CD30 is a marker of activated effector regulatory T cells in solid tumors providing clinical rationale for the combination of brentuximab vedotin and PD-1 inhibitors

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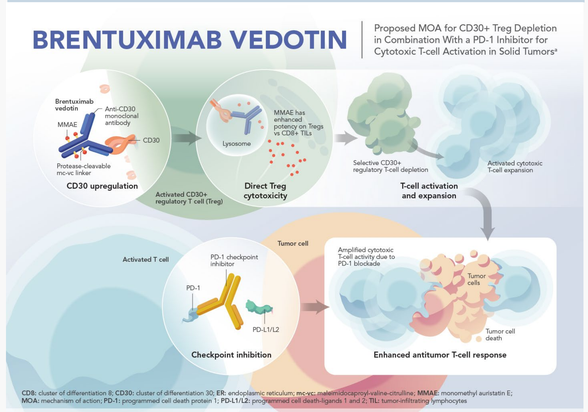
Background

- Brentuximab vedotin (BV) is comprised of a CD30-directed monoclonal antibody conjugated to the highly potent microtubule-disrupting agent monomethyl auristatin E (MMAE).
- CD30 (*TNFRSF8*), a member of the TNF receptor superfamily, is enriched on suppressive tumor-resident T regulatory (Treg) cells in multiple solid tumor types and may be a therapeutic target for tumor-selective depletion of activated Tregs (1,2,3,4).
- An emerging mechanism of resistance to anti-PD-1 therapy in solid tumors is the activation and expansion of regulatory T cells (5,6).
- Here we characterize CD30 expression by Tregs in tumor samples and provide additional rationale for the clinical combination of PD-1 inhibition and intratumoral CD30+ Treg depletion.
- An ongoing Phase 2 clinical trial (NCT04609566) is underway to investigate the potential anti-tumor effect of BV (1.8 mg/kg, Q3W) in combination with pembrolizumab (200 mg, Q3W) in metastatic melanoma and NSCLC patients who have relapsed or are refractory to αPD-1 therapy.

Proposed Mechanism of Action



References

References:
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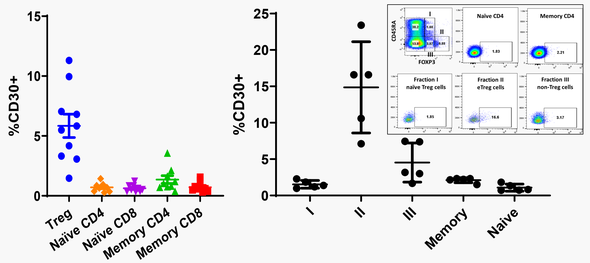
Disclosures
-All authors are employees and have equity interest in Seagen Inc. RH and BG are inventors on patent WO2022120084A1.
-This study is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

Results

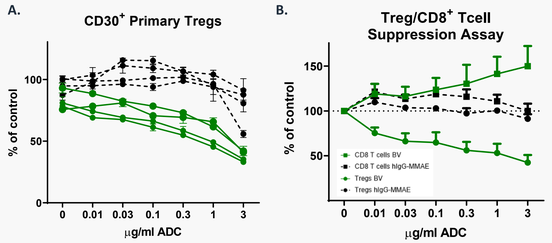
CD30 expression by peripheral blood Tregs

CD30 is found on a subset of activated Fraction II eTregs



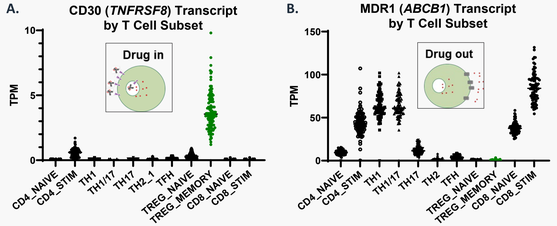
Characterization of CD30 expression in healthy PBMC. Among peripheral blood T cells, CD30 is enriched on a sub-population of CD45RA^{neg} FOXP3^{hi} (FR.II) effector Tregs (eTregs).

BV selectively kills CD30+ Tregs over CD8 T cells in vitro



BV kills Tregs in vitro. Primary Tregs were activated with CD3/CD28 beads + IL-2 in the presence of BV or a control ADC. CD30$^+$ cells were counted by flow cytometry. (A) BV drove dose-dependent killing of CD30$^+$ Tregs (n=4). (B) BV treatment of co-cultured Tregs and CD8$^+$ T cells resulted in depletion of CD30+ Tregs and concomitant expansion of CD8$^+$ T cells (n=3).

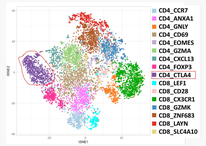
The selectivity of BV for activated effector Tregs may be due to CD30 expression and MDR1 expression differences



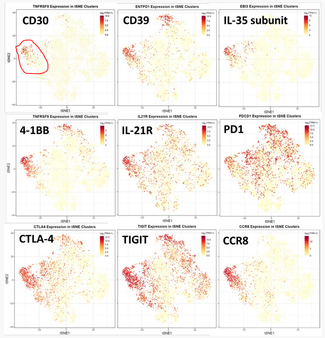
RNA expression among T cell subsets from 100 healthy PBMC donors. *(A)* TNFRSF8 is only expressed at low levels by bulk Tregs consistent with the low-frequency expression seen by flow. (B) ABCB1 is absent in Tregs, but high on cytotoxic T cells. TPM: Transcript per million. *(source:* https://dice-database.org).

CD30 expression by solid tumor Tregs

CD30 is associated with suppressive intratumoral Tregs

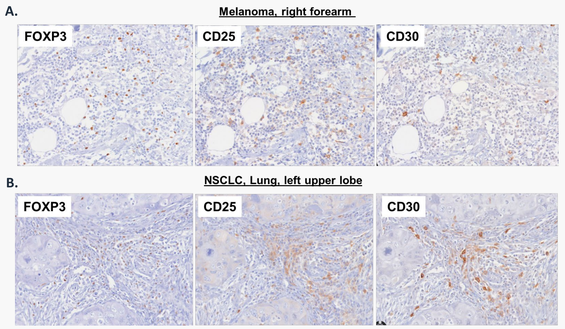


Gene	Pearson's R	p-value
TNFRSF9	0.351	<0.0001
EBI3	0.336	<0.0001
TNFRSF18	0.302	<0.0001
CCR8	0.256	<0.0001
IL21R	0.256	<0.0001
ENTPD1	0.212	<0.0001
IL2RA	0.161	<0.0001
STAT5A	0.156	<0.0001
CTLA4	0.143	<0.0001
IRF4	0.141	<0.0001
ICOS	0.132	<0.0001
TBET	0.112	0.0009
FOXP3	0.110	0.0011
GATA3	0.106	0.0016
CCR4	0.102	0.0026
PD1	0.086	0.0107
GZMB	0.069	0.0397
IL10	0.053	0.115
PDL1	0.041	0.2235
BLIMP1	0.037	0.2743
TIGIT	0.036	0.2903
BCL2	0.007	0.8407
CD38	0.000	0.9963
TGFB1	-0.006	0.852
IL7R	-0.086	0.0103
CD69	-0.130	0.0001



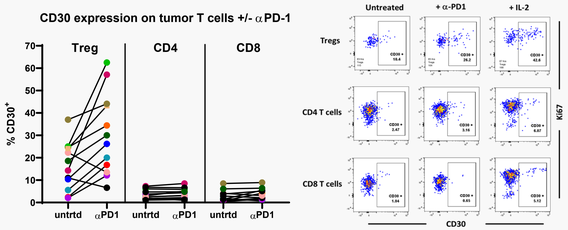
T-SNE plots of scRNA transcript expression across peripheral blood, normal tissue, and tumor T cells pooled from 14 NSCLC patients (7) (http://lung.cancer-pku.cn/). 882 individual Tregs within cluster CD4_CTLA4 were used to assess the correlation of known transcripts of Treg function with *TNFRSF8* (Table).

CD30$^+$ cells are found in T cell regions in NSCLC and melanoma tumors by IHC



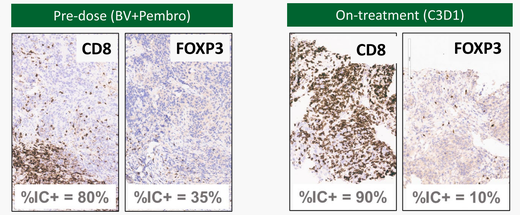
CD30 expression in tumors. Representative examples of IHC staining for FOXP3, CD25, and CD30 from serial sections of non-study melanoma (A) and NSCLC (B) samples.

Treatment of dissociated NSCLC tumor cells with anti-PD-1 or IL-2 upregulates CD30 on intratumoral Tregs



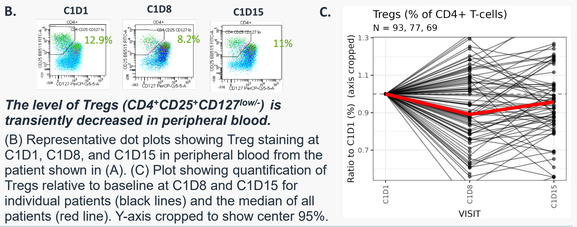
CD30 expression on tumor T cells with and without anti-PD-1 treatment. (A) CD30 expression on dissociated tumor sample T cells from NSCLC and melanoma treated with anti-PD1 for 4 days. (B) Representative CD30 vs Ki67 flow plots after treatment with αPD-1 (20ug/ml) or IL-2 (10ng/ml).

Preliminary biomarker data from NCT04609566 shows pharmacodynamic effects supportive of the proposed MOA



(A) IHC analysis of paired baseline and on-treatment tumor biopsies (cycle 3 day 1, C3D1) from a melanoma patient who progressed on prior anti-PD-1 therapy showed increased CD8 T cell infiltration and CD8 to Foxp3 ratio after treatment with BV plus pembro. IC: "Immune cells"

Partial decrease in Tregs in peripheral blood



The level of Tregs (CD4$^+$CD25$^+$CD127$^{low/-}$) is transiently decreased in peripheral blood.
(B) Representative dot plots showing Treg staining at C1D1, C1D8, and C1D15 in peripheral blood from the patient shown in (A). (C) Plot showing quantification of Tregs relative to baseline at C1D8 and C1D15 for individual patients (black lines) and the median of all patients (red line). Y-axis cropped to show center 95%.

Conclusions

- CD30 is restricted to a subset of activated effector Tregs that are enriched in solid tumors and correlates with known markers of heightened suppressive function (*TNFRSF9, ENTPD1, EBI3, IL21R*).
- Treatment with anti-PD-1 (or IL-2) in NSCLC and melanoma DTC spheroids upregulates CD30 on Tregs, but not other intratumoral T cell subsets.
- Preliminary biomarker data from NCT04609566 show PD effects consistent with the proposed mechanism of action for the combination.
- Together, these data support the ongoing Phase 2 clinical trial (NCT04609566) evaluating the combination of brentuximab vedotin + pembrolizumab in metastatic solid malignancies after progression on prior anti-PD-1 therapy.

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