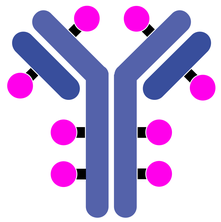
SGN-35C: a Novel Topoisomerase 1-inhibitor Antibody-Drug Conjugate for the Treatment of Lymphomas

Kevin J. Hamblett, Julia Cochran, Katie Snead, Steven Jin, Roma Yumul, Jessica K. Simmons, Ivan Stone, Ryan Lyski, Jason P. Schrum, Andrea R. Lim, Astrid Clarke
Seagen Inc., Bothell, WA

Background

- **CD30** (TNFRSF8), a member of the TNF receptor superfamily, is a validated clinical target based on the efficacy of brentuximab vedotin (BV), a CD30-directed antibody drug conjugate, which has been approved for used in multiple CD30-expressing lymphomas.
- **Antibody-drug conjugates (ADCs)** employing the vedotin drug linker are effective anti-cancer agents in a wide variety of solid and hematologic cancers. While vedotin ADCs are efficacious, side effects such as dose-limiting neutropenia and other toxicities may be observed[1].
- Camptothecins are a class of topoisomerase 1 (TOP1) inhibitors that bind to single-stranded DNA and interfere with replication, leading to cell cycle arrest and death[1]. Other camptothecin derivatives including irinotecan, topotecan, and exatecan demonstrate an acceptable safety profile in approved therapies for multiple indications of cancer[2-4]. ADCs utilizing camptothecins are anticipated to have distinct tolerability profile compared to vedotin ADCs.
- **SGN-35C** is a CD30-directed ADC with the same mAb backbone as brentuximab vedotin (BV), conjugated to a TOP1 inhibitor (TOP1i) camptothecin-derivative through a cleavable linker to the eight cysteines that comprise the interchain disulfide bonds.

Proposed Mechanism of Action



- SGN-35C utilizes the same CD30-directed monoclonal antibody backbone as BV and a camptothecin-derivative as the cytotoxic payload.
- SGN-35C binds to CD30 on cancer cells, internalizes, and the TOP1i is released from the antibody in the endo-lysosomal pathway. TOP1i elicits activity via the selective inhibition of TOP1 which leads to cell cycle arrest and apoptosis via the accumulation of DNA breaks.

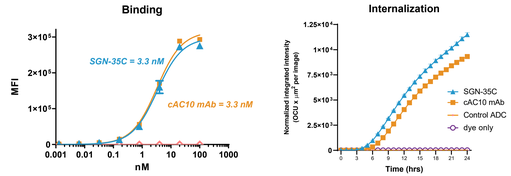
References

1. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer. 2006a;6(10):789-802.
2. Keam SJ. Trastuzumab Deruxtecan: First Approval. Drugs. 2020;80(5):501-08.
3. Li F, Jiang T, Li Q, Ling X. Camptothecin (CPT) and its derivatives are known to target topoisomerase I (TOP1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer? Am J Cancer Res. 2017;7(12):2350-94.
4. Modi S, Jacot W, Yamashita T, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med. 2022;387(1):9-20.

Results

In vitro Characterization of SGN-35C

SGN-35C Binds CD30⁺ Cells and Internalizes Rapidly



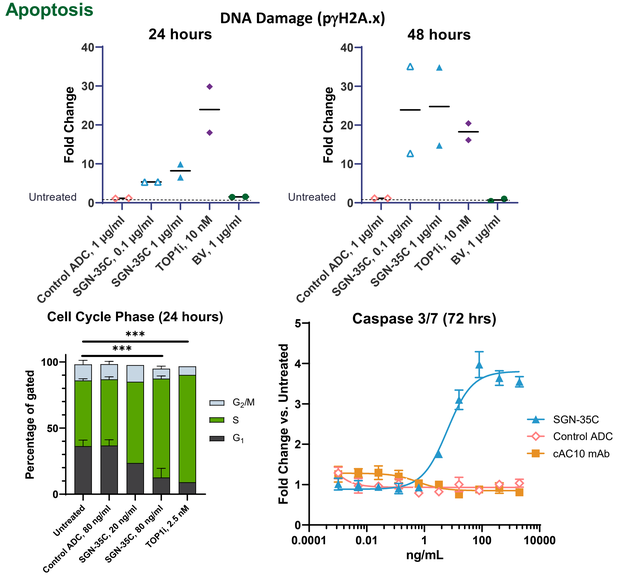
(**Left**) Saturation binding of SGN-35C, parental mAb cAC10, and a non-binding control antibody conjugated to TOP1i (Control ADC) to CD30-expressing Karpas-299 lymphoma cells, measured by flow cytometry (N=2). Error bars represent SEM. (**Right**) Fluorescent intensity reflecting internalization of each antibody complexed with a pH-sensitive reporter (N=3). Data were normalized to the percent confluency per well. Error bars represent SD.

Following Internalization TOP1i is Released From SGN-35C

Test article	fmol/1x10⁶ cells
SGN-35C	22 ± 1
Control ADC	0

Released TOP1i was quantified in Karpas-299 cells incubated with 100 ng/mL of SGN-35C or non-binding control antibody conjugated to TOP1i (Control ADC) for 24 hours by tandem mass spectrometry. Intracellular TOP1i was calculated as a percentage of total free drug measured in the cell pellet plus supernatant (N=2). The concentration of TOP1i per million cells is reported (mean ± SD).

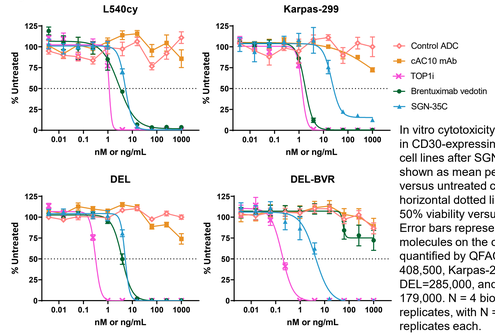
SGN-35C Induces DNA damage, S-phase Cell Cycle Arrest, and Apoptosis



(**Top**) DNA damage in Karpas-299 cells treated with test articles was quantified by assessing the mean fluorescence intensity (MFI) from DNA damage marker γH2A.X at 24 (**top left**) and 48 hours (**top right**) post-treatment by flow cytometry in live cells (N=2). MFI values were normalized to the untreated group (dotted line) and reported as fold change. **(Bottom, left)** The percentage of live Karpas-299 cells in each phase of the cell cycle after 24 hours of treatment (N=2). The results of a two-way ANOVA comparing frequency of cells in G1 and S phase amongst treatment groups are shown. Error bars denote SD. **(Bottom, right)** Caspase 3/7 activity was measured upon treating Karpas-299 cells with a 10-point, 5-fold dilution series for 72 hours. Caspase 3/7 activity in treated groups was normalized to the untreated control. Error bars represent SEM. Non-binding control antibody conjugated to TOP1i (Control ADC)

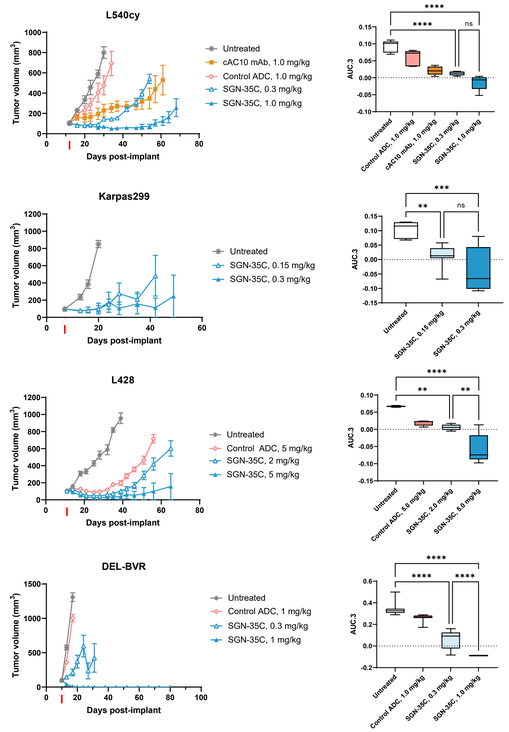
SGN-35C Induces Direct Cytotoxicity In Vitro and In Vivo

Lymphoma Cell Lines are Sensitive to SGN-35C In Vitro



In vitro cytotoxicity was evaluated in CD30-expressing ALCL and HL cell lines after SGN-35C treatment, shown as mean percent viability versus untreated cells. The horizontal dotted line indicates 50% viability versus untreated. Error bars represent SEM. CD30 molecules on the cell surface were quantified by QFACS: L540cy = 408,500, Karpas-299 = 318,900, DEL=285,000, and DEL-BVR = 179,000. N = 4 biological replicates, with N = 4 technical replicates each.

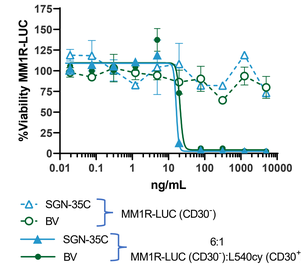
SGN-35C Demonstrates Efficacy in Xenograft Models

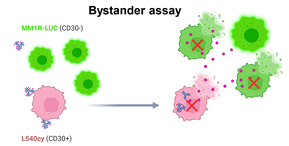


Human xenografts were subcutaneously implanted into female SCID mice. After tumors were established (N=5-12 mice/group), mice were treated once by i.p. (red arrow). Tumor growth curves (left panels) show mean tumor volumes for each group. Non-binding control antibody conjugated to TOP1i (Control ADC). Error bars represent SEM. Right, normalized area under the curve of tumor volume was measured for each animal (AUC.3), AUC.3 of each treatment group was compared by one-way ANOVA followed by Tukey's post-hoc test. The box-and-whisker plots represent the median and range for each treatment group. Asterisks indicate significance of selected comparisons from Tukey's post-hoc test. ****P<0.0001; ***P<0.001; **P<0.01; n.s., not significant.

SGN-35C Displays Bystander Activity In Vitro and In Vivo

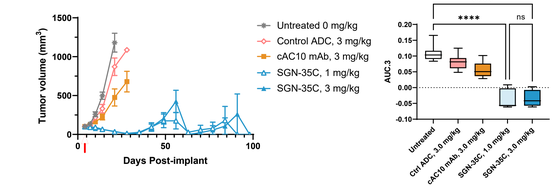
Bystander activity in a CD30-heterogenous co-culture model





CD30-negative MM1R multiple myeloma cells were engineered to express luciferase (MM1R-LUC) and co-cultured at a 6:1 ratio with CD30-expressing L540cy cells. Co-cultures were dosed once with SGN-35C or brentuximab vedotin (BV) for 96 hours, then measured for viability as a percent of untreated cells N=2 technical replicates. Error bars represent SD.

Bystander Activity in a Mixed Model of CD30 Expression In Vivo



(**Left**) Karpas-299 ALCL cells were admixed at a 1:1 ratio with a daughter line selected for brentuximab vedotin resistance, Karpas-BVR. 5 × 10⁶ total cells were subcutaneously implanted into female SCID mice (N = 10 mice per group). After tumors were established at 100 mm³, mice received one dose of test article by i.p. injection (red arrow). Tumor volume growth curves were plotted (left). Error bars indicate SEM. (**Right**) normalized area under the curve of tumor volume was measured for each animal (AUC.3) and compared across treatment groups by one way ANOVA and Tukey's post-hoc pairwise comparisons test (P<0.0001). The dashed line indicates an AUC of 0. Asterisks reflect select comparisons from Tukey's post-hoc test. Bars indicate median and quartiles. ****P<0.0001; n.s., not significant.

SGN-35C is Well Tolerated in Non-Human Primates

- 10 mg/kg Q3Wx4 highest non-severely toxic dose (HNSTD) in non-human primates
- Dose-limiting toxicity: reversible intestinal mucosal degeneration
- No hematologic toxicities at HNSTD
- No pulmonary toxicity observed in NHP

Summary

- SGN-35C displays efficient binding, internalization, and cytotoxicity in CD30⁺ tumor cells.
- SGN-35C is active in a wide variety of cell lines in vitro and in vivo, including the BV-resistant line DEL-BVR in which underscores the unique MOA of SGN-35C.
- SGN-35C induces direct cytotoxicity as well as bystander killing of heterogenous tumor cell populations in vitro and in vivo.
- SGN-35C demonstrates a tolerability profile in non-human primates that is consistent with camptothecins.
- A Phase 1, first-in-human study is planned to evaluate the safety and antitumor activity of SGN-35C in lymphoid malignancies.

Poster No. 1440
ASH; December 9–12, 2023

Seagen