SGN-35T: Activated regulatory T cells in solid tumors express CD30, which are selectively targeted by the novel anti-CD30 antibody drug conjugate SGN-35T

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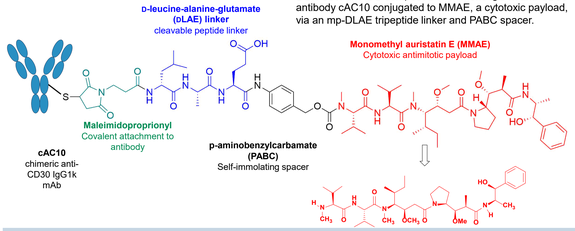
Background

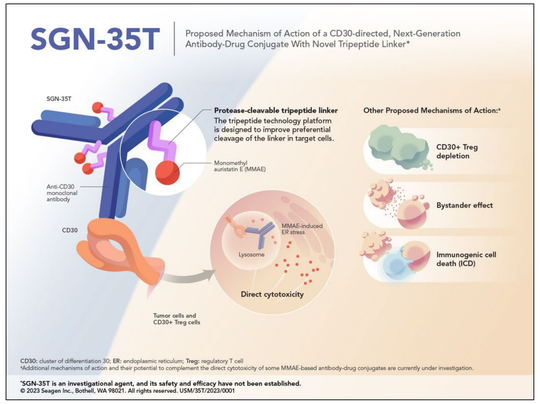
- While antibody-drug conjugates (ADCs) are effective anti-cancer agents in a wide variety of solid and hematologic cancers, side effects such as dose-limiting neutropenia may be observed[1].
- SGN-35T is a CD30-directed ADC with the same mAb backbone and cytotoxic monomethyl auristatin E (MMAE) payload as BV but with a novel cleavable tripeptide DLAE linker designed to improve the therapeutic window.
- CD30 (TNFRSF8) is a TNF receptor superfamily member that is expressed by intra-tumoral Tregs with an activated profile.
- Treg proliferation is associated with CD30 expression, resistance to immune checkpoint inhibition, and tumor growth.
- Potential of targeting CD30+ Tregs for solid tumor treatment has been demonstrated by a Phase 2 Trial of Brentuximab Vedotin (BV) With Pembrolizumab (Pembro) in Patients With Metastatic Non-Small Cell Lung Cancer or Metastatic Cutaneous Melanoma After Progression on Anti-PD-1 Therapy (Abstract # 699).

SGN-35T Structure



SGN-35T: an ADC composed of chimeric IgG1k anti-CD30 antibody cAC10 conjugated to MMAE, a cytotoxic payload, via an mp-DLAE tripeptide linker and PABC spacer.

Proposed Mechanism Of Action



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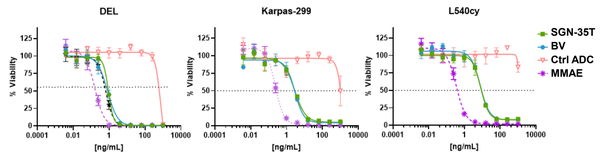
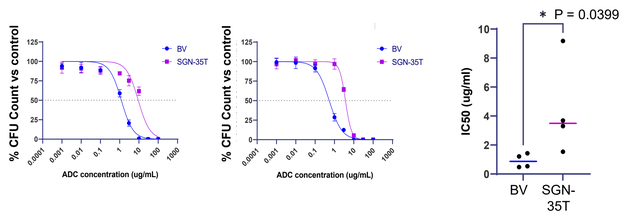
Results

SGN-35T Displays Anti-lymphoma Activity Similar To BV



CD30+ Lymphoma cell lines were sensitive to SGN-35T in vitro. In vitro cytotoxicity was evaluated in CD30-expressing ALCL and HL cell lines after SGN-35T treatment, shown as mean percent viability versus untreated cells. The horizontal dotted line indicates 50% viability versus untreated. Error bars represent SD. CD30 expression on the cell surface was quantified by QFACS: DEL=285,000, Karpas-299=318,900, and L540cy=408,500. Ctrl ADC = Control Ab-DLAE-MMAE.

SGN-35T Demonstrates Improved Bone Marrow Tolerability In Vitro

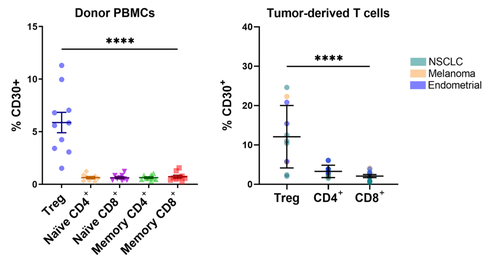


The cytotoxic effects of SGN-35T on human bone marrow progenitor cells were assessed using human granulocyte-monocyte colony-forming units (CFU-GM). Left, representative donor normalized dose responses (N=4 donors total). Right, IC_{50} of BV and SGN-35T. Each dot represents the IC_{50} value calculated from one donor. Mean IC_{50} values were compared using an unpaired Student's t-test. *P<0.05.

Comparison Of SGN-35T Vs BV Characteristics

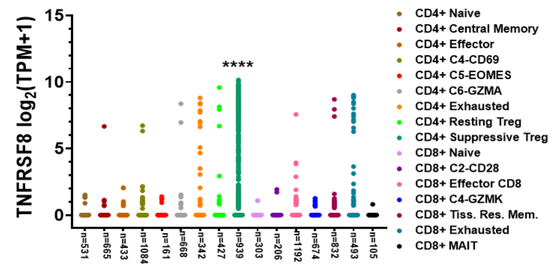
SGN-35T similarities with BV	SGN-35T differences with BV
Linker differences do not change target-mediated lymphoma cytotoxicity.	SGN-35T is tolerated better than BV in non-human primates.
Similar drug release, bystander effect, and induction of immunogenic cell death markers preclinically.	Tripeptide linker reduces hydrophobicity and reduces bone marrow toxicity preclinically.
Same clinically-validated MMAE payload.	No payload difference.

Preferential Expression Of CD30 By Treg Vs Other T Cells



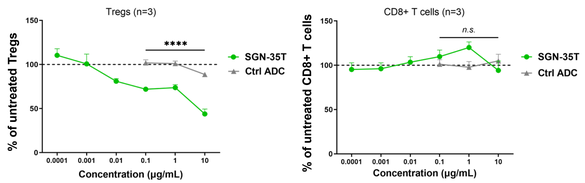
A) PBMC samples from healthy donors (N=9-10 per group) were stained for T cell lineage markers and frequency of CD30 expression. Tregs displayed the highest CD30 frequency compared to other T cell subpopulations (P<0.0001, one-way ANOVA). Each dot represents the fraction of T cells from one donor. B) T cells pooled from patient tumors including NSCLC (N=6), endometrial cancer (N=3) and melanoma (N=2) were evaluated for CD30-positivity. A significantly greater percentage of tumor-associated Tregs was CD30+ versus CD4+ and CD8+ T cells (P<0.0001 by one-way ANOVA). Bars represent mean and standard deviation. ****P<0.0001.

CD30 mRNA Is Highly Expressed By Intratumoral Treg Vs Other T Cell Subsets Within The TME



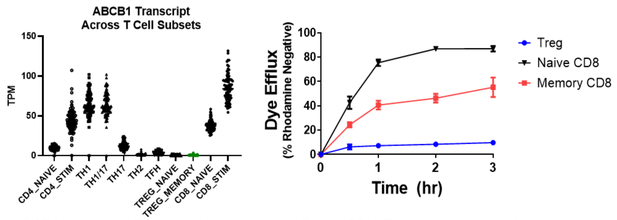
CD30 (TNFRSF8) transcript expression in peripheral and tumor T cell subsets from publicly available scRNAseq data of human NSCLC patient T cells (peripheral blood, normal tissue, and tumor T cell subsets)[2]. Each point represents an individual T cell from N=14 NSCLC donors. The number of individual cells in each cell type cluster is displayed. One-way ANOVA was performed followed by Tukey's post-hoc multiple comparisons test. Error bars represent mean and SD. Asterisks indicate significance from Tukey's post-hoc test, comparing each group to the intratumoral Treg group. ****P<0.0001.

SGN-35T Selectively Depletes Tregs Vs. CD8+ T Cells



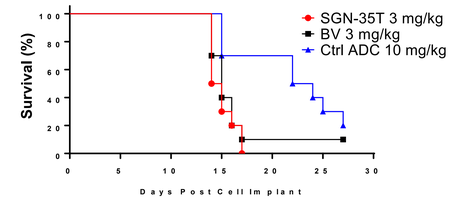
The percentage of CD30+ Tregs (left panel) and CD30+ CD8+ T cells (right panel) after treatment with increasing doses of SGN-35T or non-binding control ADC, relative to untreated cells. Tregs were significantly reduced by SGN-35T at matching doses versus non-binding control ADC (P<0.0001, two-way ANOVA with treatment as the source of variance), whereas no significant difference was observed for CD8+ T cells (P=0.2892, two-way ANOVA). Error bars represent the standard deviation. ****P<0.0001; n.s., not significant. Ctrl ADC = Control Ab-DLAE-MMAE.

Tregs Express Lower Levels Of ABCB1 mRNA And Demonstrate Less Drug Pump Efflux Compared To CD8+ T Cells



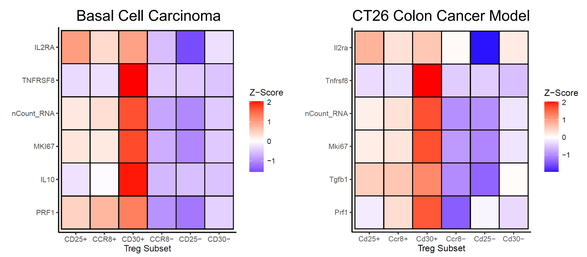
ABCB1 is absent in Tregs, but high on cytotoxic T cells. TPM: Transcript per million.(source: https://dice-database.org). The efflux potential of Tregs and CD8+ T cells was compared by quantifying signal retention of cells treated with a cell-permeable dye over time. This assay demonstrated that Tregs retain significantly more dye than naïve ($p < 0.0001$ at 3 hrs) or memory CD8+ T cells ($P < 0.0014$ at 3 hrs) (n=4).

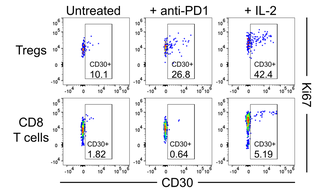
SGN-35T Inhibits Treg Function Similarly to BV In A GVHD Model



Previous studies demonstrated that a CD30+, proliferating subpopulation of Treg mediate protection in GVHD models and that decreased survival is correlated with depletion of Treg and enhanced activity of CD8+ T cells[3]. A xenogenic GVHD model was treated with BV, SGN-35T, or control ADC to test whether targeting of CD30+Tregs by SGN-35T enhanced inflammation induced mortality. Compared with the control ADC, BV and SGN-35T induced similar accelerated mortality. Ctrl ADC = Control Ab-DLAE-MMAE.

CD30+ Tregs Are Proliferative And Transcriptionally Activated Compared To Other Intratumoral Tregs





(Top panel) Relative gene expression comparison of activated, intratumoral Treg subsets from basal cell carcinoma (left[4]) or CT26 model (right[5]) via a re-analysis of public scRNA-seq data. Compared with other subsets, CD30+ (TNFRSF8+) Tregs express high levels of the proliferation marker Mki67, mRNA transcripts, and suppression effector genes. (Bottom panel) Treatment of dissociated NSCLC tumor cells with anti-PD-1 or IL-2 preferentially induces a CD30+,Ki67+ phenotype in intratumoral Tregs vs CD8+ T cells.

Summary

- **In preclinical models, SGN-35T demonstrates similar efficacy with the potential for reduced toxicity compared with BV.**
- **SGN-35T selectively depletes Tregs vs other T cell subsets.**
- **CD30+ intratumoral Treg represent a highly activated, proliferating, and suppressive population.**
- **In comparison to other T cell subsets, Tregs express high levels of CD30 but lower efflux capacity.**
- **SGN-35T, similar to BV, targeted Tregs in a model of GVHD resulting in accelerated T cell mediated mortality.**
- **Overall, these observations support the hypothesis that SGN-35T can selectively target a potent population of intratumoral Tregs to bolster anti-tumor immunity similar to BV but with a potential for reduced toxicity.**

References

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