AGEN1571 is a novel high-affinity ILT2 antagonist antibody that promotes adaptive and innate immune responses

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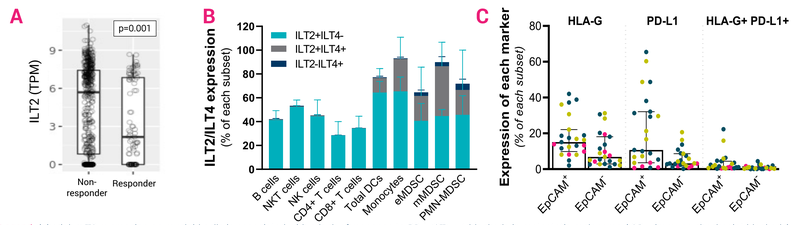
ILT2 is a major suppressor of anti-tumor immune responses and is associated with resistance to immune checkpoint blockade therapy

- Engages classical and non-classical MHC I molecules including tumor neo-expressed HLA-G
- Broadly expressed co-inhibitory receptor that suppresses myeloid and lymphoid cellular responses
- Antagonizes NFAT, NF-κB, and IRF pro-inflammatory signaling pathways *via* 4x ITIM domains
- Elevated expression in anti-PD-1- & anti-CTLA-4-treatment resistant patients
- Predominant expression compared to other HLA-G receptors (*i.e.* ILT4)
- Distinct expression of ILT2 and PD-1 on CD8 T cell subsets supports combination rationale

Elevated myeloid ILT2 is associated with poor responses to ICB therapy

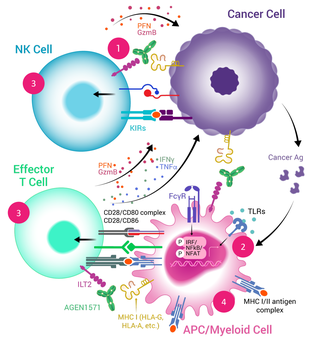
ILT2 is more prominently expressed than ILT4 in TME

Distinct expression of HLA-G and PD-L1 on dissociated tumor cells, supports combinational rationale



Legend. (A) High ILT2 expression on myeloid cells is associated with a lack of response to PD-1/CTLA-4 blockade in metastatic melanoma (ICB = immune checkpoint blockade). Single-cell RNA-seq analysis of tumor infiltrating immune cells in checkpoint-treated metastatic melanoma (* p<0.001) TPM – transcripts per million. (B) Profile of ILT2 and ILT4 expression on hematopoietic (CD45+) subpopulations in dissociated tumor samples. Cumulative data for gastric (n=4), kidney (n=11) and lung (n=8) cancer specimens are shown. (C) Profile of HLA-G and PD-L1 expression on non-hematopoietic (CD45-) subpopulations in dissociated gastric (n=4, pink dots), kidney (n=11, khaki dots) and lung (n=8, teal dots) samples. TME – tumor microenvironment.

AGEN1571 is an ILT2 antagonist antibody designed to block ILT2 immune suppression and enhance innate and adaptive anti-tumor immunity



AGEN1571:

1. Binds with high-affinity to ILT2, and blocks ILT2 interaction with its ligands expressed on tumor and immune cells
2. Potentiates NF-κB, NFAT, and IRF signaling to enhance pro-inflammatory responses
3. Enhances NK, NKT and CD8+ T effector cytotoxicity and release of pro-inflammatory cytokines
4. Promotes APC/myeloid cell re polarization to a pro-inflammatory phenotype via potentiation of FcγR, TLR, and cGAS signaling pathways

Best-in-class molecular features of AGEN1571

- Higher binding affinity to all isoforms of ILT2 and superior binding to cells expressing low ILT2 levels as compared to 15G8 (analogue competitor anti-ILT2 mAb)[4]
- Complete blockade of HLA-A, HLA-B, HLA-C, and HLA-G ligands in contrast to 15G8
- IgG4 Fc format selected to minimize FcγR-induced ILT2 forward signaling and FcγR-dependent effector activity (i.e. ADCC/ADCP of ILT2-expressing cells)
- Fully-human antibody: Low immunogenicity risk
- Optimized biophysical properties including low risk of aggregation and PTMs

References

1. Banham, *et al.*, *J Leukoc Biol*, 1999
2. Shiroshi *et al.*, *PNAS*, 2003
3. Sade-Feldman *et al.*, *Cell*, 2018
4. WO2021028921 (15G8 sequence; BIOND BIOLOGICS LTD.)

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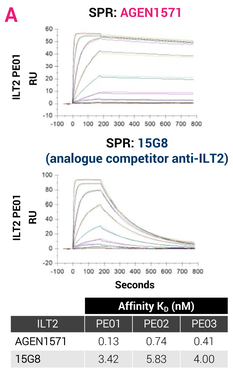
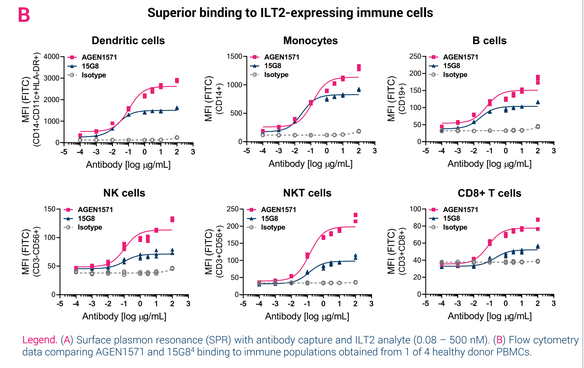
AGEN1571 demonstrates potent and high-affinity binding to ILT2

Superior binding properties compared to competitor ILT2 mAb (15G8)



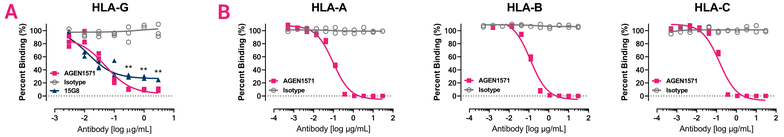
	Affinity K_D (nM)		
ILT2	PE01	PE02	PE03
AGEN1571	0.13	0.74	0.41
15G8	3.42	5.83	4.00



Legend. (A) Surface plasmon resonance (SPR) with antibody capture and ILT2 analyte (0.08 – 500 nM). (B) Flow cytometry data comparing AGEN1571 and 15G8[4] binding to immune populations obtained from 1 of 4 healthy donor PBMCs.

AGEN1571 completely blocks ILT2-ligand interactions

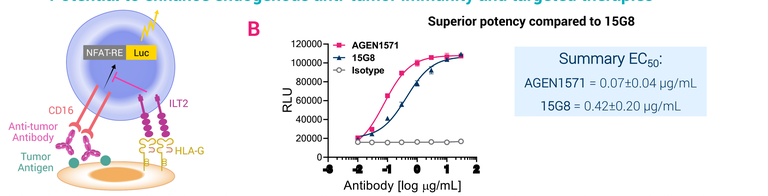
Superior blockade of ILT2-HLA-G compared to competitor ILT2 mAb (15G8)



Legend. (A) ELISA-based blocking assay showing superior blocking activity of AGEN1571 compared to 15G8[4]. Recombinant human ILT2-Fc and HLA-G-His were used. Statistical analysis for each concentration point was performed by t-test (** p<0.01) A dose range of AGEN1571 (squares), 15G8 (triangles) or IgG4 isotype control (circles) was tested as indicated. (B) Ligand blocking assay using recombinant human HLA-A/B/C/G and CHO-ILT2 cells. A dose range of AGEN1571 (squares) or IgG4 isotype control (circles) was tested as indicated.

AGEN1571 blocks ILT2-mediated inhibition to enhance FcγR-signaling

Potential to enhance endogenous anti-tumor immunity and targeted therapies



Summary EC_{50}:

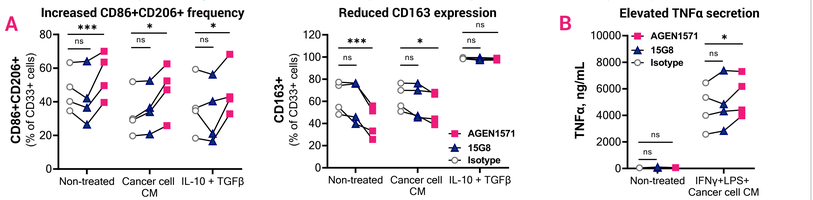
AGEN1571 = 0.07±0.04 μg/mL

15G8 = 0.42±0.20 μg/mL

Legend. (A) Illustration of reporter cell assay. (B) Effector Jurkat cells expressing ILT2 and an FcγR-dependent luciferase reporter, were co-cultured overnight with target cells (HLA-G-expressing Ramos cells) in the presence of rituximab and a dose titration of AGEN1571 (pink square), 15G8[4] (navy triangle) or isotype control antibodies (gray circle). Luciferase activity was measured and expressed as Relative Luminescence Units (RLU).

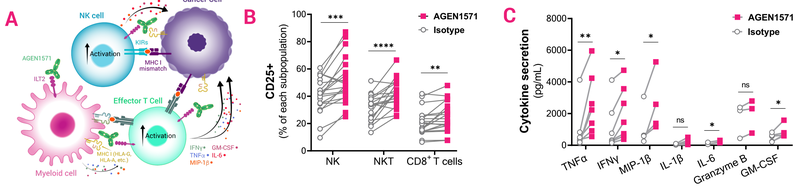
AGEN1571 polarizes macrophages to a pro-inflammatory phenotype

Superior myeloid activation compared to competitor ILT2 mAb (15G8)

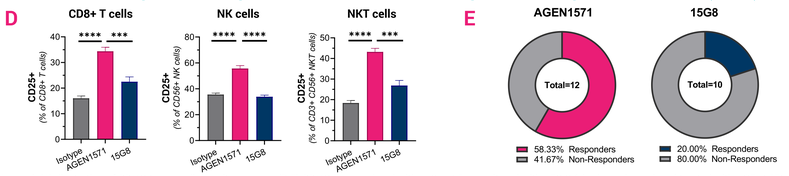


Legend. Monocytes were isolated from PBMCs and differentiated into macrophages in presence of AGEN1571, 15G8[4], or isotype control. (A) Macrophages were left unstimulated or were treated with JEG-3 (choriocarcinoma) conditioned media (Cancer cell CM) or IL-10 and TGFβ cytokine cocktail. Surface expression of CD86 and CD206 (left) or CD163 (right) was analyzed by flow cytometry. (B) Macrophages were left unstimulated or were treated with LPS + IFNγ and JEG-3 (choriocarcinoma) conditioned media (Cancer cell CM). Cytokine release was analyzed by Luminex. Statistical analysis performed by paired t-test (ns – not significant, *p<0.05, **p<0.01, ***p<0.001).

AGEN1571 enhances CD8 T, NK and NKT cell activation and cytokine secretion in PBMCs co-cultured with tumors

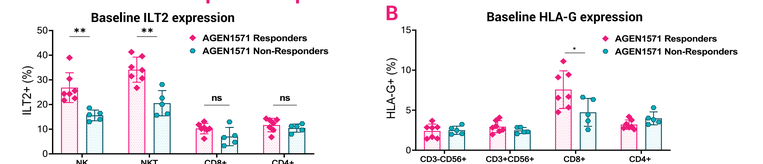


AGEN1571 promotes superior immune activation compared to competitor ILT2 mAb (15G8)



Legend. (A) Illustration of PBMC-tumor cell co-culture assay. (B-E) PBMCs were primed with IL-2/IL-15, co-cultured with JEG-3 (choriocarcinoma) and treated with AGEN1571, anti-ILT2 (15G8) or isotype control antibodies for 72 hours. (B) Summarized CD25 expression across 12 donors and 7 independent experiments. Each dot represents a single donor in a separate experiment. (C) Cytokine secretion in cell supernatants from 6 donors and 3 independent experiments. (D) Representative data demonstrating superior activity of AGEN1571 over 15G8[4] in PBMC-tumor cell co-culture assay (n=10 donors). (E) Pie chart showing the fraction of donors responding to AGEN1571 or 15G8. ***Ex vivo* Responder criteria:** At least one of analyzed immune subpopulations responded to ILT2 blockade by ≥ 20% increase of CD25 surface expression over isotype control. Statistical analysis was performed by paired t-test or one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001).

AGEN1571 *ex vivo* responders have higher baseline ILT2 and HLA-G expression – potential patient selection biomarker



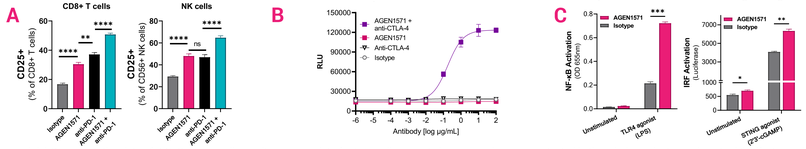
Legend. PBMC donors were assigned to "Responders" or "Non-responders" group based on the response to AGEN1571 in PBMC-tumor cell co-culture assay. ***Ex vivo* Responder criteria:** 1+ analyzed immune subpopulations (NK, NKT, CD8+ T cells) showed ≥ 20% increase of CD25 surface expression by AGEN1571 over isotype control. Baseline expression of (A) ILT2 and (B) HLA-G analyzed by flow cytometry. Statistical analysis performed by t-test (*p<0.05, **p<0.01).

AGEN1571 combines with checkpoint blockade and TLR/STING agonists to enhance immune cell activation and pro-inflammatory myeloid cell polarization

Combines with anti-PD-1 to further enhance CD8+ T, NK and NKT cell activation

Combines with Fc-enhanced anti-CTLA4 to increase FcγR-mediated cell activation

Combines with TLR4&STING agonists to promote NFκB and IRF signaling



Legend. (A) Representative data of PBMC-Tumor cell co-culture assay; data for NKT cells are not shown. Ten donors were tested in three independent experiments. (B) Effector Jurkat cells expressing ILT2 and an FcγR-dependent luciferase reporter were co-cultured overnight with CTLA-4-expressing Raji cells in the presence of botensilimab (Fc-enhanced anti-CTLA-4) and a dose titration of AGEN1571 or isotype control antibodies. Luciferase activity was measured and expressed as Relative Luminescence Units (RLU). (C) Reporter assay using THP-1-Dual™ NF-κB-SEAP and IRF-Lucia monocytes. Statistical analysis was performed by (A) one-way ANOVA or (C) t-test (*p<0.05, **p<0.01, ***p<0.001, ***p<0.0001).

Conclusions

- AGEN1571 is a high-affinity, fully human, ILT2 antagonist antibody with potential best-in-class activity.
- AGEN1571 demonstrates more effective HLA-G blocking activity and superior innate and adaptive immune activation potential compared to competitor ILT2 antibody (15G8).
- AGEN1571 demonstrates single agent activity and broad combination potential to enhance anti-tumor immunity.
- AGEN1571's mechanism-of-action and potent pharmacologic properties support its development as a therapeutic agent for patients with solid tumors. Clinical trials expected to start in 2022.