

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



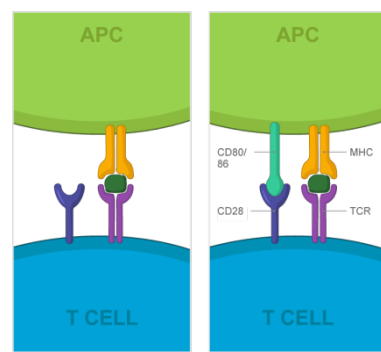
Elise Drouin¹, Ana Gonzalez¹, Hao Tang¹, David Savitsky¹, Randi Gombos¹, Jeremy Waight¹, Benjamin Duckless¹, Andrea Schuster^{1,2}, Lili Wang¹, Shiwen Lin¹, Cornelia Mundt^{1,2}, Gerd Ritter³, Taha Merghoub⁴, Kyle Draletau⁴, Jedd Wolchok^{3,4}, Marc van Dijk^{1,2}, John M. Goldberg¹, Daniel Levey¹, Jennifer Buell¹, Robert Stein¹ and Nicholas S. Wilson¹

¹Agenus Inc., Lexington MA, USA. ²4-Antibody AG, Basel, Switzerland. ³The Ludwig Institute for Cancer Research, NY, USA. ⁴Memorial Sloan Kettering Cancer Center, NY, USA.

#5005

Presented at the American Association for Cancer Research Annual Meeting 2016 New Orleans, LA, USA • April 16-20, 2016

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.

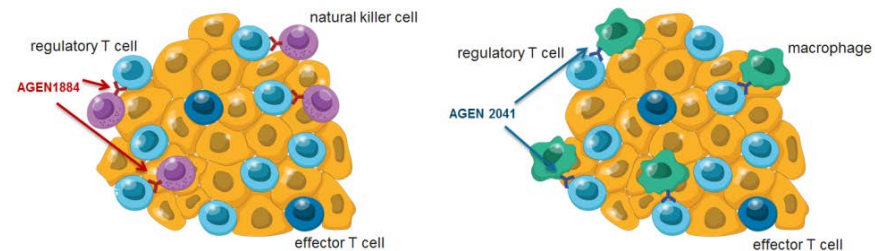
Distinct effector cell activity of anti-CTLA-4 antagonist antibodies (IgG1 *versus* IgG2)

AGEN1884 (IgG1)

Natural killer cell-mediated intratumoral regulatory T cell depletion

AGEN2041 (IgG2)

Macrophage-mediated intratumoral regulatory T cell depletion

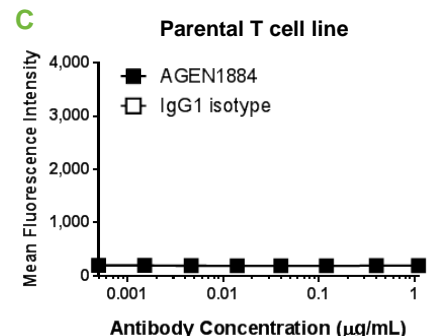
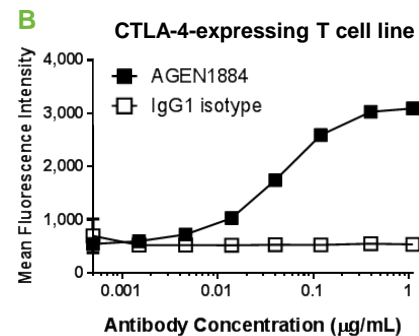


AGEN1884 and AGEN2041 are fully human anti-CTLA-4 antibodies with identical Fab regions, but distinct Fc regions (IgG1 *versus* IgG2). Differences in the Fc region may impact the ability of each antibody to elicit effector cell activities, based upon the tumor immune cell composition.

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

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

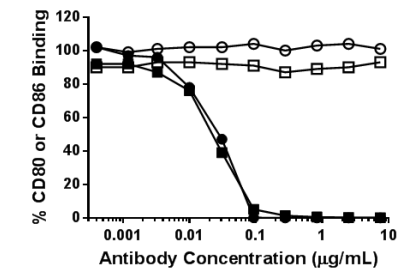
Human CTLA-4	ka [1/Ms]	1.9 x 10 ⁶
	kd [1/s]	2.7 x 10 ⁻³
	K _D [M]	1.5 x 10 ⁻⁹



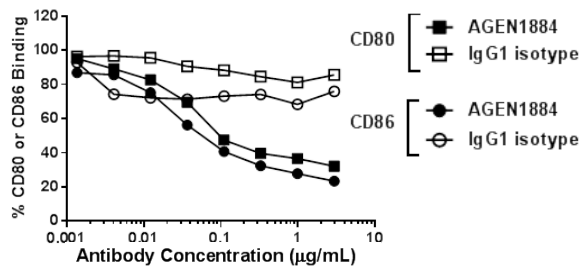
- A. Affinity of anti-CTLA-4 antibody to recombinant human CTLA-4 (SPR).
 B. Anti-CTLA-4 antibody binding to CTLA-4 overexpressing human T cells.
 C. Anti-CTLA-4 antibody binding to parental human T cells (CTLA-4 negative).

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



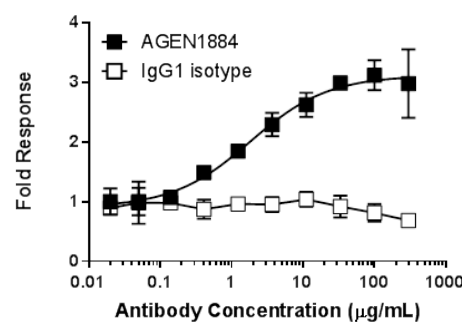
B Anti-CTLA-4 antibody blocks CD80 and CD86 from binding to cell expressed CTLA-4



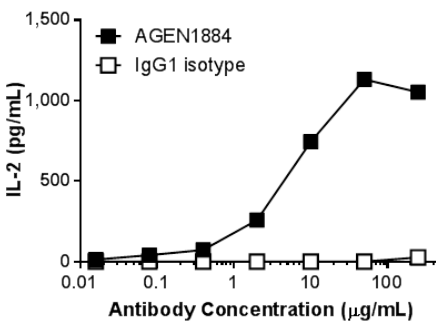
- A. Recombinant CTLA-4 was coupled to microsphere beads and incubated with increasing concentrations of the anti-CTLA-4 antibody or an isotype control antibody. Fluorescently labeled CD80 or CD86 were added and binding to beads was quantitated.
 B. Human T cells engineered to overexpress CTLA-4 were 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

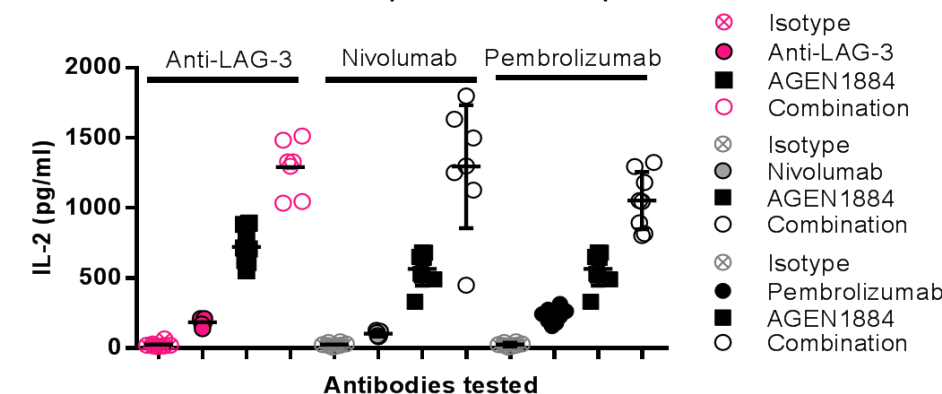
A T cell reporter assay: AGEN1884 promotes CD80/CD86 binding to CD28 to reactivate the IL-2 pathway



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

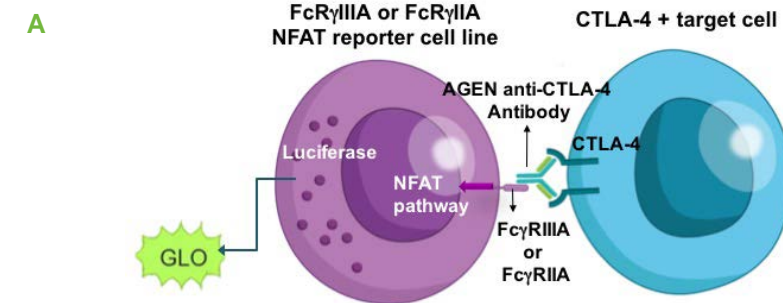


C AGEN1884 combines effectively with anti-LAG-3 and anti-PD-1 antagonist antibodies to enhance T cell responsiveness to suboptimal TCR stimulation

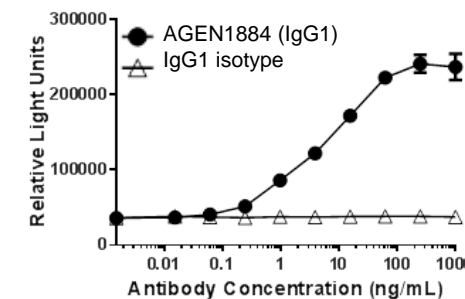


- A. Reporter cell assay: AGEN1884 and AGEN2041 (not shown) potently block CTLA-4 binding to CD80 and CD86, leading to enhanced IL-2 promoter gene activation *via* CD28.
 B. Peripheral blood mononuclear cells (PBMCs) were sub-optimally stimulated with the SEA superantigen together with a dose-response 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).

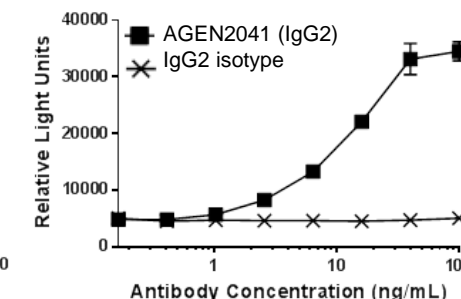
AGEN1884 or AGEN2041 mediate FcγRIIA and FcγRIIIA signaling in the context of target cells



B AGEN1884 (IgG1) mediates FcγRIIIA signaling

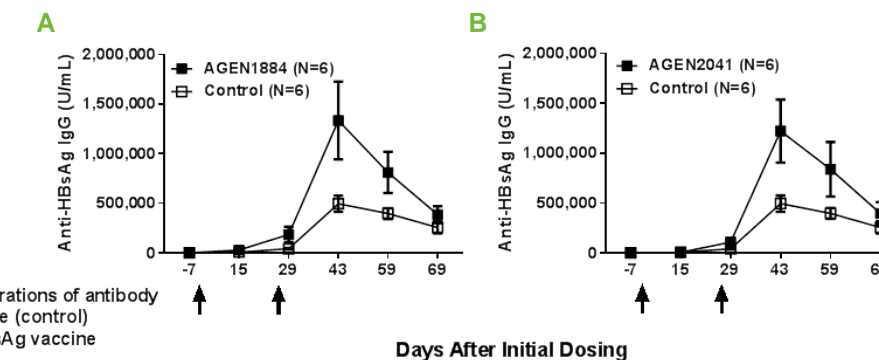
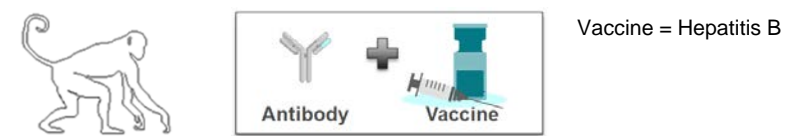


C AGEN2041 (IgG2) mediates FcγRIIA signaling



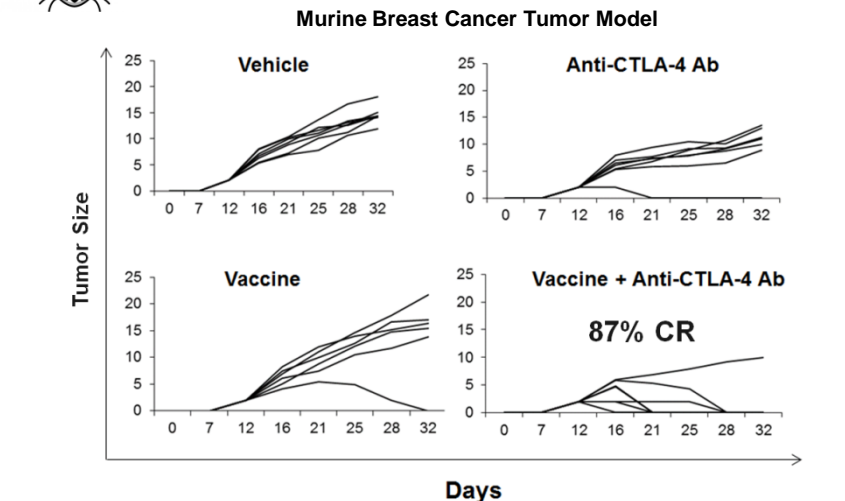
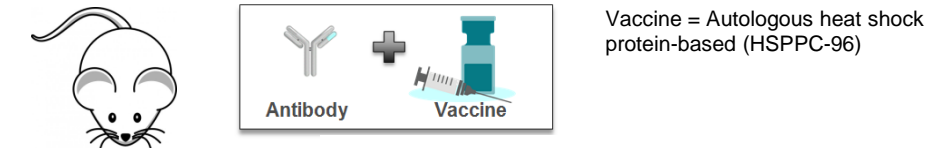
- A. CTLA-4 expressing T cells were co-cultured with FcγRIIIA or FcγRIIA reporter cells expressing nuclear factor of activated T-cells response element (NFAT-RE) upstream of the firefly luciferase gene.
 B. Co-engagement of CTLA-4 and FcγRIIA by AGEN1884 induced dose dependent luciferase expression.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



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



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 HSPCC-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

- Riley, J.L. *et al.* Blood. 2005; 105, 13-21.
- Krummel, M.F. *et al.* J Exp Med. 1995; 182, 459-465.
- Curran, M.A. *et al.* Proc Natl Acad Sci. 2010; 107, 4275-4280.
- Grosso, J.F. *et al.* Cancer Immunol. 2013; 13.
- Leach DR *et al.* Science. 1996; 271, 1734-1736.
- Azuma, H *et al.* Nature. 1993; 366, 76-79.
- Freedman, A.S. *et al.* Cell Immunol. 1991; 137, 429-437.
- Hathcock, K.S. *et al.* Science. 1993; 262, 905-907.
- Selby, M.J. *et al.* Cancer Immunol Res. 2013; 1, 32-42.
- Schadendorf, D. *et al.* J Clin Oncol. 2015; 33, 1889-1894.

Author Disclosures

Elise Drouin, Ana Gonzalez, Hao Tang, David Savitsky, Jeremy Waight, Randi Gombos, Benjamin Duckless, Andrea Schuster, Lili Wang, Shiwen Lin, Cornelia Mundt, Marc van Dijk, John Goldberg, Daniel Levey, Jennifer Buell, Robert Stein, Nicholas S. Wilson: Agenus Inc. Employment and Stock ownership.

Kyle Draletau, Taha Merghoub, Gerd Ritter and Jedd Wolchok: No competing interests declared.

Acknowledgments

The authors would like to thank Rebecca Woelfle for her assistance in preparing poster material and Joseph Connolly and Zhenyu Li for their help characterizing and producing the AGEN anti-CTLA-4 antibodies.