AGEN2373 is a conditionally-active agonist antibody targeting the co-stimulatory receptor CD137 for the treatment of human malignancies

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Rationale for CD137 agonists in cancer

CD137 (TNFRSF9, 4-1BB) is a member of the tumor necrosis factor receptor superfamily that functions as a potent co-stimulator of adaptive and innate immune cells[1] (Figure 1). The antitumor activity from targeting the CD137 pathway in preclinical models[2] has provided rationale for pharmacologic modulation of the CD137 axis in cancer patients. Antibody-mediated stimulation of CD137 is anticipated to augment T cell co-stimulation, enhance NK cell cytotoxicity, promote maturation of antigen presenting cells (APCs), and suppress T regulatory cells (Tregs)[1]. Despite signs of clinical activity, the development of first-generation anti-CD137 antibodies has been hampered by on-target, dose-limiting hepatotoxicity[3,4]. Emerging data also suggest that pharmacologic optimization of epitope and Fc interactions is critical for realizing maximal efficacy for this class of molecule[5].

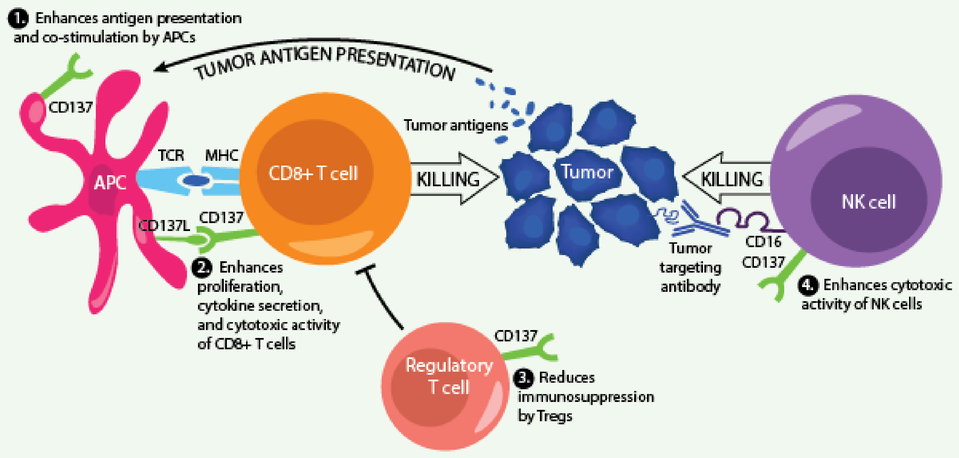


Figure 1. CD137 holistic action on immune cells. CD137 signaling promotes cytotoxicity of CD8+ T cells and antibody-dependent cellular cytotoxicity (ADCC) of NK cells, suppresses Tregs, and induces maturation of APCs.

Translating CD137 co-stimulation to the clinic

To overcome clinical limitations, Agenus has developed AGEN2373, a novel anti-CD137 antibody designed to stimulate CD137 signaling only in the context of ongoing immune cell activation.

A

Anti-CD137 clone	X-link dependence	Ligand blocking	Fc	T cell stimulation	Human liver toxicity
AGEN2373	+	-	IgG1	++	TBD
Reference #1	-	-	IgG4	++	Yes
Reference #2	+/-	+	IgG2	+	No

B

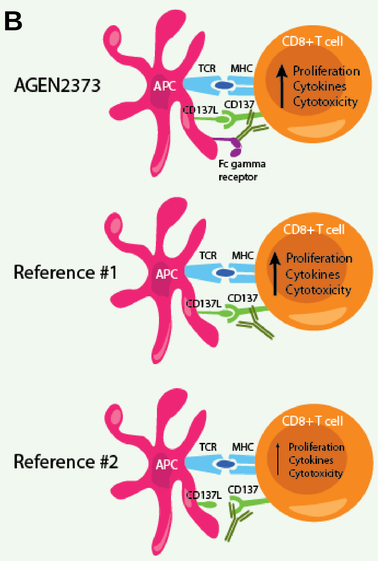


Figure 2. Anti-CD137 agonistic strategies. (**A**) Comparative table showing the characteristics of AGEN2373 versus Reference #1 and Reference #2. (**B**) Representation of the mechanism of actions of AGEN2373 versus Reference #1 and Reference #2 in the context of an immune synapse between an APC and a CD8+ T cell.

Conclusion

The pharmacologic and non-clinical safety profile of AGEN2373, a novel anti-CD137 antibody designed to provide potent yet restricted CD137 pathway co-stimulation, supports the potential for a therapeutic window in patients as a monotherapy or in combination with other therapeutic modalities.

AGEN2373 binds to a non-ligand blocking epitope

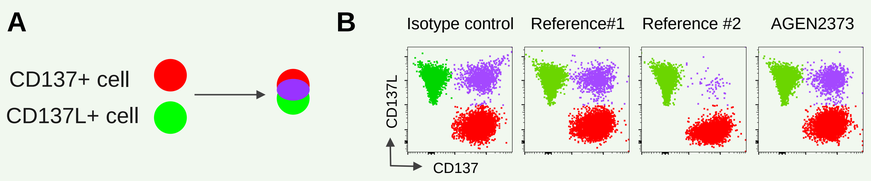


Figure 3. AGEN2373 binds CD137 without disruption of ligand binding. (A) Cell conjugation assay principle. PKH26-stained Jurkat cells expressing CD137 (red) and PKH67-stained Jurkat cells expressing CD137L (green) were pre-incubated with anti-CD137 antibodies or isotype control, and then incubated with Jurkat-CD137L. **(B)** Cell conjugates appear double positive for CD137 and CD137L (purple).

AGEN2373 conditionally stimulates CD137 under receptor clustering conditions

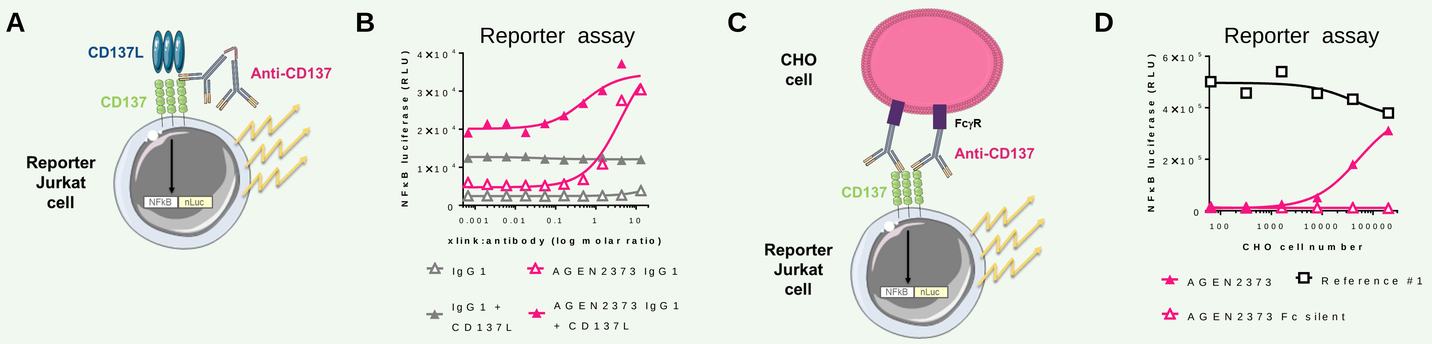


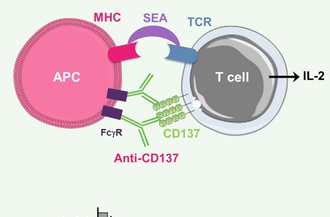
Figure 4. Two antibody crosslinking methods demonstrate that AGEN2373 requires antibody clustering to induce CD137 forward signaling. (A and C) Assay principles. Antibody function was tested in a Jurkat-CD137-NFκB-luciferase reporter cell assay under increasing crosslinking conditions. CD137 signaling was read out as relative luciferase expression (RLU). (**B**) CD137 signaling with AGEN2373 or an isotype control incubated with a dose range of anti-human IgG F(ab)'$_2$ in the absence or presence of soluble human CD137L-His. (**D**) CD137 signaling with anti-CD137 antibodies incubated with a dose range of CHO cells expressing CD16/FcγRIIA.

Optimal activity of AGEN2373 on IgG1 Fc backbone in cytokine release assays

A

Fc Format	FcγRI	FcγRIIA	FcγRIIB	FcγRIIIA
IgG1	++	+	+	++
IgG2	-	+	-	-
IgG4	+	-	+/-	-

B



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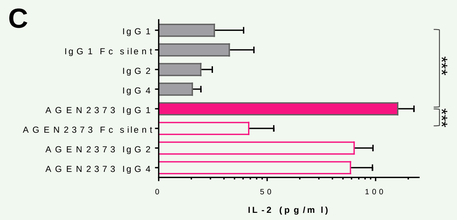

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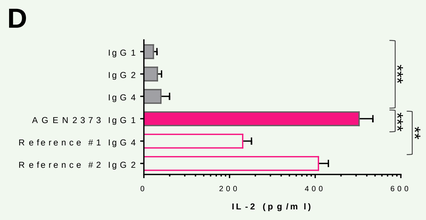


Figure 5. Sub-optimally superantigen-stimulated T cells release cytokines upon activation by Fc-competent AGEN2373. (**A**) Human Fc backbone binding to human FcγR sub-types[6]. (**B**) Assay principle. T cell receptor (TCR) cross-bridging to the Major Histocompatibility Complex class II (MHC II) on an APC *via* the superantigen staphylococcal enterotoxin A (SEA) stimulates T cell signaling. IL-2 production in supernatants was analyzed by AlphaLISA after a 5-day incubation. **(C)** IL-2 production from human PBMCs incubated with SEA peptide and AGEN2373 on multiple Fc backbones (pink bars) or respective isotype controls (grey bars). (**D**) IL-2 production from human PBMCs incubated with SEA peptide and anti-CD137 antibodies (pink bars) or respective isotype controls (grey bars) One-way ANOVA test: **, p<0.01; *** p<0.001.

AGEN2373 combines with other checkpoint modulators to enhance T cell activity

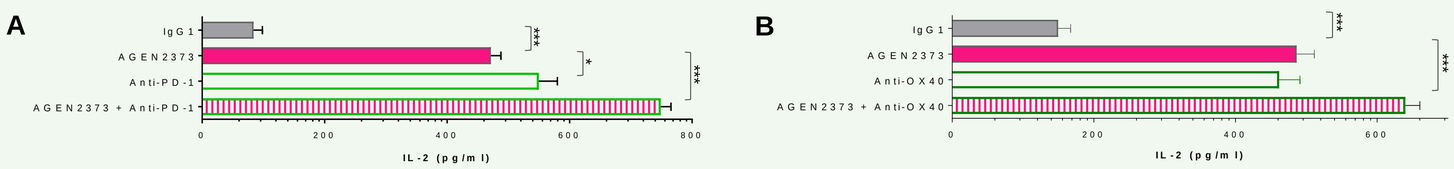
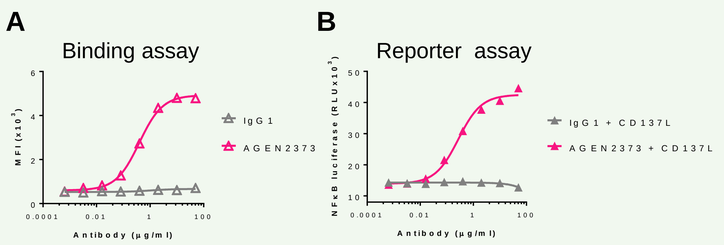
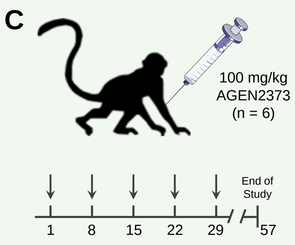


Figure 6. AGEN2373 combines with PD-1 antagonist and OX40 agonist. T cell stimulation is induced by cross-bridging TCR and MHC II as in Fig. 5B. IL-2 production from human PBMCs incubated with Staphylococcal Enterotoxin A (SEA) and AGEN2373 and/or anti-PD-1 (**A**), and/or anti-OX40 (**B**) or their respective isotype controls. One-way ANOVA test: **, p<0.01; ***, p<0.001.

Non-clinical safety of AGEN2373 in non-human primates





- No changes in clinical observations following weekly 100 mg/kg on study
- A transient and recoverable increase, in normal range, in mean alanine aminotransferase (ALT) observed at Day 8. No related histopathology changes observed.
- No changes in cellularity or frequency of immune cell subsets consistent with a lack of indiscriminate cell activation or cell depletion (*e.g.* ADCC)

Figure 7. AGEN2373 binds and induces signaling through cynomolgus monkey CD137. (A) Binding of AGEN2373 antibody or a human IgG1 isotype control antibody to activated cynomolgus monkey primary CD8$^+$ T cells. **(B)** NFκB-luciferase reporter activity in Jurkat cells expressing cynomolgus monkey CD137 and incubated with serial dilutions of the AGEN2373 antibody or a human IgG1 isotype control antibody. Design **(C)** and summary **(D)** of a toxicology study in cynomolgus monkey. Six animals received 100 mg/kg AGEN2373. AGEN2373 was given at Day 1, 8, 15, 22, and 29. Serum and PBMCs were harvested weekly.

Disclosures

Galand, Xiao, Mundt, Morin, Chand, Riordan, Venkatraman, Ward, Gombos, Lim, Costa, Joyce, Ignatovich, Findeis, Underwood, Stein, van Dijk, Wilson, Savitsky: Present and former employment and stock ownership – *Agenus Inc.*

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1. Yonezawa A et al. *Clin Cancer Res*. 2015;21(14):3113-20. **2**.Bartkowiak T et al. *Proc Natl Acad Sci U S A*. 2015;112(38):E5290-9. **3**. Segal NH et al. *Clin Cancer Res*. 2018;24(8):1816-1823. **4**. Seagal NH et al. *Clin Cancer Res*. 2017;23(8):1929-1936. **5**. Mimoto F et al. *Protein Eng Des Sel*. 2013 Oct;26(10):589-98. **6**. Arce Vargas F et al. Cancer Cell. 2018;33(4):649-663.