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A Novel Anti-CTLA-4 Checkpoint Inhibitor Prodrug to Address On-target Off-tumor Toxicity for Cancer Immunotherapy

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Abstract

As the first FDA approved immune checkpoint inhibitor targeting CTLA-4, ipilimumab has proven the clinical benefit of checkpoint blockade in cancer immunotherapy, especially in combination with anti-PD-1 therapy. However, ipilimumab can induce severe and sometimes fatal immune-mediated adverse reactions due to autoimmune responses in normal tissues which may involve many organ systems, thus restricting its clinical applications. Consequently, development of next generation anti-CTLA-4 antibodies with enhanced antitumor efficacy and improved safety profiles are urgently needed.

ADG126 is a novel anti-CTLA-4 fully human IgG1 antibody prodrug engineered using Adagene SAFEbody technology. ADG126 is designed for an antibody to be preferentially activated in the tumor microenvironment, limiting its on-target off-tumor toxicities in normal tissues. As shown in Fig 1, a SAFEbody is masked by a covalently linked N-terminal peptide, followed by protease cleavage sites. In normal tissues, the SAFEbody masking moiety functions to block ADG126 binding to CTLA-4, however, after cleavage by proteases known to be highly expressed and active in tumor tissues, the masking moiety is released enabling the activated ADG126 antibody to bind and inhibit CTLA-4 function in situ.

Nonclinical pharmacological studies demonstrate that unmasked or activated ADG126 binds to a unique and conserved epitope of CTLA-4 with species cross-reactivity and partially blocks the CTLA-4/B7 ligand interactions. Activated ADG126 potentiates T cell activation by inducing IL-2 cytokine production in the presence of a primary stimulatory signal, depletes immunosuppressive T_{regs} through enhanced ADCC, and reduces immunosuppressive T_{reg} activity specifically in the tumor microenvironment to mediate anti-tumor responses. ADG126 demonstrates efficacious anti-tumor activity in multiple syngeneic murine tumor models as a single agent, as well as in combination with other immune modulatory agents. Nonclinical toxicology studies demonstrate that ADG126 is well-tolerated in cynomolgus monkeys. These results suggest that activation of the masked ADG126 prodrug preferentially in the local tumor microenvironment may allow treatments that deliver higher efficacious dose levels to the tumor concomitant with a superior systemic safety profile compared with other traditional anti-CTLA-4 antibodies. Thus, ADG126 holds great promise to achieve much better efficacy and safety profiles for immunotherapy against a broad spectrum of human cancers.

SAFEbody: Proprietary differentiated precision antibody masking technology

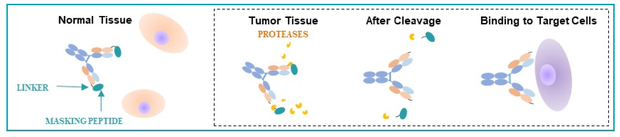

Fig 1. An Adagene SAFEbody is masked by a covalently linked N-terminal peptide, followed by protease cleavage sites. In normal tissues, a SAFEbody remains masked and unable to bind to its targets. However, in the tumor microenvironment, the mask is cleaved by proteases overexpressed in various tumor tissues, resulting in activation of the SAFEbody and enabling binding to its targets as depicted below for ADG126.

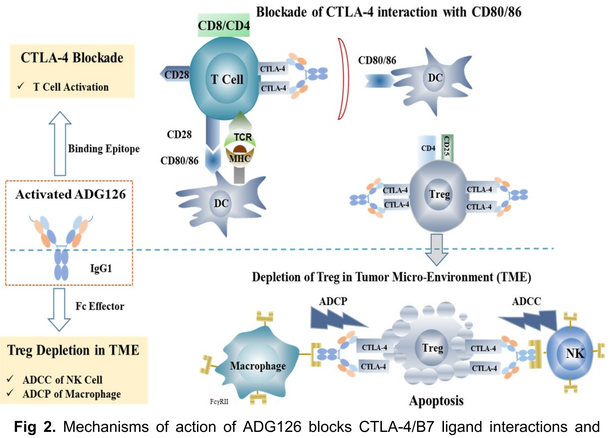

Fig 2. Mechanisms of action of ADG126 blocks CTLA-4/B7 ligand interactions and potentiates T cell activation. *For a comprehensive review of CTLA-4 targeted therapy, see Ref 1.*

Activatable high target-binding affinity across species

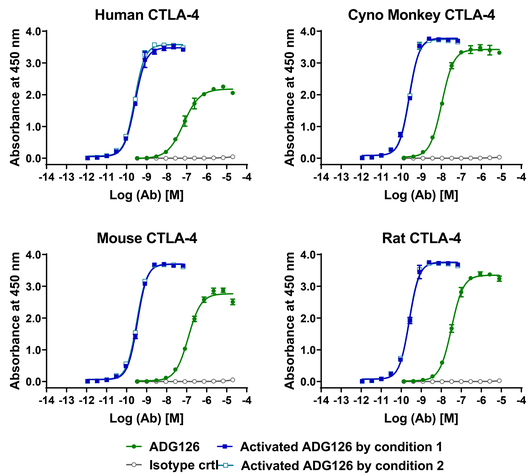

Fig 3. The target binding site of the ADG126 SAFEbody is highly masked and binds weakly to CTLA-4 protein. However, once proteases cleave off the masking peptide, ADG126 is activated and can bind at high affinity to the cross species CTLA-4 proteins of human, monkey, and rodent origins.

Activation of CD28 signaling by CTLA-4 blockade

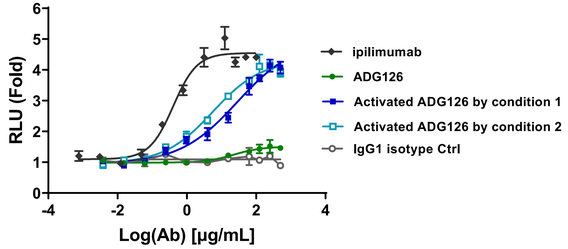

Fig 4. Protease-activated ADG126 is a softer CTLA-4 checkpoint inhibitor than ipilimumab. A cell-based CTLA-4 blockade bioassay (Promega) was employed to evaluate anti-CTLA-4 mediated CD28 signaling activation.

In vitro Fc effector function

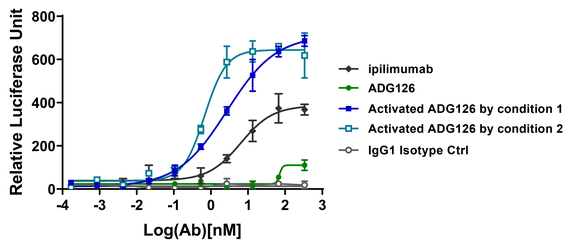

Fig 5. Protease activated, but not the masking peptide-intact ADG126, exhibits ADCC signaling activity that is stronger than ipilimumab. An in vitro ADCC reporter assay using CTLA-4 expressing target cells and Jurkat NFAT-Luc/CD16 reporter cells was employed to evaluate ADG126 mediated ADCC signaling activation.

Human T cell activation in vitro

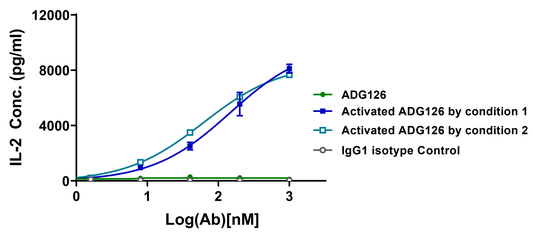

Fig 6. Protease activated ADG126, but not the masking peptide-intact ADG126, can enhance the SEA (staphylococcus enterotoxin A) induced human T cell activation. IL-2 was measured as a function of T cell activation.

In vivo antitumor efficacy of ADG126 monotherapy

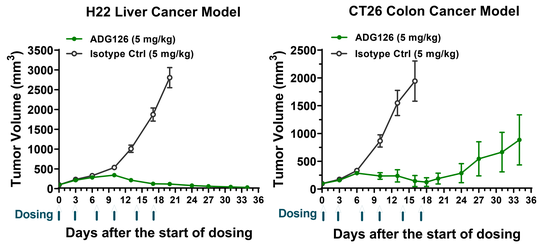

Fig 7. ADG126 (5 mg/kg) demonstrates efficacious anti-tumor activity in vivo as a single agent in syngeneic mouse tumor models.

In vivo efficacy of ADG126 combined with anti-PD-1

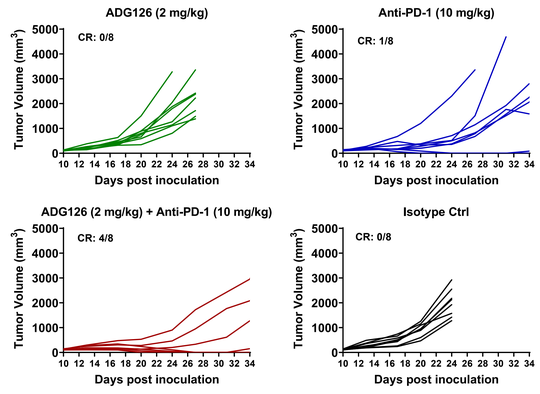

Fig 8. ADG126 (2 mg/kg) demonstrates efficacious anti-tumor activity in vivo in combination with anti-PD-1 in the mouse Lewis lung cancer model. Combination therapy significantly slowed tumor growth and caused complete regression (CR) in four of the eight tumors.

Intra-tumoral T_{reg} depletion

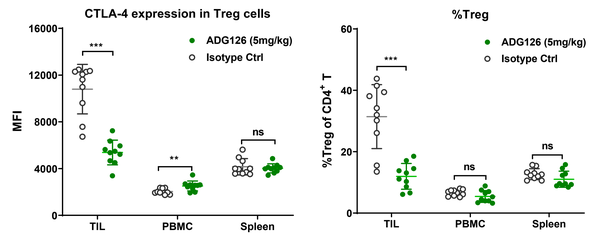

Fig 9. T_{reg} cells in tumor tissues exhibit higher CTLA-4 expression than that in peripheral tissues and are efficiently depleted upon treatment with ADG126 (5mg/kg) in the CT26 colon tumor model..

Preclinical Toxicology

- In the nonobese diabetic (NOD) mouse model, all mice survived after six treatments of ADG126 at 50 mg/kg, No abnormal findings attributable to ADG126 were observed.
- In a 4-week GLP repeat-dose toxicology study, intravenous infusion of ADG126 to cynomolgus monkeys at 5, 30, and 200 mg/kg/dose once weekly for five doses, followed by a 28-day recovery period, was well tolerated. Adverse, but reversible, microscopic findings of minimal to moderate mixed perivascular infiltrates were observed at 200 mg/kg in both sexes in multiple organs and tissues.
- The no-observed-adverse-effect-level (NOAEL) was determined to be 30 mg/kg/dose in both rats and cynomolgus monkeys, and the highest non-severely toxic dose (HNSTD) was determined to be 200 mg/kg/dose.

Summary

- ADG126 is an activatable prodrug, that binds specifically to CTLA-4 across multiple species upon protease cleavage to remove the masking peptide.
- Activated ADG126 is a softer ligand blocker than ipilimumab but has more potent ADCC activity targeting high density CTLA-4 expressing cells.
- Activated ADG126, but not the masking peptide-intact ADG126 SAFEbody, can potently enhance T cell activation.
- ADG126 exhibits potent antitumor activity as a single agent in different mouse tumor models.
- ADG126 combined synergistically with other IO agents, such as anti-PD-1 antibody, to inhibit tumor growth in vivo.
- ADG126 can efficiently deplete T_{reg} cells in tumors, which express higher levels of CTLA-4, but not T_{reg} cells in peripheral tissues.
- ADG126 is well tolerated in animals, including NOD mice and cynomolgus monkeys, suggesting the potential for a high therapeutic index.

References

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

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