Brentuximab Vedotin, a CD30-Directed Antibody-Drug Conjugate, Selectively Depletes Activated Tregs In Vitro and In Vivo

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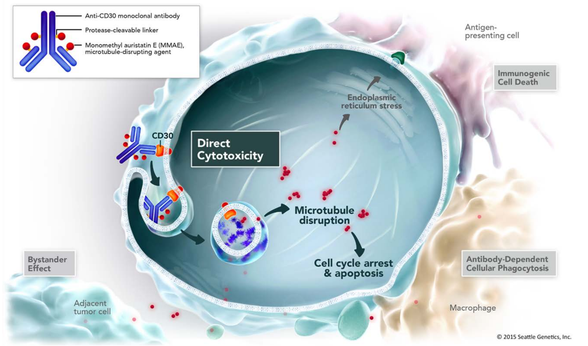
Background

- Brentuximab vedotin (BV) is comprised of a CD30 directed monoclonal antibody conjugated to the highly potent microtubule-disrupting agent monomethyl auristatin E (MMAE).
- Brentuximab vedotin (BV) is approved for classical Hodgkin lymphoma (cHL) across multiple lines of therapy including frontline use in stage III/IV cHL in combination with chemotherapy. BV is also approved for certain CD30 expressing T-cell lymphomas.
- CD30 (TNFRSF8) is a member of the tumor necrosis factor superfamily of immune receptors with diverse roles in regulation of lymphocyte proliferation and apoptosis.
- CD30 expression is enriched on suppressive tumor-resident T regulatory (Treg) cells (1,2), IRF4-expressing effector Tregs (3), and tumor-resident TH2-like effector Tregs (4).

Proposed Mechanism of Action



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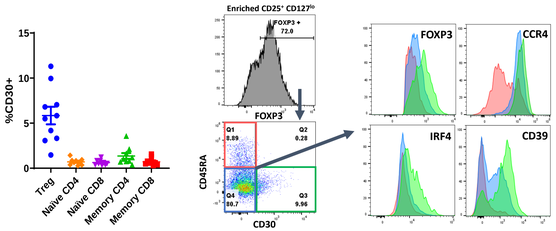
Disclosures

All authors are employees and have equity interest in Seagen, Inc. RH is an inventor on patent W/O 2019/075188 A1.

Results

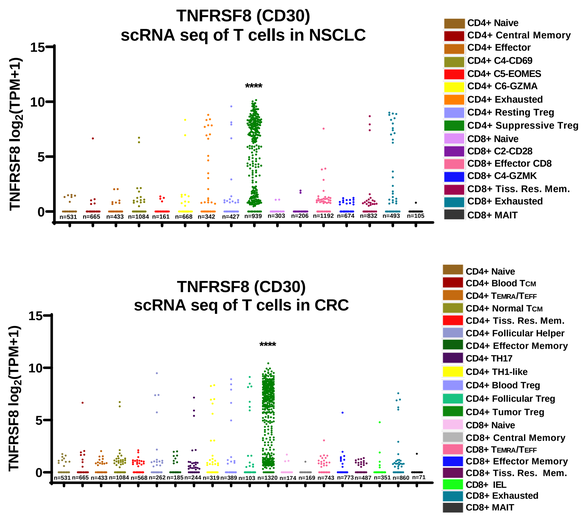
CD30 Expression on T cells

CD30 is expressed on a subset Tregs with an activated effector phenotype



Among healthy donor peripheral blood T cells, CD30 is restricted to a sub-population of CD45RA^{neg} "effector" Tregs. Among sorted Tregs, CD30 is found co-expressed on the activated (FOXP3^{++}, IRF4^{+}, CD39^{HI}) effector CD45RA^{neg}, CCR4^{HI} subset.

CD30 expression is restricted to suppressive intratumoral T regs

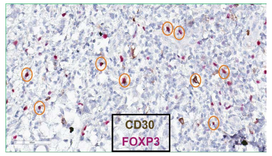


Box plots showing the $\log_2$(TPM + 1) expression of TNFRSF8 transcript across peripheral blood, normal tissue, and tumor T cell subsets identified by unsupervised clustering analysis of single-cell RNA-seq datasets from NSCLC and CRC (4,5).

Each dot represents individual T cells pooled from 14 (NSCLC) and 12 (CRC) donors. Number of individual cells in clusters is listed.

Statistical analysis was performed using a one-way ANOVA with multiple comparisons test. P-value **** <0.0001

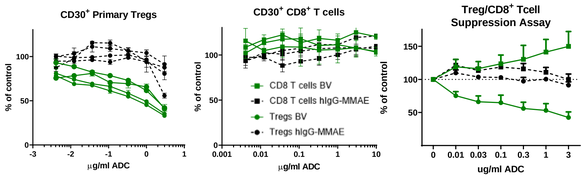
Tumor IHC shows CD30/FOXP3 co-localization



Example of dual IHC staining of a heavily infiltrated pancreatic tumor showing co-localization of CD30 with a subset of FOXP3^{+} cells.

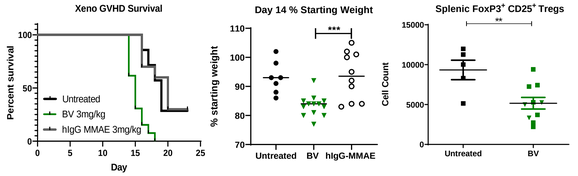
BV Selectively Kills Tregs in vitro and in vivo

BV kills activated Tregs, but not activated CD8 T cells in vitro



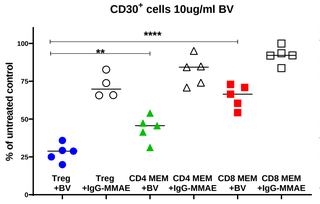
Primary Tregs were activated with CD3/CD28 beads and IL-2 for 4-5 days in the presence of BV or control ADC and CD30^{+} cells were counted by flow cytometry. BV showed dose-dependent cytotoxicity on CD30^{+} Tregs but not CD30^{+} CD8^{+} T cells (n=4). Treatment of co-cultured inducible Tregs and CD8^{+} T cells with BV in an in vitro suppression assay results in depletion of Tregs with a concomitant expansion of CD8^{+} T cells. (n=3)

BV accelerates xeno-GVHD by depleting human Tregs in vivo



Human PBMCs were adoptively transferred into irradiated NSG mice and given a single dose of BV or control ADC (3mg/kg, day 5). BV significantly accelerated mortality and weight loss. Spleens harvested from treated mice showed significant depletion of FOXP3^{+} CD25^{+} Tregs. Statistical analysis was performed using a two-tailed t-test. P-value *** <0.001, ** <0.01

BV has increased potency on Tregs compared to memory T cells



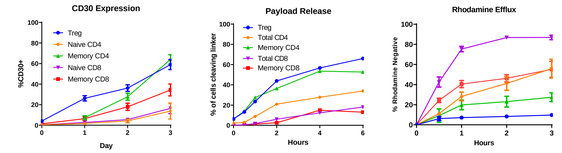
CD30 has been linked to activation of memory T cells. To evaluate the activity of BV on Tregs relative to memory T cell subsets, sorted populations were activated with CD3/CD28 and IL-2 and treated with a clinically relevant concentration of BV (6).

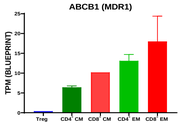
BV shows increased potency on Tregs relative to memory CD4^{+} and CD8^{+} T cells. Naïve cells were insensitive to BV (not shown).

Statistical analysis was performed using a two-tailed t-test. P-value **** <0.001, ** <0.01

T cell Subset Characteristics Drive Selectivity

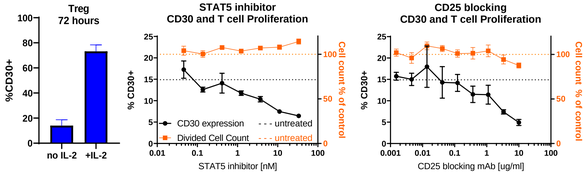
CD30 expression and reduced efflux drive BV potency on Tregs





Stimulation of cell subsets in vitro (CD3/CD28 and IL-2) leads to rapid upregulation of CD30 on Tregs and memory CD4^{+} T cells (n=5). Tregs and memory CD4^{+} T cells rapidly internalized and cleaved a conditionally-fluorescent CD30 ADC, consistent with efficient drug delivery. Tregs failed to efflux rhodamine relative to other T cell subsets and lack the major efflux pump transcript, MDR1 (n=4). Together, increased CD30 and the inability to efflux for Tregs may result in greater intracellular drug accumulation and cell death.

CD30 expression in vitro is tied to CD25-mediated STAT5 signaling, a key driver of Treg identity and function



CD30 expression on Tregs in vitro is driven by activation and IL-2 driven STAT5 signaling. T cells were stimulated with CD3/CD28 +/- IL-2, and CD30 expression was measured by flow. Inhibition of STAT5 or IL-2RA (CD25) directly impairs CD30 expression.

Conclusions

- CD30 expression on T cells is restricted to a subset of activated effector Tregs that are enriched in solid tumors.
- In in vitro and in vivo models of human T cell activation, brentuximab vedotin (BV) shows selective depletion of activated Tregs over cytotoxic CD8^{+} T cells.
- The preferential potency of BV on Tregs likely results from enhanced CD30 expression and decreased efflux pump activity resulting in greater intracellular drug accumulation.
- Preclinical data supports the therapeutic potential of BV as a targeted approach to selectively deplete highly activated Tregs in solid tumors.

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