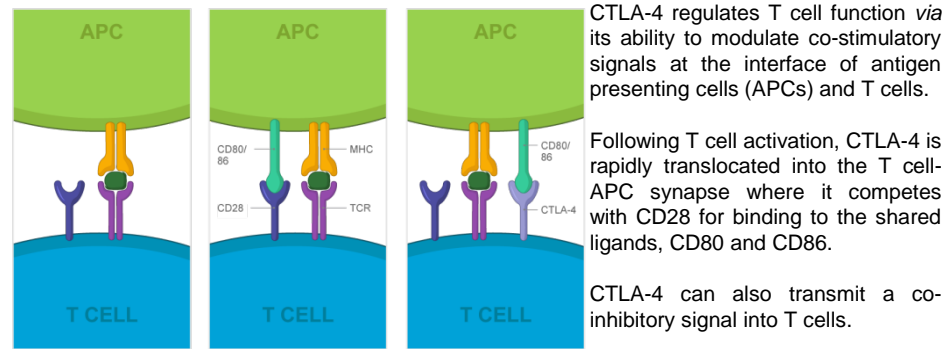# AGEN1884 and AGEN2041: Two functionally distinct anti-CTLA-4 antagonist antibodies

Elise Drouin[1], Ana Gonzalez[1], Hao Tang[1], David Savitsky[1], Randi Gombos[1], Jeremy Waight[1], Benjamin Duckless[1], Andrea Schuster[1,2], Lili Wang[1], Shiwen Lin[1], Cornelia Mundt[1,2], Gerd Ritter[3],Taha Merghoub[4], Kyle Draleau[4], Jedd Wolchok[3,4], Marc van Dijk[1,2], John M. Goldberg[1], Daniel Levey[1], Jennifer Buell[1], Robert Stein[1] and Nicholas S. Wilson[1]

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Control of T cell responsiveness *via* the CTLA-4 (CD152)/CD28 signaling pathways

CTLA-4 regulates T cell function *via* its ability to modulate co-stimulatory signals at the interface of antigen presenting cells (APCs) and T cells.

Following T cell activation, CTLA-4 is rapidly translocated into the T cell-APC synapse where it competes with CD28 for binding to the shared ligands, CD80 and CD86.

CTLA-4 can also transmit a co-inhibitory signal into T cells.

Distinct effector cell activity of anti-CTLA-4 antagonist antibodies (IgG1 *versus* IgG2)

AGEN1884 (IgG1)
Natural killer cell-mediated intratumoral regulatory T cell depletion

AGEN2041 (IgG2)
Macrophage-mediated intratumoral regulatory T cell depletion
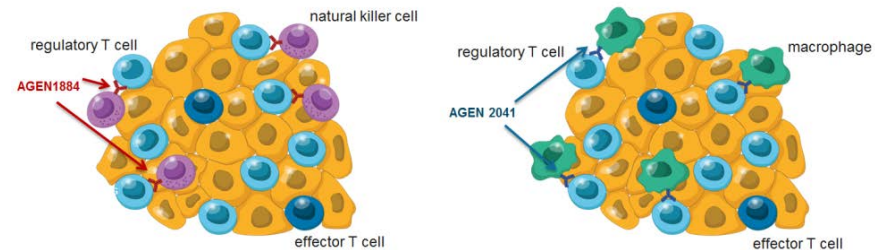
AGEN1884 and AGEN2041 are fully human anti-CTLA-4 antibodies with identical Fab regions, but distinct Fc regions (IgG1 *versus* IgG2). Differences in the Fc region may impact the ability of each antibody to elicit effector cell activities, based upon the tumor immune composition.

## Anti-CTLA-4 antibody binding affinity and selectivity for cell expressed CTLA-4

A

Anti-CTLA-4 antibody binding affinity to recombinant CTLA-4

Human CTLA-4	ka [1/Ms]	1.9 x10^6
	kd [1/s]	2.7 x 10^-3
	K_D [M]	1.5 x 10^-9

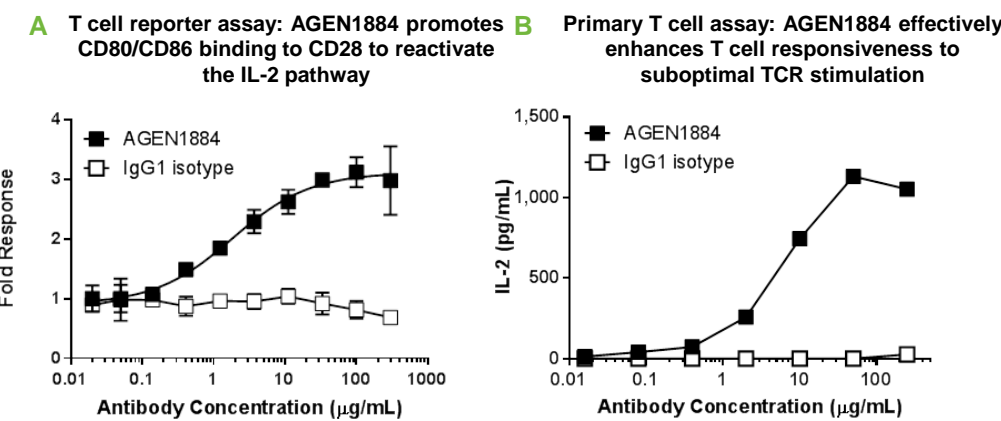**B** CTLA-4-expressing T cell line
C Parental T cell line
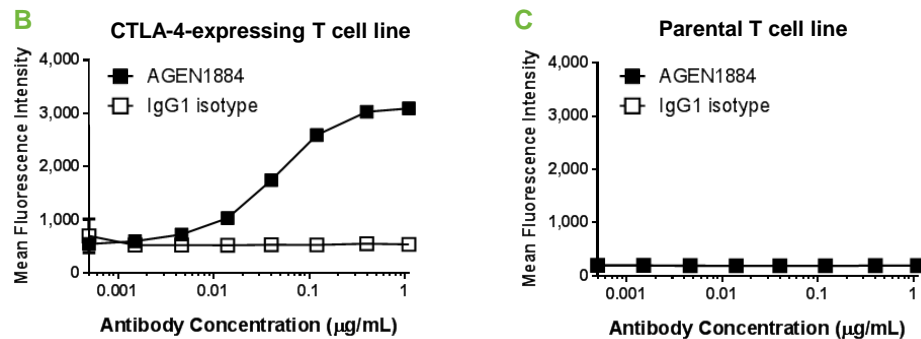
A. Affinity of anti-CTLA-4 antibody to recombinant human CTLA-4 (SPR).
B. Anti-CTLA-4 antibody binding to CTLA-4 overexpressing human T cells.
C. Anti-CTLA-4 antibody binding to parental human T cells (CTLA-4 negative).

Anti-CTLA-4 antibody binding to CTLA-4 potently inhibits CD80 and CD86 engagement

A Anti-CTLA-4 antibody blocks CD80 and CD86 from binding recombinant CTLA-4

B Anti-CTLA-4 antibody blocks CD80 and CD86 from binding to cell expressed CTLA-4
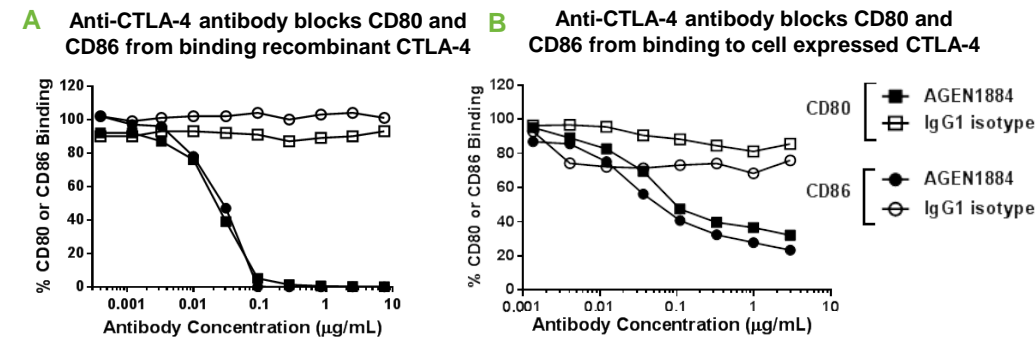
A. Recombinant CTLA-4 was coupled to microsphere beads and incubated with increasing concentrations of the anti-CTLA-4 antibody or an isotype control antibody. Fluorescently labeled CD80 or CD86 were added and binding to beads was quantitated.
B. Human T cells engineered to overexpress CTLA-4 were incubated with increasing concentrations of the anti-CTLA-4 antibody or an isotype control antibody. Fluorescently labeled CD80 or CD86 were added and their binding to cells was measured using flow cytometry.

Anti-CTLA-4 antibody inhibits CD80 and CD86 binding to CTLA-4 and enhances T cell activation

A T cell reporter assay: AGEN1884 promotes CD80/CD86 binding to CD28 to reactivate the IL-2 pathway

B Primary T cell assay: AGEN1884 effectively enhances T cell responsiveness to suboptimal TCR stimulation

C AGEN1884 combines effectively with anti-LAG-3 and anti-PD1 antagonist antibodies to enhance T cell responsiveness to suboptimal TCR stimulation
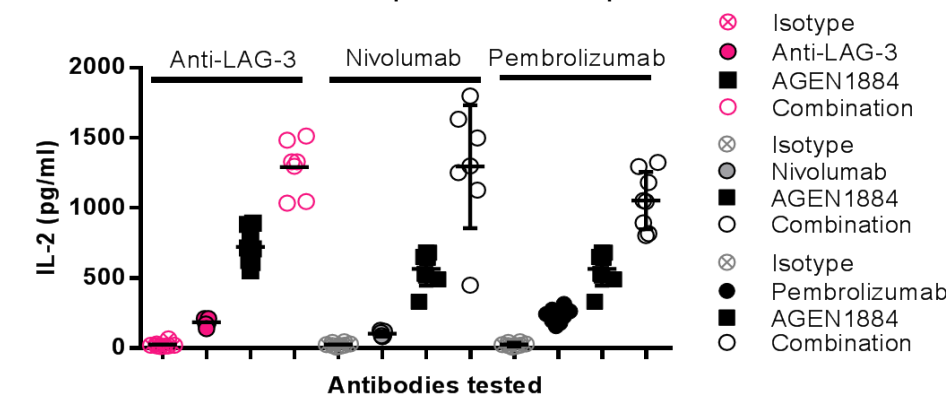

A. Reporter cell assay: AGEN1884 and AGEN2041 (not shown) potently block CTLA-4 binding to CD80 and CD86, leading to enhanced IL-2 promoter gene activation *via* CD28.
B. Peripheral blood mononuclear cells (PBMCs) were sub-optimally stimulated with the SEA superantigen together with a dose-response of AGEN1884 (AGEN2041 – not shown). IL-2 secretion was measured.
C. PBMCs sub-optimally stimulated with the SEA superantigen with AGEN1884 alone or in combination with an anti-LAG3 antibody, Nivolumab (anti-PD-1 antibody), or Pembrolizumab (anti-PD-1 antibody).

AGEN1884 or AGEN2041 mediate FcγRIIA and FcγRIIIA signaling in the context of target cells
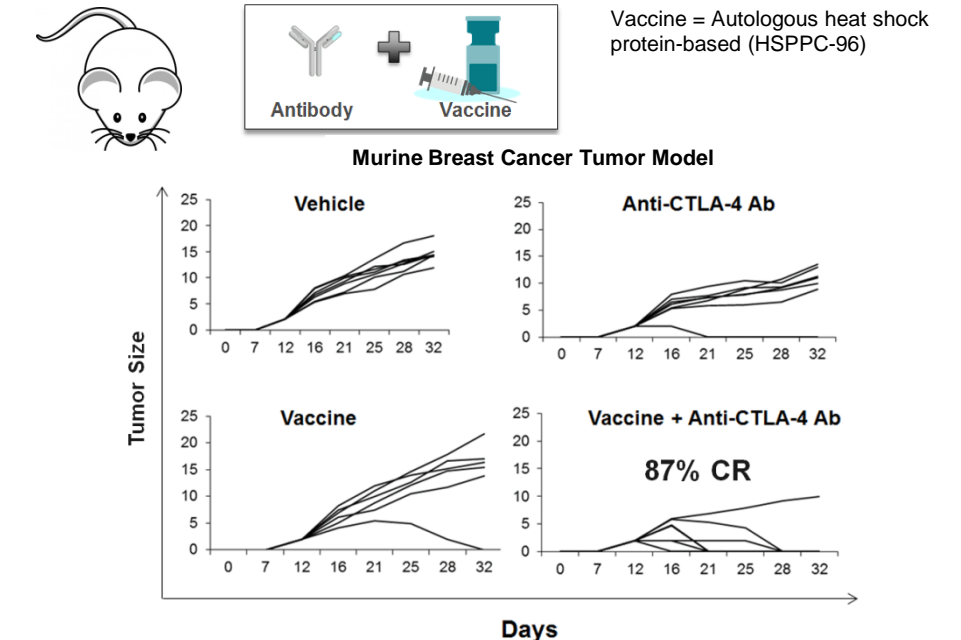
A

B AGEN1884 (IgG1) mediates FcγRIIIA signaling

C AGEN2041 (IgG2) mediates FcγRIIA signaling
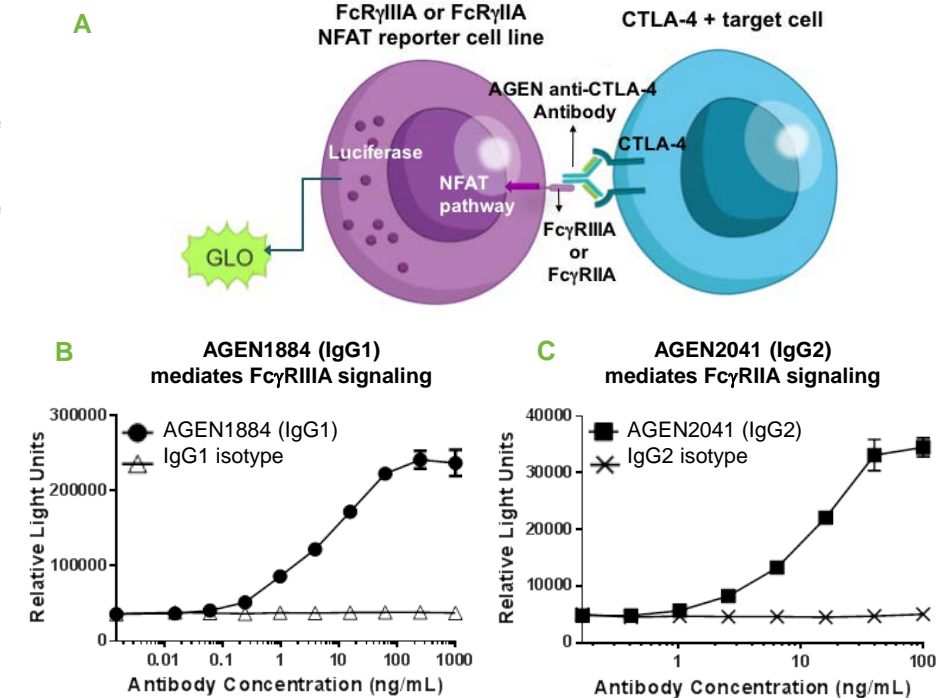
A. CTLA-4 expressing T cells were co-cultured with FcγRIIIA or FcγRIIA reporter cells expressing nuclear factor of activated T-cells response element (NFAT-RE) upstream of the firefly luciferase gene.
B. Co-engagement of CTLA-4 and FcγRIIIA by AGEN1884 induced dose dependent luciferase expression.
C. Co-engagement of CTLA-4 and FcγRIIA by AGEN2041 induced dose dependent luciferase expression.

Enhanced vaccine response in non-human primates co-administered AGEN1884 or AGEN2041

Vaccine = Hepatitis B

A **B**

Administrations of antibody or vehicle (control) plus HBsAg vaccine
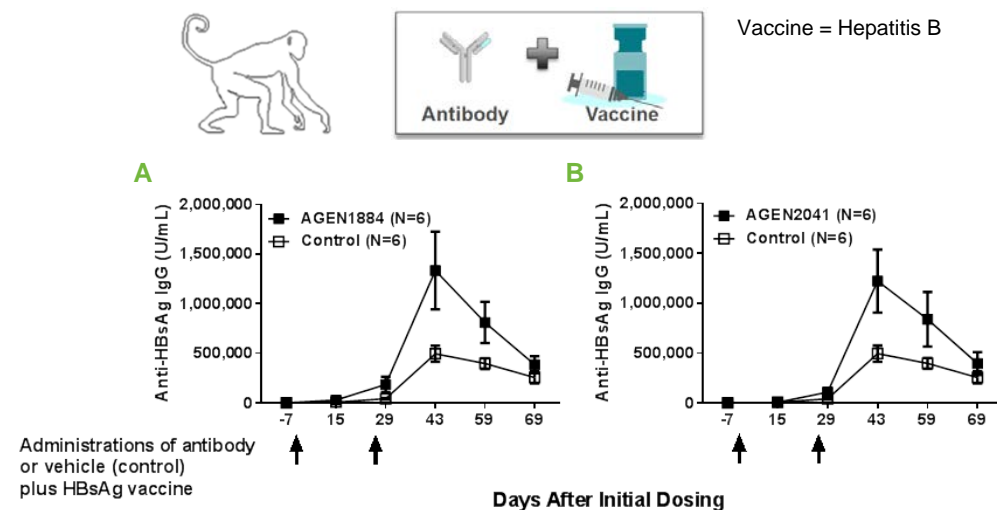
AGEN1884 and AGEN2041 potentiated a T cell dependent antibody response (TDAR) in cynomolgus monkeys vaccinated with hepatitis B surface antigen (HBsAg). Cynomolgus monkeys were administered AGEN1884 (A) or AGEN2041 (B) together with HBsAg vaccine on days 1 and 29.

An anti-mouse CTLA-4 antibody combines effectively with autologous peptide vaccine

Vaccine = Autologous heat shock protein-based (HSPPC-96)

Murine Breast Cancer Tumor Model

Vehicle / Anti-CTLA-4 Ab / Vaccine / Vaccine + Anti-CTLA-4 Ab (87% CR)

Anti-CTLA-4 antibody (clone 9D9) combined effectively with an autologous tumor vaccine. BALB/c mice were injected with SM1 tumor cells intradermally and treated with an anti-CTLA-4 antibody and/or an autologous tumor vaccine HSPPC-96; a protein peptide complex consisting of a 96 kDa heat shock protein (gp96) and gp96-associated cellular peptides derived from SM1 breast carcinoma tumors.

Summary

- AGEN1884 and AGEN2041 bind with high affinity to CTLA-4 and potently block CTLA-4 binding to its ligands CD80 and CD86.
- AGEN1884 and AGEN2041 promote CD80/CD86 signaling *via* CD28 to enhance IL-2 cytokine production.
- AGEN1884 combines effectively with other antagonist immuno-modulatory antibodies to enhance T cell responsiveness to suboptimal TCR stimulation.
- Consistent with distinct Fc regions, AGEN1884 and AGEN2041 bound to target cells activated FcγRIIIA and FcγRIIA, respectively.
- AGEN1884 and AGEN2041 enhanced a T cell dependent antibody response in cynomolgus monkeys, supporting their utility to effectively combine with immune education approaches.
- In a preclinical mouse tumor model, a surrogate anti-mouse CTLA-4 antagonist antibody combined effectively with a heat shock protein-based therapeutic tumor vaccine.

References
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