Poster # 922

Expanding the Therapeutic Potential of anti-PD-1 and anti-CTLA-4 Therapy with Innovative Fc Engineering and Rationale Combinations for the Treatment of Solid Tumors

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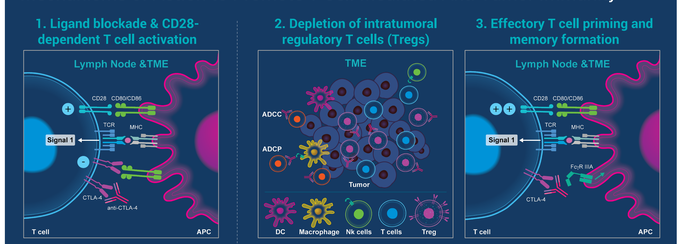
Background

AGEN1181, an Fc-Enhanced Anti-CTLA-4 Antibodies Engage Multiple Mechanisms of Action to Promote T Cell-Mediated Anti-Tumor Immunity



Hypothesis: Optimizing Fc - FcγR co-engagement enhances the activity of anti-CTLA-4 antagonist antibodies[1,2]. CTLA-4 antibodies with increased binding affinities to activating Fcγ receptors FcγRIV (CD16-2, mouse) or FcγRIIIA (CD16a, human) augment T cell priming by improving the quality of the immune synapse between a T cell and an antigen presenting cell (APC).

Fcγr binding characteristics of studied antibodies

Table 1: anti-CTLA-4

		Fc Isotype	Fc mutations	Blocking Properties	FcγR Binding Characteristics
Human	Parental	IgG1	-	+	Low
	Fc-Enhanced AGEN1181	IgG1.DLE	S239D.A330L.I332E	+	> FcγRIIIA binding ("Fc enhanced")
Murine Surrogates	Parental 9D9	mIgG2b	-	+	Low
	AGEN1181^{ms} Fc-Enhanced 9D9	mIgG2b.DLE	S241D.A332L.I334E	+	> FcγRIV binding

AGEN1181^{ms} has improved therapeutic potential against immunogenic tumors by enhancing Treg depletion and T cell priming

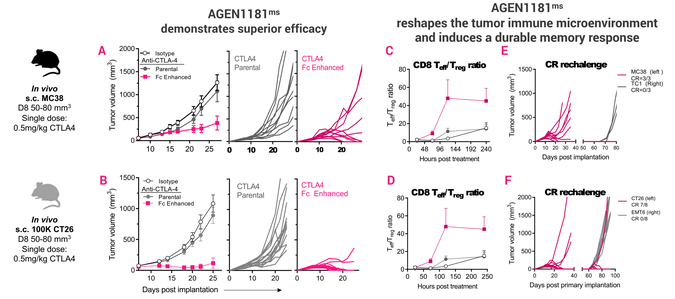


Figure 1: BALB/c and C57Bl6 mice with established CT26 and MC38 tumors received a single injection intraperitoneally (i.p.) of anti-CTLA4 clone 9D9 Parental or Fc-Enhanced or mIgG2a isotype control. **A-B:** Tumor growth are represented as average per group and single mice to emphasize the increased potency of the AGEN1181 "surrogate" **C-D:** CD8 Teff / CD4 Treg ratio changes over time is shown as average per group to emphasize the increased ability of the Fc-Enh. Antibody to deplete Treg and expend Teff. Briefly, tumor were resected and were phenotyped by flow cytometry the effector CD8 T (CD3+ CD8+ CD44+) Vs CD4 regulatory T (CD3+ CD4+ FoxP3+) ratio was calculated at different time points post treatment. **E-F:** The antitumoral memory response was assessed in complete responder (CR) mice treated with the Fc Enh. Mice were bilaterally re-challenged with 100K primary tumor CT26 and MC38 and non relevant tumors EMT 6 or TC1 .

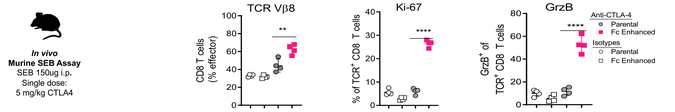


Figure 2: C57BL/6 mice were injected i.p. with the staphylococcal enterotoxin B (SEB) superantigen together with anti-CTLA-4 parental , Fc-Enhanced AGEN1181 "surrogate" or isotype control antibodies. SEB-specific (TCR Vb8) CD8+ T cells (CD3+ CD8+ CD44+), their replicative status (Ki67) and their functional profile (Grzb) were evaluated by flow cytometry on day 6; demonstrating an increase potency of the Fc-Enh. antibody to prime antigen specific effector CD8 T cells in a T reg depletion independent mechanism (not shown) . N=4 mice/group, and data are representative of at least three independent experiments.

AGEN1181^{ms} Fc Enhanced anti CTLA4 combines with ACT & Vaccines in CPM refractory tumor models

Fc-enhanced CTLA-4 combines with Adoptive T Cell Therapy (ACT) to promote robust tumor control

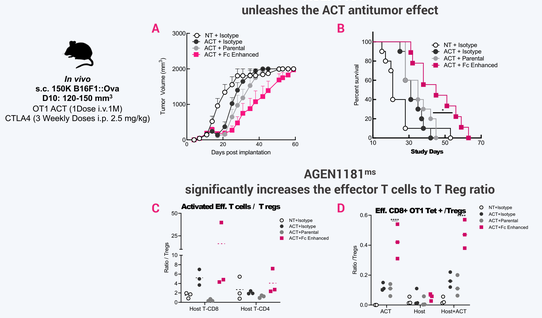


Figure 3: C57Bl6 were challenged s.c. with B16F1::OVA cells. At day 10 tumor-bearing mice were treated intravenously with OT1-specific transgenic CD8+ T cells (ACT) or PBS . CTLA4 treated groups received three doses of i.p weekly injections of Fc-Enhanced AGEN1181 "surrogate" or the reference molecule starting at D10 **A:** Tumor volumes were measured every 3-4 days and represented as average per group (N=10) **B:** Representation of the overall survival per treatment group. **C:** Tumor infiltrating CD8 & CD4 Teff / CD4 Treg ratio evaluation **D:** Host and ACT Tumor infiltrating OT1 Tet + CD8 Teff / CD4 Treg ratio evaluation. Briefly, tumor were resected and phenotyped by flow cytometry. Effector CD8 T OT1 Tet +/- (CD3+ CD8+ CD44+ PD1+ GzB+) Vs CD4 regulatory T (CD3+ CD4+ FoxP3+) ratio was calculated for Host (CD45.1) and ACT (CD45.2).

Fc-enhanced CTLA-4 promotes tumor control in combination with Tumor Antigen Vaccine + QS-21 to enhance tumor control

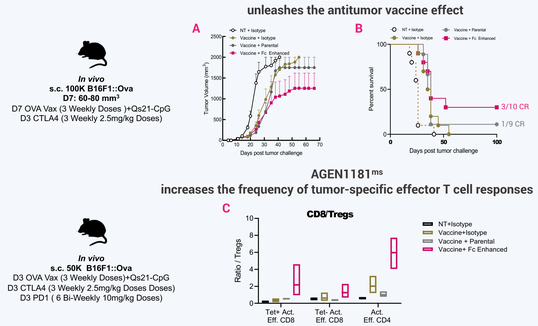


Figure 4: C57Bl6 were injected s.c. with B16F1::OVA cells. Challenged mice were subcutaneously treated with Agenus preclinical heat shock protein vaccine targeting MHC-I/II ovalbumin epitopes, (HSC70-OVA OTI/II) administered with Agenu's QS21 Stimulon and CpG adjuvant. Mice were treated i.p. with anti-CTLA-4 and anti-PD-1 or corresponding isotype control antibodies **A:** Tumor volumes were measured every 3-4 days and represented as average per group (N=10) **B:** survival curves per treatment group are shown. **C:** In an independent study tumor were resected after euthanasia at day 24 to prepare CD45+ enriched single cell solution and phenotyped by flow cytometry. Activated Eff. Cd8 T cells +/- Tetramer (OT1) (CD3+ CD8+ CD44+ PD1+ GzB+) and Eff. CD4 T cells (CD3+ CD4+ CD44+) were characterized Vs host CD4 Tregs (CD3+ CD4+ FoxP3+)

AGEN1181^{ms} Fc-Enhanced CTLA4 & PD1 triple combination with ACT and Vaccines are curative in IO refractory models

Sequential treatment with Fc-enhanced anti-CTLA-4 and PD1 promotes curative responses in combination with ACT

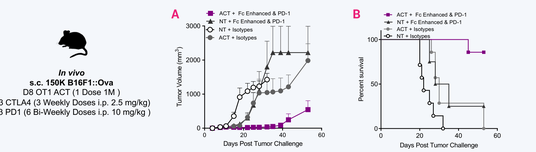


Figure 5: C57Bl6 were challenged s.c. with B16F1::OVA cells. Mice were preconditioned with early treatment (D3) using AGEN1181 anti-CTLA-4 Fc-Enhanced "surrogate" and anti-PD-1 or the corresponding isotype control. Eight days after tumor implantation, tumor-bearing mice were treated intravenously with OT1-specific transgenic CD8+ T cells (ACT) or PBS. **A:** Tumor volumes were measured every 3-4 days and represented as average per group (N=10) **B:** Representation of the overall survival per treatment group.

Concurrent treatment promotes curative responses in combination with HSC70 tumor antigen vaccine + QS-21

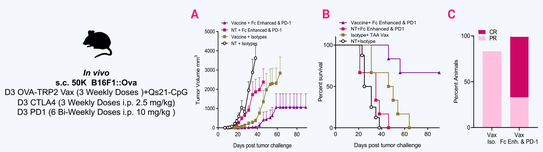


Figure 6: C57Bl6 were challenged s.c. with B16F1::OVA cells. Tumor-bearing mice were subcutaneously treated with the Agenus preclinical heat shock protein vaccine targeting MHC-I/II ovalbumin, and MHC-I TRP2 epitopes (HSC70-OVA–TRP2) administered with Agenu's QS21 Stimulon and CpG adjuvant. Mice were treated i.p. with anti-CTLA-4 and anti-PD-1 or corresponding isotype control antibodies **A:** Average tumor volumes per group (n=10/group) **B:** survival curves and **C:** treatment response evaluation per treatment group (CR: Complete Responder; PR: partial responder defined by relapsing pattern).

AGEN1181^{ms} Fc-Enhanced CTLA4 combines with adjuvant therapies

Combination with anti-PD-1 and Focal Radiation promotes significant tumor control

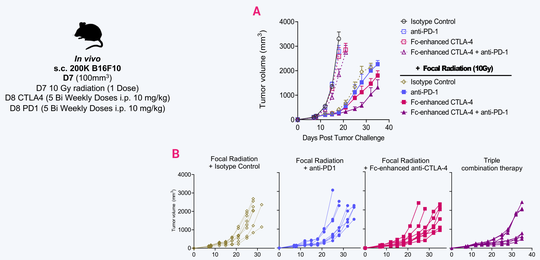


Figure 7: C57BL6 mice, were challenged s.c. with B16F10 cells. Tumor bearing mice received no radiation or a single-dose 10 Gy radiation treatment administered using a Small Animal Radiation Research Platform. Mice were subsequently treated i.p with anti-PD-1 and AGEN1181 anti-CTLA-4 Fc Enhanced "surrogate" or the corresponding isotype control . **A-B:** Tumor growth are represented as average per group and single mice to emphasize the increased efficacy of the triple combo RT with Fc-enhanced and PD1.

Combination with Alpha-GalCer as iNKT triggering therapy promotes robust tumor clearance in the lung

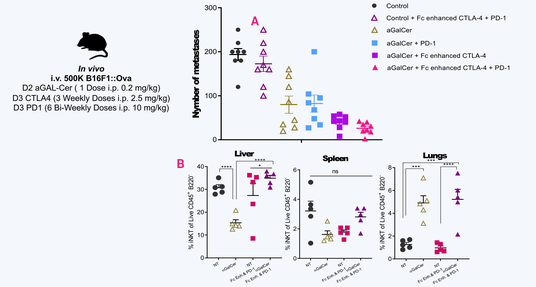


Figure 8: C57BL6 mice, were injected i.v. with B16F1::OVA cells. Challenged mice were treated i.p. with the iNKT activator lipid aGalCer or vehicle control. Mice were subsequently treated i.p with anti-PD-1 and AGEN1181 anti-CTLA-4 Fc Enhanced "surrogate". or the corresponding isotype control . **A:** Nodule counting to evaluate Pulmonary Disease burden per group (N=8) **B:** Phenotyping of the lung, spleen and liver infiltrating leukocytes by flow cytometry focusing on the iNKT infiltration.

AGEN1181 outperforms conventional IgG1 CTA-4 mAbs

Fc-enhanced CTLA-4, AGEN1181, combines with anti-PD-1 or anti-TIGIT to further enhance T cell responsiveness

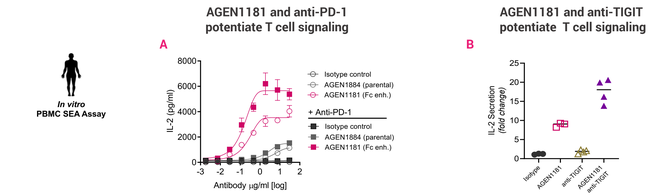


Figure 9: A: IL-2 production by human PBMCs stimulated with a suboptimal concentration of SEA peptide together with increasing concentrations of Fc-enhanced anti-CTLA-4 AGEN1181, parental anti-CTLA-4 AGEN1884 (IgG1) or isotype control alone or in combination with 10 μg/ml anti-PD-1 (Pembrolizumab). B: IL-2 production (day 4) by human PBMCs stimulated with a suboptimal concentration of SEA peptide together with 10 μg/ml of Fc-enhanced anti-CTLA-4, AGEN1181, alone or in combination with 5 μg/ml of anti-TIGIT mAb.

AGEN1181 expands the therapeutic reach and activity of CTLA-4 therapy by improved binding to both low and high affinity FcγRIIIA allele

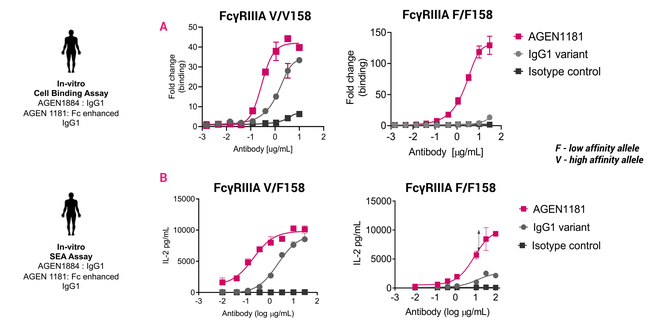


Figure 10: A: Binding profiles of the human Fc engineered antibody AGEN1181 Vs the parental AGEN1884 and Fc silent variants of the anti-CTLA4 blocking antibody to cells stably expressing hFcγRIIIA V/V haplotype, hFcγRIIIA F/F haplotype. Binding was assessed by flow cytometry, mean fluorescence intensity normalized according to standard methods and the ratio of the increase binding Vs parental was calculated. B: Evaluation of IL-2 production by human PBMCs donors that are homozygous for the high affinity hFcγRIIIA V/V haplotype, or the low affinity hFcγRIIIA F/F haplotype and stimulated with staphylococcal enterotoxin A (SEA) peptide together with increasing concentrations of anti-CTLA-4 hIgG1: AGEN1884, antiCTLA-4 Fc-enhanced hIgG1: AGEN1181 or an hIgG1 Fc-enhanced (Fc E) isotype control antibody. Polymorphism in Fcg receptor was determined by PCR followed by Sanger sequencing.

This version includes corrections to an error in the original presentation contained on the legend for Figure 10B.

Conclusions

AGEN1181 Fc-enhanced anti-CTLA-4 demonstrates superior single agent and broader IO & SOC combination activity than conventional CTLA-4 mAbs:

- Engages multiple mechanisms of action to promote optimal anti-tumor immunity
- Promotes curative responses in checkpoint resistant preclinical cancer models in combination with anti-PD-1 and Vaccine + QS-21, Adoptive T cell therapy, radiation therapy and iNKT activating Alpha-GalCer adjuvant therapy
- Enhances T cell responsiveness in combination with anti-PD-1 or anti-TIGIT mAbs
- Expands the therapeutic reach and activity of CTLA-4 therapy by improved binding to both the low and high affinity FcγRIIIA and in turn expands the therapeutic reach of anti-CTLA-4 to an additional 40% of patients[1,3,4] who express the low affinity FcγRIIIA allele and respond poorly to first generation anti-CTLA-4 mAbs.

On-Going Trial

Combination of AGEN1181 with Agenus's balstilimab (anti-PD-1) is advancing in the clinic (NCT03860272).

References:

[1]Waight et al. Cancer Cell 2018
[2]Danbee et al. PNAS 2018
[3]Vargas et al. Cancer Cell 2018
[4]Romano et al. PNAS 2015

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For a more detailed presentation: