Development of a novel antagonist anti-CTLA-4 antibody for cancer immunotherapy

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Abstract

CTLA-4 and CD28 exemplify a co-inhibitory and co-stimulatory T cell signaling axis by sharing common ligands CD80 and CD86, which regulate antigen-specific T cell immunity. CTLA-4 acts as a checkpoint to confine the magnitude of CD28 receptor mediated T cell activation and has been widely investigated as a target to boost antitumor immunity.

ADG116 is a fully human antagonistic anti-CTLA-4 IgG1 monoclonal antibody identified through our proprietary Phage display Dynamic Precision Library (DPL) technology. Different from the clinically approved Ipilimumab, ADG116 binds to a unique evolutionally conserved epitope on CTLA-4, which endows ADG116 a broad species cross-reactivity to CTLA-4 of human, monkey and rodent origins, and allows the extensive evaluation of its MOA and efficacy in various mouse cancer models. Compared to Ipilimumab, ADG116 *in vitro* is a weaker CTLA-4 checkpoint inhibitor in terms of its ligand relieving activity to co-stimulate CD28 receptor signaling, while ADG116 has much potent ADCC activity towards activated Treg cells but not effector T cells. *In vivo*, ADG116 alone exhibits robust dose-dependent single agent anti-tumor activity in established tumors and/or increases overall survival in syngeneic mouse models of liver, breast, lung and colon cancers, as well as induces durable antigen-specific memory immunity that protect animals from re-challenged tumor cells. The activity of ADG116 to enhance activation and inflammatory cytokine release of primed T cells *in vitro* and specifically deplete intra-tumoral Treg cells *in vivo*, is consistent with the MOA by which ADG116 acts through two distinct mechanisms: 1) functions as a checkpoint inhibitor to directly enhance effector T cell activation, and 2) functions as an ADCC targeting antibody to alleviate immunosuppression in tumor microenvironment through specific Treg depletion in tumors but not in peripheral tissues. Both mechanisms are involved in mediating its potent anti-tumor responses. In a human CTLA-4 knock-in mouse tumor model, ADG116 exhibits more potent anti-tumor efficacy than Ipilimumab, along with stronger intra-tumoral Treg depletion.

Weekly repeat-dose GLP toxicology studies demonstrate a relatively good safety profile for ADG116 in rats and cynomolgus monkeys. These pre-clinical results provide strong support for further clinical development of ADG116 as a novel anti-cancer immunotherapy agent for a broad spectrum of human malignancies.

Mechanisms of anti-CTLA-4 antibody treatment

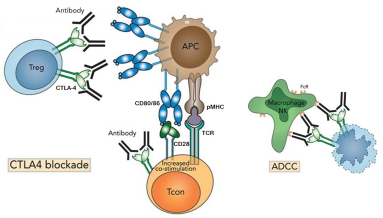


Fig. 1. Mechanisms of anti-CTLA-4 therapy. Anti-CTLA-4 antibody can directly activate CD28 co-stimulatory T cell signaling through relieving CD80/CD86 ligands from CTLA-4 sequestration, as well as mitigate the immunosuppression by depleting activated Treg cells through ADCC. Cartoon is modified from Fig.2 in Reference 1.

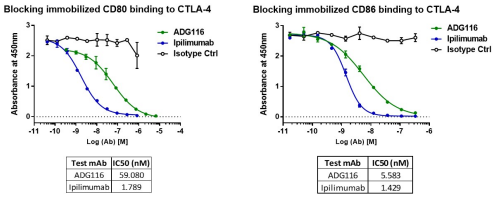
High affinity binding to CTLA-4 across species

CTLA-4 binding Affinity by SPR (Biacore)

KD (nM)	Human	Cyno Monkey	Mouse	Rat
ADG116	2.79	1.168	2.417	1.844
Ipilimumab	3.932	1.679	NB	NB

ADG116 acts as a CTLA-4 checkpoint inhibitor

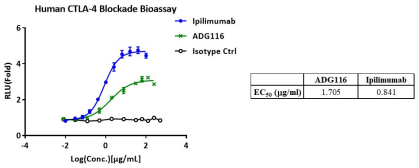
A. Ligand binding blocking by ELISA



Test mAb	IC50 (nM)
ADG116	59.080
Ipilimumab	1.789

Test mAb	IC50 (nM)
ADG116	5.583
Ipilimumab	1.429

B. CTLA-4 functional blockade in cell-based reporter assay



	ADG116	Ipilimumab
EC_{50} (μg/ml)	1.705	0.841

Fig. 2. ADG116 is a weaker CTLA-4 checkpoint inhibitor than Ipilimumab. A. Ligand blocking assay by ELISA, where recombinant protein-protein interactions were analyzed with human CTLA-4 and either CD80 or CD86 In the presence of isotype control, ADG116 or Ipilimumab. B. Cell-based CTLA-4 blockade bioassay (Promega) was employed to evaluate anti-CTLA-4 mediated CD28 signaling activation.

ADCC activity on activated Treg cells in vitro

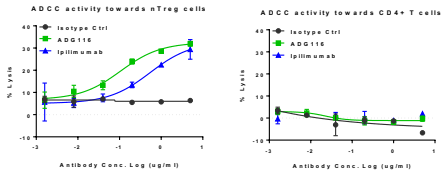
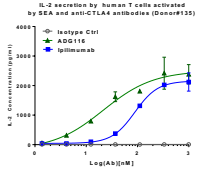


Fig. 3. ADG116 vs Ipilimumab on NK cell mediated ADCC activity towards in vitro activated human Treg or effector CD4 T cells by anti-CD3/anti-CD28 antibodies. ADG116 showed stronger ADCC activity than Ipilimumab for killing activated Treg but not CD4 effector T cells.

SEA-induced T cell activation



EC_{50} (nM) of anti-CTLA4 induced IL-2 release

Donor	ADG116	Ipilimumab
#132	3.93	36.2
#133	8.7	50.9
#135	21.5	89.1

Fig. 4. ADG116 vs Ipilimumab in the SEA (staphylococcus enterotoxin A) induced human T cells activation assay. Human PBMCs were isolated from healthy donors and cultured in the presence of SEA and various concentrations of isotype control, ADG116, or Ipilimumab. IL-2 was measured as a function of T cell activation. ADG116 is 4~9-fold more potent than Ipilimumab in this assay.

In vivo antitumor efficacy of ADG116

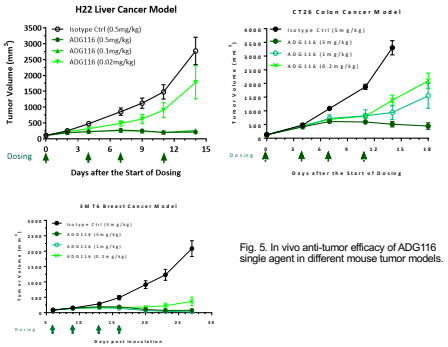


Fig. 5. In vivo anti-tumor efficacy of ADG116 single agent in different mouse tumor models.

ADG116 in combination with anti-PD-1

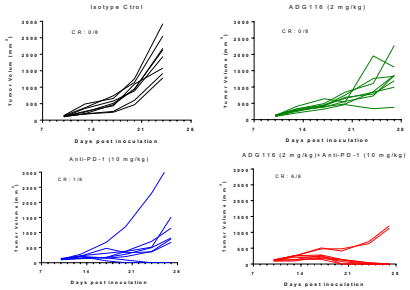


Fig. 6. In vivo anti-tumor efficacy of ADG116 in combination with anti-PD-1 in Lewis mouse lung cancer model.

Induction of antitumor memory response

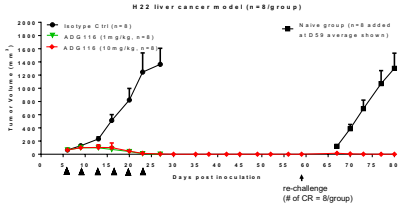


Fig. 7. Development of antitumor memory response by ADG116 treatment in mouse H22 liver cancer model.

Antitumor Efficacy of ADG116 vs Ipilimumab

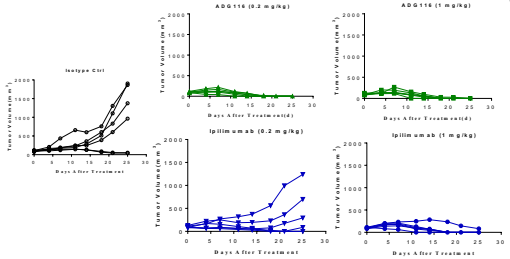


Fig. 8. Comparison of ADG116 vs Ipilimumab in MC38 tumor model in hCTLA-4 KI mice.

Treg depletion

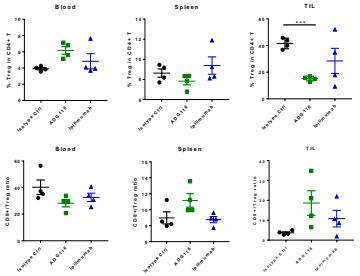


Fig. 9. Treg depletion upon ADG116 vs Ipilimumab (1mg/kg) treatment in MC38 tumor model in hCTLA-4 KI mice. % Treg cells and CD8/Treg ratio in tumor, blood or spleen were measured.

Summary

- ADG116 specifically binds to CTLA-4 across a broad species .
- ADG116 is a weaker ligand blocker than Ipilimumab, but with higher ADCC activity targeting activated Treg cells.
- ADG116 is 4~9-fold more potent than Ipilimumab to stimulate SEA-induced T cell activation.
- ADG116 has potent antitumor activity in mouse tumor models as single agent as well as in combination with anti-PD-1 and can induce long-term protective memory responses.
- ADG116 has more potent antitumor activity than Ipilimumab in human CTLA-4 KI mouse tumor model.
- ADG116 mediates stronger, compared to Ipilimumab, tumor specific Treg depletion, but not Treg cells in peripheral blood or spleen.
- GLP toxicity studies demonstrate that ADG116 is well tolerated in Rats and Cynomolgus monkeys at weekly doses up to 30 mg/kg.

References

1. Rowshanravan et al. CTLA-4: a moving target in immunotherapy. *Blood* 2018, 131: 58

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