells, stromal cells in the TME, and on many tumour cells. Ligation of LIGHT and LTβR can both directly and indirectly contribute to myeloid cell activation, enhanced antigen processing/presentation, and the induction of both adaptive and innate immune cytokines, including CCL19 (Chemokine (C-C motif) ligand 19), CCL21 (Chemokine (C-C motif) ligand 21), CXCL9 (Chemokine (C-X-C motif) ligand 9), CXCL8 (Chemokine (C-X-C motif) ligand 8), CXCL10 (Chemokine (C-X-C motif) ligand 10), and CXCL13 (Chemokine (C-X-C motif) ligand 13) (Fig. 3A) [15,43–47]. Consistently, LTβR has been associated with the differentiation and activation of follicular dendritic cells and lymphoid tissue organisation, and LTβR knockout mice fail to develop lymph nodes [48,49].

LIGHT is primarily upregulated transiently on activated T cells, but constitutive expression of LIGHT on T cells in LIGHT-transgenic mice resulted in peripheral autoimmunity, the induction of tissue destruction, and a reduction in thymic size, suggesting that mechanisms evolved to restrict LIGHT-mediated costimulation as a safeguard against autoimmune disease. The appearance of a decoy receptor, DcR3 (Decoy receptor 3), in humans was a relatively recent evolutionary event, which may have proven beneficial as an additional protection against LIGHT (as well as FasL and TL1A (TNF-like ligand 1A)) in the context of inflammatory disease [50–52]. BTLA belongs to the CD28 immunoglobulin superfamily and is a checkpoint receptor that has been shown to interact with HVEM either in *cis* or *trans*, to attenuate activation signals and facilitate immune memory formation (Fig. 3B) [53]. BTLA interactions with HVEM can block the ability of LIGHT to stimulate HVEM; however, BTLA is generally considered a weak checkpoint and trimeric LIGHT preferentially binds to HVEM through a non-competitive mechanism [54]. CD160 (Cluster of Differentiation 160) can also interact with HVEM and has been associated with both immune stimulatory and inhibitory activities [55,56]. Soluble LTα3 (Lymphotoxin(LT)α3) and T cell-expressed LTα1β2 (Lymphotoxin(LT)α1β2) can provide immune co-stimulation via HVEM and LTβR, respectively (Fig. 3B–C) [57].

Immature and abnormal vasculature is a common feature within tumours, resulting from the combined effects of rapid proliferation of tumour cells, inflammation and cytokine production within the tumour microenvironment, and localised tissue hypoxia. These effects result in an intratumoral vascular endothelium which can lack key selectins and integrins to support transendothelial migration of lymphocytes, and normalisation of vasculature within the TME is thought to be a mechanism associated with positive anti-tumour responses [58,59]. LTβR engagement on tumour stromal cells and the resulting secretion of cytokines and chemokines, contributes to these vascular responses through the formation of HEV and TLS, which can indirectly function as a chemoattractant to promote immune cell infiltration into the TME (Fig. 3C) [60–62]. Myeloid cells also appear to be critical for vasculature restructuring as the loss of F4/80+ macrophages limited TLS formation, and like LTβR knockout, prevented lymph node formation [49]. Recently, LTβR's role in HEV/TLS formation was shown to mediate infiltration of CD4/CD8 T and NK cells into the TME, which was enhanced in the presence of anti-VEGFR2 (vascular endothelial growth factor receptor 2), anti-PDL1 and an LTβR agonist antibody [63]. LIGHT/LTβR mediated remodelling or normalisation of the tumour vasculature and the associated increased infiltration of cytolytic T cells, enhanced the therapeutic activity of checkpoint inhibitor therapies that directly targeted those TIL. Along these lines, an LTβR agonist antibody was recently shown to increase the frequency and maturation of tumour-associated HEVs, resulting in an increase in

the ratio of stem-like CD8+ T cells to exhausted T cells, and improved anti-tumour efficacy in combination with CPI therapy [64].

Tagging recombinant LIGHT with a vasculature targeting peptide (VTP) was shown to preferentially localise LIGHT to the tumour vasculature through the upregulation of adhesion molecules like ICAM-1 (Intercellular Adhesion Molecule 1) and VCAM-1 (Vascular cell adhesion protein 1), which enhanced TLS formation, vasculature normalisation, and anti-tumour responses in orthotopic and metastatic preclinical tumour models [45,62]. Another preclinical approach at localising LIGHT to the TME included the adeno-associated viral (AAV) delivery of LIGHT intratumorally. This was successful in multiple preclinical models in controlling tumour growth and increasing the ratio of effector to regulatory T cells in the TME [65,66]. We developed a TIGIT-Fc-LIGHT bispecific fusion protein that was shown to stimulate T, NK, and myeloid cells in human PBMC (Peripheral blood monobuclear cells) cultures and in mice, which induced anti-tumour immunity in preclinical models of checkpoint inhibitor responsive and resistant tumours [15]. The *in vitro* engagement of tumour-expressed LTβR by recombinant LIGHT has also been shown to directly induce tumour cell apoptosis, and this activity appeared to occur in the absence of HVEM through FAS-associated death domain protein (FADD)/Caspase-8 activation, when co-treated with TNF-α [67–69].

It should be noted that direct versus indirect effects of HVEM/LTβR stimulation through LIGHT are challenging to separate in mixed PBMC cultures and *in vivo* systems, although some attempts have been made over the years to isolate the LIGHT/LTβR-specific role in myeloid cells. For example, macrophages stimulated *ex vivo* with LIGHT-VTP and adoptively transferred into tumour-bearing mice stimulated intratumoural TLS and expression of CCL21 and TNFα, suggesting that direct LIGHT/LTβR interactions altered the function of macrophages in a manner which influenced *in vivo* function [60]. In another study, LIGHT was shown to regulate the magnitude of CD40L (Cluster of Differentiation 40 ligand) activity on peripheral blood-derived dendritic cell maturation and the associated secretion IL-12 (interleukin 12), IL-6 (interleukin 6), and TNFα (Tumor necrosis factor alpha), compared to the activity of CD40L alone [70]. LTβR was also shown to play a role in dendritic cell homoeostasis, where CD11c (Cluster of Differentiation 11c) DCs (dendritic cell) accumulating around newly formed HEVs were dependent upon LTβR, as LTα, LTβ, or LTβR deficient animals demonstrated reduced DC numbers and reduced formation of HEVs [71].

The anti-tumour activities of LIGHT discussed thus far suggest that therapeutically targeting the LIGHT signalling axis, has the potential to provide broad immune co-stimulatory activities that could promote anti-tumour responses. Despite this, clinical efforts to develop LTβR agonists in oncology or antagonists in autoimmunity have not advanced and strategies to target HVEM or BTLA remain in early development [15,43,72]. Modest monotherapy activity was reported with the BTLA-blocking antibody icatolimab, with one confirmed PR and six SD (standard deviation) out of 19 evaluable patients. [73]. Icatolimab is now being pursued in advanced solid tumours in combination with anti-PD1 (anti-Programmed cell death protein 1 receptor) [74]. We have shown that a dual checkpoint blocking/immune stimulating bispecific fusion protein consisting of TIGIT-Fc-LIGHT blocked all poliovirus receptor (PVR)-ligand checkpoint molecules and provided broad immune co-stimulation via LIGHT, that controlled the growth of aggressive CPI-acquired resistance tumours. The observed anti-tumour immune responses were achieved through the combined activation of cytolytic T/NK cells, and also through LIGHT's activation of myeloid cells via LTβR, and the upregulation of MHC class II (Major histocompatibility complex class II), CD80 (Cluster of Differentiation 80), and CD86 (Cluster of Differentiation 86) on these cells, along with the induction of both adaptive and innate immune cytokines, including IL-2, TARC, MDC, MCP-1, MIP-2, and IL-12p70 [15]. The on-target pharmacodynamic activity of TIGIT-Fc-LIGHT translated to similar immune cell-activating and cytokine-inducing activities in non-human primate studies. However, maximum anti-tumour activity of TIGIT-Fc-LIGHT still required combination with anti-PD1 or anti-PDL1 [15]. Together, these findings highlight the importance of combination strategies that maximise the activation of both the adaptive and innate immune systems. In addition, further preclinical model and biomarker development efforts are needed to better inform on combination strategies that have the potential to (1) provide therapeutic benefit to patients with CPI-resistant tumours and/or (2) re-sensitise a resistant tumour to conventional checkpoint blockade.

4. LIGHT/HVEM/LTβR signalling axis in CPI-resistant tumours

While much of the data commonly mined to identify targets of interest preceded the widespread clinical use of anti-PD1, anti-PDL1, or anti-CTLA4 treatment, and/or when patient response data was not available or associated with treatment outcomes (e.g. TCGA), a growing body of data currently being generated allows for the assessment of mechanisms that drive resistance to CPI therapy where these mechanisms can be directly correlated with patient response. The development of new preclinical models has also advanced over the last several years and our confidence that these models more accurately mimic resistance mechanisms observed in human disease is growing, as preclinical and clinical

phenotypes of disease yield similar results. For example, the transcriptomic analysis of a murine tumour conditioned to develop anti-PD1 therapy resistance, revealed an unexpected hyperactivation in genes associated with interferon responsiveness, Jak/Stat signalling, and antigen processing/presentation pathways; however, the hyperactivation of these genes did not translate into increased protein levels, but instead defects in protein translation and trafficking pathways. Interestingly, this transcriptional phenotype was shared with that of NSCLC (non-small cell lung cancer) patients that initially responded to CPI therapy but later developed CPI-acquired resistance [75].

To assess the abundance of HVEM, LTBR, and other CPI/co-stim genes in CPI-resistant patients, we analysed publicly available scRNA-seq data on CD45+ leucocytes isolated from melanoma tumour biopsies in 32 patients that contributed to 30 sequenced lesions classified as 'non-responders' and 17 sequenced lesions characterised as 'responders' to anti-PD1, anti-CTLA4, or anti-PD1+anti-CTLA4 therapy [76]. UMAP (Uniform Manifold Approximation and Projection) demonstrated the cell type-specific expression of LTBR, HVEM (tumor necrosis factor receptor superfamily (TNFRSF14)), and LIGHT (TNFSF14) (Fig. 4A). LTBR expression was exclusive to myeloid cells and the magnitude of expression was greater in non-responding patients. HVEM was expressed at high levels across a range of cell types, including T, NK, and myeloid cells, and the expression of HVEM was also observed to be higher in non-responding patients. Interestingly, LIGHT was seen in T/NK cells, however, at low levels in both responding and non-responding patients (Fig. 4A–B). To assess whether other CP/co-stim genes were differentially expressed between responders and non-responders, genes associated with myeloid activation were compared between responders and non-responders on combined populations of monocytes, macrophages, and dendritic cells; referred to as MoMacDc. The pattern of expression of CD40 (Cluster of Differentiation 40), CD80, CD86, and CXCL10 was similar to that of LTBR and TNFRSF14, and higher expression was observed in non-responder MoMacDc as compared to responder populations (Fig. 4B). To assess more broadly, genes that were found to significantly differ between responding and non-responding groups with an adjusted p-value ≤0.05 in at least one of the following cell populations—T cells, B cells, monocytes, NK cells, macrophages, and DC (dendritic cells)—were identified and plotted in a heatmap that depicts the $\log_2$ fold-change in responders versus non-responders (Fig. 4C). At a high-level, these results demonstrated that very few T/NK CP or co-stim genes were appreciably different in expression between responders and non-responders (Fig. 4C; note the colours pink and white correspond to modest changes in expression). The primary cell type in which responding patients expressed higher levels of CP/co-stim genes than non-responding patients were B cells, and consistently, B/plasma cell presence in the TME has previously been reported to serve as a positive prognostic marker for response to CPI therapy [77,78].

What was striking here, was that the largest fold-change in CP/co-stim gene expression was observed on myeloid cells, where the expression of several myeloid checkpoints and co-stims were higher in non-responding patients as compared to responding patients (Fig. 4C; blue colour indicates higher expression in non-responders). This included LILRB2 and SIRPA on macrophages and monocytes, and LILRB1, LTBR, HAVCR2 (TIM-3), SELPLG (PSGL-1), TNFRSF14 (HVEM), CD40, CD80, CD86, and CXCL10 on macrophages, monocytes, and dendritic cells (Fig. 4B–C). The myeloid receptors TREM1 and TREM2 were also highly expressed on monocytes and macrophages; however, they were not differentially expressed between responders and non-responders (data not shown).

Overall survival clearly differentiated between responders and non-responders, with CPI-responding patients demonstrating a clear survival advantage (Fig. 4D). When the median values of either all CP or co-stim genes were used to bifurcate patients into high- and low-expressing populations, the level of CP/co-stim gene expression on T/NK cells did not predict differences in overall survival. Interestingly, CP/co-stim expression on myeloid cells (MoMacDc) did delineate overall survival between groups with high-expressing patients faring worse than CP/co-stim low patients, with a mortality rate of ~50% by 600 d (Fig. 4D). Patients in the high CP expression group were the same as the high co-stim expression group, therefore the Kaplan-Meir graphs are identical.

Together, these results suggest that the expression of CP and co-stim markers like LTBR and HVEM on non-responder myeloid cells could be predictive of response to CPIs and might present an opportunity to target these abundant receptors/ligands to convert non-responders into responders, particularly if their expression in CPI resistant patients correlates with the expression of other myeloid activation markers. This analysis does not provide any evidence of causality, or whether the increased expression of LTBR and HVEM corresponds to tonic activation or absence of downstream signalling from LTBR or HVEM; however, previous studies have suggested a negative feedback where LIGHT engagement resulted in the downregulation of HVEM [79]. This suggests that the high expression of HVEM in the TME is an indicator that costimulation through LIGHT is not present, and that the provision of a LIGHT activation signal could tip the balance back to reinitiate an adaptive anti-tumour immune response (Fig. 3D). Given the wide use of anti-PD1/L1 and anti-CTLA4 checkpoint inhibitor antibodies across many tumour types, CP acquired resistance is a growing medical problem and

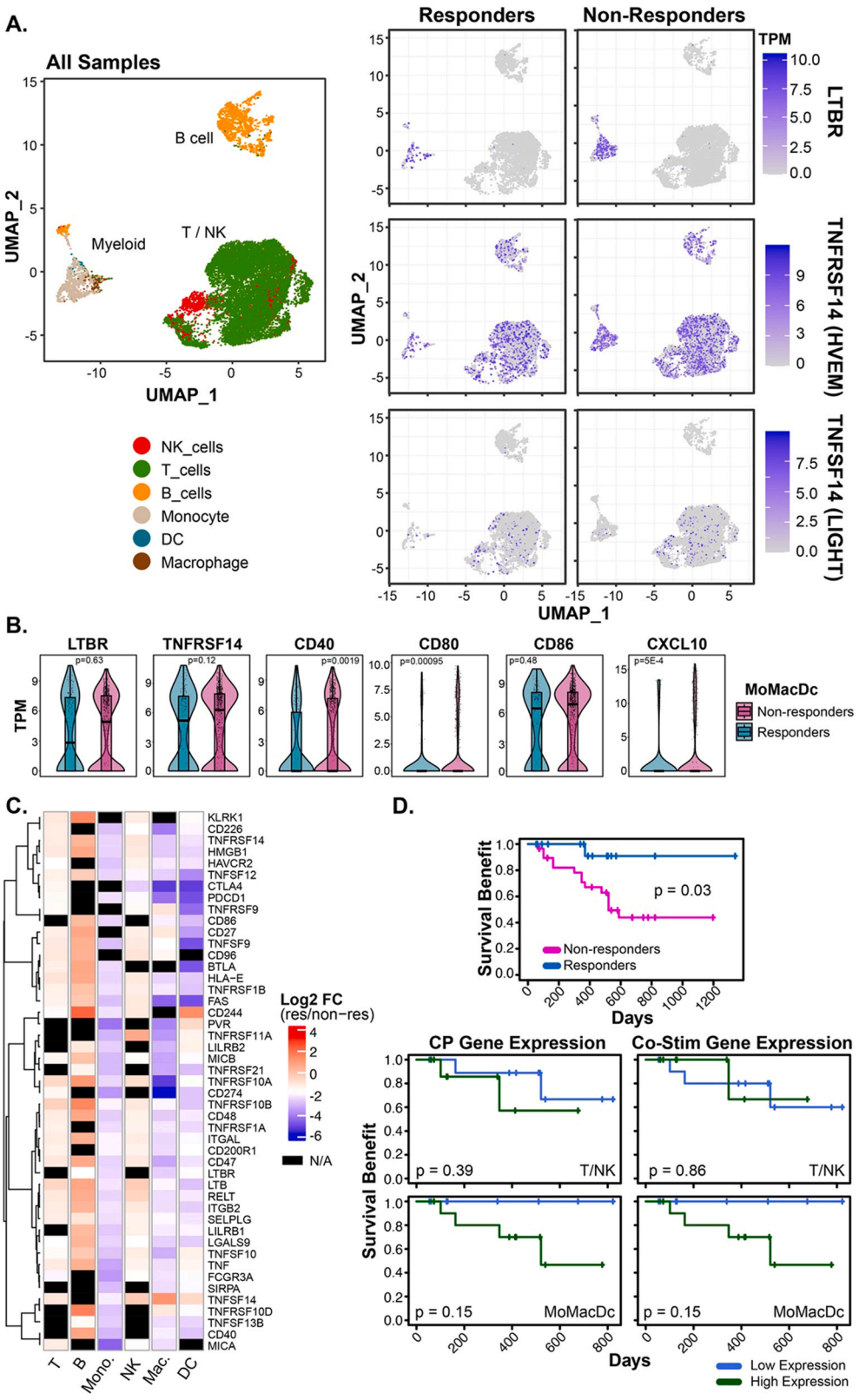
A.
All Samples
B cell
Myeloid
T / NK
UMAP_2
UMAP_1
NK_cells
T_cells
B_cells
Monocyte
DC
Macrophage
Responders
Non-Responders
TPM
LTBR
TNFRSF14 (HVEM)
TNFSF14 (LIGHT)
B.
LTBR
TNFRSF14
CD40
CD80
CD86
CXCL10
p=0.63
p=0.12
p=0.0019
p=0.00095
p=0.48
p=5E-4
MoMacDc
Non-responders
Responders
C.
KLRK1
CD226
TNFRSF14
HMGB1
HAVCR2
TNFSF12
CTLA4
PDCD1
TNFRSF9
CD86
CD27
TNFSF9
CD96
BTLA
HLA−E
TNFRSF1B
FAS
CD244
PVR
TNFRSF11A
LILRB2
MICB
TNFRSF21
TNFRSF10A
CD274
TNFRSF10B
CD48
TNFRSF1A
ITGAL
CD200R1
CD47
LTBR
LTB
RELT
ITGB2
SELPLG
LILRB1
LGALS9
TNFSF10
TNF
FCGR3A
SIRPA
TNFSF14
TNFRSF10D
TNFSF13B
CD40
MICA
T
B
Mono.
NK
Mac.
DC
Log2 FC
(res/non−res)
N/A
D.
Survival Benefit
p = 0.03
Non-responders
Responders
Days
CP Gene Expression
Co-Stim Gene Expression
p = 0.39
T/NK
p = 0.86
T/NK
p = 0.15
MoMacDc
p = 0.15
MoMacDc
Low Expression
High Expression

Fig. 4. The evaluation of CP and co-stim gene expression in checkpoint inhibitor responder and non-responder melanoma patients. Melanoma single-cell RNA sequencing (scRNA-seq) (GSE120575) from CD45+ leucocytes isolated from dissociated metastatic biopsy samples in 47 tumours from patients treated with anti-PD1, anti-CTLA4, or anti-PD1+ anti-CTLA4 was analysed. Cells were pruned if the gene count was above the 3*median absolute deviation (MAD) or below 250, if the unique molecular identifier (UMI) count was above 3*MAD or below 500, if the percentage of mitochondrial genes was above 3*MAD, contained an estimated contamination of ambient RNA ≥50% per DecontX, or was assessed as a doublet as having a hybrid doublet score ≥1. Expression data were scaled and clustered using the Louvain algorithm, and cell types were annotated using SingleR and the human primary cell atlas as a reference. RECIST (Response evaluation criteria in solid tumors) criteria were used to stratify patients as 'Responders' (complete or partial responses) and 'Non-Responders' (stable or progressive disease). Differential expression by response was performed using a Wilcoxon test for each cell type individually. (A) UMAP plot of immune cell populations (left) and the tissue-specific expression of TNFRSF14, LTBR, and LIGHT (right). (B) Expression of genes of interest between responders and non-responders in monocytes, macrophages, and dendritic cells (MoMacDc) with associated p-values. Horizontal lines indicate median expression levels. (C) Log_2 fold-change values in expression at significant CP and co-stim genes in at least one of the cell types assessed (adjusted p-value ≤0.05) between responders and non-responders. Fold-change was calculated using summations of TPM expression values for each cell type of responders and non-responders. Black boxes indicate values that were not available (N/A) due to lack of power. (D) Overall survival was plotted based on responders/non-responders and CP or co-stim high/low gene expression on T/NK cells and MoMacDc if the total sum of the CP or co-stim gene counts was greater than the median of the entire cohort.

identifying targets and strategies to re-sensitise tumours and immune systems to CPI therapy such as these, could be transformative.

5. Conclusion

The results presented throughout this review are an example of the complex nature of cancer disease aetiology, which is multifactorial and requires further study in order to design elegant strategies to effectively target CPI non-responding or acquired resistance populations. The integrated analysis of existing NGS data allows for the development of models to prioritise candidates of interest, that can be further optimised as the availability of relevant data continues to grow and experimental models to validate hypotheses are developed in the lab. Here, using publicly available TCGA and scRNA-seq datasets from a range of tumours, we identified the TNF-receptors HVEM and LTβR as two of the most highly expressed co-stimulatory receptors on immune cells in the TME, and propose that the therapeutic targeting of HVEM/LTβR by their shared ligand known as LIGHT, could represent a therapeutic strategy to improve responses in patients that have failed previous lines of CPI therapy and in the process acquired various resistance mechanisms. Therapies directed towards other targets identified through these exercises have recently entered clinical development (e.g. CD47, TREM1, TREM2, LILRB1, LILRB2, and PSGL-1) and it will be interesting to watch these clinical trials evolve. This review has highlighted the importance of combinatorial approaches of myeloid-targeting agents with conventional checkpoint blockade of T/NK cells as a strategy to effectively treat patients that have acquired resistance mechanisms to CPI therapy. Targeting the LIGHT/HVEM/LTβR axis, due to its broad immune activating potential, may be one such approach to improve response rates in cancer patients.

Funding

Provided by Shattuck Labs, Inc.

CRediT authorship contribution statement

CWS, THS, and GF wrote the review, analysed data, and generated figures. VMP and SRS analysed data and generated figures.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Casey Shuptrine, George Fromm, and Taylor Schreiber are all employees and shareholders of Shattuck Labs, Inc. Vincent Perez and Sara Selitsky are employees of QuantBio, part of Tempus Labs, and are paid as contractors by Shattuck Labs, Inc.

Acknowledgements

We would like to acknowledge Kevin Bullaughey of DataHelix for help with the bioinformatic analysis of publicly available scRNA-seq datasets.

References

[1] Wei SC, Anang NAS, Sharma R, Andrews MC, Reuben A, Levine JH, et al. Combination anti-CTLA-4 plus anti-PD-1 checkpoint blockade utilizes cellular mechanisms partially distinct from monotherapies. Proc Natl Acad Sci U S A 2019;116(45):22699–709.

[2] Curigliano G, Gelderblom H, Mach N, Doi T, Tai D, Forde PM, et al. Phase I/Ib clinical trial of sabatolimab, an anti-TIM-3 antibody, alone and in combination with spartalizumab, an anti-PD-1 antibody, in advanced solid tumors. Clin Cancer Res 2021;27(13):3620–9.

[3] Brunner AM, Kimmo Porkka JE, Knapper S, Vey N, Scholl S, Garcia-Manero G, et al. Efficacy and safety of sabatolimab

(MBG453) in combination with Hypomethylating Agents (HMAs) in patients with Acute Myeloid Leukemia (AML) and High-Risk Myelodysplastic Syndrome (HR-MDS): Updated Results from a Phase 1b Study. Blood 2020;136:1–2.
[4] Cho BC, Abreu DR, Hussein M, Cobo M, Patel AJ, Secen N, et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol 2022;23(6):781–92.
[5] Niu J, Maurice-Dror C, Lee DH, Kim DW, Nagrial A, Voskoboynik M, et al. First-in-human phase 1 study of the anti-TIGIT antibody vibostolimab as monotherapy or with pembrolizumab for advanced solid tumors, including non-small-cell lung cancer(.). Ann Oncol 2022;33(2):169–80.
[6] Tawbi HA, Hodi FS, Long GV. Nivolumab with or without relatlimab in untreated advanced melanoma. Reply. N Engl J Med 2022;386(19):1860–1.
[7] FDA approves anti-LAG3 checkpoint. Nat Biotechnol. 2022;40(5):625.
[8] van Hall T, Andre P, Horowitz A, Ruan DF, Borst L, Zerbib R, et al. Monalizumab: inhibiting the novel immune checkpoint NKG2A. J Immunother Cancer 2019;7(1):263.
[9] Tang K, Wu YH, Song Y, Yu B. Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors in clinical trials for cancer immunotherapy. J Hematol Oncol 2021;14(1):68.
[10] Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity 2016;44(5):989–1004.
[11] Tian Z, Liu M, Zhang Y, Wang X. Bispecific T cell engagers: an emerging therapy for management of hematologic malignancies. J Hematol Oncol 2021;14(1):75.
[12] Khan M, Arooj S, Wang H. Soluble B7-CD28 family inhibitory immune checkpoint proteins and anti-cancer immunotherapy. Front Immunol 2021;12:651634.
[13] de Silva S, Fromm G, Shuptrine CW, Johannes K, Patel A, Yoo KJ, et al. CD40 enhances Type I interferon responses downstream of CD47 blockade, bridging innate and adaptive immunity. Cancer Immunol Res 2020;8(2):230–45.
[14] Fromm G, de Silva S, Johannes K, Patel A, Hornblower JC, Schreiber TH. Agonist redirected checkpoint, PD1-Fc-OX40L, for cancer immunotherapy. J Immunother Cancer 2018;6(1):149.
[15] Yoo KJ, Johannes K, Gonzalez LE, Patel A, Shuptrine CW, Opheim Z, et al. LIGHT (TNFSF14) costimulation enhances myeloid cell activation and antitumor immunity in the setting of PD-1/PD-L1 and TIGIT checkpoint blockade. J Immunol 2022;209(3):510–25.
[16] Kucka K, Wajant H. Receptor oligomerization and its relevance for signaling by receptors of the tumor necrosis factor receptor superfamily. Front Cell Dev Biol 2020;8:615141.
[17] Garber K. Immune agonist antibodies face critical test. Nat Rev Drug Discov 2020;19(1):3–5.
[18] Karathanasis C, Medler J, Fricke F, Smith S, Malkusch S, Widera D, et al. Single-molecule imaging reveals the oligomeric state of functional TNFalpha-induced plasma membrane TNFR1 clusters in cells. Sci Signal 2020;13(614).
[19] Cao J, Meng F, Gao X, Dong H, Yao W. Expression and purification of a natural N-terminal pre-ligand assembly domain of tumor necrosis factor receptor 1 (TNFR1 PLAD) and preliminary activity determination. Protein J 2011;30(4):281–9.
[20] Smulski CR, Beyrath J, Decossas M, Chekkat N, Wolff P, Estieu-Gionnet K, et al. Cysteine-rich domain 1 of CD40 mediates receptor self-assembly. J Biol Chem 2013;288(15):10914–22.
[21] Lee HS, Chang JS, Baek JA, Chung MY, Lee HC, Rhim BY, et al. TNF-alpha activates death pathway in human aorta smooth muscle cell in the presence of 7-ketocholesterol. Biochem Biophys Res Commun 2005;333(4):1093–9.
[22] Wajant H. Principles of antibody-mediated TNF receptor activation. Cell Death Differ 2015;22(11):1727–41.
[23] Kabelitz D, Serrano R, Kouakanou L, Peters C, Kalyan S. Correction to: cancer immunotherapy with gammadelta T cells: many paths ahead of us. Cell Mol Immunol 2020;17(10):1118.
[24] Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends Immunol 2016;37(3):208–20.
[25] Butowski N, Colman H, De Groot JF, Omuro AM, Nayak L, Wen PY, et al. Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. Neuro Oncol 2016;18(4):557–64.
[26] Cassier PA, Italiano A, Gomez-Roca CA, Le Tourneau C, Toulmonde M, Cannarile MA, et al. CSF1R inhibition with emactuzumab in locally advanced diffuse-type tenosynovial giant cell tumours of the soft tissue: a dose-escalation and dose-expansion phase 1 study. Lancet Oncol 2015;16(8):949–56.
[27] Lin CC. Clinical development of colony-stimulating factor 1 receptor (CSF1R) inhibitors. J Immunother Precis Oncol. 2021;4(2):105–14.
[28] Kim SK, Cho SW. The evasion mechanisms of cancer immunity and drug intervention in the tumor microenvironment. Front Pharmacol 2022;13:868695.
[29] Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 2017;18(1):220.
[30] Ma L, Wang L, Khatib SA, Chang CW, Heinrich S, Dominguez DA, et al. Single-cell atlas of tumor cell evolution in response to therapy in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. J Hepatol 2021;75(6):1397–408.
[31] Qian J, Olbrecht S, Boeckx B, Vos H, Laoui D, Etlioglu E, et al. A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. Cell Res 2020;30(9):745–62.
[32] Bassez A, Vos H, Van Dyck L, Floris G, Arijs I, Desmedt C, et al. A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. Nat Med 2021;27(5):820–32.
[33] Lee HO, Hong Y, Etlioglu HE, Cho YB, Pomella V, Van den Bosch B, et al. Lineage-dependent gene expression programs influence the immune landscape of colorectal cancer. Nat Genet 2020;52(6):594–603.
[34] Uhlitz F, Bischoff P, Peidli S, Sieber A, Trinks A, Luthen M, et al. Mitogen-activated protein kinase activity drives cell trajectories in colorectal cancer. EMBO Mol Med 2021;13(10):e14123.
[35] Regner MJ, Wisniewska K, Garcia-Recio S, Thennavan A, Mendez-Giraldez R, Malladi VS, et al. A multi-omic single-cell landscape of human gynecologic malignancies. Mol Cell 2021;81(23):4924–41. e10.
[36] Sathe A, Grimes SM, Lau BT, Chen J, Suarez C, Huang RJ, et al. Single-Cell genomic characterization reveals the cellular reprogramming of the gastric tumor microenvironment. Clin Cancer Res 2020;26(11):2640–53.
[37] Chan JM, Quintanal-Villalonga A, Gao VR, Xie Y, Allaj V, Chaudhary O, et al. Signatures of plasticity, metastasis, and immunosuppression in an atlas of human small cell lung cancer. Cancer Cell 2021;39(11):1479–96. e18.
[38] Kim N, Kim HK, Lee K, Hong Y, Cho JH, Choi JW, et al. Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. Nat Commun 2020;11(1):2285.
[39] Laughney AM, Hu J, Campbell NR, Bakhoum SF, Setty M, Lavallee VP, et al. Regenerative lineages and immune-mediated pruning in lung cancer metastasis. Nat Med 2020;26(2):259–69.
[40] Peng J, Sun BF, Chen CY, Zhou JY, Chen YS, Chen H, et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and

malignant progression in pancreatic ductal adenocarcinoma. Cell Res 2019;29(2):725–38.
[41] Wang J, Chun T, Lo JC, Wu Q, Wang Y, Foster A, et al. The critical role of LIGHT, a TNF family member, in T cell development. J Immunol 2001;167(9):5099–105.
[42] Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity. 1998;8(1):21–30.
[43] Lukashev M, LePage D, Wilson C, Bailly V, Garber E, Lukashin A, et al. Targeting the lymphotoxin-beta receptor with agonist antibodies as a potential cancer therapy. Cancer Res 2006;66(19):9617–24.
[44] Ware CF. Targeting the LIGHT-HVEM pathway. Adv Exp Med Biol 2009;647:146–55.
[45] Johansson-Percival A, Ganss R. Therapeutic induction of tertiary lymphoid structures in cancer through stromal remodeling. Front Immunol 2021;12:674375.
[46] Wang Y, Zhu M, Miller M, Fu YX. Immunoregulation by tumor necrosis factor superfamily member LIGHT. Immunol Rev 2009;229(1):232–43.
[47] So T, Lee SW, Croft M. Tumor necrosis factor/tumor necrosis factor receptor family members that positively regulate immunity. Int J Hematol 2006;83(1):1–11.
[48] Peduto L, Dulauroy S, Lochner M, Spath GF, Morales MA, Cumano A, et al. Inflammation recapitulates the ontogeny of lymphoid stromal cells. J Immunol 2009;182(9):5789–99.
[49] Furtado GC, Pacer ME, Bongers G, Benezech C, He Z, Chen L, et al. TNFalpha-dependent development of lymphoid tissue in the absence of RORgammat(+) lymphoid tissue inducer cells. Mucosal Immunol 2014;7(3):602–14.
[50] Shaikh RB, Santee S, Granger SW, Butrovich K, Cheung T, Kronenberg M, et al. Constitutive expression of LIGHT on T cells leads to lymphocyte activation, inflammation, and tissue destruction. J Immunol 2001;167(11):6330–7.
[51] Wang J, Lo JC, Foster A, Yu P, Chen HM, Wang Y, et al. The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. J Clin Invest 2001;108(12):1771–80.
[52] Hsieh SL, Lin WW. Decoy receptor 3: an endogenous immunomodulator in cancer growth and inflammatory reactions. J Biomed Sci 2017;24(1):39.
[53] Cheung TC, Oborne LM, Steinberg MW, Macauley MG, Fukuyama S, Sanjo H, et al. T cell intrinsic heterodimeric complexes between HVEM and BTLA determine receptivity to the surrounding microenvironment. J Immunol 2009;183(11):7286–96.
[54] Cheung TC, Steinberg MW, Oborne LM, Macauley MG, Fukuyama S, Sanjo H, et al. Unconventional ligand activation of herpesvirus entry mediator signals cell survival. Proc Natl Acad Sci U S A 2009;106(15):6244–9.
[55] Rodriguez-Barbosa JI, Schneider P, Weigert A, Lee KM, Kim TJ, Perez-Simon JA, et al. HVEM, a cosignaling molecular switch, and its interactions with BTLA, CD160 and LIGHT. Cell Mol Immunol 2019;16(7):679–82.
[56] Sedy JR, Ramezani-Rad P. HVEM network signaling in cancer. Adv Cancer Res 2019;142:145–86.
[57] Mintz MA, Felce JH, Chou MY, Mayya V, Xu Y, Shui JW, et al. The HVEM-BTLA axis restrains T cell help to germinal center B cells and functions as a cell-extrinsic suppressor in lymphomagenesis. Immunity. 2019;51(2):310–23. e7.
[58] Yang T, Xiao H, Liu X, Wang Z, Zhang Q, Wei N, et al. Vascular normalization: a new window opened for cancer therapies. Front Oncol 2021;11:719836.
[59] Quezada SA, Peggs KS, Simpson TR, Shen Y, Littman DR, Allison JP. Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. J Exp Med 2008;205(9):2125–38.
[60] Johansson-Percival A, He B, Li ZJ, Kjellen A, Russell K, Li J, et al. De novo induction of intratumoral lymphoid structures and vessel normalization enhances immunotherapy in resistant tumors. Nat Immunol 2017;18(11):1207–17.
[61] Lu TT, Browning JL. Role of the lymphotoxin/LIGHT system in the development and maintenance of reticular networks and vasculature in lymphoid tissues. Front Immunol 2014;5:47.
[62] He B, Johansson-Percival A, Backhouse J, Li J, Lee GYF, Hamzah J, et al. Remodeling of metastatic vasculature reduces lung colonization and sensitizes overt metastases to immunotherapy. Cell Rep 2020;30(3):714–24. e5.
[63] Hua Y, Vella G, Rambow F, Allen E, Antoranz Martinez A, Duhamel M, et al. Cancer immunotherapies transition endothelial cells into HEVs that generate TCF1(+) T lymphocyte niches through a feed-forward loop. Cancer Cell 2022;40(12):1600–18. e10.
[64] Asrir A, Tardiveau C, Coudert J, Laffont R, Blanchard L, Bellard E, et al. Tumor-associated high endothelial venules mediate lymphocyte entry into tumors and predict response to PD-1 plus CTLA-4 combination immunotherapy. Cancer Cell 2022;40(3):318–34. e9.
[65] Washburn ML, Kovalev GI, Koroleva E, Fu YX, Su L. LIGHT induces distinct signals to clear an AAV-expressed persistent antigen in the mouse liver and to induce liver inflammation. PLoS One 2010;5(5):e10585.
[66] Yu P, Lee Y, Wang Y, Liu X, Auh S, Gajewski TF, et al. Targeting the primary tumor to generate CTL for the effective eradication of spontaneous metastases. J Immunol 2007;179(3):1960–8.
[67] Hu X, Zimmerman MA, Bardhan K, Yang D, Waller JL, Liles GB, et al. Lymphotoxin beta receptor mediates caspase-dependent tumor cell apoptosis in vitro and tumor suppression in vivo despite induction of NF-kappaB activation. Carcinogenesis. 2013;34(5):1105–14.
[68] Bechill J, Muller WJ. Herpesvirus entry mediator (HVEM) attenuates signals mediated by the lymphotoxin beta receptor (LTbetaR) in human cells stimulated by the shared ligand LIGHT. Mol Immunol 2014;62(1):96–103.
[69] Fernandes MT, Dejardin E, dos Santos NR. Context-dependent roles for lymphotoxin-beta receptor signaling in cancer development. Biochim Biophys Acta 2016;1865(2):204–19.
[70] Morel Y, Truneh A, Sweet RW, Olive D, Costello RT. The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. J Immunol 2001;167(5):2479–86.
[71] Kabashima K, Banks TA, Ansel KM, Lu TT, Ware CF, Cyster JG. Intrinsic lymphotoxin-beta receptor requirement for homeostasis of lymphoid tissue dendritic cells. Immunity. 2005;22(4):439–50.
[72] Aubert N, Brunel S, Olive D, Marodon G. Blockade of HVEM for prostate cancer immunotherapy in humanized mice. Cancers (Basel) 2021;13(12).
[73] Schilder RJ, Powderly JD, Park H, Bilen MA, McKean M, May R, et al. Phase Ia dose-escalation study of the anti-BTLA antibody icatolimab as a monotherapy in patients with advanced solid tumor. J Clin Oncol 2022;40(16):2643.
[74] Ma JXY, Zhang H, Song Y, Zhao W, Pan Y, Ran F, et al. Phase I study of the anti-BTLA antibody icatolimab as a single agent or in combination with toripalimab in relapsed/refractory lymphomas. J Clin Oncol 2022;40(16):7578.
[75] Memon DRH, Fromm G, Lihm J, Schoenfeld AJ, Sauter JL, Luo J, et al. Clinical and molecular features of acquired resistance to immunotherapy in non-small cell lung cancer. bioRxiv 2022. https://doi.org/10.1101/2021.07.21.452854v1.
[76] Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. Cell 2018;175(4):998–1013. e20.

[77] Patil NS, Nabet BY, Muller S, Koeppen H, Zou W, Giltnane J, et al. Intratumoral plasma cells predict outcomes to PD-L1 blockade in non-small cell lung cancer. Cancer Cell 2022;40(3):289–300. e4.

[78] Selitsky SR, Mose LE, Smith CC, Chai S, Hoadley KA, Dittmer DP, et al. Prognostic value of B cells in cutaneous melanoma. Genome Med 2019;11(1):36.

[79] Morel Y, Schiano de Colella JM, Harrop J, Deen KC, Holmes SD, Wattam TA, et al. Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. J Immunol 2000;165(8):4397–404.