# AGEN2034, a novel anti-PD-1 antibody that combines effectively with CTLA-4 pathway blockade to enhance T cell activity

Dhan Chand[1], David Savitsky[1], Ana Gonzalez[1], Mariana Manrique[1], Christopher Clarke[1], Andrea Schuster[1], Elise E. Drouin[1], Jeremy D. Waight[1], Cornelia Mundt[1], Gerd Ritter[2], Taha Merghoub[3], David Schaer[3], Rikke B. Holmgaard[3], Roberta Zappasodi[3], Jedd Wolchok[3], Marc van Dijk[1], Jennifer S. Buell[1], Jean-Marie Cuillerot[1], Robert Stein[1] and Nicholas S. Wilson[1]

[1] Current or former employee of Agenus Inc., Lexington, MA, USA, or subsidiary thereof; [2]The Ludwig Institute for Cancer Research; [3]Memorial Sloan Kettering Cancer Center, New York, NY

agenus

**Poster: #P312**
SITC Annual Meeting
Washington, DC, USA • November 9-12, 2017

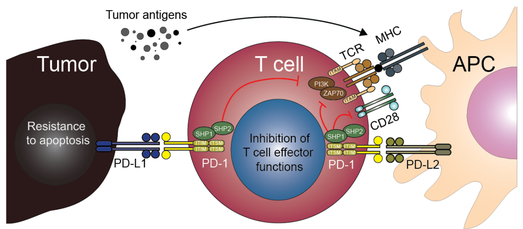
## Abstract

PD-1 (or CD279) is a co-inhibitory receptor that suppresses T cell function upon binding to its ligands, PD-L1 or PD-L2. PD-1 signaling functions cooperatively with CTLA-4 to limit T cell activation during priming by antigen presenting cells, leading to reduced proliferation, cytokine and chemokine production and cell survival. Anti-PD-1 antibody therapies that block the interaction between PD-1 and its ligands have shown durable clinical benefit both as single agents, but particularly in combination with antibodies that antagonize CTLA-4.

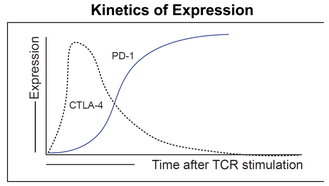
AGEN2034, a novel human IgG4 anti-PD-1 antagonist antibody, potently inhibits PD-1 binding to PD-L1 and PD-L2, resulting in enhanced T cell responsiveness *in vitro* as well as in a non-human primate model. AGEN2034 combined effectively with AGEN1884, a human IgG1 anti-CTLA-4 antibody, anti-TIGIT or anti-LAG-3 to further enhance T cell responsiveness. Furthermore, the combination of AGEN2034 and anti-CTLA-4 blockade promoted a pharmacodynamic response in cynomolgus monkeys, including a transient increase in proliferation and ICOS (inducible co-stimulator molecule) expression in a subset of central memory and effector memory T cells.

AGEN2034 was well tolerated, and a no-observed-adverse-effect level (NOAEL) could be established up to 40 mg/kg in non-human primates. AGEN2034 is currently under evaluation in a Phase 1/2 study in subjects with advanced tumors and cervical cancer (NCT03104699) and clinical studies to evaluate AGEN2034 in combination with AGEN1884 are planned.

## PD-1: A Key Pathway Of Immune Evasion




**Kinetics of Expression**




## Rationale Behind Anti-PD-1 And Anti-CTLA-4 Combination Therapy

### Anti-PD-1 Blockade

**Ligands**: PD-L1 is expressed on immune cells, most normal tissues and tumor cells; PD-L2 is expressed on dendritic cells and macrophages

**T cell expression**: TILs commonly express elevated levels of PD-1 due to chronic tumor antigen stimulation

**Anti-PD-1 blockade** functions to restore antigen-specific T cell effector function mainly in the tumor microenvironment.

### Anti-CTLA-4 Blockade

**Ligands**: CD80 (B7-1) and CD86 (B7-2) are expressed on professional APCs, but not on non-hematologic tumor cells

**T cell expression**: CTLA-4 is predominately expressed on CD4 "helper," not CD8 "killer" cells

**Anti-CTLA-4 blockade** is believed to enhance the priming phase of T cell activation mainly in lymphoid organs regardless of TCR clonality

Anti-PD-1 and anti-CTLA-4 combination therapy have demonstrated robust antitumor efficacy in preclinical mouse tumor models[a] and improved response rates in the clinic, such as in patients with metastatic melanoma[b], advanced small cell lung cancer (SCLC)[c] and metastatic renal cell carcinoma (RCC)[d]

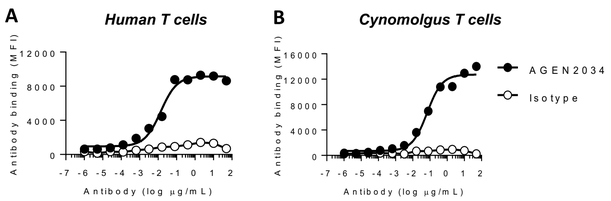
## Agenus Therapeutic Antibodies In Phase I / II Clinical Trials

| | AGEN2034 | AGEN1884 |
|---|---|---|
| **Target** | PD-1 | CTLA-4 |
| **Characterization** | Fully human IgG4-S228P | Fully human IgG1 |
| **Discovery Platform** | Retrocyte Display™ | Retrocyte Display™ |
| **Mechanism of Action** | Antagonist | Antagonist |
| **Clinical Trial #** | NCT03104699* | NCT02694822 |

**Clinical activity of AGEN2034 in subjects with metastatic or locally advanced solid tumors, with a consecutive Phase 2 expansion to evaluate efficacy in subjects with recurrent, unresectable, or metastatic (advanced) cervical cancer that has progressed after a platinum doublet.*

**References**
**a.** Curan MA. et al. PNAS. 2010; 107(9):4275-80.
**b.** Wolchok JD. et al. N Engl J Med 2017; 377:1345-1356.
**c.** Hellmann MD. *et al.* J Clin Oncol. 2017; 35:15 suppl. 8503.
**d.** Escudier B. *et al.* Checkmate 214: ESMO 2017 congress. Abstract LBA5

## AGEN2034 Binds With High Affinity to Human and Cynomolgus Monkey PD-1

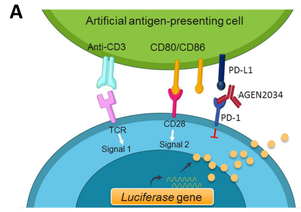



C

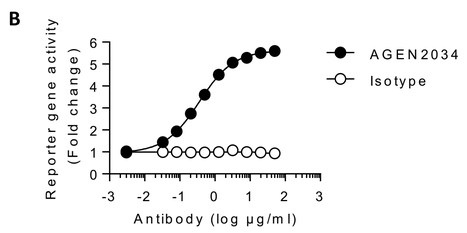
| Binding Affinity of AGEN2034 | | | |
|---|---|---|---|
| **Ligand** | **$k_d$ ($s^{-1}$)** | **$k_a$ ($Ms^{-1}$)** | **$K_D$ (nM)** |
| Human PD-1 | $5.7x10^{-5}$ | $4.1x10^{5}$ | 0.14 |
| Cyno PD-1 | $4.4x10^{-5}$ | $3.8x10^{5}$ | 0.12 |

***Legend:*** AGEN2034 binding to **(A)** activated human T cells and (**B**) activated cynomolgus T cells from human or cynomolgus peripheral blood mononuclear cells (PBMCs) stimulated with Staphylococcal enterotoxin A bacterial peptide for 5 days. Binding of increasing doses of AGEN2034 or isotype control antibody was assessed by flow cytometry. **(C)** Representative surface plasmon resonance (SPR) experiment for the binding of AGEN2034 to human PD-1-Fc or cynomolgus monkey PD-1-Fc recombinant proteins.

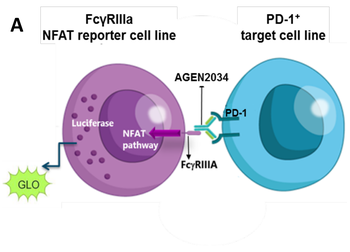
## AGEN2034 Antagonizes Ligand Binding to PD-1 to Restore T cell activation



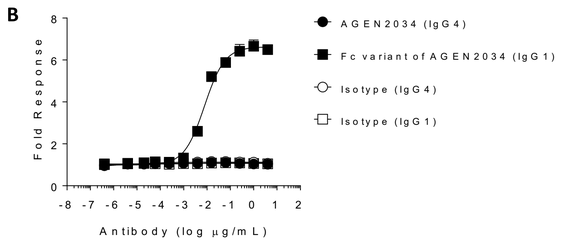

***Legend:*** (**A**) Illustration of reporter cell line assay. A human T cell line (Jurkat) were engineered to express PD-1 and a luciferase reporter gene driven by an NFAT promoter. Jurkat cells were incubated with Chinese hamster ovarian (CHO) cells engineered to express PD-L1 and a membrane-bound anti-CD3 antibody. (**B**) A dose titration of AGEN2034 or an IgG4 isotype control antibody were added to the T cell:CHO co-culture for 6 hours.



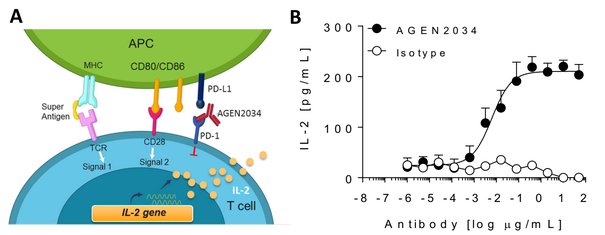

## AGEN2034 Does Not Activate FcγR Receptor IIIA Signaling, Consistent with an IgG4 Fc Region




***Legend:*** **(A)** Illustration of antibody-mediated Fc receptor signaling assay. **(B)** FcγRIIIA signaling was evaluated by luciferase expression from a Jurkat cell line engineered to over express FcγRIIIA and an NFAT- luciferase reporter gene incubated in the presence of a dose titration of AGEN2034 IgG4 (black circles), AGEN2034 IgG1 (black squares), isotype IgG4 (white circles), and isotype IgG4 (white squares) antibodies and Jurkat cells expressing PD-1 target.

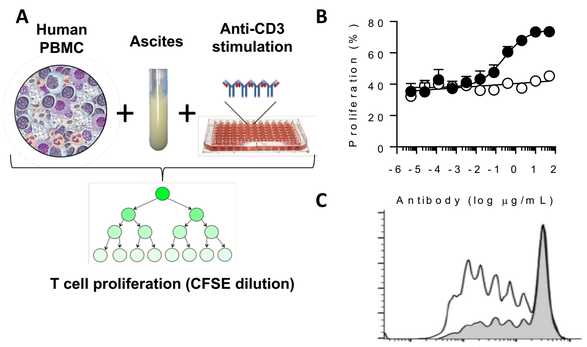



## AGEN2034 Enhances Primary Human T Cell Responsiveness



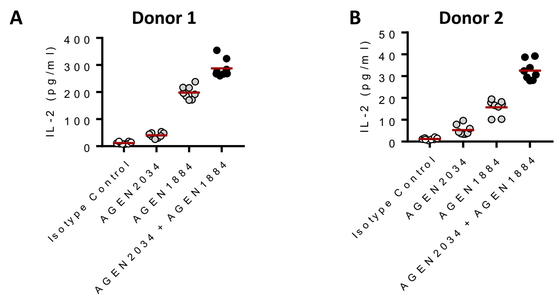

***Legend:*** **(A)** Illustration of primary cell stimulation assay. **(B)** Healthy human PBMCs were stimulated with Staphylococcal enterotoxin A peptide in the presence of dose titration of AGEN2034 or IgG4 isotype antibody for 5 days. Cytokine production (example: IL-2) was measured in the culture supernatant at day 5.

## AGEN2034 Promotes T cell Proliferation and Reverses Ovarian Ascites-Mediated T cell Suppression



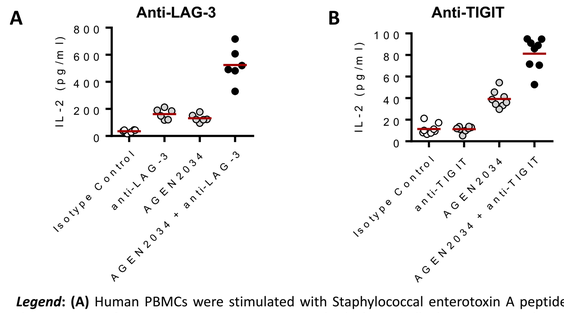

***Legend:*** (**A**) Illustration of primary cell suppression assay. (**B**) The percentage of CD4+ CFSE low (*i.e.* proliferated T cells) after 4 days in PBMC cultures stimulated with plate-bound anti-CD3 antibody, a fixed concentration of human ovarian cancer ascites, and a dose titration of AGEN2034 or an IgG4 isotype antibody. **(C)** Representative histogram for CFSE dilution at 10 µg/mL of AGEN2034 (gray) or isotype antibody (white) treated cultures is shown.

## AGEN2034 Effectively combines with AGEN1884 To Further Enhance Cytokine Secretion



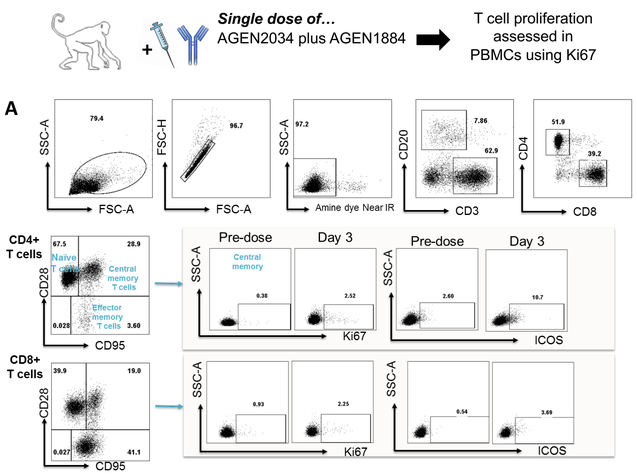

***Legend***: Human PBMCs from two healthy donors were stimulated with Staphylococcal enterotoxin A peptide in the presence of AGEN2034 or IgG4 isotype antibody (10µg/ml) alone or in combination with anti-CTLA-4, AGEN1884, antibody (10µg/mL) for 5 days. Cytokine production (example: IL-2) was measured in the culture supernatant at day 5.

## AGEN2034 Cooperates with other immunomodulatory antibodies to Enhance T cell Responsiveness




***Legend:*** **(A)** Human PBMCs were stimulated with Staphylococcal enterotoxin A peptide in the presence of AGEN2034 or IgG4 isotype antibody (10µg/ml) alone or in combination with anti-LAG-3 **(A)** or anti-TIGIT **(B)** antibodies (10µg/mL) for 5 days. Cytokine production (example: IL-2) was measured in the culture supernatant at day 5.

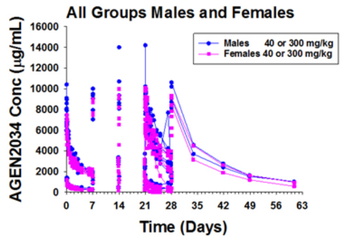
## AGEN2034 Combines With Anti-CTLA-4 To Enhance Activation and Proliferation of Central Memory T cells in Non-human Primates




***Legend:*** Cynomolgus monkeys were injected once with 10 mg/kg of AGEN1884 in combination with 3 mg/kg of AGEN2034. Blood samples were collected bi-weekly for 30 days and analyzed by flow cytometry. **(A)** Representative data for the frequencies of CD4+ and CD8+ naïve, central memory, and effector memory T cells are shown. Frequency of CD8+ ICOS+ **(B)** and CD8+ Ki67+ **(C)** central memory T cells 3 days following antibody treatment (n=4 animals).

## AGEN2034 Is Safe And Well-Tolerated

***Four Weeks, IV injection of AGEN2034***
*Day 1, 8, 15, 22 and 29; **Dosage**: 40 or 300 mg/Kg*




- AGEN2034 was well-tolerated up to the NOAEL of 40 mg/kg
- TK analysis showed dose proportionality with some accumulation; no gender differences.
- Estimated AGEN2034 half-life of ~12 days

## Summary

- AGEN2034 (anti-PD-1, IgG4) and AGEN1884 (anti-CTLA-4, IgG1) antibodies were discovered using Agenus' proprietary mammalian display antibody platform (Retrocyte Display™)
- AGEN2034 potently blocks ligand binding to enhance T cell activation and rescue T cell responsiveness in peptide stimulation and T cell suppression assays
- AGEN2034 cooperates with AGEN1884 and other immuno-modulatory pathways to further enhance T cell responsiveness
- In cynomolgus monkey, AGEN2034 in combination with AGEN1884, also promoted a pharmacodynamic proliferative and activation response in circulating T cells *in vivo*
- Clinical trials (NCT03104699) evaluating AGEN2034 in patients with advanced solid tumors (Phase 1) with expansion to second-line cervical cancer (Phase 2) is ongoing.

**Author Disclosures**
**Dhan Chand, David Savitsky, Ana Gonzalez, Mariana Manrique, Christopher Clarke, Andrea Schuster, Elise E. Drouin, Jeremy D. Waight, Cornelia Mundt, Marc van Dijk, Jennifer S. Buell, Jean-Marie Cuillerot, Robert Stein and Nicholas S. Wilson**: *Agenus Inc. and subsidiaries thereof*: Current or former employment and stock ownership.
**Jedd Wolchok, Gerd Ritter, Taha Merghoub, David Schaer, Rikke B. Holmgaard, Roberta Zappasodi:** No competing interests declared.

**Acknowledgments**
The licensed antibodies AGEN1884 and AGEN2034 were originally developed under a Collaborative Research and Development Agreement between Ludwig Cancer Research, 4-Antibody AG (now Agenus Switzerland Inc.) and Recepta Biopharma S.A. These antibodies are partnered with Recepta Biopharma S.A. for certain South American rights.