Characterization of the anti-CTLA-4 antibody AGEN1884, including toxicology and pharmacology assessments in non-human primates

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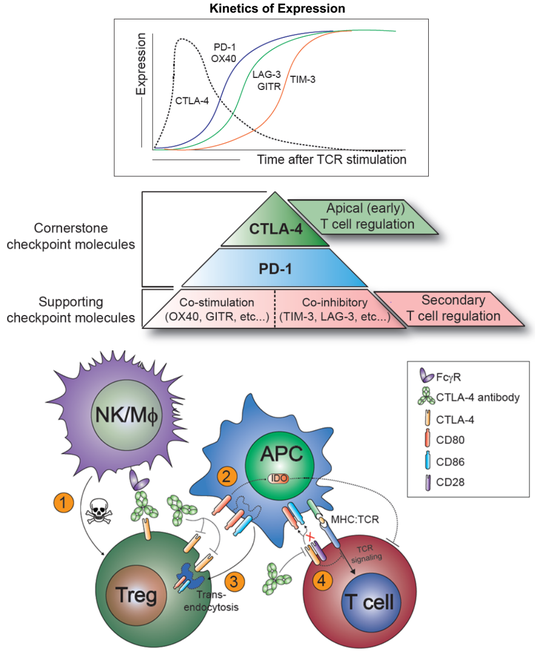
Abstract

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an important negative regulator of T cell function. Together with CD28, these receptors exemplify a co-inhibitory and co-stimulatory signaling axis that dynamically sculpts the interaction of antigen-specific T cells with antigen presenting cells (APCs). Preclinical studies have demonstrated that anti-CTLA-4 antibodies can enhance tumor-specific immunity through a variety of mechanisms including: i) blockade of CD80 or CD86 binding to CTLA-4; ii) preventing CTLA-4-expressing regulatory T cells from physically removing CD80 and CD86 from the surface of APCs; and iii) selective elimination of CTLA-4-expressing intratumoral regulatory T cells by an Fcγ receptor-dependent mechanism. Here we describe the pharmacological and toxicological characterization of a novel human IgG1 anti-CTLA-4 antagonist antibody, AGEN1884. AGEN1884 potently enhanced T cell responsiveness *in vitro*, and combined effectively with other immunomodulatory antibodies targeting co-inhibitory and co-stimulatory receptors on T cells. AGEN1884 was well-tolerated in non-human primates and was confirmed to modulate cellular and humoral immune responses to co-administered reporter vaccines. In addition to the activity of AGEN1884 as a monotherapy, a memory T cell proliferative response was observed in peripheral blood of animals when co-administered with an anti-PD-1 antibody. Finally, we provide a comparison of the *in vitro* and *in vivo* functional properties of an IgG2 variant of AGEN1884, revealing important antibody isotype differences that may have an impact on the design of optimal dosing regimens in patients. Taken together, the pharmacologic properties of AGEN1884 support its clinical investigation as both a single therapeutic agent and in combination therapies..

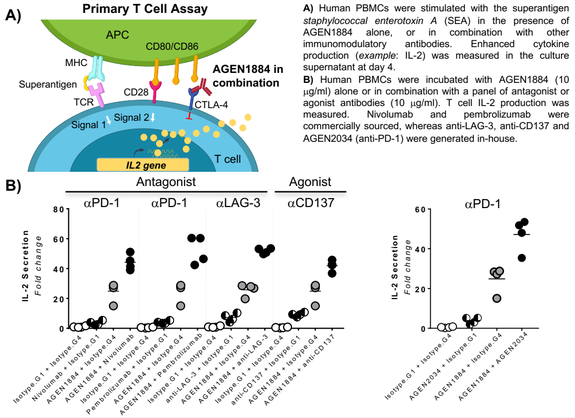
CTLA-4: A Master Regulator of the Immune Synapse



Potential MoAs and therapeutic interventions

1. ADCC/P of Tregs
2. CTLA-4 induced IDO production
3. Trans-endocytosis of CD80/86
4. Prevention of CD28 co-stimulation

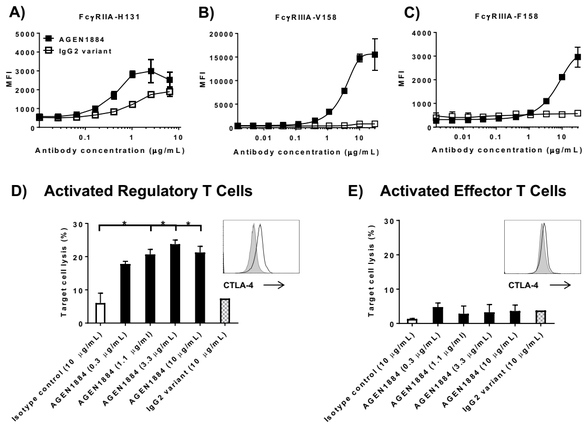
AGEN1884 Cooperates With Other Immunomodulatory Antibodies to Enhance T cell Responsiveness



A) Human PBMCs were stimulated with the superantigen *staphylococcal enterotoxin A* (SEA) in the presence of AGEN1884 alone, or in combination with other immunomodulatory antibodies. Enhanced cytokine production (*example:* IL-2) was measured in the culture supernatant at day 4.

B) Human PBMCs were incubated with AGEN1884 (10 μg/ml) alone or in combination with a panel of antagonist or agonist antibodies (10 μg/ml). T cell IL-2 production was measured. Nivolumab and pembrolizumab were commercially sourced, whereas anti-LAG-3, anti-CD137 and AGEN2034 (anti-PD-1) were generated in-house.

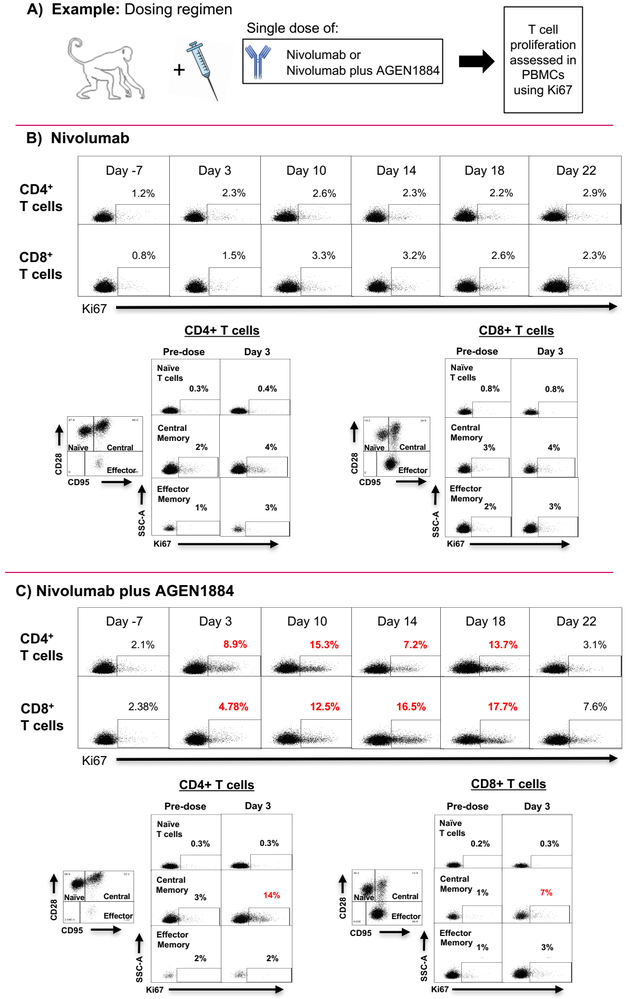
AGEN1884 Engages Fc Gamma Receptors and Has the Potential to Mediate ADCC Toward Activated Regulatory T cells



A-C) Binding of increasing doses of AGEN1884 or an IgG2 Fc variant to **A)** rJurkat-huFcγRIIA-H131*, **B)** rCHO-huFcγRIIIA-V158*, and **C)** rCHO-huFcγRIIIA-F158* engineered cell lines. The mean fluorescence intensity (MFI) was determined based on binding of an anti-F(ab')2-PE labeled secondary F(ab')2 fragment to AGEN1884 (black squares) compared to the IgG2 variant (white squares) are shown.

D-E) Primary effector T cells or regulatory T cells were activated with anti-CD3/CD28 beads for 7 days. After stimulation, CTLA-4 and Foxp3 expression were confirmed by flow cytometry. **D)** CTLA-4^{high} target CD3$^+$FoxP3$^+$ T cells or **E)** CTLA-4$^+$ target CD3$^+$FoxP3$^-$ T cells were co-cultured with primary NK cells at an effector:target ratio of 5:1 in the presence of increasing concentrations of AGEN1884. Cell-specific lysis was assessed as a percentage of CD3+ target cells that stained positive for the non-viable cell marker 7-aminoactinomycin D when assessed using flow cytometry. As a control, cells were incubated with 10 μg/mL of an IgG2 variant. Data were analyzed using a Student's t-test for each dose of AGEN1884 compared to the isotype. Significant differences depicted were p<0.05 (*).

AGEN1884 Cooperates with PD-1 Blockade to Enhance Proliferation of Central Memory T cells in Non-human Primates

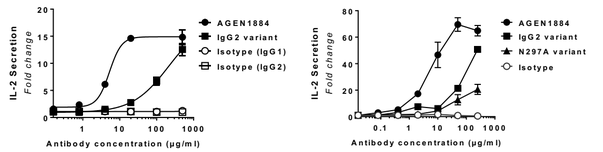


A) Cynomolgus monkeys were administered nivolumab alone (3 mg/kg) or in combination with AGEN1884 (10mg/kg). PBMCs were isolated pre and post antibody administration. PBMCs were thawed and surface stained with T cell lineage markers and a viability dye, followed by permeabilization and staining for a cellular marker of proliferation (Ki67). Cells were analyzed using flow cytometry.

B) Representative histograms and dot plots from an individual animal administered nivolumab alone.

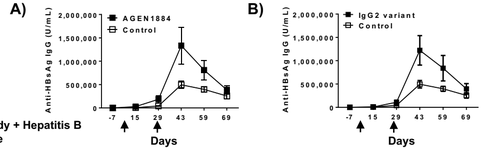
C) Representative histograms and dot plots from an individual animal administered nivolumab together with AGEN1884.

AGEN1884 (IgG1) is More Potent than an IgG2 or Fc Silent Variant at Enhancing T cell Responsiveness



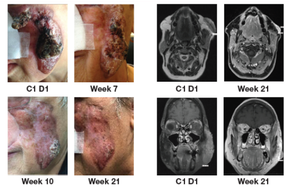
Human PBMCs were stimulated with a sub-maximal concentration of the SEA superantigen (100 ng/mL) and increasing doses of AGEN1884, an IgG2 variant, an N297A variant or isotype controls. Representative data indicate the mean ± SEM of multiple replicates in each treatment group.

Immune Responses in Non-human Primates to Reporter Vaccines Co-administered with AGEN1884 or an IgG2 Fc Variant



Cynomolgus monkeys (n=6 per group) were administered 10 mg/kg of **A)** AGEN1884 or **B)** an IgG2 Fc variant *via* IV administration with a Hepatitis B vaccine on days 1 and 29. Duplicate samples were analyzed for anti-HBsAg-specific IgG serum titers.

Preliminary Clinical Data Reveal Signs of Clinical Activity with Complete Response of Angiosarcoma After Treatment with AGEN1884



In Phase 1 clinical trials (NCT02694822), early clinical data observations of one confirmed complete response (July 21, 2017) by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria. Patient 189-002 was a 62-year-old female being treated for angiosarcoma of nose and cheeks with 0.1mg/kg of AGEN1884 (n=5 in cohort). At week 33, a 92% reduction of target lesion was observed (Wilky BA, *et al.* Poster presented at: 53rd Annual Meeting of the American Society of Clinical Oncology; 2017 June 2-6; Chicago, IL)

Summary

- AGEN1884 cooperates with antibodies targeting the PD-1 pathway and other immuno-modulatory co-inhibitory and co-stimulatory pathways to promote T cell responsiveness
- In combination with anti-PD-1, AGEN1884 also promoted a PD proliferative response in circulating T cells *in vivo*
- By virtue of its human IgG1 Fc region, AGEN1884 demonstrated selective depletion of CTLA-4-expressing intratumoral Treg cells and increased potency at enhancing IL-2 production *in vitro*
- At maximal doses, AGEN1884 and the IgG2 variant demonstrated similar enhancement of vaccine-specific antibody responses in non-human primates
- Phase 1 clinical trial (NCT02694822) evaluating AGEN1884 in patients with advanced solid tumors has demonstrated acceptable safety profile at 0.1, 0.3, 1.0 and 3.0mg/kg dose levels

Author Disclosures

Randi B. Gombos, Ana Gonzalez, Mariana Manrique, Dhan Chand, David Savitsky, Benjamin Morin, Ekaterina Breous-Nystrom, Christopher Dupont, Rebecca A. Ward, Cornelia Mundt, Benjamin Duckless, Hao Tang, Mark A. Findeis, Andrea Schuster, Jeremy D. Waight, Dennis Underwood, Christopher Clarke, Marc van Dijk, Jennifer S. Buell, Jean-Marie Cuillerot, Robert Stein, Elise E. Drouin and Nicholas S. Wilson: *Agenus Inc. and subsidiaries thereof:* Current or former employment and stock ownership.
Breelyn Wilky, David Schaer, Jedd Wolchok, Taha Merghoub and Gerd Ritter: No competing interests declared.

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Acknowledgments

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