Abundance of *KLRB1*+ (CD161) T cells in anti-PD1 non responders coupled with enhanced tumor cytotoxicity of anti-CD161 (IMT-009) with anti-PD1 makes it a rational target for combination with anti-PD-(L)1 immunotherapy

Poster #1375

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Background

CD161 (*KLRB1*), a C type lectin related transmembrane protein, and its ligand CLEC2D make a key immunomodulatory pathway in both T and NK cells that suppresses their activation and cytotoxic function[1,2]. Immunitas has developed a fully human anti CD161 monoclonal antibody called IMT-009 that can restore both NK mediated cytotoxicity and polyfunctionality of CD4 and CD8 T cells. We have also shown monotherapy activity of IMT-009 in an *in vivo* humanized mouse model of B cell lymphoma that is unresponsive to anti-PD-(L)1. Furthermore, we have shown that IMT-009 enhances NK mediated antibody dependent cellular cytotoxicity (ADCC) in a Raji Lymphoma model that endogenously expresses CLEC2D.

CD161 is highly expressed in a subset of tumor infiltrating lymphocytes in both solid tumors such as CRC, HNSCC, and NSCLC and in hematological malignancies. Increased abundance of CD161+ T cells has been correlated to relapse in HCC tumors[3] and chemotherapy resistance in breast cancer[4] suggesting CD161 expression to be a potential immune evasion mechanism in patients with high unmet need.

IMT-009 is currently being tested in a Phase 1/2a monotherapy clinical trial. The data described here provides support for potential efficacy of IMT-009 in combination with anti-PD-(L)1 therapy in patients who have previously progressed on, or are refractory to, anti-PD-(L)1 therapy:

- scRNA seq analysis using publicly available data for anti-PD(L)1 treated patients shows higher abundance of *KLRB1*+ T cells in non responder patients compared to responders
- *KLRB1* is expressed more broadly in anti PD1 non-responsive tumor types such as MSS CRC than *PDCD1*
- Combination of IMT-009 with anti-PD1 enhances antigen specific T cell mediated cytotoxicity *in vitro*
- Transcriptomic changes upon treatment with IMT-009 and combination of anti-PD1 show further increase in T cell activation and cytotoxicity.

Hypothesis for therapeutically combining unique pathways of CD161 and PD1 in Immuno-oncology

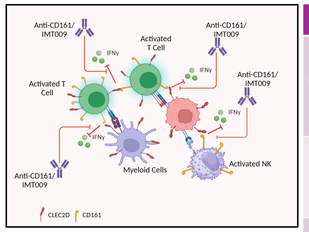

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Figure 1. Mechanisms of CLEC2D/CD161 mediated inhibition in the TME. CLEC2D ligand on tumor cells/immune cells interacts with CD161 to inhibit T cell and NK cell function.

Table 1. Differentiating characteristics of the CD161 and PD1 pathways

	CD161	PD1
Expression profile	• Expressed on NK cells, T cells, Th17, Innate like T cells including Tc1, MAIT and γδ T cells[5] • In cancer, CD161 is expressed by clonally expanding, cytotoxic memory CD8 T cells that have tissue resident phenotype[2,6] • CD161+ CD4 T cells are associated with enhanced secretion of IL-17a, IL-22, IFN-g. • Minimal expression by FoxP3+ Tregs[5]	• PD-1 is upregulated on almost all T cells post TCR activation and inhibits effector function of T cells • PD1 expression is correlated to T cell exhaustion • Upregulated transiently on NK cells
Ligand	• CLEC2D is the only known ligand for CD161[5] • It is expressed on germinal center B cells, activated T cells, NK cells, macrophages and dendritic cells[5] • B cell lymphomas express high levels of CLEC2D[7] • Solid tumors express variable levels of CLEC2D	• PD-L1 and PD-L2 are the two known ligands[8] • PD-L1 is constitutively expressed in a wide range of cells, including hematopoietic and non-hematopoietic cells such as tumor • PD-L2 expression is restricted to professional antigen-presenting cells • Expression of both PD-L1 and PD-L2 is regulated by Interferons
Signaling	• CD161 interacts and activates acid sphingomyelinase (ASM) that produces the second messenger molecule ceramide[9]. This regulates calcium influx and mTOR/STAT3[10] signaling affecting NK and T cell function	• Cytoplasmic domain has ITIM and ITSM motifs[11] • Upon interaction with PD-L1, recruitment of SHP phosphatases to the phosphorylated tyrosine residues to these motifs leads to inhibition of signaling downstream of CD28 and TCR affecting T cell activation
Targeting relevance in cancer	• Enhanced cytotoxicity, tissue homing, memory phenotype, broad expression in TME and dual inhibition of both T and NK cells make **CD161/CLEC2D as an important immunomodulatory pathway in cancer** • Anti-CD161 antibody IMT009 is in Phase I clinical trail for hematological and solid tumor malignancies (NCT05565457).	• Anti PD(L)1 have been FDA approved for treatment of a variety of solid tumors. • Certain tumors such as MSS colorectal cancer do not respond to anti PD1 therapy and have high unmet need • Acquired resistance to anti-PD1 treatment is also prevalent and supports rationale for targeting the immune evasion mechanisms

High *KLRB1* expression and *KLRB1*+ T cell abundance in anti-PD1 refractory MSS CRC and in anti-PD1 non responder patients in multiple solid tumors

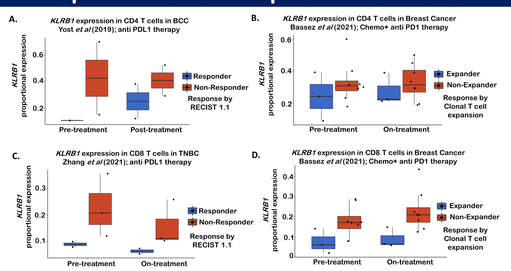

Figure 2. Trends of High *KLRB1* expression and *KLRB1*+ CD4 (**A, B**) and CD8 T cell (**C, D**) abundance in anti-PD1 non-responsive patient tumors were observed. *KLRB1* expression in tumor infiltrating CD4 and CD8 T cells was re-analysed from the following datasets: Yost K. *et al*[12] (**A**), Bassez A. *et al*[13] (**B,D**) and Zhang Y. *et al*[14] (**C**).

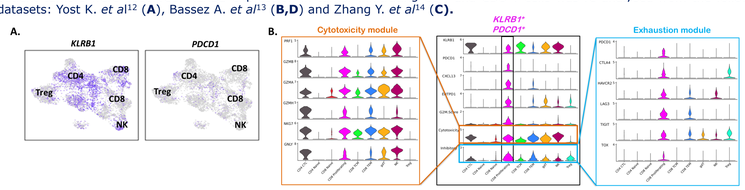

Figure 3. (**A**) scRNAseq analysis reveals broad expression of *KLRB1* in comparison to *PDCD1* in MSS CRC. Data reanalyzed from Zhang L. *et al*[15]. (**B**) scRNAseq in MSS CRC identifies population of *KLRB1*+ *PDCD1*+ exhausted CD8 T cells with high cytotoxicity and *KLRB1*+ CD4 cytotoxic T cells. Data from Pelka K. *et al*[16] re-analyzed by Immunitas.

IMT-009 mediated activation of antigen specific CD8 T cells leads to enhanced killing of tumor target cells

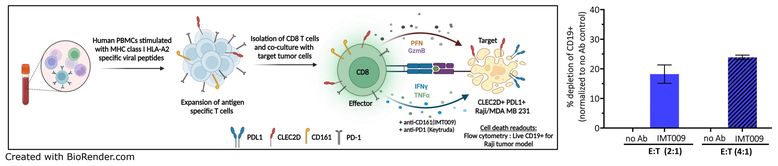

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Figure 4. Antigen-expanded CD8 T cells were co-cultured with antigen-primed Raji lymphoma cell lines at E:T ratio of 2:1 or 4:1 (E: CD8 T cells; T: Raji cells). Tumor cell death was measured by flow cytometry 48 hours post co-culture. IMT009 treatment reverses CLEC2D/CD161 mediated inhibition and enhanced CD8 T cell mediated cytotoxicity.

Treatment of fresh NSCLC tumor with IMT-009 leads to enhanced activation of tumor infiltrating lymphocytes

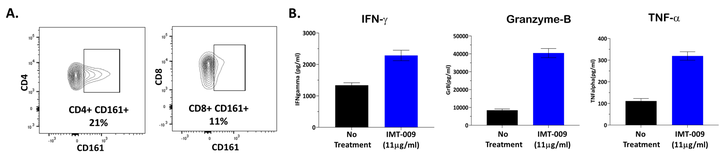

Figure 5. (**A**) Surgically resected NSCLC tumor was dissociated into single cells and analyzed for expression of CD161 and PD1 in CD4 and CD8+ T cells. (**B**) Dissociated tumor cells were then treated with IMT-009 (11μg/ml). T cell activation was determined by measuring cytokines in culture supernatants by MSD 3 days post-treatment. IMT-009 enhanced T cell activating cytokine production.

IMT-009 in combination with anti-PD1 enhances CD8 T cell cytotoxicity in a Raji HLA-A2/PD-L1 lymphoma model system with endogenous CLEC2D and overexpression of PD-L1

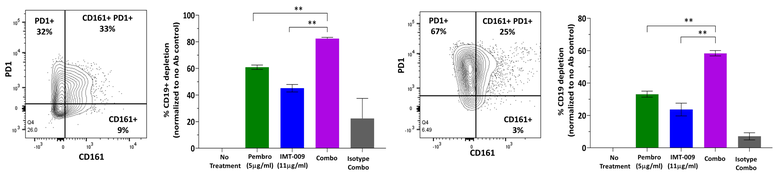

Figure 6. PBMCs from specific donors were expanded on MHC class I HLA-A2 restricted viral peptides (**A**) or the covid spike peptide (**B**) and set up in a cytotoxicity assay with HLA-A2 and PDL1 overexpressing Raji tumor cells expressing endogenous CLEC2D. The co-culture was treated with either Pembrolizumab (anti-PD1) (5μg/ml) or IMT-009 (11μg/ml) or combination. No treatment and combination of isotypes were used as controls. 48 hrs post co-culture live CD19+ Raji tumor cells was determined by flow cytometry providing % measure of cytotoxicity. Both antibodies show increased cytotoxicity over controls with additive effect in the combination treatment. (** p<0.01)

scRNAseq analysis of CD8 T cells and Raji tumor cells co-culture reveals increase in %*KLRB1*+ CD8 and CD4 T cells upon treatment with IMT-009 alone

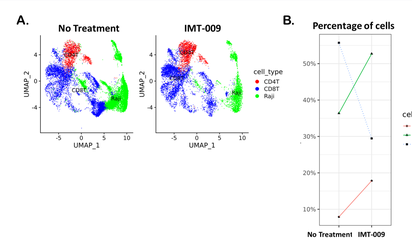

Figure 7. (**A**) UMAP visualization of clusters from 10X genomics scRNA-seq analysis of a co-culture of antigen specific T cells with Raji tumor cells with or without treatment of IMT-009. Colors indicate subtypes of CD4, CD8 and Raji tumor cells. Equal number of cells are depicted. (**B**) IMT009 treatment led to increase in % CD4 and CD8 T cells and a decrease in Raji tumor cell population indicating blocking of CD161 pathway leads to T cell activation and proliferation and enhanced tumor cell killing.

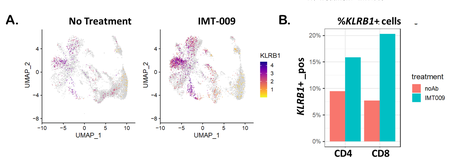

Figure 8. (**A**) UMAP visualization of *KLRB1*+ clusters of T cells between no treatment and IMT009 treatment shows increased *KLRB1*+ T cells. Equal number of cells are depicted. (**B**) Quantification of percent increase in *KLRB1*+ CD4 T cells and *KLRB1*+ CD8 T cells after IMT-009 treatment.

Transcriptomic changes induced in T cells upon IMT-009 treatment include enhanced TCR signaling, Cytotoxicity, NK cell like activity and Interferon signaling

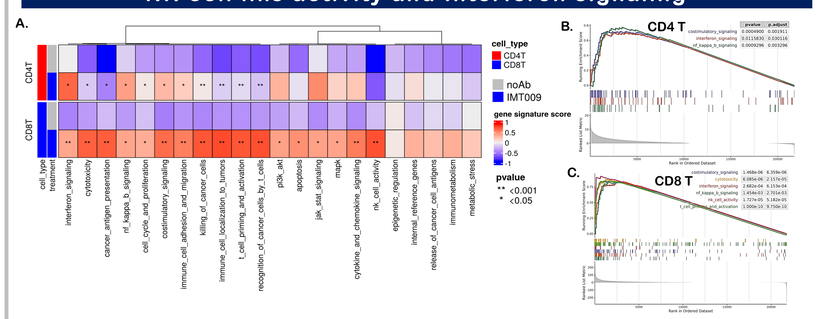

Figure 9. (**A**) Heat map showing functional enrichment in key biological processes after IMT-009 treatment using NanoString's pan cancer IO 360 panel pathways. These include antigen specific proximal TCR activation and signaling, distal PI3K_Akt signaling and NF-KB activation. Control internal reference genes remain insignificant with IMT009 treatment.(**B,C**) GSEA enrichment analysis of genes related to key signaling pathways in CD4 T cells and CD8 T cells upon IMT-009 treatment.

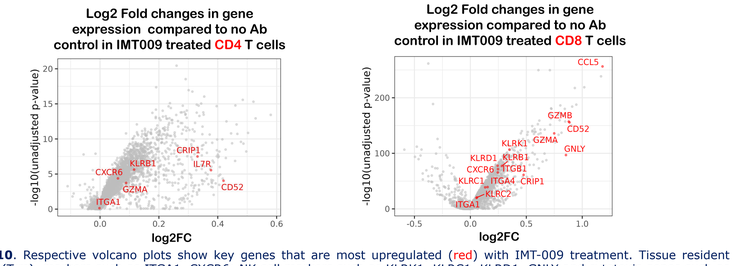

Figure 10. Respective volcano plots show key genes that are most upregulated (red) with IMT-009 treatment. Tissue resident memory (Trm) markers such as ITGA1, CXCR6; NK cell markers such as KLRK1, KLRC1, KLRD1, GNLY and cytotoxic genes such as GZMB and GZMA are among the most statistically significant differentially expressed genes in CD8 T cells supporting enhanced cytotoxicity in presence of IMT-009.

scRNAseq analysis of T cells upon treatment with IMT-009 in combination with anti-PD1 shows further enrichment in T cell activation and cytotoxicity gene signatures

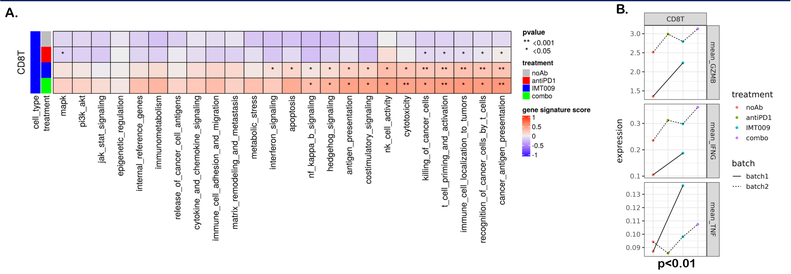

Figure 11. (**A**) Heat map showing functional enrichment in key biological processes across different treatment conditions using NanoString's pan cancer IO 360 panel pathways. Significant enrichment is observed with either treatments in pathways involved in TCR activation and signaling. (**B**) Treatment with combination compared to either drugs alone enhances mean expression of *IFNG, TNF,* and *GZMB* in CD8 T cells (p<0.01 between anti PD1 and combination) underscoring enhanced T cell activation in combination with anti-PD1 (pembrolizumab).

Conclusions

Functional activity of IMT-009

- IMT-009 is a fully human anti-CD161 antibody that restores T and NK cell function including tumor killing and cytokine production
- In this study, we show that IMT-009 can activate T cells in human tumor samples providing evidence of CD161's role as an inhibitory checkpoint in tumor infiltrating T cells.
- scRNAseq analysis shows that treatment with IMT-009 enhanced key T cell functions including enhanced TCR signaling, T cell cytotoxicity and interferon signaling. Furthermore, there is an increase in tissue residency program of both CD4 and CD8 T cells.

Rationale for combination of IMT-009 with anti-PD1

- CD161 and PD1 are unique pathways of immunomodulation in cancer.
- scRNAseq analysis of tumors from anti-PD1 responders and non-responders reveals higher abundance of *KLRB1*+ T cells (gene encoding for CD161) in non-responders.
- Anti-PD1 non-responding tumor types such as MSS CRC show broader expression of *KLRB1* than *PDCD1*. *KLRB1*+ cells show high expression of a cytotoxicity module and low expression of an exhaustion module as compared to *PDCD1*+ cells
- We further show that combination of anti-CD161 (IMT-009) and anti-PD1 can enhance T cell mediated tumor cell killing *in vitro*.

The IMT-009-101 is a Phase 1/2a, First-in-Human (FIH), Open-Label, Dose-Escalation and Dose Expansion Study of the Monoclonal Antibody IMT-009 in Patients with Advanced Solid Tumors or Lymphomas (Clinical trial.gov identifier: NCT05565417). It is currently enrolling in the dose escalation and expansion phase. Contact: BD@immunitastx.com

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