LIGHT (TNFSF14) Co-stimulation with TIGIT blockade broadens the activity of checkpoint inhibitors (CPIs) into CPI refractory and resistant tumors through targeted myeloid cell and effector lymphocyte activation

#4214

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Abstract

Although increased TIGIT expression on TILs is associated with poor survival in patients with cancer, monotherapy TIGIT antibody blockade has not yet demonstrated significant clinical activity. PD-1 is commonly co-expressed on TILs, and PD-1 mediated SHP-2 signaling inhibits DNAM-1 (CD226), preventing PVR co-stimulation in the setting of TIGIT blockade. The interaction between PD-1 and CD226 explains why TIGIT blockade only translates to clinical benefit in the setting of co-blockade of PD-1/L1, however does not explain why co-blockade of TIGIT and PD-1/L1 does not provide benefit in advanced and PD-1/L1 resistant tumors. In advanced and PD-1/L1 resistant tumors, progressive downregulation of CD226 has been reported. Thus, we hypothesized that the lack of CD226 may underlie the lack of clinical responses to combined TIGIT/PD-1/L1 blockade in PD-1/L1 experienced tumors. We therefore sought to identify alternative costimulatory receptors with high expression.

Analysis of TCGA, as well as single-cell RNA-seq from TILs, identified HVEM and LTβR as two costimulatory receptors with higher expression in advanced cancers as compared to CD226. HVEM and LTβR directly activate CD8+ T, natural killer (NK), and myeloid cells when bound by their TNF superfamily ligand, known as LIGHT. LIGHT potentiates effector lymphocyte function through signaling that obviates the costimulatory role of DNAM-1. We demonstrated that when the extracellular domain (ECD) of LIGHT is linked to the ECD of TIGIT on a TIGIT-Fc-LIGHT bi-functional fusion protein, the simultaneous blockade of PVR, PVRL2, PVRL3, and Nectin-4 and immune co-stimulation by LIGHT increased CD8+ T and NK infiltration into tumors. This translated into tumor cell killing, regression of established tumors, and improved survival in preclinical models of checkpoint primary and acquired resistance.

The translation of TIGIT-Fc-LIGHT activity was evaluated in cynomolgus macaques and was well tolerated at doses up to 40 mg/kg. A series of adaptive immune and proinflammatory cytokines, including IL-2, CCL2, CCL4, IL-10, CXCL10, and CCL17, were induced within two hours of TIGIT-Fc-LIGHT infusion. Additionally, receptor engagement led to the rapid margination of HVEM+CD8+ T cells from the periphery into secondary immune tissues. This network of cytokines and post-dose immune cell margination identified in monkey, was identical to findings in murine models that ultimately translated into significant anti-tumor responses.

Lastly, the combination of TIGIT-Fc-LIGHT with anti-PD(L)1 broadened anti-tumor activity of the checkpoint antibodies in preclinical aggressive CPI-resistant tumors. These results suggest that LIGHT could be the differentiator that extends the clinical activity of conventional CPIs into PD-L1^{low} or CPI acquired resistance tumors.

Costimulatory Receptor Identification

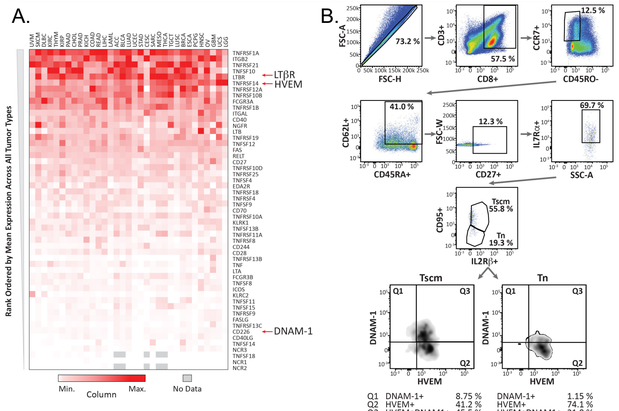


Figure 1. Nomination of HVEM and LTβR as TNF-receptors highly expressed in tumors and on a range of immune cells. (A) The expression of 53 immune co-stimulatory genes was rank ordered across all TCGA cancers, identifying HVEM and LTBR in the top 10. (B) HVEM expression was characterized in relation to DNAM-1 expression on T stem cell memory (Tscm) cells induced for 9 days with CD3/CD28, IL-2, and a GSK3β inhibitor.

Generation of TIGIT-Fc-LIGHT

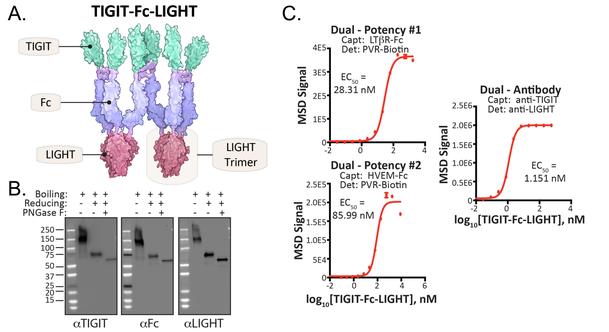


Figure 2. TIGIT-Fc-LIGHT characterization and receptor binding. (A) Hexameric TIGIT-Fc-LIGHT structure consisting of six TIGIT extracellular domains linked to two sets of active LIGHT trimers. (B) Each domain of TIGIT-Fc-LIGHT was detected using Western blot under native, reduced (BME), and reduced/deglycosylated conditions. (C) MSD demonstrated simultaneous binding of TIGIT-Fc-LIGHT to checkpoint targets (PVR) and immune co-stimulatory receptors found on myeloid, CD8+ T, and NK cells (LTβR and HVEM).

Fc-Independent Immune Activation

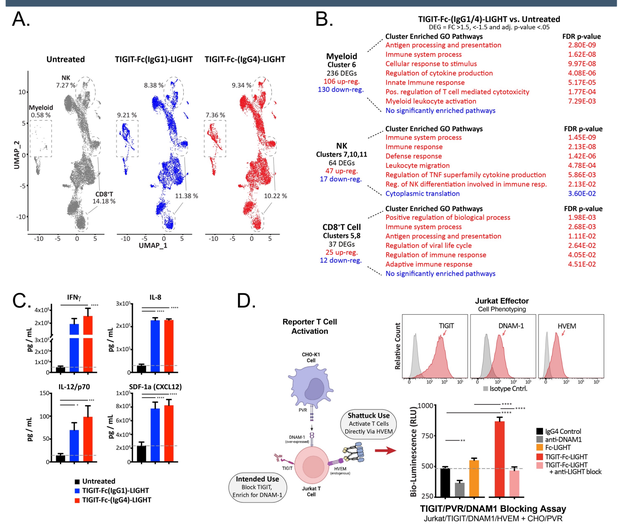
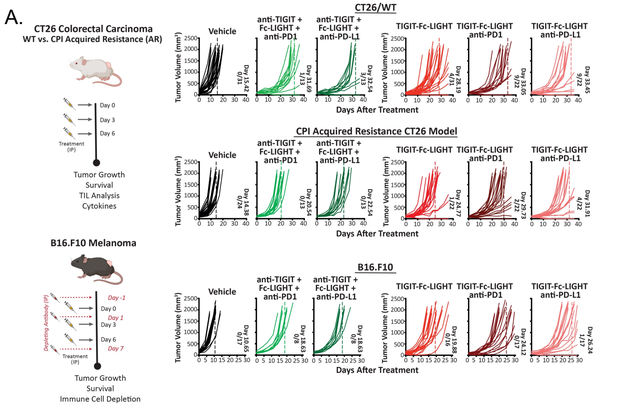


Figure 3. TIGIT-Fc-LIGHT stimulated myeloid, CD8+ T, and NK cells with both FcγR competent and inert Fc domains. (A) Single cell RNA-seq was performed on human PBMC cultured with IgG1 or IgG4 variants of TIGIT-Fc-LIGHT for 2 days in AIMV media. UMAP was used to visualize cell populations that corresponded to myeloid, CD8+ T, or NK cells (identified elsewhere using SingleR and ImmuneExp). (B) PANTHER was used to identify pathways associated with TIGIT-Fc-LIGHT induced DEGs. (C) After 2 days, cytokines were assessed in the culture supernatant using MSD. (D) Jurkat effector cells from a commercially available PVR:DNAM1 reporter assay were found to also express human HVEM. TIGIT-Fc-LIGHT bypassed the need for DNAM-1 co-stimulation and directly activated downstream signaling via HVEM.

TIGIT-Fc-LIGHT Anti-Tumor Activity



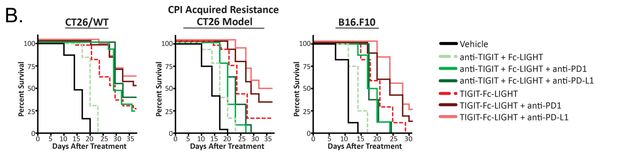


Figure 4. Anti-tumor activity of the murine TIGIT-Fc-LIGHT surrogate. (A) BALB/c mice were implanted with wild-type (WT) CT26 tumors, or tumors that were conditioned *in vivo* to develop anti-PD1 acquired resistance (CT26/AR), and C57BL/6 mice were implanted with B16.F10 melanoma tumors. When tumors established, mice were treated with vehicle (PBS), anti-TIGIT (1G9), Fc-LIGHT, anti-PD1 (RMP1-14), anti-PD-L1 (10F.9G2), or mTIGIT-Fc-LIGHT (controls at 100 μg and TIGIT-Fc-LIGHT at 200 μg per dose). Shown are the individual animal tumor growth curves, the average day in which each group reached tumor burden, and the number of mice that completely rejected the tumor in response to treatment. (B) Kaplan-Meir survival curves for various treatment groups in the CT26/WT, CT26/AR, and B16.F10 preclinical tumor models.

Enhanced Lymphocyte Infiltration into Tumors

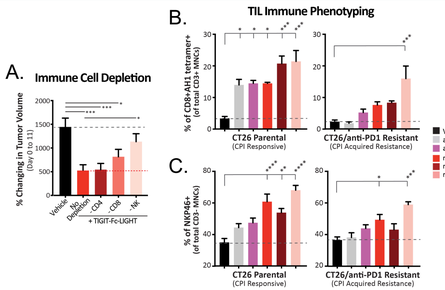
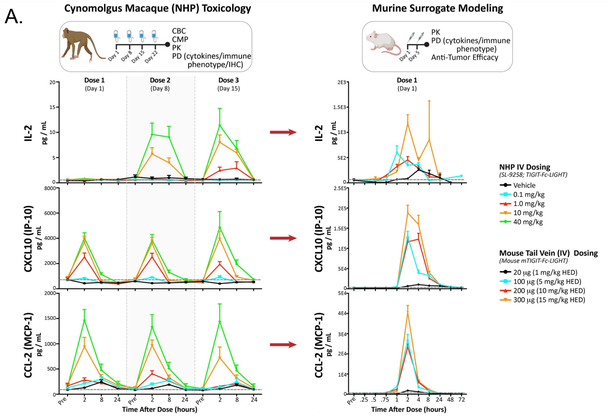


Figure 5. TIGIT-Fc-LIGHT *in vivo* activity. (A) The impact of CD4+ T, CD8+ T, or NK cells on TIGIT-Fc-LIGHT was evaluated in the B16.F10 model, through immune cell depletion studies. (B) Tumors were isolated from a cohort of treated animals 9 days after the initial treatment. Tumors were dissociated and assessed by flow cytometry for populations of antigen-specific CD8+ T cells (AH1 tetramer+) and (C) NKP46+ NK cells.

Translation of Pharmacodynamic Activity



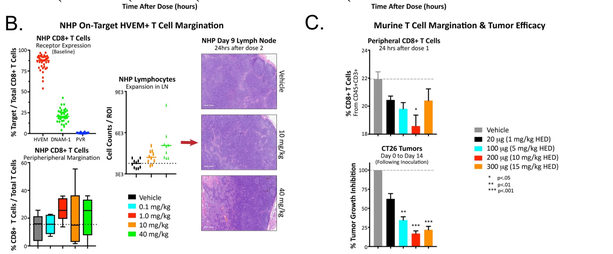


Figure 6. TIGIT-Fc-LIGHT pharmacodynamic (PD) activity in cynomolgus macaque. Cynomolgus macaques were given 4 weekly IV infusions (days 1, 8, 15, 22) of vehicle or 0.1, 1.0, 10, or 40 mg/kg of SL-9258 (TIGIT-Fc-LIGHT), which was well-tolerated and did not significantly impact organ function, hemoglobin, hematocrit, or peripheral counts of neutrophils, basophils, eosinophils, or platelets. (A) Both human TIGIT-Fc-LIGHT in NHP (left) and the murine surrogate (right, given by tail vein injection on days 1 and 5) rapidly induced both adaptive and innate immune serum cytokines. (B) Like human and mouse, HVEM was widely expressed on NHP CD8+ T cells and on-target binding by TIGIT-Fc-LIGHT resulted in dose-dependent margination of CD8+ T cells out of the periphery, which was associated with an expansion of lymphocytes in the lymph nodes on day 9. (C) A similar dose-dependent margination of CD8+ T cells out of the periphery, was observed in mice, and was accompanied by anti-tumor activity in CT26 colorectal carcinoma bearing animals.

Conclusions

TIGIT-Fc-LIGHT was designed to overcome the limitations of TIGIT blocking antibodies through:

- Preserved co-stimulation in advanced tumors (through HVEM)
- Direct myeloid cell activation (via LTβR)
- Co-stimulation that is not dependent on PD-1 blockade (as it is for DNAM-1)
- Blockade of all known TIGIT ligands (PVR, PVRL2, PVRL3, and Nectin-4)
- No risk of depleting effector lymphocytes (no dependence on Fc composition)
- Translation of on-target pharmacodynamic activity into NHP

Pre-clinical data indicate that these goals were achieved, and further development is warranted.