AGEN1884, an IgG1 anti-CTLA-4 antibody, combines effectively with PD-1 blockade in primary human T cell assays and in a non-human primate pharmacodynamic (PD) model

Elise E. Drouin[1], David Savitsky[1], Ana M. Gonzalez[1], Randi Gombos[1], Dhan Chand[1], Jeremy Waight[1], Xia Yang[1], Mithun Khattar[1], Benjamin Morin[1], Mark Findeis[1], David Schaer[2], Taha Merghoub[2], Gerd Ritter[3], Antoine Tanne[1], Marc van Dijk[4], John M. Goldberg[1], Daniel Levey[1], John Castle[4], Jean-Marie Cuillerot[1], Jennifer S. Buell[1], Robert Stein[1], Nicholas S. Wilson[1]. [1]Agenus Inc., Lexington, MA; [2]Memorial Sloan Kettering Cancer Center, New York, NY; [3]The Ludwig Institute for Cancer Research; [4]Agenus Switzerland Inc.

Poster: #27
Abstract: #3654
AACR Annual Meeting 2017
Washington, DC, USA • April 1-5, 2017

Abstract

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) play important non-redundant roles in negatively regulating T cell immune responses. Therapeutic blockade of CTLA-4 or PD-1 pathways has been demonstrated to enhance T cell reactivity to tumor-specific antigens, translating to a significant improvement in overall survival in subsets of patients. This tumoricidal effect can be further augmented when PD-1 and CTLA-4 antagonist antibodies are co-administered.

AGEN1884, a human IgG1 antibody directed against CTLA-4, potently inhibits CTLA-4 binding to CD80 and CD86, resulting in enhanced T cell responsiveness *in vitro*, as well as in a vaccination model in non-human primates. A Phase 1 clinical study (NCT02694822) is currently ongoing to evaluate the safety and pharmacokinetic (PK)/pharmacodynamic (PD) relationships in patients with advanced solid tumors. AGEN2034 is a human IgG4 antibody that binds selectively to PD-1 with high affinity and potentiates T cell responsiveness *via* the blockade of PD-L1 and PD-L2 binding to PD-1. Here we evaluated the pharmacologic effect of combining AGEN1884 with AGEN2034 and other molecules targeting the PD-1/PD-L1 pathway on primary human T cell immune responses. Consistent with these *in vitro* findings, the co-administration of AGEN1884 with an anti-PD-1 antibody in cynomolgus monkeys (*Macaca fascicularis*) induced a dynamic PD effect, including a proliferative T cell response in peripheral blood, as compared to animals receiving either antibody alone. Finally, co-administration of an anti-mouse CTLA-4 antibody together with Agenus' neoepitope-based AutoSynVax™ vaccine in mice induced effective amplification of vaccine-driven T cell responses, compared to animals that received the vaccine alone. These data further exemplify the versatility of harnessing antibody-mediated CTLA-4 blockade to influence apical events involved in T cell priming by antigen presenting cells.

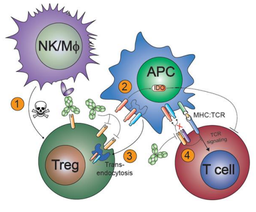
Taken together, these *in vitro* and *in vivo* findings demonstrate that the combination of AGEN1884 with PD-1 pathway blockade or with neoepitope-based vaccines has the potential to provide therapeutic activity that is superior to that of either checkpoint- or vaccine-based monotherapies.

AGEN1884 and AGEN2034 Clinical Trials

	AGEN1884	AGEN2034
Target	CTLA-4	PD-1
Characterization	Human IgG1	Human IgG4-S228P
Discovery Platform[1]	Retrocyte Display™	Retrocyte Display™
Mechanism of action	Antagonist	Antagonist
Phase 1 Identifier	NCT02694822*	NCT03104699*

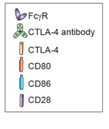
Note: *Clinical studies evaluating AGEN1884 in combination with AGEN2034 are expected to be initiated in the second half of 2017

Anti-CTLA-4 Mechanisms of action

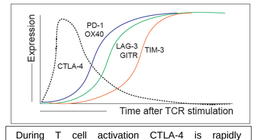


Potential MoAs and therapeutic interventions

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



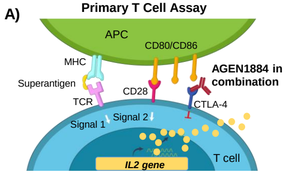
Kinetics of Expression



During T cell activation CTLA-4 is rapidly translocated into the immunological synapse and functions as critical early regulator during T cell priming and recall responses. CTLA-4 antagonist antibodies cooperate with other modalities that further sculpt the magnitude and quality of the T cell response.

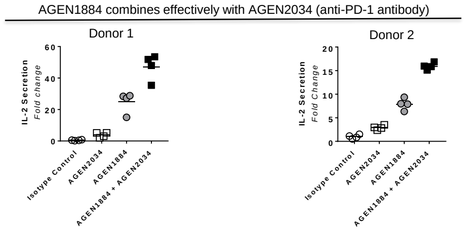
AGEN1884 Combines Effectively With Immunomodulatory Antibodies to Enhance T cell Responsiveness

A)

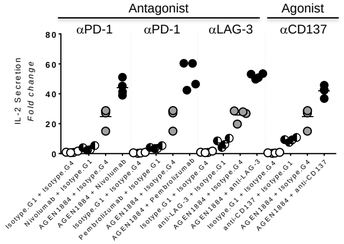


A) Human PBMCs were stimulated with the superantigen *staphylococcal enterotoxin A* (SEA) in the presence of AGEN1884 alone, or in combination with other immunomodulatory antibodies. Enhanced cytokine production (*example*: IL-2) was measured in the culture supernatant at day 4-to-5.
B) Human PBMCs were incubated with AGEN1884 (10 μg/ml) alone or in combination with AGEN2034 (anti-PD-1 antibody) (10 μg/ml). T cell IL-2 production was measured. Two donors shown.
C) 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 and anti-CD137 were generated in-house.

B)

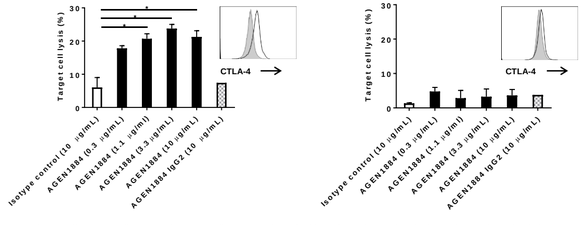


C)



AGEN1884 Preferentially Targets Activated Regulatory T cells for NK Cell-Mediated ADCC

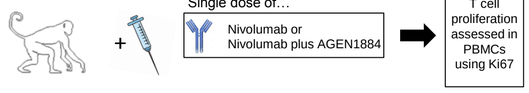
A) Activated Regulatory T Cells **B) Activated Effector T Cells**



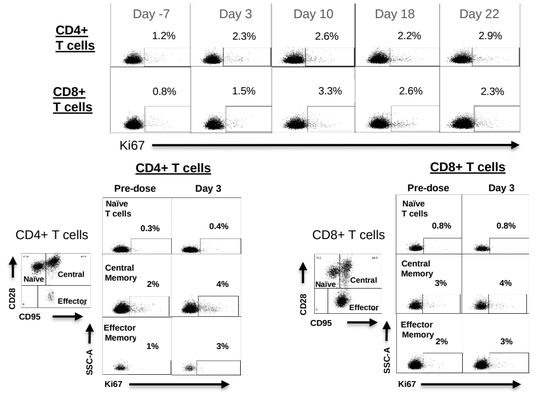
Primary effector T cells or regulatory T cells were activated with anti-CD3/CD28 beads for 7 days. After stimulation, CTLA-4 and Foxp3 expression were confirmed by flow cytometry. **A)** CTLA-4^{high} target CD3$^+$FoxP3$^+$ T cells or **B)** CTLA-4$^+$ target CD3$^+$FoxP3$^-$ T cells were co-cultured 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 (7-ADD) when assessed using flow cytometry. As a control, cells were incubated with 10 μg/mL of an IgG2 AGEN1884 variant (AGEN2041). 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 when Combined with Nivolumab Induces Proliferation of Central Memory T cells

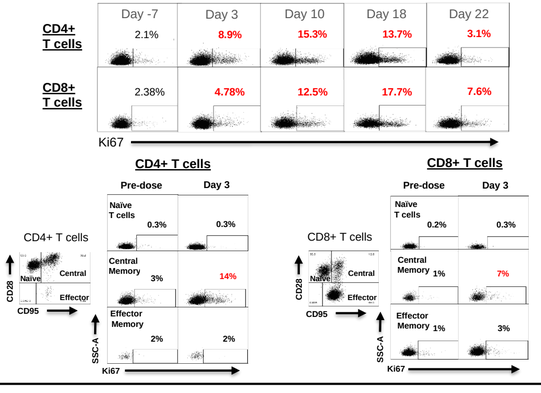
A) Example: Dosing regimen



B) Nivolumab



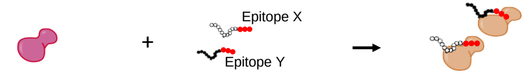
C) Nivolumab plus AGEN1884



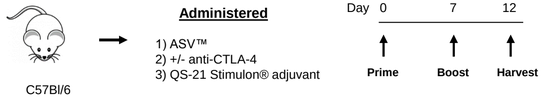
A) Cynomolgus monkeys were administered nivolumab alone (3 mg/kg) or in combination with AGEN1884 (10mg/kg). PBMCs were isolated pre and post antibody administration. PBMCs were thawed and surface stained with T cell lineage markers and a viability dye, followed by permeabilization and staining for a 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 dots plot from an individual animal administered nivolumab together with AGEN1884.

Anti-CTLA-4 Blockade Enhances T cell Responses to the Tumor Neoepitope-Based AutoSynVax™ Vaccine

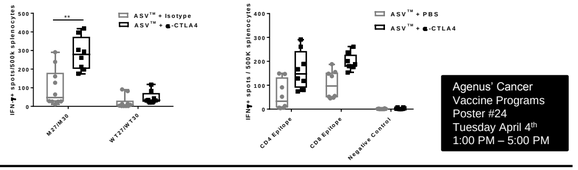
A) Generation of AutoSynVax™ vaccine



B) Dosing regimen



C) CTLA-4 blockade promotes CD4 and CD8 T cell immunity



Agenus' Cancer Vaccine Programs
Poster #24
Tuesday April 4th
1:00 PM – 5:00 PM

A & B) Naïve 6-8 week old female C57BL/6 mice were administered on days 0 and 7, 30 to 60 μg of Hsc70 complexed with 38mer peptides corresponding to the B16.F10 tumor neoantigens M27 and M30*, or in a separate study, a mixture of CD4 and CD8 epitopes of model viral antigens. Each peptide was linked to a heptameric sequence known to have a high affinity for the Hsc70 binding site. The ASV vaccine comprising the peptides was mixed with 10 μg of QS-21 Stimulon® adjuvant (Agenus) and co-administered 300 μg of the mouse anti-CTLA-4 antibody or isotype control.
C) Five days after the boost vaccination (Day 12) an ELISpot assay was used to measure the T cell response in lymphoid cells pooled from draining brachial, cervical lymph nodes and spleens of individual mice. Left panel: peptides containing the tumor-associated neoepitopes (M27 and M30) were tested, and their immunoreactivity was compared to the corresponding wild-type sequences (WT27 and WT30) as a control. Right panel: immunoreactivity was tested to peptides corresponding to the minimal viral CD4 and CD8 epitopes or an irrelevant peptide.

Summary

- AGEN1884 (anti-CTLA-4, IgG1) and AGEN2034 (anti-PD-1, IgG4) antibodies were discovered using Agenus' proprietary mammalian display antibody platform (Retrocyte Display™)[1]
- AGEN1884 and AGEN2034 have the ability to concurrently target the CTLA-4 and the PD-1 T cell inhibitory pathways augmenting T cell responsiveness more than either single agent
- AGEN1884 cooperated with other antibodies targeting the PD-1 pathway (nivolumab and pembrolizumab) *in vitro*, and promoted a PD proliferative response in circulating T cells *in vivo*
- AGEN1884 cooperated with other immuno-modulatory antibodies targeting co-inhibitory and co-stimulatory pathways to promote T cell responsiveness, which highlights the potential utility of using AGEN1884 in multiple clinical combination regimens
- In addition to antibodies, anti-CTLA-4 blockade combined effectively with Agenus' proprietary tumor neoepitope-based AutoSynVax™ vaccine and enhanced both CD4+ and CD8+ T cell immune responses
- Phase 1 clinical trials evaluating AGEN1884 or AGEN2034 in patients with advanced solid tumors have been initiated to evaluate safety and establish pharmacokinetic (PK) and pharmacodynamic (PD) relationships
- Clinical studies to evaluate AGEN1884 in combination with AGEN2034 are planned, as well as AGEN1884 in combination with AutoSynVax™

Author Disclosures
Elise E. Drouin, David Savitsky, Ana M. Gonzalez, Randi Gombos, Dhan Chand, Jeremy Waight, Xia Yang, Mithun Khattar, Benjamin Morin, Mark Findeis, Antoine Tanne, Marc van Dijk, John Goldberg, Daniel Levey, John Castle, Jean-Marie Cuillerot, Jennifer Buell, Robert Stein, Nicholas S. Wilson: *Agenus Inc:* Employment and Stock ownership.
David Schaer, Taha Merghoub and Gerd Ritter: No competing interests declared.

References
1. Breous-Nystrom E *et al.* Methods. 2014; 65:57-67.
2. Tykodi, SS. OncoTargets and Therapy. 2014; 4:1345
2. Buchbinder, EI and Desai, A. Am J Clin Oncol. 2016;
3. Mo A. *et al.* Vaccine. 2011; 29:8530-8541.
4. Kreiter S. *et al.* Nature. 2015; 520:692-696.