Anti-CD161 antibody IMT-009 is a novel immunotherapeutic agent that reinvigorates T and NK cell function and anti-tumor efficacy through blocking interaction of CD161 with its ligand CLEC2D

Poster # 1332

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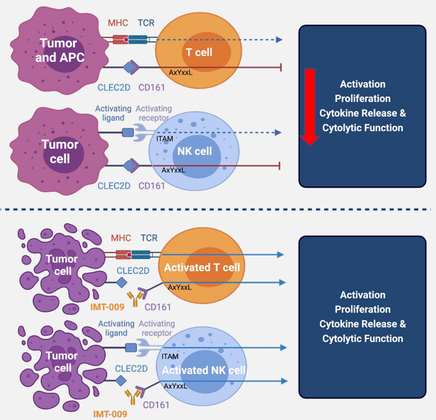
BACKGROUND

The CLEC2D/CD161 axis is a novel ligand-receptor pathway for immunotherapeutic intervention. CD161 is a C-type lectin-like receptor, which is broadly expressed on NK cells and subsets of both CD4$^+$ and CD8$^+$ T cells. Its cognate ligand, CLEC2D, is expressed on the surface of both malignant cells and immune cells, including activated B cells and myeloid cells.

Blocking the interaction between CLEC2D and CD161 has been reported to enhance NK cell-mediated lysis against TNBC cells in vitro[1]. CD161 also marks a distinct memory T cell population that is significantly increased in cancer patients and may regulate immune response[2]. It has been recently reported that gene expression of KLRB1 (encoding CD161) marked a distinct and innate-like CD8$^+$ T cell population in glioma that is largely independent of PD-1 expression, suggesting an opportunity for complementary therapeutic combinations with PD-1 blockade therapy[3].

Figure 1. IMT-009 has a differentiated, first in class, dual mechanism of activation of both T and NK cells by blocking interaction of CD161 with CLEC2D



ANTIBODY DISCOVERY

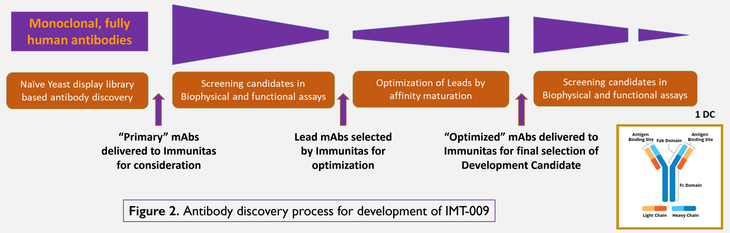


Figure 2. Antibody discovery process for development of IMT-009

IMT-009 is a fully human monoclonal, IgG1 antibody directed against CD161 that prevents its interaction with its ligand, CLEC2D. IMT-009 has an inactive Fc domain due to the introduction of the N297A mutation to avoid inadvertent and undesirable depletion of CD161$^+$T and NK cells. IMT-009 also has an expected pharmacokinetic (PK) profile of a typical IgG1 molecule.

IMT-009 binds with high affinity to both human and cynomolgus CD161, on both engineered and primary cells. It binds CD161 with high degree of selectivity, blocking its interaction with CLEC2D at an IC50 of 0.94 nM (Figure 3). **IMT-009 is a high affinity and selective molecule for CD161 that potently blocks interaction of CD161 with its ligand CLEC2D.**

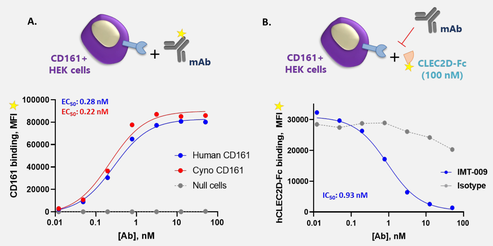


Figure 3. High affinity binding of IMT-009 to CD161 leads to potent blockade of interaction of CD161 with CLEC2D

FUNCTIONAL ACTIVITY

In presence of CLEC2D-expressing target cells K562, NK cell degranulation (**A**), cytokine production (**B**) and cellular cytotoxicity (**C**) towards tumor targets is highly suppressed; IMT-009 can overcome this inhibition with an EC50 of 0.2 nM. **Hence, potent blocking of CD161/CLEC2D interaction by IMT-009 restored the functional activity of NK cells.**

Figure 4. IMT-009 restores immune function of NK cells

Similarly, IMT-009 reversed CLEC2D-mediated inhibition and restored T cell receptor signaling and IL-2 production in a Jurkat cell reporter system (EC50 = 3.5 nM), as well as enhanced effector cytokine production of primary antigen-specific human T cells, including secretion of IFNγ (**A**), IL-2 (**B**), and TNF-α (**C**), (EC50 = 1.4 nM, 0.4 nM, and 0.2 nM, respectively), and direct T cell mediated cytotoxicity (**D, E**). **Overall, functional inhibition of CD161 by IMT-009 in primary T cells led to enhanced polyfunctionality and target cell death.**

Figure 5. IMT-009 leads to increased polyfunctionality and direct T cell mediated cytotoxicity of antigen specific primary human CD8 T cells

IMT-009 also released CD161 mediated suppression on effector memory CD161$^+$ CD4$^+$ T cells, resulting in an increase in their proliferation (Ki67$^+$) (**A**) and increased frequency of IFN-γ$^+$ cells (**B**) indicative of a stronger recall response to antigenic peptides. **Hence, IMT-009 provided benefit by enhancing memory recall responses of CD161$^+$ CD4$^+$ cells.**

Figure 6. IMT-009 enhances antigen recall responses of CD161+ CD4+ fffector memory T cells

Neither immobilized nor soluble IMT-009 induced cytokine release in unstimulated human PBMCs from healthy donors when tested up to 1000 µg/ml. This data suggests that that there is a low risk of cytokine release in blood of patients treated with IMT-009.

Furthermore, in a GLP toxicology study in cynomolgus monkeys, IMT-009 administered IV once weekly in 5 doses over 4 weeks demonstrated no significant signs of toxicity. **Taken together, IMT-009 demonstrated a clean safety profile for testing in FIH trials.**

Figure 7. IMT-009 does not induce cytokine release in human PBMCs

Figure 4

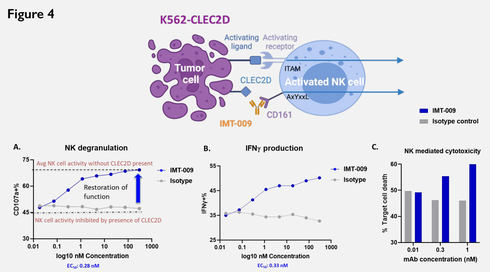


Figure 5

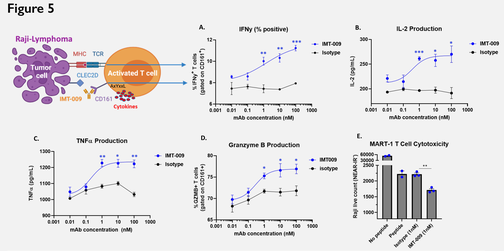


Figure 6

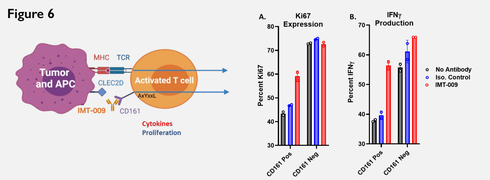
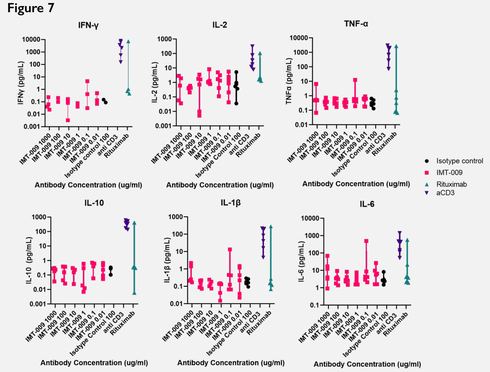


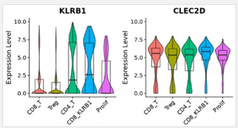
Figure 7



TRANSLATION INTO CLINIC

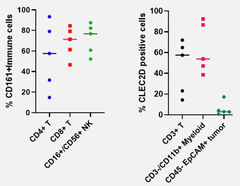
The mechanism of action of anti CD161 antibody IMT-009 to restore T and NK cell function is by blocking interaction of CD161 with its inhibitory ligand CLEC2D. Thus, it is hypothesized that expression of CLEC2D (by the tumor cells or cells in the tumor microenvironment [TME]), T cell infiltration, and CD161 expression on tumor infiltrating T cells and/or NK cells are ideal prerequisites for patients to derive benefit from IMT-009 treatment. Orthogonal approaches were used to characterize various tumor indications based on CLEC2D and CD161 expression to help identify and prioritize the ones to be included in the FIH clinical trial. These approaches included (**A**) single cell RNA seq analyses to assess transcriptomic expression of KLRB1 and CLEC2D in tumor infiltrating immune cells, (**B**) flow cytometry for CD161 and CLEC2D expression within dissociated tumor cells and (**C**) multiplexed immunofluorescence for CD161, CLEC2D and other immune markers. Multiplexed immunofluorescence data of over 30 solid tumor types showed the highest density of CLEC2D+ and CD161+ cells in the following indications: NSCLC-squamous cell carcinoma, NSCLC- adenocarcinoma, head and neck squamous cell carcinoma (HNSCC), triple negative breast cancer (TNBC), cutaneous squamous cell carcinoma and colorectal carcinoma.

A. scRNA-seq analysis of NSCLC TILs

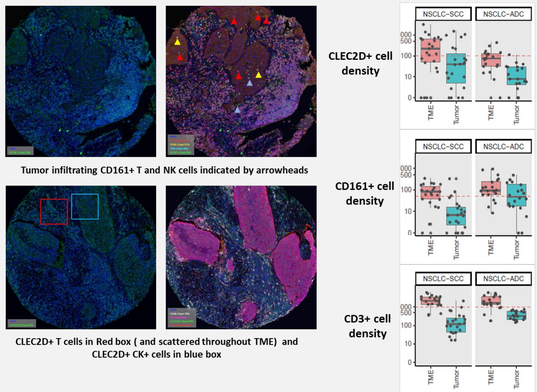


Guo et al; Nature Medicine (2018)
11 ADC + 3 Sq/14

B. Flow cytometry analyses of NSCLC TILs



C. Multiplexed Immunofluorescence Analyses of NSCLC Tumor Samples



Tumor infiltrating CD161+ T and NK cells indicated by arrowheads

CLEC2D+ T cells in Red box (and scattered throughout TME) and CLEC2D+ CK+ cells in blue box

Figure 8. Non-small cell lung cancer (both adenocarcinoma and squamous cell carcinoma) show consistent expression of CD161 and CLEC2D across multiple analyses supporting its inclusion in the FIH trial for IMT-009

DISCUSSION/CONCLUSION

- The results described above support the development of IMT-009 as a novel cancer immunotherapy for application in several solid tumor indications and lymphomas
- IMT-009 has received FDA clearance of IND in solid tumors and hematological malignancies
- The trial has initiated patient recruitment (ClinicalTrials.gov Identifier: NCT05565417)
- For additional information, please contact BD@immunitastx.com

1. Marrufo A, Mathew P. Blocking LLT1-NKRP1A (CD161) interaction enhances natural killed cell-mediated lysis of triple negative breast cancer cells. Am J Cancer Res. 2018; 8(6):1050-1063. 2. Iliopoulou IG, Karamouzis M-V, Missitzis I, Increased Frequency of CD4+ Cells Expressing CD161 in Cancer Patients. Clin Cancer Res 2006;12:6901-6909. 3. Mathewson N, Ashenberg O, Tirosh I, Wucherpfennig K, Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. Cell. 2021;184:1281–1298.