SGN-B6A Induces Immunogenic Cell Death as an Additional Mechanism of Action

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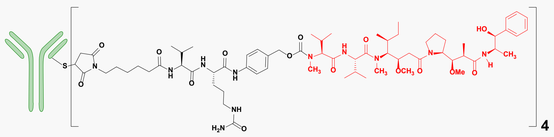
Vedotin Antibody-Drug Conjugate SGN-B6A

- SGN-B6A is an investigational vedotin antibody-drug conjugate (ADC) directed to integrin beta-6 that is currently being evaluated in a phase I study (NCT04389632)
- SGN-B6A is comprised of the humanized antibody h2A2, highly specific for integrin beta-6 over other beta integrins, paired with the vedotin ADC technology that delivers the potent cytotoxin MMAE



h2A2
humanized anti-integrin beta-6 antibody

monomethyl auristatin E (MMAE)
cytotoxic antimitotic payload

Figure 1. Composition of SGN-B6A.

- Other vedotin ADCs delivering the clinically validated MMAE payload (including those ADCs based on the antibodies brentuximab, enfortumab, tisotumab, and ladirituzumab) have been shown to induce immunogenic cell death (ICD) in preclinical models [1-5] and have demonstrated promising clinical activity in combination with immunotherapy [6-8]
- Here, we present data to support immunogenic cell death as an additional mechanism of action for SGN-B6A

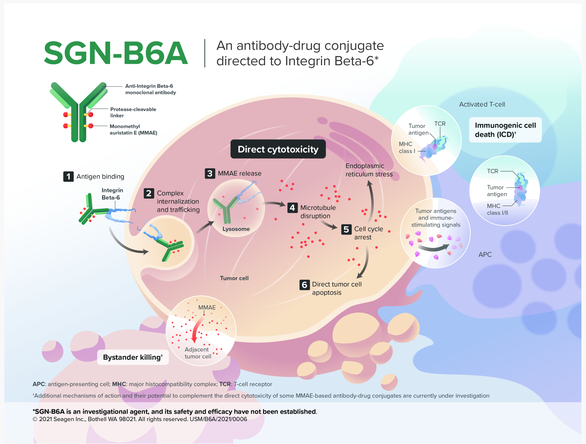
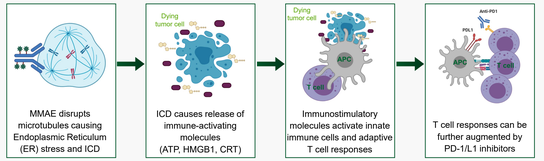


Figure 2. Proposed mechanisms of action of SGN-B6A.

Vedotin ADC-induced Immunogenic Cell Death

ADCs linked to MMAE induce cell killing in a manner consistent with immunogenic cell death (ICD), and may enhance antitumor immunity



SGN-B6A Displays Anti-tumor Activity in BxPC3 and HPAFII Xenograft Models

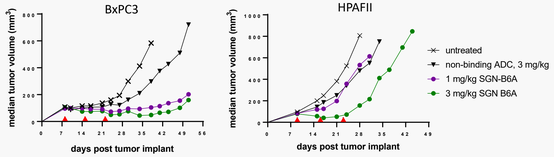


Figure 3. SGN-B6A demonstrates anti-tumor activity in xenograft models used for ICD studies. Nude mice bearing BxPC3 or HPAFII xenografts were dosed with 1 mg/kg or 3 mg/kg of SGN-B6A or non-binding ADC weekly for three doses (indicated by ▲). Points are median values of 8 mice per group. SGN-B6A induced transient tumor volume regression in both models at 3 mg/kg.

SGN-B6A Induces Apoptosis and ICD Markers In Vitro

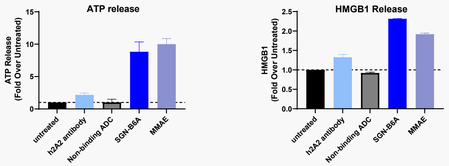


Figure 4. SGN-B6A induces secretion of ATP and HMGB1. SGN-B6A, h2A2 antibody, and non-binding ADC were incubated with BxPC3 cells for 48 hours (Ab and ADCs at 1 µg/mL, MMAE at 10 nM). SGN-B6A and free MMAE demonstrated increased release of ATP and HMGB1 compared to cells treated with naked Ab or non-binding ADC.

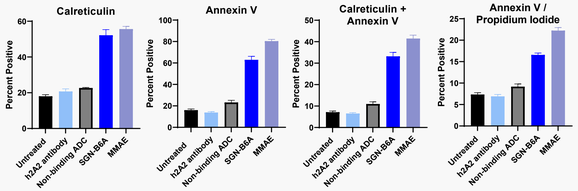
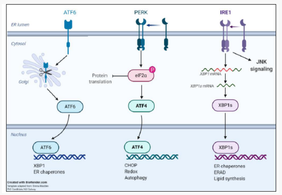


Figure 5. SGN-B6A induces apoptosis and cell surface translocation of calreticulin, assessed by flow cytometry. SGN-B6A, h2A2 antibody, and non-binding ADC were incubated with BxPC3 cells for 40 hours (Ab and ADCs at 1 µg/mL, MMAE at 10 nM). SGN B6A and free MMAE demonstrated increased staining via flow cytometry for the early ICD marker calreticulin. Cells treated with SGN-B6A and MMAE also showed increased apoptosis via Annexin V and Propidium Iodide staining.

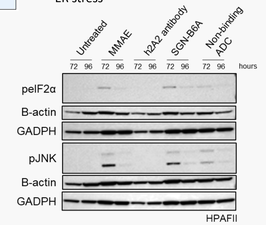
SGN-B6A Induces ER Stress In Vitro

The Endoplasmic Reticulum (ER) is a key organelle that has evolved complex signaling cascades to help maintain its homeostasis when undergoing stress. This process is known as the unfolded protein response and is regulated by three sensors: ATF, PERK, IRE1α



- In ICD, all three branches are activated with PERK as a mandatory pathway
- Downstream signaling of PERK leads to a proapoptotic program, largely controlled by CHOP
- Alternatively, IREα can independently contribute to apoptosis via activation of JNK
- Phosphorylation of JNK (pJNK) and eIF2a (peIF2a) are two downstream markers of ER stress

Figure 6. SGN-B6A induces ER stress signaling. SGN-B6A and MMAE elicit ER stress pathways through multiple branches of signaling. Integrin beta-6-expressing HPAFII tumor cells were incubated with 2nM MMAE free drug or 2µg/mL SGN-B6A, naked mAb, or non-binding MMAE ADC for 72 and 96 hours. Phosphorylated JNK and eIF2α were detected in SGN-B6A treated cells and not in cells treated with naked mAb.



SGN-B6A Induces ICD-Related Genes In Vivo

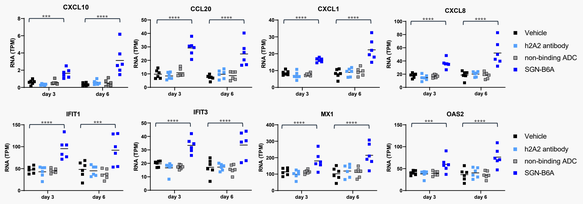
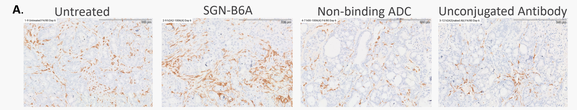


Figure 7. SGN-B6A induces upregulation of human chemokine and type I IFN response genes in HPAFII xenograft tumor cells. HPAFII tumors in nude mice were treated with a single dose of 3 mg/kg SGN-B6A, non-binding ADC, or h2A2 antibody. Tumors were harvested at days 3 and 6 post-treatment and processed for RNAseq. Transcripts encoding human chemokines as well as Type I interferon (IFN) response genes were upregulated in tumor cells following treatment with SGN-B6A. Statistical analysis was performed using a one-way ANOVA with Sidak's multiple comparison test. P-values shown for SGN-B6A vs vehicle control: **** <0.0001, ***<0.001

SGN-B6A Recruits Effector Cells In Vivo



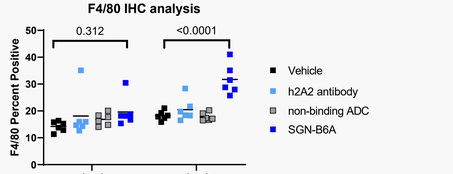
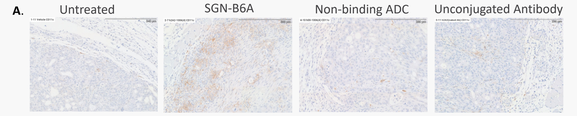


Figure 8. SGN-B6A recruits F4/80-expressing mouse macrophages to HPAFII xenograft tumors HPAFII tumors in nude mice were treated with a single dose of 3 mg/kg SGN-B6A, non-binding ADC, and naked mAb. Tumors were harvested at days 3 and 6 post-treatment and processed for IHC. Staining of tumors demonstrated an increase in F4/80+ macrophages at the tumor site 6 days after treatment with SGN-B6A (**A**). F4/80 percent positive cells (brown) were quantified using Halo image analysis and show a significant (one-way ANOVA) increase of F4/80+macrophages at day 6 post-dose (**B**).



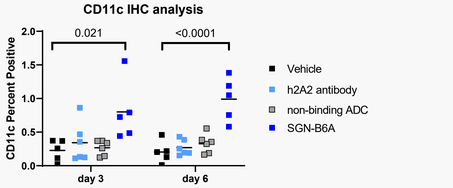


Figure 9. SGN-B6A recruits mouse CD11-expressing APCs to HPAFII xenograft tumors. HPAFII tumors in nude mice were treated with a single dose of 3 mg/kg SGN-B6A, non-binding ADC, and naked mAb. Tumors were harvested at days 3 and 6 post-treatment and processed for IHC. Staining of tumors demonstrated an increase in CD11+ antigen presenting cells (APCs) at the tumor site 6 days after treatment with SGN-B6A (**A**). CD11 percent positive cells (brown) were quantified using Halo image analysis and show a significant (one-way ANOVA) increase of CD11+ APCs at day 6 post-dose (**B**).

Conclusions

- **SGN-B6A exhibits hallmarks of inducing immunogenic cell death in both *in vitro* and *in vivo* assays**
- **We have previously reported similar preclinical findings for other vedotin ADCs that have also shown promising clinical combination data with immune checkpoint inhibitors [1-8]**
- **SGN-B6A is currently being evaluated as monotherapy in NSCLC, HNSCC, ESCC, and other tumors in a phase I study (NCT04389632)**
- **This work provides additional preclinical rationale for exploring SGN-B6A in combination with immune checkpoint inhibitors in the clinic**

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DISCLOSURES: All authors are employees and shareholders of Seagen Inc.

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