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Poster # **1677**

Characterization of The Pharmacodynamic Activity of AGEN1181, an Fc-enhanced CTLA-4 Antibody, Alone and in Combination With the PD-1 Antibody Balstilimab

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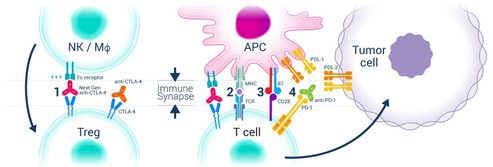
Background

AGEN1181 leverages a novel Fc-mechanism of action to promote:

- ✓ Superior efficacy: Enhanced T cell priming, Treg depletion and T cell memory formation for durable anti-tumor immune response
- ✓ Improved safety: Avoid complement mediated toxicity associated with many current immune checkpoint inhibitors
- ✓ Expand therapeutic reach: by improved binding to CD16 (FcγRIIIA) for both low and high affinity allele - expressing patients



Enhanced intratumoral Treg Depletion | Superior T cell Priming, and Memory formation | Improved tumor killing and combination efficacy

AGEN1181 mechanism of action

1. Optimized Fc to enhance Treg depletion
2. Optimized Fc to enhance immune synapse quality and T cell priming & activation
3. Reverse T cell dysfunction and restore tumor targeting T cell responses
3. Superior T cell memory responses & improve durability of response

Study Design

Phase I study assessing the safety and efficacy of AGEN1181 (anti-CTLA-4), both as monotherapy and in combination with balstilimab (anti-PD-1), in patients with advanced solid tumors (NCT03860272)

End Points

Primary: Safety and tolerability

Secondary: Pharmacokinetics profile, ORR per RECIST 1.1

Exploratory: Pharmacodynamics, CD16/FcγR polymorphism expression

AGEN1181 Exhibits Single-Agent and Combination Clinical Activity in Patients With Advanced Solid Tumors

Table 1. Responses observed in Phase I trial of AGEN1181as monotherapy and in combination with balstilimab

Subject #	Cancer Diagnosis	Dose and Schedule	Best Overall Response
Subject I	Ovarian	AGEN1181 2 mg/kg Q6W + balstilimab 3 mg/kg Q2W	PR, CR*
Subject E	Ovarian	AGEN1181 0.1 mg.kg Q3W then AGEN1181 1 mg/kg Q6W + balstilimab 3 mg/kg Q2W	PR
Subject J	Melanoma	AGEN1181 2 mg/kg Q3W	PR*
Subject F	Endometrial (MSS, PD-L1$^-$)	AGEN1181 1 mg/kg Q3W	CR
Subject C	Endometrial (MSS, PD-L1$^-$)	AGEN1181 0.1 mg/kg Q6W + balstilimab 3 mg/kg Q2W	PR (CR by PET)
Subject H	Colorectal cancer (MSS)	AGEN1181 1 mg/kg Q6W + balstilimab 3 mg/kg Q2W	PR
Subject G	Colorectal cancer-MANEC (MSS)	AGEN1181 1 mg/kg Q6W + balstilimab 3 mg/kg Q2W	PR

*, unconfirmed response (as of March 26, 2021)

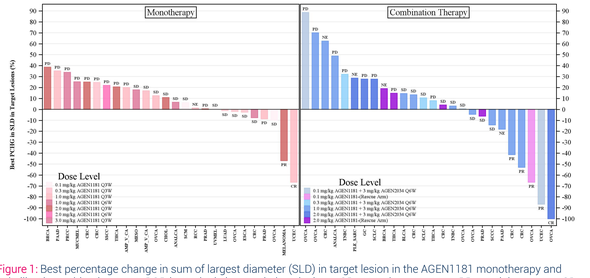


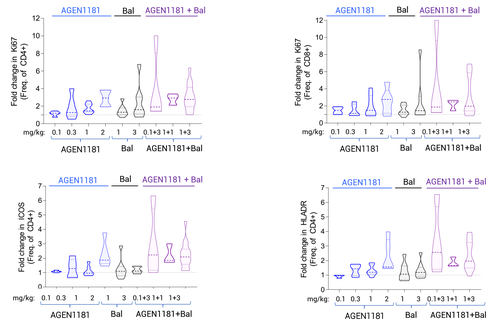
Figure 1: Best percentage change in sum of largest diameter (SLD) in target lesion in the AGEN1181 monotherapy and balstilimab combination arms. BOR is marked above or below the bars. CR- complete response; PR- partial response; SD – stable disease; PD – partial disease.

Pharmacodynamic Analysis at The Periphery

Enhanced Peripheral T Cell Activation With AGEN1181 as Monotherapy and in Combination With Balstilimab

Dose-dependent increase in Ki67$^+$ CD4$^+$ and CD8$^+$ T-cells and ICOS$^+$, HLA-DR$^+$ CD4$^+$ T cells 7 days post first dose

A)



Sustained CD4$^+$ T cell activation by AGEN1181 monotherapy and in combination with balstilimab

B)

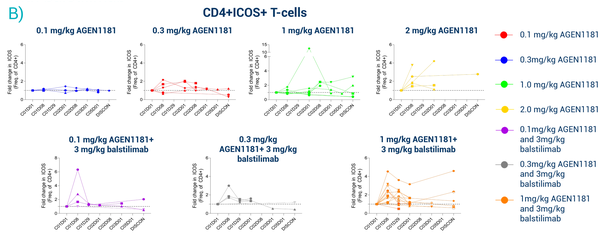
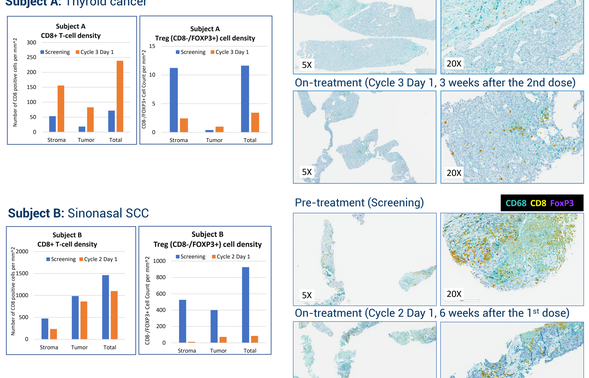


Figure 2: Peripheral flow cytometry analysis using PBMCs from patients treated with AGEN1181 monotherapy or in combination with balstilimab collected at Day 7 after the first dose A) or at various timepoints, as indicated. B) Data is represented as a fold change over baseline (pre-treatment, Day 1) of frequency of activated CD4+ or CD8+ T cells out of total CD4+ or CD8+ cells (frequency of parent).

Pharmacodynamic Analysis in the TME

Selective Depletion of Intratumoral Tregs With AGEN1181 Monotherapy

Subject A: Thyroid cancer

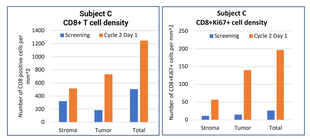


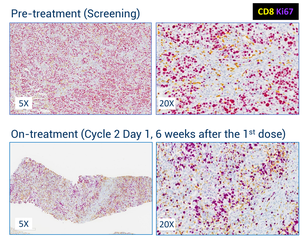
Subject B: Sinonasal SCC

Figure 3: CD8 (yellow)/ FOXP3 (purple) /CD68 (turquoise) triplex chromogenic IHC of FFPE tumor biopsy samples collected at screening and on-treatment (cycle 2 Day 1 for Q6W cohort; cycle 3 Day 1 for Q3W cohort). Images were analyzed using Flagship Biosciences image analysis software for quantification of CD8+, FoxP3+, CD68+ cells as positive or negative. Tregs were defined as FoxP3+/CD8- cells. Machine learning algorithms were implemented to separate out lymphocyte-like cells and to stratify all cells as belonging to the 'tumor' or 'stromal' compartments. Outputs of the digital analysis included CD8+, FoxP3+, CD68+ cell density (cells per mm2) depicted on the graphs in A) and B).

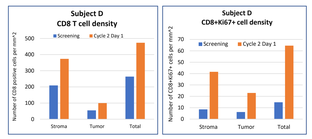
AGEN1181 in Combination With Balstilimab Promotes Intra-Tumoral Recruitment and Activation of CD8 T Cells

AGEN1181 Q6W 0.1mg/kg + balstilimab Q2W 3 mg/kg
Subject C: Endometrial cancer





AGEN1181 Q6W 1mg/kg + balstilimab Q2W 3 mg/kg
Subject D: Ovarian cancer



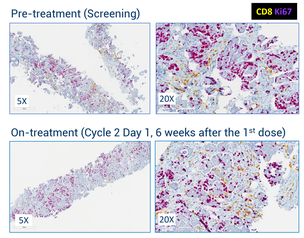


Figure 4: CD8 (yellow)/ Ki67(purple) duplex chromogenic IHC of FFPE tumor biopsy samples collected at screening and on-treatment (cycle 2 Day 1, 6 weeks after the 1st dose). Images were stained and analyzed as described in Figure 3.

Enhanced Fc Design of AGEN1181 Induces Clinical Activity in Patients With Low Affinity or Heterozygous FCγR SNP

Responses observed in patients with low affinity or heterozygous FCγRIIIA SNP genotype

- 43 PGx blood samples evaluated for FCgRIIIa SNP
- 6 responders: 2 low affinity, 3 heterozygous, 1 high affinity FCgRIIIA SNP

Subject	Genotype	FCγRIIIA affinity	BOR
Subject E	F/F	low affinity	PR
Subject F	F/F	low affinity	CR
Subject C	V/F	heterozygous	PR
Subject G	V/F	heterozygous	PR
Subject H	V/F	heterozygous	PR
Subject I	V/V	high affinity	PR

FCγR affinity	FCγRIIIA rs396991	FCγRIIIA V158F
High	C/C	V/V
Mixed	C/A	V/F
Low	A/A	F/F

Figure 5: FCgRIIIA genotype was assessed in the whole blood samples collected from patients treated with AGEN1181 monotherapy or in combination with balstilimab. Validated real-time SNP genotyping assay specific to human FCGR3A rs396991 V158F SNP was used. Pharmacogenomics consent was obtained from all patients included in this analysis.

Cytokine Expression is Not Significantly Induced By Single Agent or Combination AGEN1181 Treatment

Cytokine expression levels were low overall (0.1 up to 100 pg/ml) for most cytokines and overall did not change longitudinally

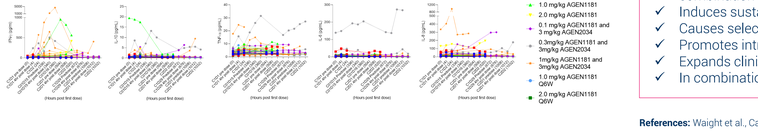


Figure 6: Cytokine expression in the longitudinal plasma samples from patients treated with AGEN1181 monotherapy and in combination with balstilimab was tested using Mesoscale Discovery (MSD) Technologies' V-Plex Plus Proinflammatory Panel to quantify the following cytokines: IFN-γ, IL-10, IL-12p70, IL-13, IL1β, IL-2, IL-4, IL-6, IL-8 and TNF-α. Graphs depict absolute cytokine levels (pg/ml) over time.

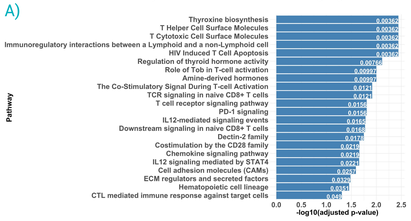
Case Study

Durable Partial Response in a Patient With Endometrial Cancer Treated With AGEN1181 in Combination with Balstilimab

Upregulation of immune regulatory signaling pathways and central/memory T cell signature

Subject C: AGEN1181 Q6W **0.1mg/kg** + balstilimab Q2W **3mg/kg**

A)



B)

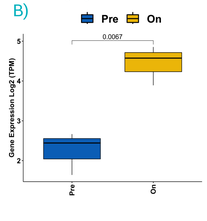


Figure 7: A) Gene set enrichment representing significantly upregulated pathways in subject C on treatment with AGEN1181 in combination with balstilimab. B) Difference in expression of Central Memory T Cell signature upon treatment in Subject C (p-value= 0.0067, calculated using t-test).

Increased TCRβ diversity in the TME and increased tumor-specific TCRβ diversity in the blood

A)

B)

Figure 8: A) Comparison of tumor TCR beta chain diversity index between pre-treatment and on-treatment with AGEN1181 in combination with balstilimab in subject C. B) Differences in frequency of TCR beta chains shared between tumor and blood upon treatment with balstilimab in Subject C.

Tumor-specific TCR clone frequency expansion at the periphery and in the TME

A)

B)

Figure 9: Correlation of TCR beta chains (red: blood only, green: shared in blood and tumor, blue: tumor only) in tumor and periphery in pre-treatment (Cycle 1 Day 1, A)) and on-treatment (Cycle 2 Day 1, B)) samples from Subject C obtained from the comparison of TCRSeq of the tumor biopsy and blood. Note: blood TCR count is the same in the pre-treatment and on-treatment samples and is limited by the maximum capacity of the TCRSeq library (Adaptive Technologies) reached due to abundance of TCRs in the blood.

Conclusions

AGEN1181:

- ✓ Demonstrates clinical activity in heavily pretreated patients as monotherapy or in combination with balstilimab
- ✓ Induces sustained T cell activation at the periphery
- ✓ Causes selective Treg depletion in the tumor microenvironment (TME)
- ✓ Promotes intra-tumoral recruitment of activated TILs in combination with balstilimab
- ✓ Expands clinical responses to patients with low affinity or heterozygous FCγRIIIA
- ✓ In combination with balstilimab, expands TCRβ diversity intra-tumorally and at the periphery

References: Waight et al., Cancer Cell 2018; Arce-Vargas et al., Cancer Cell 2018

Acknowledgment: We thank the patients and their families in this study and the clinical caregivers for the dedication to improve their patient's lives. We thank Flagship Biosciences, Precision for Medicine, Adaptive Technologies and Personalis for help with clinical sample testing.

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