Anti-TIGIT antagonist antibody EOS884448 shows dual mechanism of action by restoration of T cell effector functions and preferential 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 Teff and NK cell function and marks a highly suppressive 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.
- 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.
- iTeos developed an anti-human/cyno TIGIT blocking mAb (EOS884448) and a surrogate anti-mouse TIGIT mAb with comparable properties.
- 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
  - ✓ Strong antitumor efficacy in monotherapy and combination with aPD(L)-1 (with surrogate mAb) in mouse CT26 model.

HIGH TIGIT EXPRESSION ON IMMUNE CELLS, FURTHER INCREASED IN CANCER PATIENTS

TIGIT phenotyping

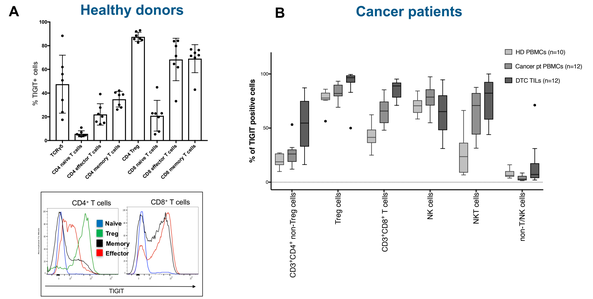
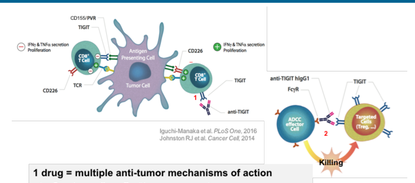


Figure 1. TIGIT expression was analyzed by flow cytometry, gating on different lymphocyte subsets in (A) healthy donor PBMCs (n=7). Frequency of TIGIT expressing cells is highest on CD4+ Tregs and effector and memory T cells on healthy donors and (B) further increases in cancer patient PBMCs or tumor infiltrated lymphocytes (TILs) (n=12).

TIGIT-DRIVEN IMMUNOSUPPRESSION

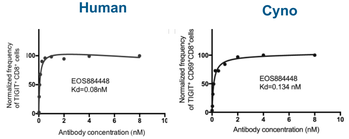


1 drug = multiple anti-tumor mechanisms of action

1. Reactivates effector T cell immune response by
   - Suppressing TIGIT-mediated inhibitory signaling
   - Increasing ligand availability for CD226 co-stimulatory receptor
2. Depletes 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

A Binding to CD8+ T cells



B Competition with hCD155

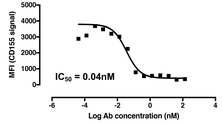


Figure 2. (A) Human and cynomolgous PBMCs were incubated with dose escalating concentrations of EOS884448. Kd of binding was calculated based on normalized frequency of TIGIT+CD8+ at the different concentrations tested. (B) EOS884448 prevents CD155 ligand binding, showing IC50 values at sub-nM range.

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

Functional assay

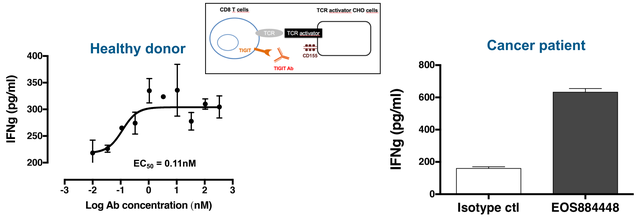


Figure 3. EOS884448 aTIGIT mAb increases IFNγ production by primary human T cells from healthy donors (CD8+ T cells) and cancer patients (CD3+ T cells). Human primary CD3+ or CD8+ T cells were stimulated with APC-like cells (CHO-TCR-CD155). Addition of aTIGIT during stimulation prevents CD155-induced inhibition and increases IFNγ production in T cells from healthy donors and cancer patients.

EOS884448 PREFERENTIALLY DEPLETES TREG IN HUMAN PBMCs FROM HEALTHY DONORS

ADCC *in vitro* assay

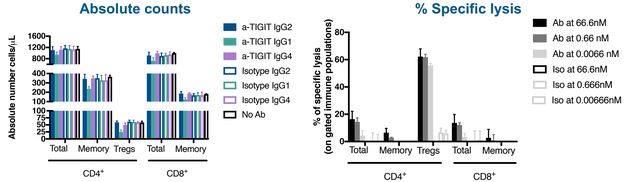
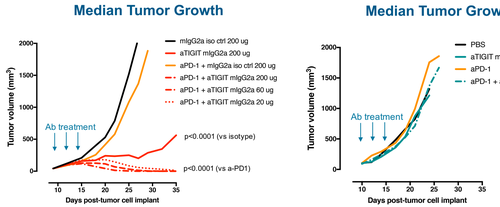


Figure 4. Absolute cell counts and depletion of different T cell populations expressing TIGIT is shown when PBMCs are incubated in presence of EOS884448 for 20h and quantified by FACS. EOS884448 shows a preferential depletion of Treg cells as compared to memory CD4+ and CD8+ T cells at 66.6 nM or lower concentrations.

iTEOS SURROGATE Ab SHOWS STRONG ANTITUMOR EFFICACY IN MONOTHERAPY OR COMBO WITH aPD-1

CT26 syngeneic tumor model

A



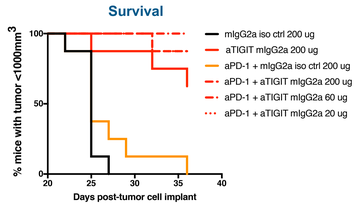
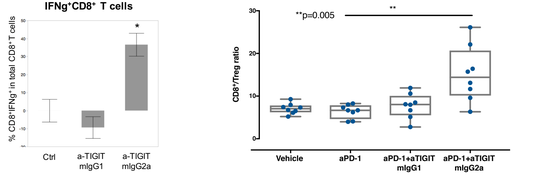


Figure 5. Surrogate mouse anti-TIGIT demonstrates potent isotype-dependent antitumor efficacy. The efficacy of mouse surrogate aTIGIT mAb antagonist was evaluated in established CT26 syngeneic tumors. Only aTIGIT ADCC permitting isotype, mIgG2a, delays CT26 tumor growth in monotherapy on established mouse tumors. In combination with 10mg/kg aPD-1 mAb (clone RMP1-14; BioXcell), complete tumor regression occurs in most of the animals treated with mIgG2a aTIGIT mAb.

ITEOS SURROGATE Ab INCREASES CD8+ T CELLS AND CYTOTOXICITY SIGNATURES IN VIVO

A Flow cytometry analysis



B

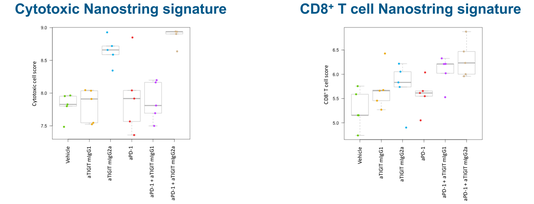


Figure 6. (A) Flow cytometry analysis of dissociated tumors, done 1 day after the last antibody treatment, demonstrated strong increase of IFNγ producing CD8+ T cells and of the CD8+/ Tregs ratio within tumors when treated with the aTIGIT mIgG2a isotype (** p<0.005). (B) CT26 syngeneic tumors were collected at day 18 after inoculation, 2 days after last antibody treatment, for Nanostring analysis of gene expression and signature changes. Transcriptomic signature shows an increase in the cytotoxic and CD8+ T-cell scores, observed in combination of aTIGIT mIgG2a and aPD-1 treatment.

CONCLUSIONS

EOS884448 is a highly potent antagonist aTIGIT mAb that:

- ❖ Binds with high affinity to human and cynomolgus TIGIT+ T cells.
- ❖ Competes for binding with TIGIT ligands.
- ❖ Increases IFNγ secretion on human primary T cells from healthy donors and cancer patients *in vitro*

  ⇒ **All at sub-nM concentrations**
- ❖ Preferentially depletes human T regs *in vitro.*

When replaced by a mouse surrogate with identical properties:

- ❖ Inverts the immunosuppressive Treg/CD8+ ratio and restores CD8+ T cell IFNγ secretion *in vivo*.
- ❖ Promotes antitumor immunity as monotherapy or in combination with aPD-1 in CT26 colon carcinoma mouse model.

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