Interim biomarker analysis of etigilimab (OMP-313M32), an anti-TIGIT antibody, in advanced solid tumors supports TIGIT-associated mechanisms of action

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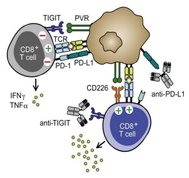
SUMMARY

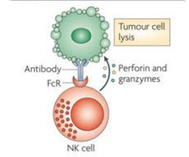
- TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is an immune checkpoint receptor shown to inhibit T cell and NK cell activation and suppress anti-cancer immune response.
- Etigilimab (OMP-313M32) is a humanized IgG1 monoclonal anti-TIGIT antibody. It was designed to inhibit TIGIT signaling and is hypothesized to deplete T regulatory cells (Treg) expressing high levels of TIGIT through antibody directed cellular cytotoxicity (ADCC).
- In a Phase I clinical trial (NCT03119428) in advanced solid tumors, etigilimab was dosed from 0.3 to 20 mg/kg every other week. Initial pharmacodynamic biomarker analysis results are presented here.
- Flow cytometry of patients' peripheral blood mononuclear cells (PBMCs) showed significant reduction of peripheral Tregs and an increase in the CD8/Treg ratio after etigillimab treatment, but no significant decreases in total CD4, CD8 T cells and NK cells were observed.
- The reduction of Tregs and the increase of CD8/Treg ratio in blood were confirmed by epigenetic quantification of immune cells.
- Etigilimab reduced the frequency of peripheral TIGIT+ cells among CD4+, CD8+ and Treg populations.
- Etigilimab increased proliferation of T cell subsets in peripheral blood.
- Etiglimab increased intracelluar IL2 levels in patients' T effector memory cells.
- The increased proliferation and cytokine function of immune cells correlated with immune-related adverse effects in patients, supporting the proposed mechanism of action of etigilimab.

TIGIT signaling



Cancer Cell 2014; **26** (6), 923-937

ADCC



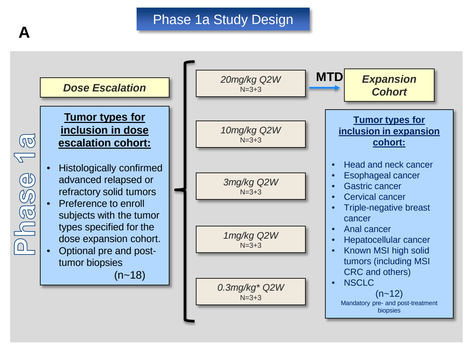
Nat Rev Immunol 2010; **10** (5), 317-327

MATERIALS AND METHODS

- Patient biomarker samples were collected at initial screening visit (SCR), Day 1 (C1D1) pre-dose, Day 8 (C1D8), Day 22 (C2D8), Day 36 (C3D8), Day 78 (C6D8), Day 120 (C9D8), Day 162 (C12D8), Day 204 (C15D8), and treatment termination (TT).
- Flow cytometry: PBMCs from patients' whole blood were isolated by Ficoll density gradient and cyropreserved for flow cytometry batch analysis at Primity Bio (Fremont, CA). Two multicolor flow cytometry panels were run on PBMCs for immunophenotyping and activation markers. Panel 1: viability dye, CD45, CD3, CD4, CD8, CD19, CD56, FoxP3, CD226, PD1 Ki67, TIGIT. Panel 2: viability dye, CD3, CD4, CD8, CD19, CD56, CD45RA, CCR7, TIGIT, IFNγ, IL2, TNFα, IL17, Granzyme B. For panel 2, cells were stimulated with Phorbol 12-myristate 13-acetate and ionomycin in the presence of GolgiStop for 4 hours prior to staining. Cell staining was quantified on a PD LSR II flow cytometer. Results are reported as frequency (percent live leukocytes), fold change from C1D1 levels, or percent change from C1D1 levels.
- Epigentic immunophenotyping was performed using whole blood samples measuring DNA methylation patterns specific for Treg and CD8 T cells at Epiontis/Precision for Medicine (Berlin, Germany).
- RNA Seq: RNA was extracted from whole blood samples. RNA Seq reads (Almac Diagnostics) were mapped to human genome GRCh38 (HG38) primary assembly, and gene counts were obtained using RSEM, TxImport, and Gencode v27 annotation. Gene filtering and normalization was performed using edgeR (Bioconductor). Differential expression analysis between post-dose and pre-dose time points was performed using the voom method from the limma package (Bioconductor).
- Patients were considered as having immune-related adverse events if they showed rash, pruritus, oral mucositis, or other immune system disorders.

BACKGROUND

313M32-001 Phase1a trial of Etigilimab in Advanced Solid Tumors



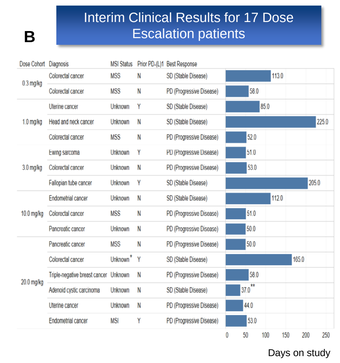
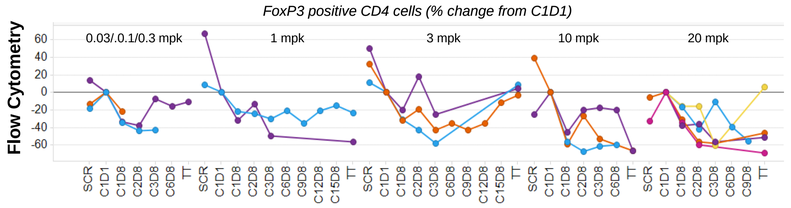


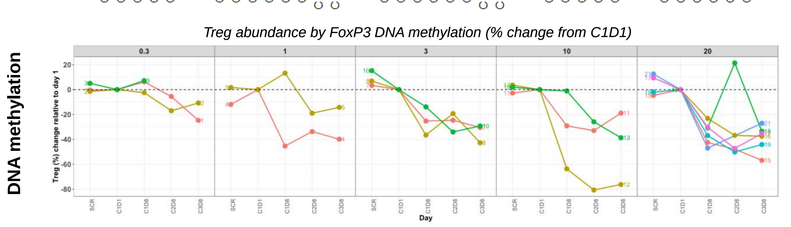
Figure 1. (A) Study design for Phase1a trial of etigilimab in advanced solid tumors. (B) Swim plot of study duration, presented at SITC 2018. * Subject noted to be MSI post data-cut for SITC 2018 presentation. ** Subject discontinued treatment due to AE (autoimmune hepatitis), and subsequently had Day 56 CT scan with stable disease.

RESULTS

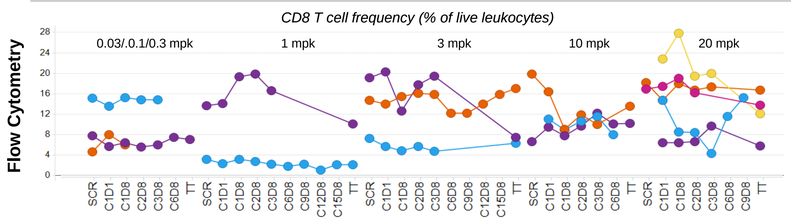
Etigilimab reduces Tregs in blood of Phase1a pts, consistent with ADCC mode of action

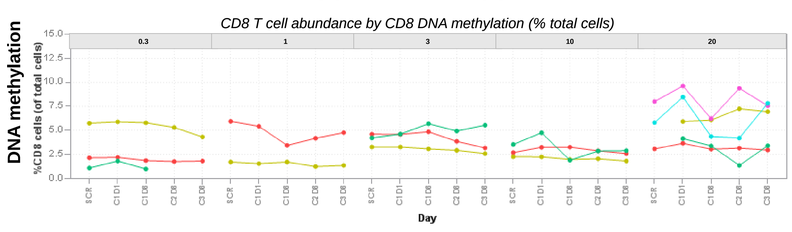
A. **Etigilimab decreases Treg frequency in peripheral blood**



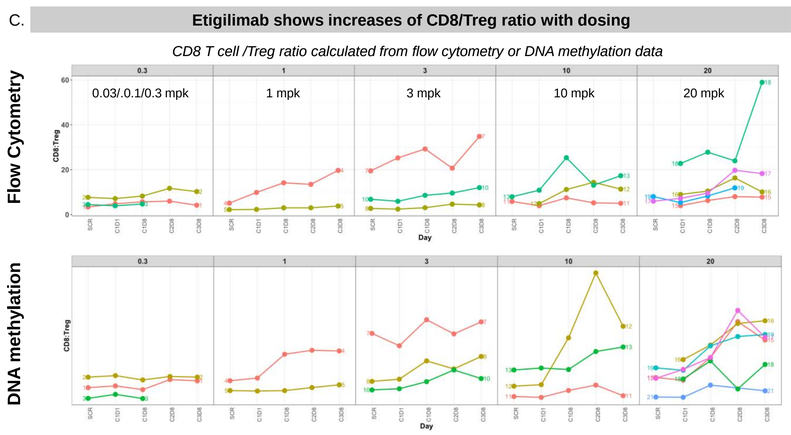


B. **No substantial changes in CD8 cells with etigilimab treatment**





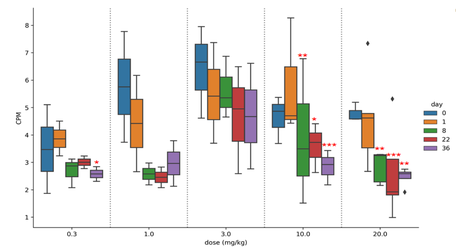
C. **Etigilimab shows increases of CD8/Treg ratio with dosing**



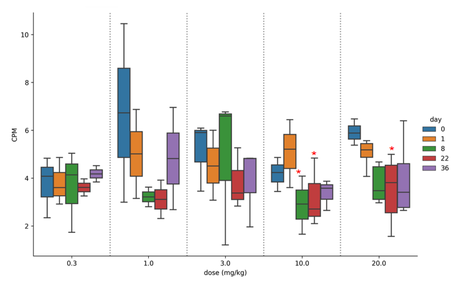
Biomarker analysis of 313M32-001 patient PBMC samples: A. Etigilimab treatment decreases Treg frequency (FoxP3+CD4+ cells) as measured by flow cytometry and DNA methylation immunophenotyping. Subjects are grouped by dose cohort. Each line represents results from an individual subject. B. No significant changes in CD8+ cell frequency were observed with etigilimab treatment by flow cytometry or DNA methylation. C. The CD8/Treg ratios calculated from flow cytometry data and DNA methylation data increase with etigilimab treatment.

Gene expression of peripheral blood suggests reduction of Tregs of Phase1a pts

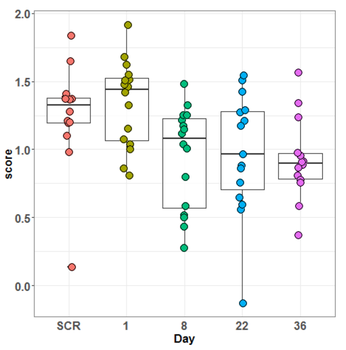
A. **Gene expression of RTKN2 (a Treg-associated gene) is significantly down-regulated by etigilimab**



B. **Gene expression of CTLA4 (an exhausted T cell and Treg-associated gene) is significantly down-regulated by etigilimab**



C. **Gene expression score of Treg-associated gene signature is significantly down-regulated by etigilimab**



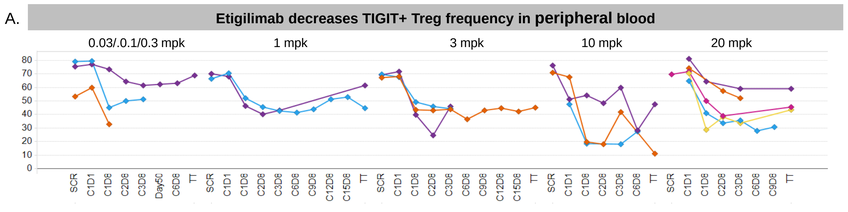
Treg gene signature
TNFRSF4
RTKN2
IL2RA
TNFRSF18
BATF
FOXP3
CTLA4

dose_timepoint	p_value
SCR vs Day1	0.1071
SCR vs Day8	<0.001
SCR vs Day22	<0.001
SCR vs Day36	<0.001

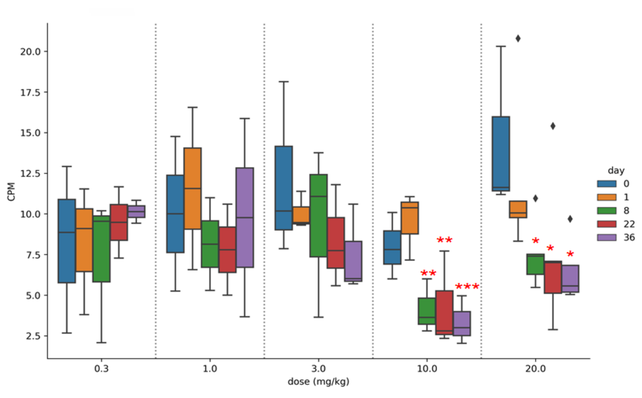
RNAseq CPM gene expression abundance shows decreases of RTKN2 (A) and CTLA4 (B). Gene expression changes calculated versus Day 1 (* p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001). C. Treg gene signature from Savas *et al* 2018 is down-regulated in post treatment blood samples. Similar results observed when normalized to CD3e gene levels (data not shown).

Etigilimab reduces the frequency of peripheral TIGIT+ cells as well as TIGIT gene expression levels, demonstrating target engagement

A. **Etigilimab decreases TIGIT+ Treg frequency in peripheral blood**



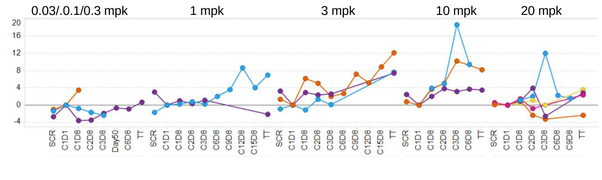
B. **TIGIT gene expression is significantly down-regulated by etigilimab at 10 mg/kg and 20 mg/kg dose groups**



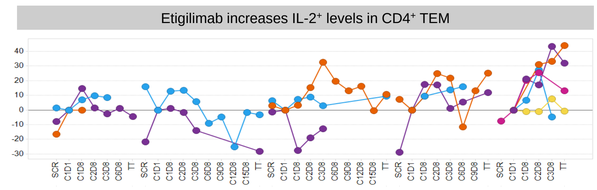
Biomarker analysis of 313M32-001 patient PBMC samples:
A. Etigilimab treatment decreases TIGIT+ cell frequency among Tregs (shown) and among CD4, CD8, and NK cells (data not shown) by flow cytomtery.
B. TIGIT gene expression (CPM counts from blood RNAseq) is down-regulated by etigilimab in 10 and 20 mg/kg dose cohorts. Gene expression changes calculated versus Day 1 (* p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001). Similar results are observed when TIGIT CPM are normalized to CD3e (data not shown).

Etigilimab enhances proliferation and intracellular IL-2 in immune subpopulations

Etigilimab increases Ki67+ of TIGIT+ CD4 cells

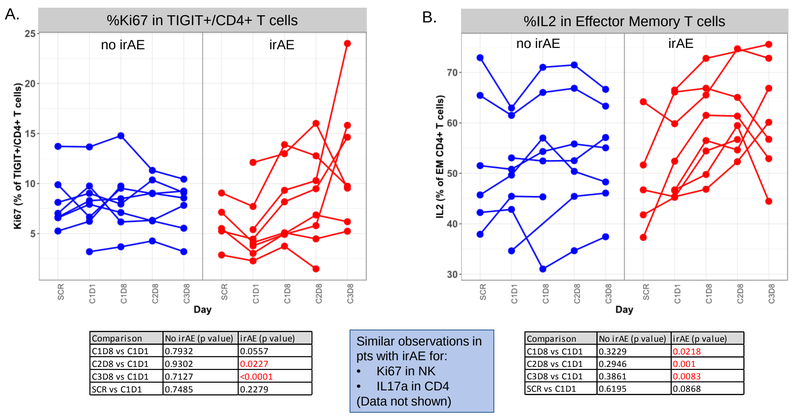


Etigilimab increases IL-2⁺ levels in CD4⁺ TEM



A. Etigilimab increases Ki67 levels in TIGIT+/CD4+ cells by flow cytometry.
B. Etigilimab increases the frequency of IL-2+ cells among T Effectory Memory (TEM) cells.

Etigilimab-enhanced proliferation and intracellular IL-2 increases are observed preferentially in patients with immune-related adverse events



Comparison	No irAE (p value)	irAE (p value)
C1D8 vs C1D1	0.7932	0.0557
C2D8 vs C1D1	0.9302	0.0227
C3D8 vs C1D1	0.7127	<0.0001
SCR vs C1D1	0.7485	0.2279

Similar observations in pts with irAE for:
- Ki67 in NK
- IL17a in CD4

(Data not shown)

Comparison	No irAE (p value)	irAE (p value)
C1D8 vs C1D1	0.3229	0.0218
C2D8 vs C1D1	0.2946	0.001
C3D8 vs C1D1	0.3861	0.0083
SCR vs C1D1	0.6195	0.0868

A. %Ki67 in TIGIT+/CD4+ T cells in subjects grouped by presence of immune related adverse events (irAEs).
B. %IL2 in effector memory T cells in subjects grouped by presence of irAEs. P values were calculated (linear mixed model) for patients in the "no irAE" and "irAE" groups for each time point relative to C1D1.

CONCLUSIONS

- Etigilimab treatment reduced Tregs in peripheral blood, more pronounced at >10mpk, with a corresponding increase in the Treg/CD8 ratio. This was observed both by flow cytometry and epigentic immune quantification.
- Etigilimab decreased Treg-related gene expression in blood, including *RTKN2* and *CTLA4*.
- Etigilimab also reduced TIGIT staining on cell surface by flow cytometry, and decreased TIGIT gene expression in blood RNA.
- Etigilimab activated immune cells as measured by increases in Ki67+TIGIT+CD4 cells and intracellular cytokines (IL17, IL2).
- Activation of immune cells correlated with immune-related adverse events, including patient rash.