23ME-01473, an Fc-enhanced anti-ULBP6/2/5 antibody, restores NK cell function through NKG2D and FcγRIIIa activation

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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. *RAETTL* (gene encoding ULBP6) exhibits this I/O signature **(Figure 1)**.

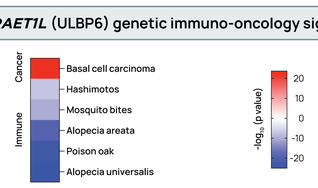
ULBP6

- UL16 binding protein 6 (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 2 left panel)**.

23ME-01473 ('1473)

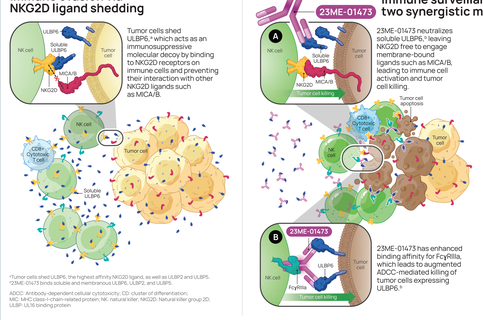
- 23ME-01473 is a high-affinity Fc-enhanced humanized monoclonal antibody that binds with high specificity to ULBP6, ULBP2, and ULBP5 and blocks their soluble forms from interacting with NKG2D to restore the binding of membrane-bound NKG2DLs to NKG2D **(Figure 2A)**.
- To leverage the binding of 23ME-01473 to ULBP6/2/5 on the surface of cancer cells, the Fc domain of 23ME-01473 has enhanced affinity for FcγRIIIa to augment 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 tumor cell recognition via MHC I.
- 23ME-01473 is currently being evaluated in a Phase 1 clinical trial (NCT06290388) as a monotherapy for patients with advanced solid tumors.

Figure 1: *RAETTL* (ULBP6) genetic immuno-oncology signature



A variant of *RAETTL* (ULBP6) exhibits the I/O genetic signature, with significant ($p < 5 \times 10^{-8}$) opposing risks for cancer (shown in red) and immune phenotypes (shown in blue).

Figure 2: Proposed Mechanism of Action of 23ME-01473



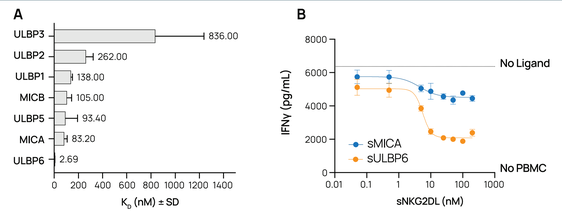
*Tumor cells shed ULBP6, the highest affinity NKG2D ligand, as well as ULBP2 and ULBP5. †23ME-01473 binds soluble and membranous ULBP6, ULBP2, and ULBP5.

ADCC: Antibody-dependent cellular cytotoxicity; CD: cluster of differentiation; MIC: MHC class I chain-related protein; NK: natural killer; NKG2D: Natural killer group 2D; ULBP: UL16 binding protein.

RESULTS

ULBP6 is the Highest Affinity and Most Immunosuppressive NKG2D Ligand

Figure 3: ULBP6 is a high affinity NKG2D ligand that suppresses anti-tumor immunity

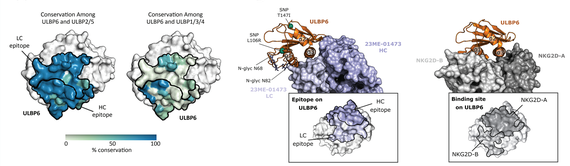


A) ULBP6 has the highest binding affinity for NKG2D. Equilibrium dissociation constant K_D (nM) for all human NKG2DLs binding to NKG2D as measured by Biacore. ULBP4 is not shown, as no binding to NKG2D was detected.

B) sULBP6 is more suppressive than sMICA. IL-2/15-primed healthy donor PBMCs were cultured with COV644 cells and soluble MICA (sMICA) or ULBP6 (sULBP6) and the supernatant harvested for IFNγ analysis via Luminex.

23ME-01473 Binds with High Affinity to ULBP6, ULBP2, and ULBP5

Figure 4: 23ME-01473 paratope is highly conserved among ULBP6/2/5 and overlaps with NKG2D binding site



A) The 23ME-01473 paratope is conserved among ULBP6/2/5. The colors represent the % amino acid conservation between ULBP6, ULBP2, and ULBP5 (left panel) and between ULBP6, ULBP1, ULBP3, and ULBP4 (right panel).

B) The 23ME-01473 paratope overlaps with the NKG2D binding site to block the ULBP6/2/5-NKG2D interaction. Crystal structure of 23ME-01473 Fab domain (right, in purple) or NKG2D (left, in gray) in complex with ULBP6 (orange). The coding SNPs are shown as green spheres, and the ULBP6 epitope that is recognized by 23ME-01473 (left) or NKG2D (right) are shown in the insets.

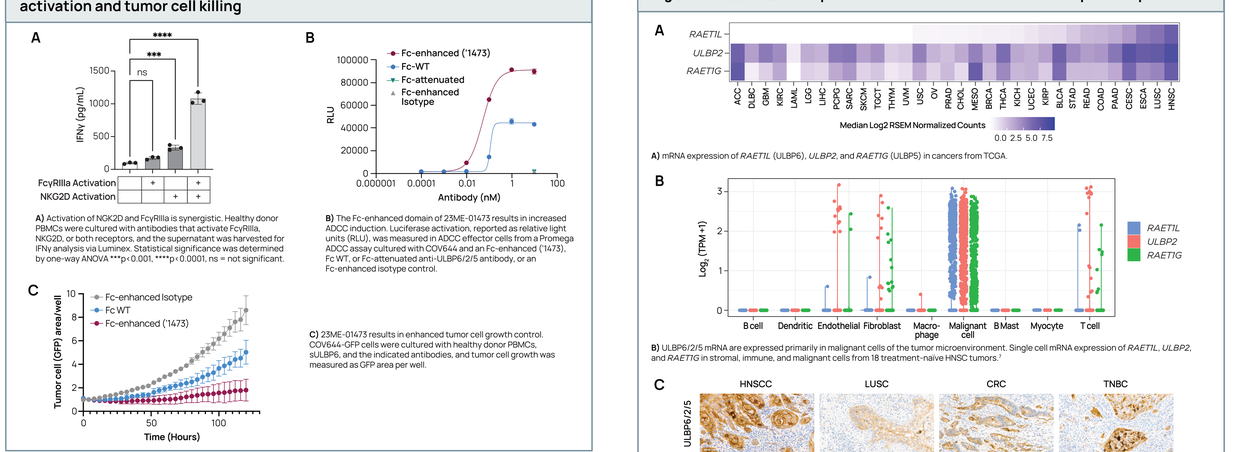
Table 1: 23ME-01473 ('1473) binds ULBP6/2/5

Ligand	'1473 Affinity to ULBPs K_D ± SD (nM) n=4[1]
ULBP2	0.23 ± 0.009
ULBP5	1.92 ± 0.071
ULBP6	0.053 ± 0.006

[1]K_D measured by Biacore SPR
No binding to ULBP1, ULBP3, and ULBP4 was observed.

23ME-01473 Augments Immune Cell-mediated Tumor Cell Killing Through Dual NKG2D and FcγRIIIa Activation

Figure 5: NKG2D and FcγRIIIa activation synergistically enhances immune activation and tumor cell killing



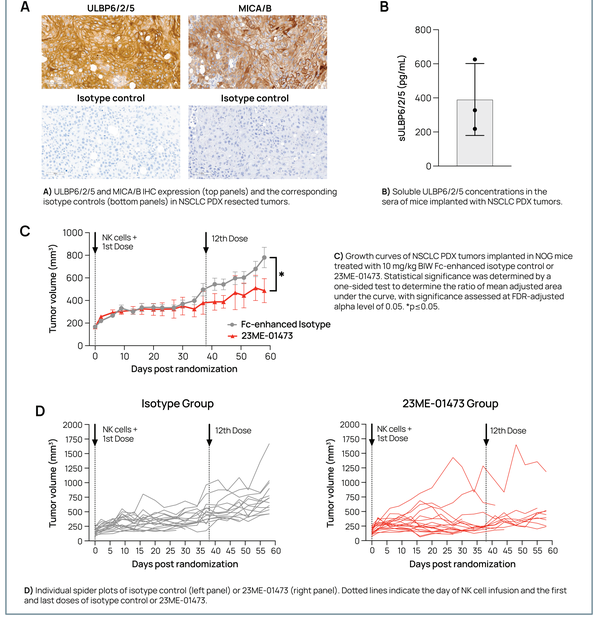
A) Activation of NGK2D and FcγRIIIa is synergistic. Healthy donor PBMCs were cultured with antibodies that activate FcγRIIIa, NKG2D, or both receptors, and the supernatant was harvested for IFNγ analysis via Luminex. Statistical significance was determined by one-way ANOVA ***p<0.001, ****p<0.0001, ns = not significant.

B) The Fc-enhanced domain of 23ME-01473 results in increased ADCC induction. 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.

C) 23ME-01473 results in enhanced tumor cell growth control. COV644-GFP cells were cultured with healthy donor PBMCs, sULBP6, and the indicated antibodies, and tumor cell growth was measured as GFP area per well.

23ME-01473 Restores NK Cell-mediated Tumor Growth Control

Figure 6: 23ME-01473 inhibits growth of a NSCLC PDX model *in vivo*



A) ULBP6/2/5 and MICA/B IHC expression (top panels) and the corresponding isotype controls (bottom panels) in NSCLC PDX resected tumors.

B) Soluble ULBP6/2/5 concentrations in the sera of mice implanted with NSCLC PDX tumors.

C) Growth curves of NSCLC PDX tumors implanted in NOG mice treated with 10 mg/kg BIW Fc-enhanced isotype control or 23ME-01473. Statistical significance was determined by a one-sided test to determine the ratio of mean adjusted area under the curve, with significance assessed at FDR-adjusted alpha level of 0.05. *p≤0.05.

D) Individual spider plots of isotype control (left panel) or 23ME-01473 (right panel). Dotted lines indicate the day of NK cell infusion and the first and last doses of isotype control or 23ME-01473.

ULBP6/2/5 Are Expressed in Squamous Cell Carcinomas and a Subset of Adenocarcinomas

Figure 7: ULBP6/2/5 are present on tumor cells and in cancer patient plasma

A) mRNA expression of *RAETTL* (ULBP6), *ULBP2*, and *RAETTG* (ULBP5) in cancers from TCGA.

B) ULBP6/2/5 mRNA are expressed primarily in malignant cells of the tumor microenvironment. Single cell mRNA expression of *RAETTL*, *ULBP2*, and *RAETTG* in stromal, immune, and malignant cells from 18 treatment-naive HNSC tumors.[7]

C) Consistent with mRNA data, ULBP6/2/5 and MICA/B proteins are highly expressed in squamous cell carcinomas and a subset of adenocarcinomas as detected by IHC.

D) ULBP6-positive tumor volume correlates with ULBP6 plasma levels in preclinical models. Correlation of plasma sULBP6/2/5 concentrations and tumor volume of mice inoculated with MC38 cells overexpressing ULBP6.

E) Soluble ULBP6/2/5 (sULBP6/2/5) are elevated in the plasma of diverse tumors. Percent of plasma samples from each tumor type with sULBP6/2/5 concentrations greater than the median of that in healthy individuals as detected by MSD.

CONCLUSIONS

- 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.
- Tumor ULBP6 expression and plasma soluble ULBP6 are elevated in diverse tumors, independent of ICI sensitivity or MHC I genetic alterations (Poster FPN #1069TiP).
- 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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