# SGN-B6A May Induce Immunogenic Cell Death as an Additional Mechanism of Action

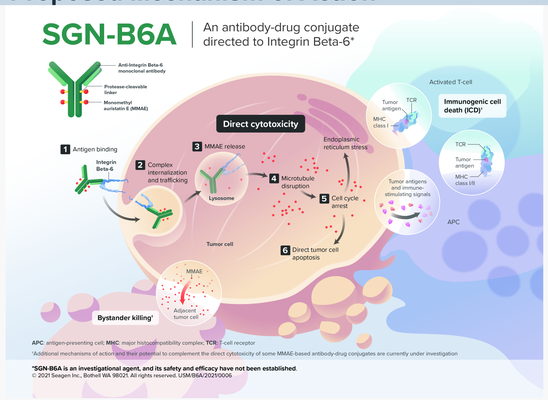
Vivian H. Trang[1], Rebecca C. Mazahreh[1], John J. Gosink[1], Michelle Ulrich[1], Devra Olson[1], Allana Ubben[1], Sean Allred[2], Li-Ya Huang[2], Kelly Hensley[2], Piper M. Treuting[2], Kerry Klussman[1], Shaylin Higgins[1], Patrick Younan[1], Roma Yumul[1], Natalya Nazarenko[3], Robert P. Lyon[1]

[1]Research, [2]Translational Sciences, and [3]Development, Seagen Inc., Bothell WA

## Vedotin Antibody-Drug Conjugate SGN-B6A

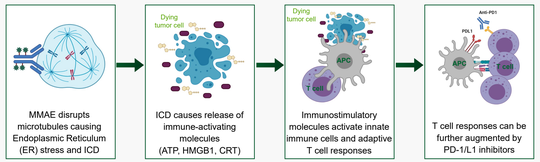
- SGN-B6A is an investigational vedotin antibody-drug conjugate (ADC) directed to integrin beta-6 that is currently being evaluated in a phase I study (NCT04389632)
- SGN-B6A is comprised of the humanized antibody h2A2, highly specific for integrin beta-6 over other beta integrins, paired with the vedotin ADC technology that delivers the potent cytotoxin MMAE
- 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 [6-9]
- Here, we present data to support immunogenic cell death as an additional mechanism of action for SGN-B6A and combination data with a PD1 checkpoint inhibitor that provides antitumor activity enhancement in a syngeneic murine model

## Proposed Mechanism of Action




## 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 Induces Apoptosis and ICD Markers In Vitro

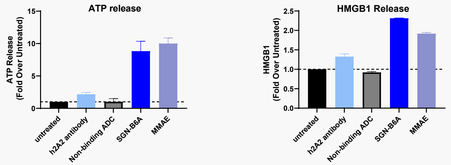



**Figure 1. SGN-B6A induces secretion of ATP and HMGB1**. SGN-B6A, h2A2 antibody, and non-binding ADC were incubated with BxPC3 cells for 48 hours (Ab and ADCs at 1 µg/mL, MMAE at 10 nM). SGN-B6A and free MMAE demonstrated increased release of ATP and HMGB1 compared to cells treated with naked Ab or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

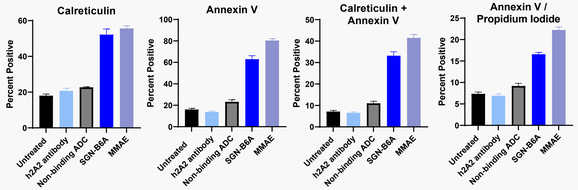



**Figure 2. SGN-B6A induces apoptosis and cell surface translocation of calreticulin, assessed by flow cytometry**. SGN-B6A, h2A2 antibody, and non-binding ADC were incubated with BxPC3 cells for 40 hours (Ab and ADCs at 1 µg/mL, MMAE at 10 nM). SGN B6A and free MMAE demonstrated increased staining via 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.

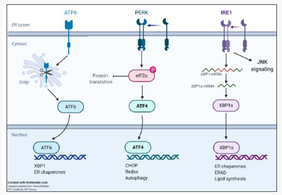
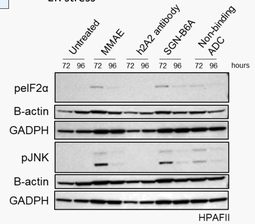



**Figure 3. SGN-B6A induces ER stress signaling.** SGN-B6A and MMAE elicit ER stress pathways through multiple branches of signaling. Integrin beta-6-expressing HPAFII tumor cells were incubated with 2nM MMAE free drug or 2µg/mL SGN-B6A, naked mAb, or non-binding MMAE ADC for 72 and 96 hours. Phosphorylated JNK and eIF2α were detected in SGN-B6A treated cells and not in cells treated with naked mAb.

- 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




## SGN-B6A Induces ICD-Related Genes In Vivo

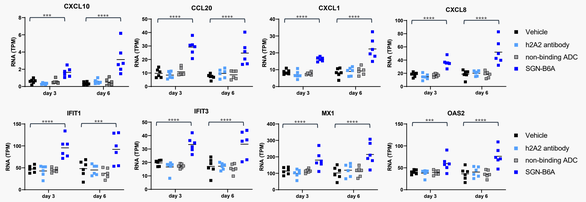



**Figure 4. SGN-B6A induces upregulation of human chemokine and type I IFN response genes in HPAFII xenograft tumor cells.** HPAFII tumors in nude mice were treated with a single dose of 3 mg/kg SGN-B6A, non-binding ADC, or h2A2 antibody. Tumors were harvested at days 3 and 6 post-treatment and processed 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

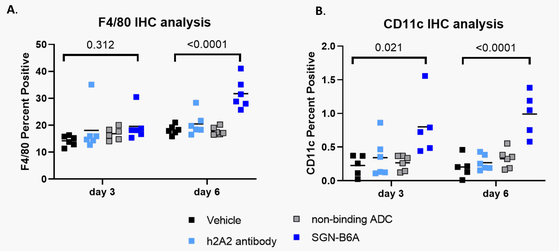



**Figure 5. SGN-B6A recruits F4/80-expressing mouse macrophages & mouse CD11-expressing APCs to HPAFII xenograft tumors** HPAFII tumors in nude mice were treated with a single dose of 3 mg/kg SGN-B6A, non-binding ADC, and naked mAb. Tumors were harvested at days 3 and 6 post-treatment and processed for IHC. (**A**) Staining of tumors demonstrated an increase in F4/80+ macrophages at the tumor site 6 days after treatment with SGN-B6A. F4/80 percent positive cells were quantified using Halo image analysis and show a significant (one-way ANOVA) increase of F4/80+macrophages at day 6 post-dose. (**B**) Staining of tumors demonstrated an increase in CD11+ antigen presenting cells (APCs) at the tumor site 6 days after treatment with SGN-B6A. CD11 percent positive cells were quantified using Halo image analysis and show a significant (one-way ANOVA) increase of CD11+ APCs at day 6 post-dose.

## mSGN-B6A Activity in Combination With Anti-mPD1

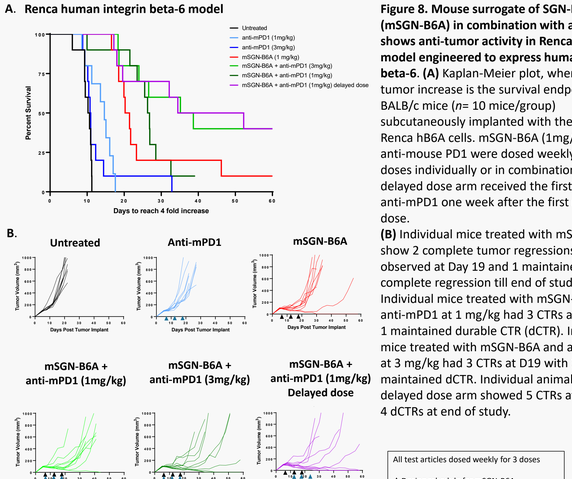



**Figure 8. Mouse surrogate of SGN-B6A (mSGN-B6A) in combination with anti-mPD1 shows anti-tumor activity in Renca syngeneic model engineered to express human integrin beta-6.** (**A**) Kaplan-Meier plot, where 4-fold tumor increase is the survival endpoint of BALB/c mice (*n*= 10 mice/group) subcutaneously implanted with the syngeneic Renca hB6A cells. mSGN-B6A (1mg/kg) and anti-mouse PD1 were dosed weekly for three doses individually or in combination. A delayed dose arm received the first dose of anti-mPD1 one week after the first mSGN-B6A dose.
(**B**) Individual mice treated with mSGN-B6A show 2 complete tumor regressions (CTR) observed at Day 19 and 1 maintained durable complete regression till end of study (Day 58). Individual mice treated with mSGN-B6A and anti-mPD1 at 1 mg/kg had 3 CTRs at D19 with 1 maintained durable CTR (dCTR). Individual mice treated with mSGN-B6A and anti-mPD1 at 3 mg/kg had 3 CTRs at D19 with 3 maintained dCTR. Individual animals in the delayed dose arm showed 5 CTRs at D19 with 4 dCTRs at end of study.

All test articles dosed weekly for 3 doses
▲ Dosing schedule for mSGN-B6A
▲ Dosing schedule for anti-mPD1

**Table 1. Tumor Growth Inhibition in Renca human integrin beta-6 syngeneic model**

| Treatment | Dose | Tumor Volume (mm³)[a] (Day 19) | TGI (%)[b] (Day 19) | CTR[c] (Day 19) | dCTR[d] (Day 58) |
|---|---|---|---|---|---|
| Untreated | - | 657.4 ± 43.1 | - | 0/10 | 0/10 |
| mSGN-B6A | 1 mg/kg | 139.9 ± 21.8 | 92.4 | 2/10 | 1/10 |
| Anti-mPD1 | 1 mg/kg | 469.2 ± 95.1 | 33.7 | 0/10 | 0/10 |
| mSGN-B6A<br>Anti-mPD1 | 1 mg/kg<br>1 mg/kg | 135.3 ± 38.0 | 93.5 | **3/10** | **1/10** |
| mSGN-B6A<br>Anti-mPD1 | 1 mg/kg<br>3 mg/kg | 112.7 ± 26.3 | 97.0 | **3/10** | **3/10** |
| mSGN-B6A<br>Anti-mPD1<br>delayed dose | 1 mg/kg<br>1mg/kg | 98.8 ± 23.7 | 99.8 | **5/10** | **4/10** |

[a]Mean ± SEM
[b]TGI: tumor growth inhibition; TGI (%) = 100 x [1- ($V_{treat\text{-}t} - V_{treat\text{-}1}$)/ ($V_{control\text{-}t} - V_{control\text{-}1}$)], where $V_{treat\text{-}1}$ and $V_{control\text{-}1}$ are mean tumor volumes of the treated and control groups on grouping day, and $V_{treat\text{-}t}$ and $V_{control\text{-}t}$ are mean tumor volumes of the treated and control groups on a given day
[c]CTR: complete tumor regression; number of animals with no measurable tumors (≤ 63mm³)
[d]dCTR: durable complete tumor regression; number of animals with no measurable tumors (≤ 63mm³) at the end of study (Day 58)

## Conclusions

- **SGN-B6A induces markers of immunogenic cell death in vitro and in vivo, recruitment of effector cells in vivo and anti-tumor activity in combination with anti-PD1 in a mouse model, consistent with other vedotin ADCs**
- **The combination of SGN-B6A with anti-PD1 in a mouse model demonstrates enhanced anti-tumor activity versus either single agent alone**
- **Together, this provides additional preclinical rationale for the potential exploration of SGN-B6A in combination with immune checkpoint inhibitors in the clinic**

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