Vedotin ADCs Induce ER Stress and Elicit Hallmarks of ICD Across Multiple Cancer Indications

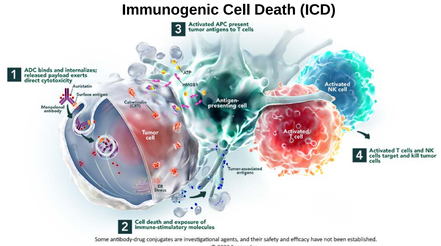
Kerry Klussman[1], Elena-Marie Tenn[1], Shaylin Higgins[1], Rebecca Mazahreh[1], Katie Snead[1], Joseph Z. Hamilton[1], Bryan Grogan[1], Johann Sigurjonsson[1], Anthony Cao[1], Shyra Gardai[1], and Bernard A. Liu[1]

[1]Seagen Inc., Bothell, WA USA

Background

Effective cancer treatment requires durable elimination of malignant cells. Cytotoxic chemotherapeutic agents used to treat cancer often show initial anti-tumor efficacy, but fail to produce long-term durable responses in patients. The elicitation of durable responses and improved survival in response to cytotoxic agents may be associated with the induction of innate and adaptive immune response to the cancer. For example, tumor cells undergoing apoptosis following exposure to some cytotoxic agents emit immunostimulatory damage-associated molecular patterns (DAMPs), this form of cell death is termed immunogenic cell death (ICD). ICD can promote the recruitment and activation of both the innate and adaptive immune system, providing an additional mechanism to drive an anti-tumor response.

Immunogenic Cell Death (ICD)



Additional Antibody-Drug Conjugate (ADC) Proposed Mechanisms of Action

Payloads of ADC Programs in the Clinic

Vedotin and other clinically validated payloads

ADC Name	Abbreviations	Antibody	Payload Class	Disease Indication	Clinical Stage
Brentuximab vedotin (SGN-35)	BV	Brentuximab	MMAE (Auristatin)	Hodgkins Lymphoma	FDA Approved
Enfortumab vedotin (ASG22ME)	EV	Enfortumab	MMAE (Auristatin)	Urothelial	FDA Approved
Tisotumab vedotin (TF-011-MMAE)	TV	Tisotumab	MMAE (Auristatin)	Endometrial	Phase II
Ladiratuzumab vedotin (SGN-LIV1A)	LV	Ladiratuzumab	MMAE (Auristatin)	Breast	Phase II
Belantamab mafodotin (GSK2857916)	BM	Belantamab	MMAF (Auristatin)	Multiple Myeloma	FDA Approved
Trastuzumab emtansine (T-DM1)	T-DM1	Trastuzumab	DM1 (Maytansine)	Breast	FDA Approved
Trastuzumab deruxtecan (DS-8201)	T-Ex	Trastuzumab	Ex (Camptothecin)	Breast	FDA Approved

Vedotin ADCs Induce ICD Markers Across Multiple Cancer Types

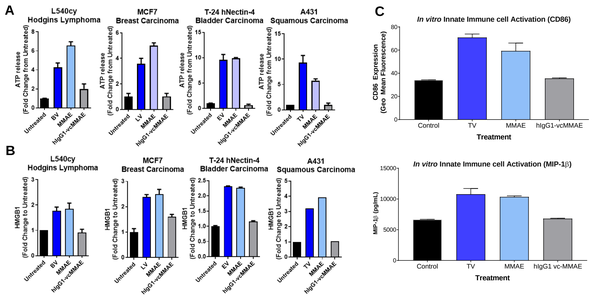


Figure 1. (A) Tumor cell lines cultured with the vedotin ADCs, free MMAE or non-binding ADC control (hIgG1-vcMMAE) demonstrate release of cytosolic ATP or (B) HMGB1 release in the media upon drug treatment, measured at 48hr. (C) Exemplary data of immune cell activation associated with ICD induced cell death is shown. Tumor cells treated with an ADC (TV) or non-binding ADC control were fed to PBMCs. Luminex cytokine analysis and flow cytometry were used to assess immune activation after 24hr.

Vedotin ADC Induces Immune Cell Recruitment and Activation *In Vivo*

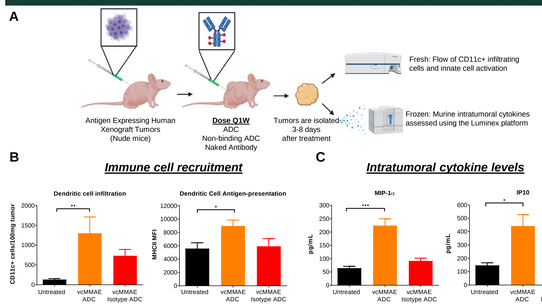


Figure 2. (A) Tumor xenografts isolated from animals treated with a vcMMAE ADC or non-binding vcMMAE Isotype ADC for 8 days, were dissociated, and subject to flow cytometry or cytokine profiling. (B) CD45 positive immune cells were stained for CD11c and activation observed by staining for the expression of MHC Class II on the cell surface. (C) Intratumoral cytokines were measured by Luminex.

Vedotin ADCs Upregulate MHC Class I in Tumors

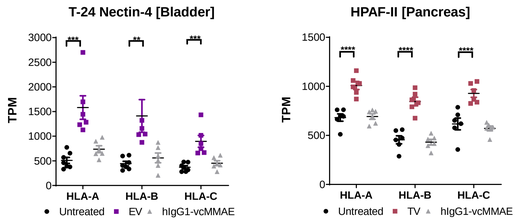


Figure 3. Tumor xenografts from animals treated with EV, TV or non-binding ADC control (hIgG1-vcMMAE) were isolated 5 days post treatment and transcripts for human MHC Class I (HLA-A,B,C) determined by RNA-seq. TPM, total reads per million.

Vedotin ADCs Promote Immune Memory for Anti-Tumor Response

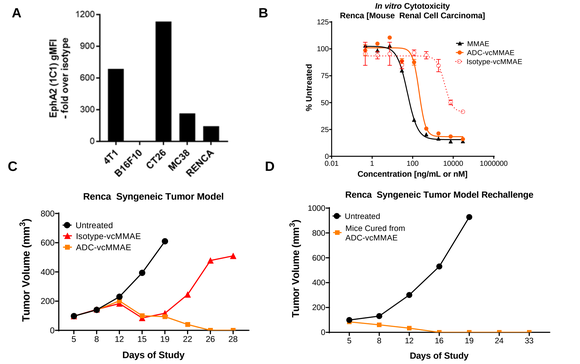


Figure 4. (A) Murine EphA2 expression on indicated syngeneic cell lines by flow cytometry. (B) Evaluation of EphA2-vcMMAE ADC in standard 96hr *in vitro* cytotoxicity assay (ng/mL). MMAE was also tested (nM). (C) Evaluation of the anti-tumor activity of EphA2-vcMMAE ADC *in vivo* in a Renca tumor model. (D) Upon re-challenge, tumor implants were rejected.

ER Stress Induction is Important for ICD and Superior for Vedotin ADCs

A

Payload Class	Members	Average Drug Load	Mechanism of Action
Auristatins	MMAE (vedotin)	4	Microtubule Disruption
	MMAF (mafadotin)	4	
Maytansines	DM1 (emtansine)	4	
Camptothecins	Ex (exatecan)	8	Topoisomerase Inhibition

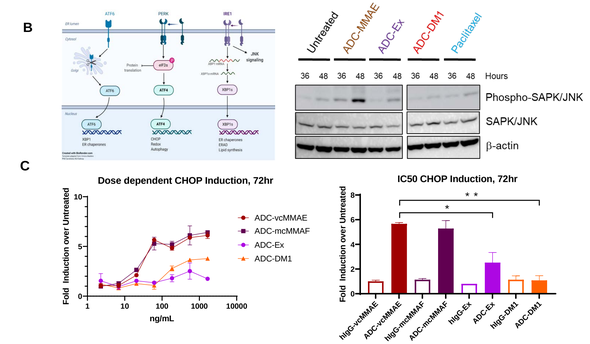


Figure 5. (A) Table of various clinically approved ADC payloads. (B) Graphic of ER stress signaling response and Western blot analysis of MIA-PaCa-2 cells treated with ADC, paclitaxel at IC50 concentrations for 36hr or 48hr. (C) MIA-PaCa-2 cells expressing CHOP-driven luciferase reporter (Signosis, Inc.) were treated with ADCs over a dose range or at IC50 dose (cytotoxicity). CHOP induction is expressed by fold induction compared to untreated cells.

ICD Potential of Clinical ADC Payloads

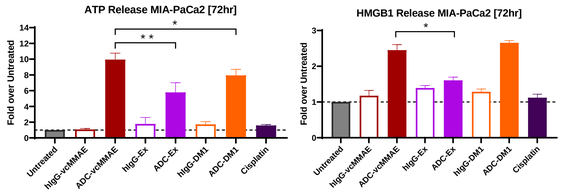


Figure 6. Supernatants were collected from MIA-PaCa-2 pancreatic tumor cells treated with 1 µg/mL ADCs for 72hr and ATP release determined by Cell Titer Glo and HMGB1 secretion by ELISA was performed.

Immune Activation Assessment of ADC Payloads

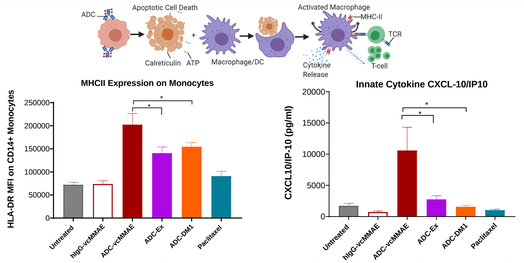


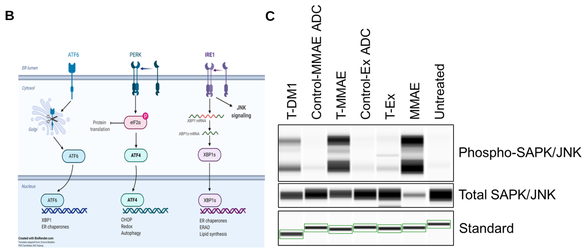
Figure 7. Upregulation of MHC Class II (HLA-DR) on myeloid cells within PBMC was assessed by flow cytometry following 48hr co-incubation of PBMC with L540cy cells dosed with ADCs (24hr IC50 concentration). 24hr supernatants were assessed by Luminex multiplex assay for cytokine levels.

Payload Evaluation on Trastuzumab Backbone

A

ADC	Antibody	Payload
T-DM1	Trastuzumab	DM1
T-MMAE	Trastuzumab	MMAE
T-Ex	Trastuzumab	Exatecan

Evaluation of ER Stress Pathway Signaling



Vedotin ADC Demonstrate Strong Activation of Multiple ICD Hallmarks

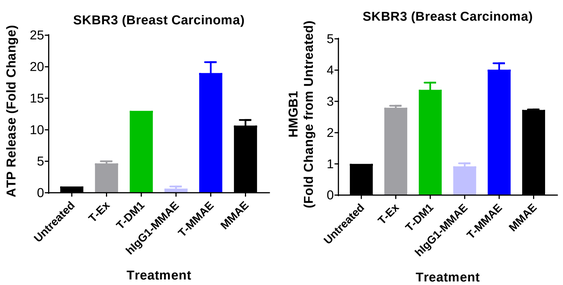


Figure 8. (A) Table of trastuzumab ADCs evaluated. (B) Graphic of ER stress signaling response. (C) Simple Western (Wes™ Protein Simple) data of BT474 cells treated with ADCs or drug for 72hr. (D) Trastuzumab ADCs dosed at 1 µg/mL or free MMAE (100nM) demonstrate differential in ICD response in SKBR3 HER2-expressing breast cancer cells. Media collected after 48hr or 72hr treatment was used to measure ATP and HMGB1 levels.

Conclusions

- Vedotin ADCs induce ER stress and tumor cell death in a manner evoking ICD hallmarks across multiple cancer indications.
- Tumor-bearing mice treated with vedotin-based ADCs resulted in the promotion of immune cell recruitment and activation in tumors.
- Vedotin-based ADCs included production of innate cytokines and upregulation of MHC Class I/II expression, supporting a role in activating both the innate and adaptive immune responses.
- To further our understanding of the potent and broad ability of vedotin ADCs to induce ICD, we have also begun to examine the ICD potential of different classes of ADC payloads including other microtubule inhibitors (auristatins and maytansines), and DNA damaging agents (DNA alkylators or topoisomerase inhibitors).
- Initial data indicate differences in ICD induction by these agents and totality of the data helps build the rationale for vedotin-based ADCs as preferred partners for immune checkpoint blockade agents.

Acknowledgements

We would like to thank our colleagues Angela Epp, Weiping Zeng and Elizabeth Gray for the Renca studies, Kaleb Smith for HPAF II study, and John Gosink for RNA-seq data and analysis.

Poster No. 618
Society for Immunotherapy of Cancer, Virtual; November 9–14, 2020

Disclosures: Seagen Inc. provided research funding, KK, EMT, SH, RM, KS, JZH, BG, JS, SG, and BAL are employees of and have equity ownership in Seagen Inc., current affiliation for AC is Celgene, Inc., San Francisco, California.

Seagen