23ME-01473, a novel anti-ULBP6/2/5 monoclonal antibody, reinvigorates anti-tumor NK cell function through NKG2D and FcγRIIIa activation

Abstract #2375

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BACKGROUND

Genetic Signature

- Using the 23andMe database, novel immuno-oncology (I/O) drug targets are identified as genetic variants with opposing effects on the risks for cancer and immune diseases, referred to as an I/O signature. *RAET1L* (gene encodes ULBP6) exhibits this I/O signature (**Figure 1**).

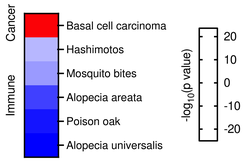
ULBP6

- UL16 binding protein (ULBP6) is a member of the stress-induced NKG2D ligand (NKG2DL) family that is upregulated on the surface of cancer cells and binds to the immune-activating NKG2D receptor on NK and T cells[1-2].
- Cancer cells shed NKG2DLs, including ULBP6, from its surface via proteolytic cleavage or exosomal release to evade immune recognition and killing, and soluble NKG2DLs are elevated in cancer patient plasma[3-6] **(Figure 2A)**.

23ME-01473 ('1473)

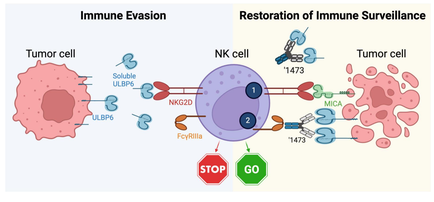
- 23ME-01473 is a high-affinity Fc-enhanced humanized monoclonal antibody that binds with high specificity to ULBP6, ULBP2, and ULBP5 and blocks their soluble forms from interacting with NKG2D to restore the binding of membrane-bound NKG2DLs to NKG2D (**Figure 2B**).
- To leverage the binding of 23ME-01473 to ULBP6/2/5 on the surface of cancer cells, the Fc domain of 23ME-01473 has enhanced affinity for FcγRIIIa to induce antibody-dependent cellular cytotoxicity (ADCC) (**Figure 2B**).
- The combined synergistic mechanisms of NKG2D and FcγRIIIa activation mediated by 23ME-01473 restore NK and T cell-mediated anti-tumor immunity, which may provide benefit to patients with cancers resistant to immune-checkpoint inhibitors due to the loss of neoantigen presentation.
- 23ME-01473 is currently being evaluated in a Phase 1 clinical trial as a monotherapy for patients with advanced solid tumors.

Figure 1. *RAET1L* (ULBP6) Genetic Immuno-Oncology Signature



A variant of *RAET1L* (ULBP6) exhibits significant genome-wide associations and opposing risks for cancer (red) and immune diseases (blue), which comprise 23andMe's proprietary immuno-oncology signature.

Figure 2. Proposed Mechanism of Action of 23ME-01473 ('1473)



23ME-01473 restores immune surveillance through synergistic mechanisms:

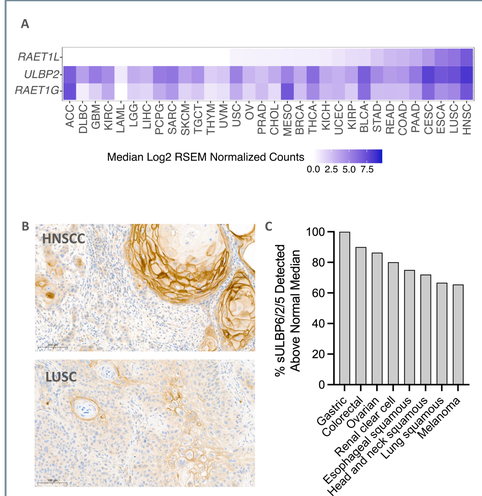
1. NKG2D activation via neutralization of soluble ULBP6 (sULBP6)[1]
2. FcγRIIIa activation via enhanced binding affinity to induce ADCC

[1]23ME-01473 binds ULBP6, 2, and 5

RESULTS

ULBP6/2/5 are upregulated in squamous cell carcinomas and a subset of adenocarcinomas

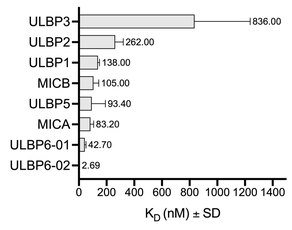
Figure 3. ULBP6/2/5 are present on tumor cells and in circulation in cancer patients



A) mRNA expression of *RAET1L* (ULBP6), *ULBP2*, and *RAET1G* (ULBP5) in cancers from TCGA. **B)** ULBP6/2/5 expression in HNSCC (top panel) and LUSC (bottom panel) tumors by IHC. **C)** Percent of cancer patients per tumor type with soluble ULBP6/2/5 (sULBP6/2/5) concentrations greater than the median sULBP6/2/5 concentration in healthy individuals as detected by MSD.

ULBP6 has the highest affinity for NKG2D among all NKG2DLs

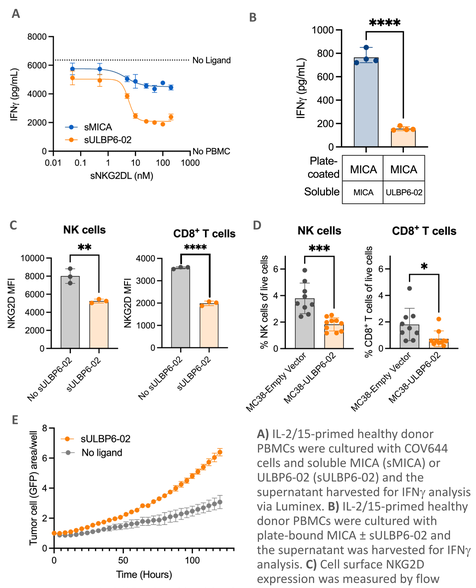
Figure 4. The two most prevalent ULBP6 isoforms have the highest binding affinity for NKG2D



Equilibrium dissociation constant K_D (nM) for all human NKG2DLs binding to NKG2D as measured by Biacore. Data represent mean ± SD of 3 technical replicates per NKG2DL. ULBP4 is not shown, as no binding to NKG2D was detected in the assay.

Soluble ULBP6 is immunosuppressive even in the presence of membrane-bound NKG2DLs

Figure 5. Soluble ULBP6 suppresses immune cell activation and tumor cell growth control



A) IL-2/15-primed healthy donor PBMCs were cultured with COV644 cells and soluble MICA (sMICA) or ULBP6-02 (sULBP6-02) and the supernatant harvested for IFNγ analysis via Luminex. **B)** IL-2/15-primed healthy donor PBMCs were cultured with plate-bound MICA ± sULBP6-02 and the supernatant was harvested for IFNγ analysis. **C)** Cell surface NKG2D expression was measured by flow cytometry on IL-2/15-primed healthy donor NK and CD8+ T cells cultured ± sULBP6-02. **D)** Percent of tumor infiltrating NK and CD8+ T cells were isolated from mice inoculated with MC38-Empty Vector or MC38 cells overexpressing ULBP6-02 (MC38-ULBP6-02). Data represent mean ± SD from 2 independent experiments. **E)** COV644-GFP cells were cultured with IL-2/15-primed healthy donor PBMCs ± sULBP6-02 and tumor growth was measured as GFP area per well. Representative data of the mean ± SD of 3-4 technical replicates per donor for A-C and E. Statistical significance was determined by unpaired Student's t test for C and D. **p<0.01, ***p<0.001, ****p<0.0001.

23ME-01473 binds with high affinity to ULBP6, ULBP2, and ULBP5

Table 1. 23ME-01473 ('1473) binds ULBP6, 2, and 5 to block their interaction with NKG2D

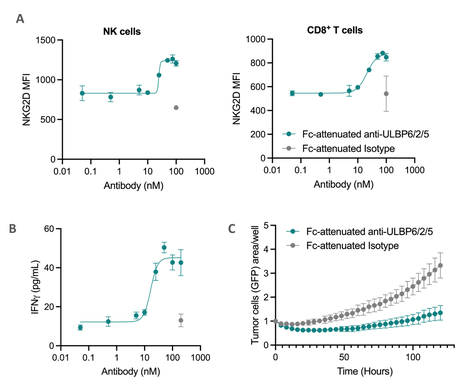
Ligand	'1473 Affinity to ULBPs K_D ± SD (nM) n=4[1]	'1473 Blockade of ULBP-NKG2D IC_{50} ± SD (nM) n=4[2]
ULPB1	No binding	N/A
ULBP2	0.23 ± 0.009	N/A
ULBP3	No binding	N/A
ULBP4	No binding	N/A
ULBP5	1.92 ± 0.071	N/A
ULBP6-01	0.066 ± 0.003	0.55 ± 0.239
ULBP6-02	0.053 ± 0.006	0.04 ± 0.024

[1]K_D measured by Biacore SPR

[2]IC_{50} determined by ELISA using EC_{80} concentration of NKG2DL binding to NKG2D

Fc-attenuated anti-ULBP6/2/5 blocks sULBP6 to restore immune activation and tumor growth control

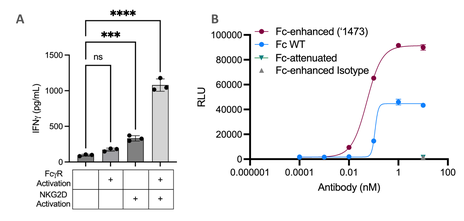
Figure 6. Fc-attenuated anti-ULBP6/2/5 antibody promotes activation of NK and CD8+ T cells



A) NKG2D expression was measured by flow cytometry on IL-2/15-primed healthy donor NK and CD8+ T cells cultured with sULBP6-02 and an Fc-attenuated anti-ULBP6/2/5 antibody or isotype control. **B)** IL-2/15-primed healthy donor PBMCs were cultured with COV644, sULBP6-02, and the indicated antibodies and the supernatant was harvested for IFNγ analysis via Luminex. **C)** COV644-GFP cells were cultured with IL-2/15-primed healthy donor PBMCs, sULBP6-02, and the indicated antibodies, and tumor growth was measured as GFP area per well. Representative data of the mean ± SD of 3-8 technical replicates per donor.

Activation of NKG2D and FcγRIIIa is synergistic

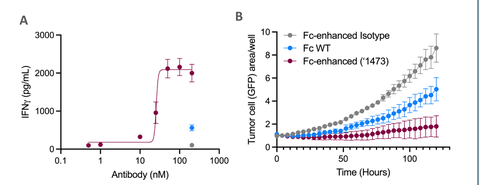
Figure 7. FcγRIIIa activation is synergistic with NKG2D activation and promotes ADCC



A) Healthy donor PBMCs were cultured with tool antibodies that activate FcγRIIIa, NKG2D, or both receptors, and the supernatant was harvested for IFNγ analysis via Luminex. Statistical significance was determined by one-way ANOVA. **B)** Luciferase activation, reported as relative light units (RLU), was measured in ADCC effector cells from a Promega ADCC assay cultured with COV644 and an Fc-enhanced ('1473), Fc WT, or Fc-attenuated anti-ULBP6/2/5 antibody, or an Fc-enhanced isotype control. Representative data of the mean ± SD of 3 technical replicates per donor. Statistical significance was determined by one-way ANOVA. *** p<0.001, ****p<0.0001, ns = not significant.

23ME-01473 (Fc-enhanced anti-ULBP6/2/5) induces superior anti-tumor immunity

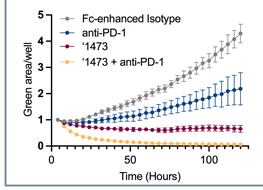
Figure 8. Fc-enhanced anti-ULBP6/2/5 antibody, 23ME-01473, augments NK and T cell activation and tumor cell killing



A) Healthy donor PBMCs were cultured with COV644, sULBP6-02, and an Fc-enhanced ('1473) or Fc WT anti-ULBP6/2/5, or an Fc-enhanced isotype control and the supernatant was harvested for IFNγ analysis via Luminex. **B)** COV644-GFP cells were cultured with healthy donor PBMCs, sULBP6-02, and the indicated antibodies, and tumor growth was measured as GFP area per well. Representative data of the mean ± SD of 2-5 technical replicates per donor.

23ME-01473 elicits enhanced tumor cell killing with anti-PD-1

Figure 9. 23ME-01473 and anti-PD-1 show increased tumor cell killing



COV644-GFP cells were cultured with healthy donor PBMCs, sULBP6-02, and the indicated antibodies, and tumor growth was measured as GFP area per well. Representative data of the mean ± SEM of 2-5 technical replicates per donor.

CONCLUSION

- **Tumor ULBP6 expression and soluble ULBP6 are elevated in cancer patients.**
- **Soluble ULBP6 is a dominant immunosuppressor compared to other (s)NKG2DLs, due to its highest binding affinity to NKG2D among all NKG2DLs.**
- **23ME-01473 is a high affinity, Fc effector-enhanced, anti-ULBP6/2/5 antibody that restores the activation and tumor cell killing capacity of NK and T cells through NKG2D activation.**
- **23ME-01473's dual synergistic activation of NKG2D and FcγRIIIa leads to optimal activation of NK cells, which may reverse immune suppression and circumvent resistance to immune-checkpoint inhibitors in tumors.**
- **The safety, pharmacokinetics, pharmacodynamics, and anti-cancer activity of 23ME-01473 are currently being evaluated in patients with advanced solid tumors in a phase I clinical trial (NCT06290388).**

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