Abstract 3824

Antitumor activity of REGN4659, a fully human anti-CTLA-4 monoclonal antibody, against MC38.Ova tumors grown in immune competent human CTLA-4 knock-in mice

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Abstract

Background:

- Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) is an inhibitory checkpoint receptor that is expressed by activated conventional T cells and regulatory T cells. CTLA-4 plays a key role in restraining T cell responses against divers antigens, including self-antigens. However, in the tumor microenvironment, it co-contributes to tumor immune escape.
- CTLA-4 shares its two ligands, CD80 and CD86, which are largely expressed on antigen-presenting cells, with CD28, a co-activator of T cells; however, CTLA-4 binds CD80 and CD86 with higher affinity than CD28.
- CTLA-4 blockade elicits effective antitumor immunity in preclinical animal models and has demonstrated favorable clinical results in several solid malignancies.

Introduction:

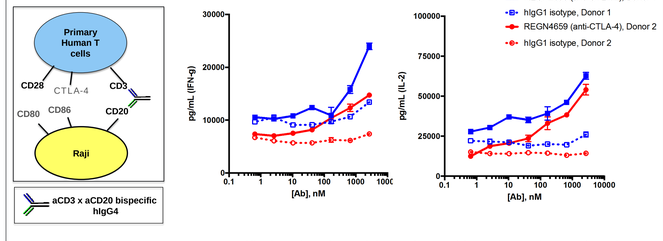
- We developed REGN4659, a human IgG1 antibody that binds to the extracellular domain of human and monkey CTLA-4 with high affinity and specificity.
- REGN4659 blocks CTLA-4 interaction with CD80 and CD86 and enhances the cytokine release of activated primary human T cells *in vitro*.
- We generated *Ctla4*$^{hum/hum}$ knock-in mice that express a human CTLA-4 chimeric protein from the endogenous mouse locus.
- *Ctla4*$^{hum/hum}$ knock-in mice were used to characterize the anti-tumor activity of REGN4659 in a prophylactic and established MC38.Ova mouse tumor model.
- The mechanism of REGN4659 action was analyzed by measuring T cell distribution and CTLA-4 expression levels on T cells within the tumor and in spleens.

Kinetic binding parameters for REGN4659 interaction with CTLA-4

Injected Protein	Surface Captured anti-hCTLA-4			
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	$t_{½}$ (min)
hCTLA-4.mmH	1.89×10^{5}	1.36×10^{-2}	7.20×10^{-8}	0.9
hCTLA-4.mFc	4.06×10^{5}	2.45×10^{-4}	6.04×10^{-10}	47.1
mfCTLA-4.mmH	ND	ND	3.57×10^{-7}	ND
mCTLA-4.mmH	NB	NB	NB	NB

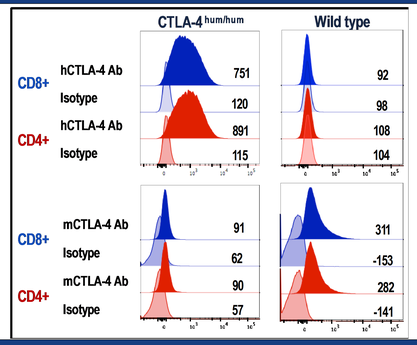
Abbreviations: CTLA-4, cytotoxic T-lymphocyte associated antigen 4; k_a, association rate constant; k_d, dissociation rate constant; K_D, equilibrium dissociation constant; h, human; m, mouse; Mf, cynomolgus monkey *Macaca fascicularis*; mFc, mouse fragment crystallizable; mmH, myc-myc-hexahistidine; NB, no detectable binding observed under the assay conditions used; ND, not determined; $t_{½}$, dissociative half-life.

REGN4659 enhances cytokines production by primary human CD4 T cells



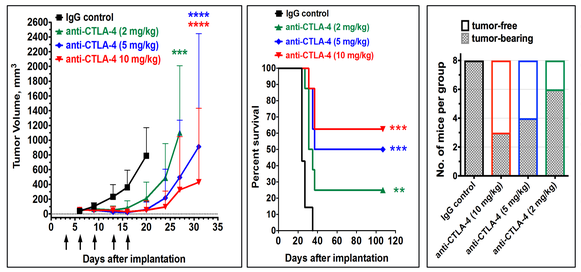
- Freshly isolated PBMC were purified and activated for 4 days with anti-CD3/CD28 beads.
- CD4+ T cells were purified and rested overnight before use.
- Raji cells were used as antigen presented cells (APC) and were inactivated with mitomycin C
- T : APC = 2 : 1 with $1x10^5$ T cells/well.
- Results are from a 3d incubation. Cytokine concentrations were measured by AlphaLISA.

Expression of human CTLA-4 on T cells from *Ctla4* $^{hum/hum}$ mice



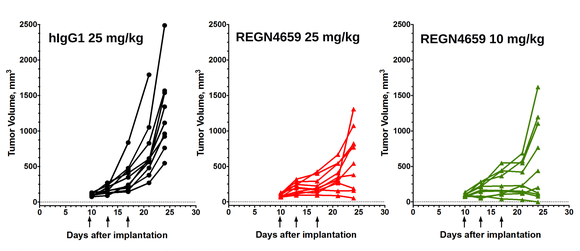
Splenocytes from *Ctla4* $^{hum/hum}$ mice or wild-type C57BL/6 mice were activated with 0.5 µg/ml Concavalin A for 72 hours and subsequently stained with LIVE/DEAD Aqua stain and fluorescent antibodies to mouse CTLA-4 (clone UC10-4F10-11) or human CTLA-4 (clone BN13). Histograms are gated on live CD4$^+$ T and CD8$^+$ T cells.

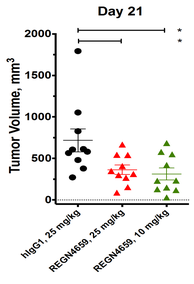
REGN4659 therapy inhibits tumor growth and improves survival of tumor-bearing *Ctla4* $^{hum/hum}$ knock-in mice



- *Ctla4* $^{hum/hum}$ knock-in mice were engrafted with MC38.Ova tumor cells s.c. on day 0
- REGN4659-hIgG1 (10 mg/kg, 5 mg/kg or 2 mg/kg) or hIgG1 control (10 mg/kg) administered IP
- 8 mice per group were used
- Treatment days are indicated by arrows.
- The number of tumor-free mice in each group by day 20 is shown, the last time point when all animals in each group were alive
- Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons post-test (**** p< 0.0001)

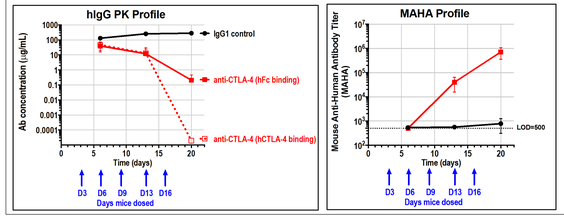
REGN4659 shows anti-tumor activity in established MC38.Ova tumors in *Ctla4* $^{hum/hum}$ knock-in mice





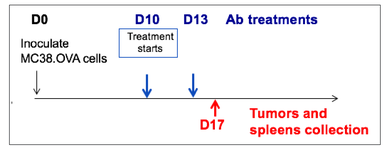
- *Ctla4*$^{hum/hum}$ mice, $5x10^5$ MC38.Ova cells/mouse
- Treatment started when tumors were 100 mm^3 average on day 10
- Antibodies were administered on days 10, 13,17
- Anti-hCTLA-4 Ab (REGN4659)- IP administration, 25 mg/kg or 10 mg/kg
- hIgG1 Ab (REGN1932)-IP administration, 25 mg/kg

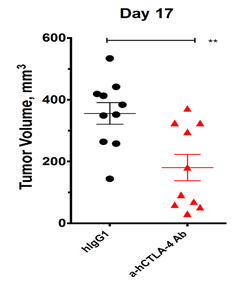
Rapid clearance of REGN4659, but not human IgG control in tumor-bearing *Ctl4* $^{hum/hum}$ mice



- Serum was collected to measure total human IgG and drug specific mouse anti-human antibody (MAHA) titers
- The concentration of the injected anti-CTLA-4 (REGN4659) or IgG1 control antibodies was determined using ELISA specific for human IgG
- The concentration of "functional" anti-CTLA-4 (REGN4659) was determined using ELISA specific for hCTLA-4
- The MAHA titers against anti-CTLA-4 (REGN4659) or IgG1 control antibody were determined using ELISA specific for each antibody

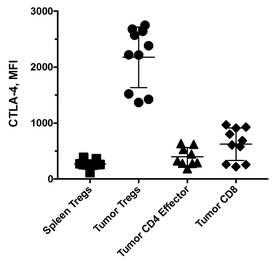
Analysis of intratumoral and peripheral T cells

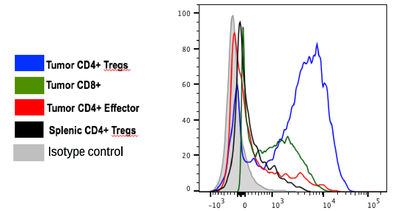




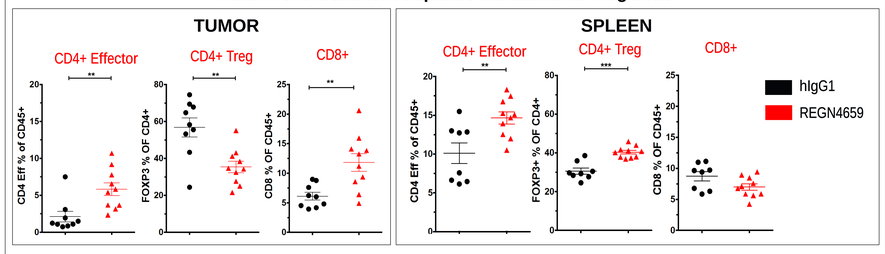
- *Ctla4* $^{hum/hum}$ mice engrafted with $5x10^5$ MC38.Ova cells/mouse on D0
- On D10 tumors with 100 mm3 size were selected
- Antibodies were administered IP on D10 and D13
- Anti-hCTLA-4 Ab (REGN4659), 25 mg/kg
- Control hIgG1 Ab (REGN1932), 25 mg/kg
- Samples were collected on D17 for flow cytometry

Expression of hCTLA-4 on intratumoral and peripheral Tregs and Teffs in *Ctl4* $^{hum/hum}$ mice

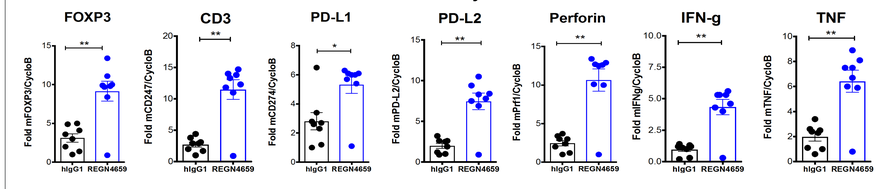




REGN4659 treatment depletes intratumoral Treg cells



REGN4659 treatment results in systemic immune activation



- MC38.Ova tumor bearing *Ctla4* $^{hum/hum}$ mice were treated with 10 mg/kg of REGN4659 or isotype control
- Spleens were collected at day 37 at the end of experiment. Expression levels of murine genes (normalized to murine cyclophilin B expression) were measured by Taqman.
- One way ANOVA with Tukey's multiple comparison test (**p<0.01, *p<0.05)

Summary

- REGN4659 is a human IgG1 antibody that binds to CTLA-4 and blocks its interaction with CD80 and CD86 ligands
- REGN4659 demonstrated anti-tumor activity in a mouse MC38.Ova tumor model in *Ctla4* $^{hum/hum}$ knock-in mice
- REGN4659 is being developed as an immunotherapeutic to treat multiple cancer type

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