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Poster # 377

AGEN2373 is a CD137 agonist antibody designed to leverage optimal CD137 and FcγR co-targeting to promote antitumor immunologic effects

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Background

AGEN2373 is a conditionally active CD137 agonist antibody designed to selectively enhance innate and adaptive immunity only in presence of Fc-gamma receptors

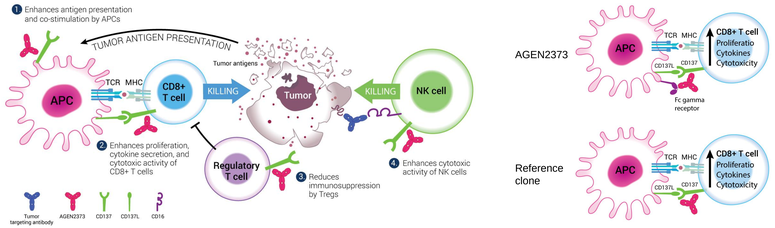
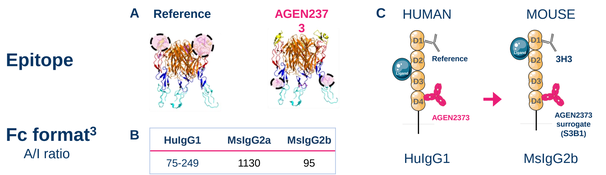


Figure 1: AGEN2373 (fully human, IgG1λ) is anticipated to enhance anti-tumor immunity through multiple mechanisms-of-action. Antibody-mediated CD137 agonist activity is expected to enhance antigen-presenting cells (APC), T cell, and natural killer (NK) cell function. In addition, AGEN2373 may target intratumoral Tregs for antibody-dependent cell cytotoxicity or phagocytosis (ADCC/ADCP)-mediated destruction[1, 2].

Figure 2: In contrast to reference clone 20H4.9, AGEN2373 agonist activity requires Fc-gamma receptor (FcγR) antibody clustering, as illustrated in the context of an APC-to-CD8+; T cell immunological synapse. This conditional activity is expected to mitigate on-target off-site immune activation potentially associated with immune-related toxicities observed for this class.

Tool building for modeling AGEN2373 responses in mice

AGEN2373 *versus* its mouse surrogate desired key characteristics

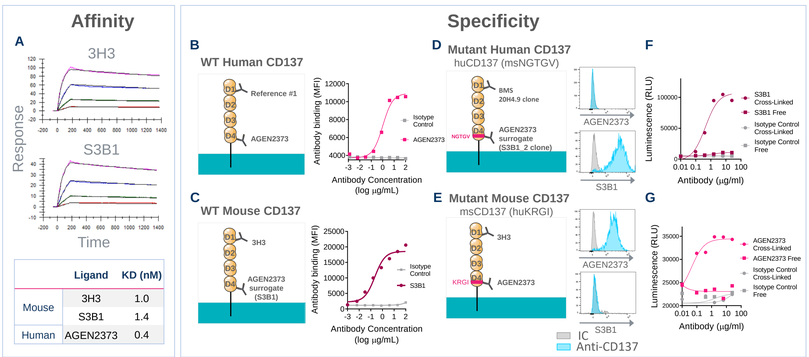


Fc format[3]
A/I ratio

B	HuIgG1	MsIgG2a	MsIgG2b
	75-249	1130	95

Figure 3: A) Crystal structure of the trimer CD137-trimer CD137L (orange) complex (PDB: 6CPR) highlighting, with circles, CD137 agonist antibody epitopes. **B)** Ratio of affinity measured by SPR for Activating-to-Inhibitory (A/I) Fc gamma-receptor reported for human IgG1 (human FcγRIIIA/FcγRIIB ratio), mouse IgG2a and mouse IgG2b (mouse FcγRIV/FcγIIB ratio). **C)** Epitope and Fc comparison of human and murine CD137 antibodies.

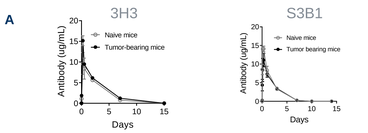
Validation of AGEN2373 true surrogate clone S3B1



	Ligand	KD (nM)
Mouse	3H3	1.0
	S3B1	1.4
Human	AGEN2373	0.4

Figure 4: AGEN2373 and AGEN2373 surrogate S3B1 binding properties were analyzed by SPR and flow cytometry. **A)** Mouse CD137-Fc was flowed over anti-mouse CD137 antibodies captured on a CM5 chip at a range of concentrations [0 to 300nM]. Dose titration of anti-CD137 antibodies binding to **B)** fully human, **C)** fully mouse, **D)** murinized-epitope, or **E)** humanized-epitope CD137. CD137 signaling in the presence and absence of antibody cross-linking was analyzed in a **F)** human- and **G)** mouse-epitope CD137ˢNFκB-luciferase reporter Jurkat cells.

PK of anti-mouse CD137 agonist antibodies supports q3-4d dosing regimen



B

Parameter	3H3 Mean [Min, Max]	S3B1 Mean [Min, Max]
CL (mL/h)	0.027 [0.026, 0.028]	0.4 [0.38, 0.41]
HL (d)	3.71 [3.72, 3.75]	2.34 [2.32, 2.38]
Cmax (µg/mL)	14.6 [14.3, 15.3]	1.48 [1.46, 1.51]
$AUC_{0\text{-}inf}$ (h*µg/mL)	1839 [1835, 1879]	125 [121, 132]

Figure 5: Balb/c mice, naïve or implanted subcutaneously with 10^5 CT26 cells, received intravenously 50µg anti-mouse CD137 surrogate clone S3B1 msIgG2b or 50µg anti-mouse CD137 reference antibody 3H3 rIgG2a (BioXcell), administered once at a tumor size range 50-100 mm^3. **A)** Antibody concentration measured in plasma from 30 min to ~14 days post drug administration by a direct ELISA with an LLoQ of 0.022 µg/mL. **B)** Pharmacokinetic parameters were analyzed using Nonlinear-Mixed-Effects (NLME) methodology (software ADAPT 5) and fitted to a 2-compartment structural model (2-CM).

AGEN2373 surrogate does not show sign of liver inflammation

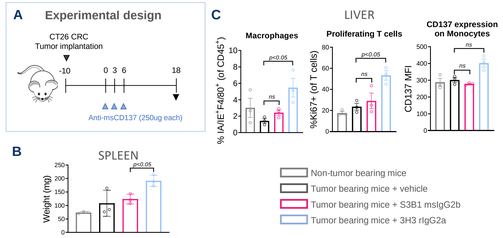


Figure 8: Balb/c mice were implanted subcutaneously with 10^5 CT26 cells. Mice received intraperitoneally three 250µg injections of AGEN2373 surrogate S3B1 msIgG2b or anti-mouse CD137 reference antibody 3H3 rIgG2a (BioXcell), when tumor size reached 50-100 mm^3. **A)** Experimental design. **B)** Spleens were harvested and weighed at day 18. A student *t* test was used to analyze differences between groups. **C)** Immune cell infiltrate of lower liver lobe was characterized by flow cytometry at day 18. A nonparametric Kruskal-Wallis test was used to analyze differences between groups.

Efficacy of anti-mouse CD137 agonist antibodies is optimal with D4 targeted/msIgG2b format

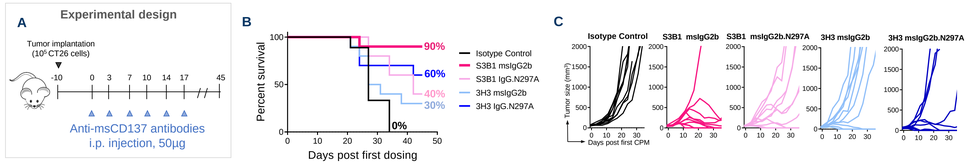


Figure 6: Balb/c mice were implanted subcutaneously with 10^5 CT26 cells. Mice were treated intraperitoneally twice weekly for 3 weeks with 50µg anti-mouse CD137 surrogate clone S3B1 msIgG2b, S3B1 msIgG2b.N297A, 3H3 msIgG2b, 3H3 msIgG2b.N297A, or a pool of isotype controls. Day 0 is the first day of treatment at tumor size of 50-100 mm^3. **A)** Experimental design. **B)** Survival curve for each treatment group. **C)** Individual tumor volumes measured every 3-4 days (N=9 or 10). MsIgG2b.N297A carry a mutation reducing binding to Fc gamma receptors.

PD signatures reflect anti-mouse CD137 epitope/Fc format combinations mechanisms-of-action

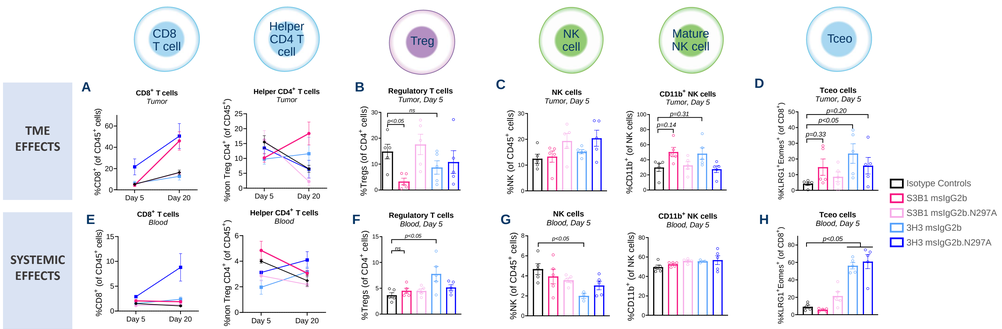


Figure 7: Immunophenotyping of the tumor microenvironment (TME) in **A-D** and blood in **E-H** in CT26 tumor-bearing mice treated twice weekly for 3 week with 50µg anti-mouse CD137 surrogate clone S3B1 msIgG2b, S3B1 msIgG2b.N297A, 3H3 msIgG2b, 3H3 msIgG2b.N297A, or a pool of isotype controls following experimental design of Figure 6. **A, E.** Frequencies of CD8 T cells and non Tregs CD4 T cells among $CD45^+$ cells. **B, F**. Frequencies of Tregs among CD4 T cells. **C, G**. Frequencies of total NK cells among CD45+ cells and mature CD11b+ NK cells among total NK cells. **D, H**. Frequencies of Tceos among CD8 T cells.

AGEN2373 is well tolerated with no sign of liver toxicity in ongoing Ph1 clinical trial

A

	Treatment-related Adverse Events
n	16
Severity, n (%)	
Grade 1 or 2	15 (93.7)
Grade 3 or higher	**0**

B

Parameter	Baseline CTCAE grade	Baseline n (%)	On Treatment (highest grade per patient) Normal n (%)	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)
ALT (U/L)	Normal	16 (100)	12 (75.0)	4 (25.0)	0 (0.0)	0 (0.0)
AST (U/L)	Normal	10 (62.5)	9 (56.3)	1 (6.3)	0 (0.0)	0 (0.0)
	Grade 1	6 (37.5)	1 (6.3)	2 (12.5)	3 (18.7)	0 (0.0)
Total Bilirubin (µmol/L)	Normal	15 (93.7)	15 (93.7)	0 (0.0)	0 (0.0)	0 (0.0)
	Grade 2	1 (6.3)*	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)*

C

Dose group	Tumor response at week 16
0.03 mg/kg	**SD**
	n/a
	SD
0.06 mg/kg	n/a
	n/a
	SD
	PD
	PD
	SD
0.3 mg/kg	PD
	PD
	PD
1 mg/kg	n/a
	PD
	n/a
	n/a
Total **SD**	4

Figure 9. An ongoing phase 1 study [NCT04121676] of AGEN2373 to evaluate AGEN2373, with a cut-off date of October 6th, 2020. Patients were enrolled in a 3+3 dose escalation in doses ranging from 0.03 to 1 mg/kg cohorts. **A)** Summary of treatment-related adverse events. **B)** Summary for liver function parameters from baseline to worst post-baseline toxicity grade (CTCAE V5). **C)** Best overall responses. SD: 4 SD at 16 weeks.*Patient with progressing liver metastases. SD: stable disease. PD: Progressive Disease. n/a: not available.

AGEN2373 surrogate antibody, in combination with other CPMs, improve responses in cold tumor mouse model

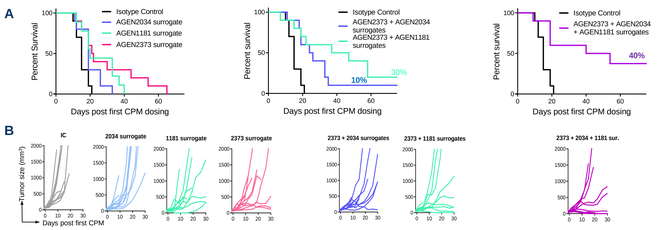


Figure 10: C57Bl/6 mice were injected subcutaneously with 10^5 B16F1-OVA cells and treated intraperitoneally twice weekly for 3 weeks as described in Figure 6 experimental design with 50µg AGEN2373 surrogate clone S3B1 msIgG2b and/or 50µg anti-mouse CTLA-4 AGEN1181 surrogate clone 9D9 msIgG2b.3M and/or 200µg of anti-mouse PD-1, AGEN2034 clone RPMI-14 rIgG2a or corresponding isotype control antibodies. Day 0 is the first day of treatment at tumor size of 50-100 mm^3. **A)** Survival curve for each treatment group. **B)** Individual tumor volumes were measured every 3-4 days (N=9 or 10).

Conclusions

Preclinical efficacy and pharmacodynamic studies illustrated antibody critical CD137-epitope targeting and FcγR interactions that optimally promote innate and adaptative immune response against solid tumors while avoiding immune-related adverse events, especially liver inflammation observed for this antibody class.

- AGEN2373 and an affinity-, epitope-, and Fc-match surrogate antibody S3B1 exhibited selective CD137 agonist activity only in the presence of FcγRs.
- AGEN2373 surrogate is an Fc competent CD137 domain 4-targeting antibody that drives intratumor-selective T cell expansion, NK cell activation, and Treg depletion but no systemic inflammation either in liver nor blood. In contrast, a CD137 domain 1-targeting antibody 3H3 induced systemic immune cell activation.
- Consistent with these findings, AGEN2373 monotherapy has shown clinical benefit in 4 patients (SD) and a favorable safety profile with no evidence of transaminitis in 16 patients dosed up a 1 mg/kg.

A phase 1 study of AGEN2373 in combination with balstilimab (Agenus anti-PD-1 antibody) is planned.

References:

1. Sugamura et al., *Nat Rev Immunol 2004*
2. Kohrt et al., *J Clin Invest 2014*
3. Vincent, Oral Presentation, PEGS 2019
4. Buchan et al., *Immunity 2018*

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