EOS884448, a high affinity fully human antibody directed against TIGIT, mediates in vitro anti-tumor activity through multiples mechanisms of action involving activation of intratumor effector cells and depletion of regulatory T cells.

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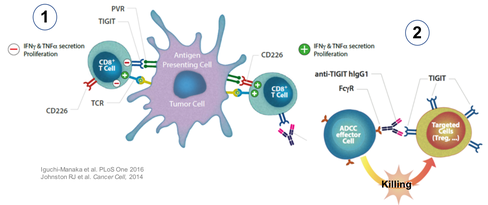
SUMMARY

- T cell Immunoreceptor with Ig and ITIM domains (TIGIT) is a negative costimulatory receptor that inhibits effector T cell and NK cell function and marks a highly suppressive regulatory T cell (Treg) subset.
- TIGIT ligands belong to the PVR/nectin family, among which PVR (CD155) shows the highest affinity and is commonly expressed on antigen presenting cells (APC) and tumor cells. *See poster #4969.*
- CD226, a co-stimulatory receptor also expressed on NK and T cells, competes with TIGIT for PVR binding but with a lower affinity.
- TIGIT expression is increased on T and NK(T) cells from cancer patients and is correlated to poor outcome and response to aPD1 therapy in some indications.
- EOS884448 properties and functionality make it an attractive Immuno-Oncology therapy candidate:
  - ✓ Strong binding to primary human and cyno T cells (sub-nM Kd)
  - ✓ Competition with natural ligands with IC_{50} in sub-nM range
  - ✓ Increase of primary T cell functions in healthy donors and cancer patients
  - ✓ Depletion through ADCC of highly suppressive TIGIT$^+$ Tregs.
  - ✓ Antitumor efficacy in animal model
  - ✓ Excellent developability profile
  - ✓ Excellent safety profile

EOS884448 PREVENTS TIGIT-DRIVEN IMMUNOSUPPRESSION



1 drug = multiple anti-tumor mechanisms of action

1. Reactivation of immune response by
   - Suppressing TIGIT-mediated inhibitory signaling
   - Increasing ligand availibility for CD226 co-stimulatory receptor
2. Depletion of TIGIT$^+$ highly suppressive Treg subpopulation and TIGIT$^+$ tumor cells with ADCC-triggering isotype (FcγR dependent)

EOS884448 BINDS WITH HIGH AFFINITY TO HUMAN AND CYNO TIGIT AND PREVENTS LIGAND BINDING TO TIGIT

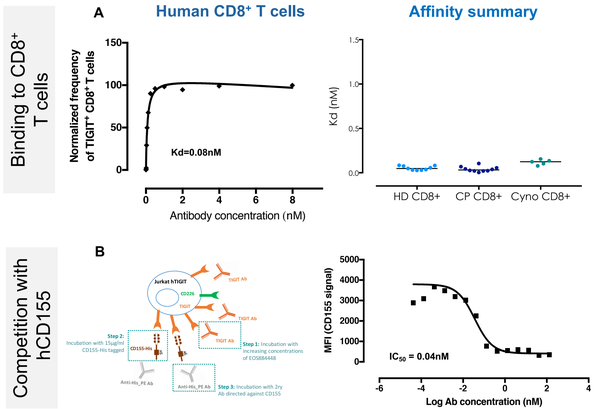


Fig. 1. EOS884448 displays a sub-nM affinity to primary T cells from healthy donors and cancer patients and potently prevents binding of TIGIT ligands. (A) Human PBMC from healthy donors (HD) and cancer patients (CP), as well as cynomolgous PBMC were incubated with dose escalating concentrations of EOS884448. Kd of binding was calculated based on normalized frequency of TIGIT$^+$CD8$^+$ at the different concentrations tested. (B) EOS884448 prevents CD155 ligand binding to TIGIT overexpressing Jurkat cells, showing IC50 values at sub-nM range.

EOS884448 POTENTLY PREVENTS TIGIT INHIBITORY SIGNALING IN PRESENCE OF CD155 LIGAND

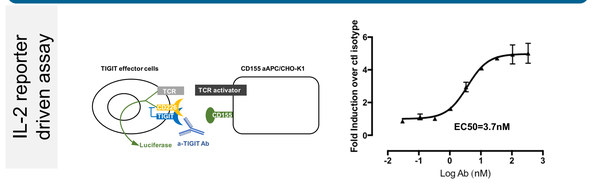


Fig. 2. EOS884448 potently blocks TIGIT-CD155 mediated immunosuppression. Jurkat T cells expressing human TIGIT with a luciferase reporter driven by a native promoter that can respond to both TCR activation and CD226 co-stimulation were stimulated with APC-like cells expressing CD155 (CHO-TCR-CD155). Addition of EOS884448 during stimulation prevents TIGIT:CD155 induced inhibition and increases T cell activation-mediated luminescence.

EOS884448 INCREASES IFNγ SECRETION IN T CELLS FROM HEALTHY AND CANCER PATIENTS

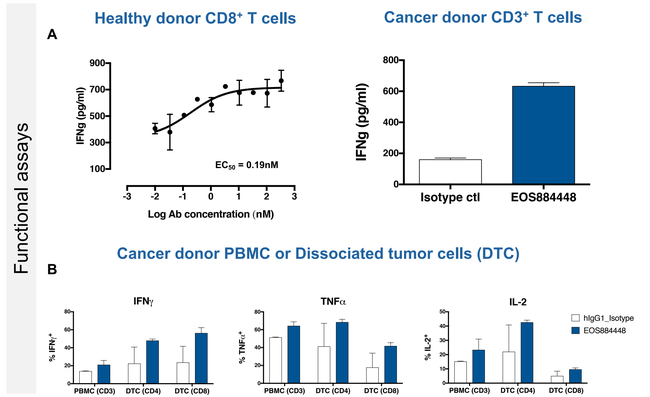


Fig. 3. EOS884448 potently restores pro-inflammatory cytokines in presence of CD155 ligand. (A) Human primary CD3$^+$ or CD8$^+$ T cells were stimulated with APC-like cells (CHO-TCR-CD155). Addition of aTIGIT during stimulation prevents CD155-induced inhibition and increases IFNγ production in T cells from healthy donors and cancer patients. (B) EOS884448 restores cytokine release from freshly isolated cancer patient PBMC or tumor infiltrated lymphocytes present among dissociated tumor when incubated overnight with aCD3/CD28 stimulation, as measured by intracellular staining.

EOS884448 POSITIVELY MODULATES ACTIVITY OF NON-CONVENTIONAL $\gamma\delta$ T CELLS

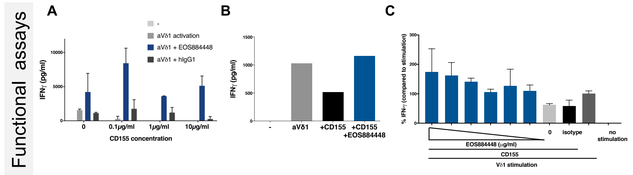


Fig. 4. EOS884448 restores IFN-γ production by $\gamma\delta$ T cells. Magnetically isolated Vδ1$^+$ $\gamma\delta$ T cells (A) or PBMC (B) from CMV$^+$ donors were activated with anti-Vδ1 and IL-15. When recombinant CD155 was added to the culture, IFN- γ production was reduced in a dose-dependant manner and EOS884448 was able to prevent this inhibition. Treatment with EOS884448 increases the secretion of IFN- γ at a level superior than the control without CD155. (C) Dose-dependant increase in IFN- γ production by EOS884448 on total PBMC activated with anti-Vδ1 and IL-15.

EOS884448 PREFERENTIALLY DEPLETES TREG IN HUMAN PBMC FROM HEALTHY AND CANCER DONORS

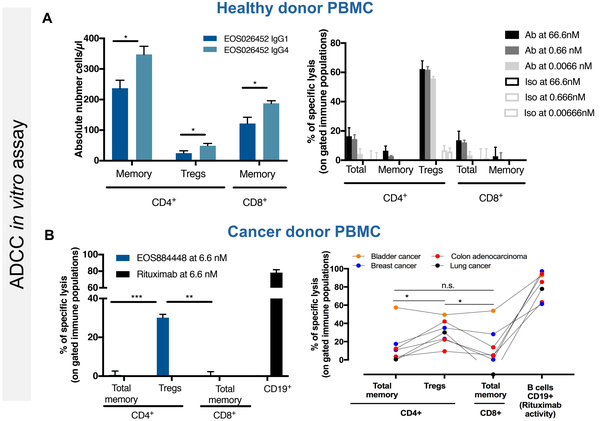


Fig. 5. EOS884448, as an hIgG1, shows preferential depletion of Tregs over memory CD4$^+$ and CD8$^+$ T cells. (A) Absolute cell counts and depletion of different T cell populations expressing TIGIT is shown when PBMC are incubated *in vitro* in presence of EOS026452 (EOS884448 parent) for 20h and quantified by FACS. EOS026452 shows a preferential depletion of Treg cells at 66.6 nM or lower concentrations in healthy donors. (B) Similar preferential depletion with EOS884448 is observed in PBMC from cancer patients suffering from different solid tumor types (*p<0.05).

CONCLUSIONS

EOS884448 is a highly potent antagonist aTIGIT mAb that:

- ❖ Binds with high affinity to human and cynomolgous TIGIT$^+$ T cells.
- ❖ Competes for binding with TIGIT ligands.
- ❖ Prevents CD155-TIGIT mediated immunosuppression and restores effector T cell function.

⇒ All at sub-nM concentrations

- ❖ Preferentially depletes human Tregs *in vitro.*
- ❖ **Preclinical data support the transition of EOS884448 to the clinic as a potent a-TIGIT antagonist Ab.**

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