MMAE Drives Immunomodulatory Changes in a Preclinical Xenograft Model That are Distinct from Other Clinical-Stage ADC Payloads

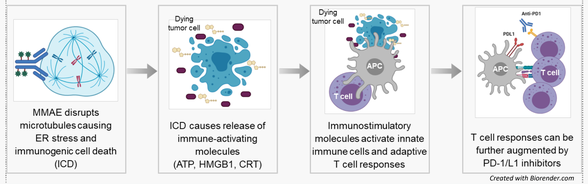
Michelle L. Ulrich[1], Kerry Klussman[1], John Gosink[1], JP Sigurjonsson[1], Sean Allred[1], Li-Ya Huang[1], Kelly Hensley[1], Piper M. Treuting[1], Anna K. de Regt[1], Nikhil J. Parekh[1], Elena Tenn[1], Shaylin Higgins[1], Rebecca Mazahreh[1], Shyra Gardai[1] and Elizabeth E. Gray[1]

[1]Seagen Inc., Bothell, WA USA

Background

- Vedotin ADCs incorporate the microtubule disrupting agent monomethyl auristatin E (MMAE) via a cleavable peptide linker that has been validated by the clinical success of ADCETRIS, PADCEV and Tivdak.
- MMAE exerts its cytotoxic effects via microtubule disruption and induction of ER stress, leading to apoptotic cell death. In addition, MMAE elicits immune modulation by inducing immunogenic cell death (ICD) and subsequent immune changes in the tumor microenvironment [1-4].
- This immune modulation may position vedotin ADCs to uniquely combine with checkpoint inhibitors, a benefit seen clinically with meaningful responses observed when vedotin ADCs are administered with anti-PD1 agents [5,6].
- Other clinical-stage and approved ADCs incorporate payloads that also cause microtubule disruption (DM1, DM4) or DNA damage through topoisomerase I inhibition (Dxd).
- While ADCs with different payloads produce clinical benefit, their long-term impact on survival and combination anti-tumor activity with anti-PD(L)1 agents may differ based on their ability to elicit immune modulation.
- In this study we investigated how DM1, DM4, and Dxd compare to MMAE in their ability to drive immune changes in the TME.

Vedotin ADC-induced Immunogenic Cell Death



MMAE disrupts microtubules causing ER stress and immunogenic cell death (ICD)

ICD causes release of immune-activating molecules (ATP, HMGB1, CRT)

Immunostimulatory molecules activate innate immune cells and adaptive T cell responses

T cell responses can be further augmented by PD-1/L1 inhibitors

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Clinical-Stage Drug-Linker Payload Systems

ADC Name	Abbreviation	Target Antigen	Payload Class	Average drug load	Disease Indication	Clinical Stage
Brentuximab vedotin (SGN-35)	BV	CD30	**MMAE** (Auristatin)	4	Hodgkin's Lymphoma	FDA Approved
Enfortumab vedotin (ASG22ME)	EV	Nectin-4			Urothelial	
Tisotumab vedotin (TF-011-MMAE)	TV	Tissue Factor			Cervical	
Trastuzumab emtansine (T-DM1)	T-DM1	HER2	**DM1** (Maytansine)	4	Breast	
Mirvetuximab soravtansine (IMGN-853)	M-DM4	FRa	**DM4** (Maytansine)	4	Ovarian	
Trastuzumab deruxtecan (DS-8201)	T-Dxd	HER2	**Dxd** (Camptothecin)	8	Breast	

References

1. Puszkiel, L.L., H., Hale, C., Grosse-Wilde, A., Specht, J., Modi, S., Han, H., Cortes, J., Oliveira, M., Garfin, P., Wang, Z., Onsum, M., *Systemic administration of ladiratuzumab vedotin alone or in combination with pembrolizumab results in significant immune activation in the tumor microenvironment in metastatic breast cancer patients.* Journal for ImmunoTherapy of Cancer, 2020. **8**.
2. Liu, B.A.O., D.; Snead, K.; Gosink, J.; Tenn, E-T.; Zaval, M.; Cao, A.; Sahetya, D.; Nesterova, A.; Hensley, K.; Cochran, J.; Gardai, S.; Lewis, T. S., *Enfortumab vedotin, an anti-Nectin-4 ADC demonstrates bystander cell killing and immunogenic cell death anti-tumor activity mechanisms of action in urothelial cancers.* Cancer Research, 2020. **80**(16_Supplement): p. 5581.
3. Gray, E.H., K., Allred, S., Trueblood, E.; Gosink, J.; Thurman, R.; Smith, K.; Jacquemont, C.; Bieda, M.; Gow, J.; Harris, J.; Brady, L.; Soumaoro, I.; Jain, S.; Nicacio, L.; Gardai, S., *Tisotumab vedotin shows immunomodulatory activity through induction of immunogenic cell death.* Journal for ImmunoTherapy of Cancer, 2020. **8**.
4. Gray, E., et al., *1281: SGN-B7H4V shows immunomodulatory activity through induction of immunogenic cell death.* Cancer Res, 2022. **82** (12_Supplement): 1281.
5. Rosenberg, J.E.F., T.W.; Friedlander, T.W.; Milowsky, M.I.; Srinivas, S.; Petrylak, D.P.; Mershan, J.R.; Bilen, M.A.; Carret, A.-S.; Yuan, N.; Sasse, C.; Holmes, C.J., *Study EV-103: Preliminary durability results of enfortumab vedotin plus pembrolizumab for locally advanced or metastatic urothelial carcinoma.* Journal of Clinical Oncology, 2020. **38**(6): p. 441-441.
6. Advani, R.H., et al., *Brentuximab vedotin in combination with nivolumab in relapsed or refractory Hodgkin lymphoma: 3-year study results.* Blood, 2021. **138**(6): p. 427-438.

In Vitro ICD Potential of Clinical ADC Payloads

MMAE elicits robust hallmarks of ICD

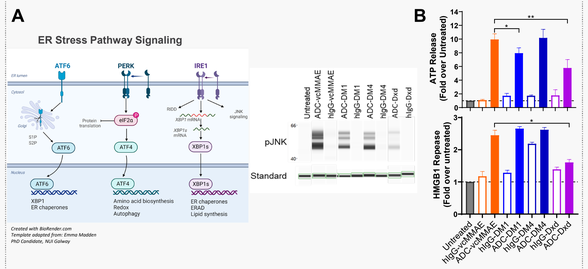

Figure 1. (A) Graphic of ER stress signaling and Simple Western (Wes™ Protein Simple) evaluation of phospho-JNK in MIA PaCa-2 tumor cells treated with 1 µg/mL ADCs for 48 hr. **(B)** Evaluation of ATP and HMGB1 release from MIA PaCa-2 cells treated with ADCs for 72 hr.

MMAE disrupts tubulin distinctly from DM1/DM4

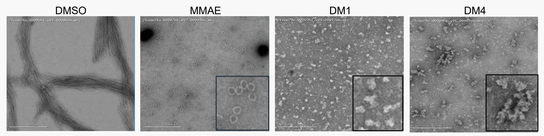

Figure 2. Electron Microscopy (EM) images of bovine brain tubulin incubated with 12% DMSO, 1 mM GTP, and 36 µM drug in 1xBRB80 buffer for 1 hr at 37°C. MMAE forces a distinct conformation compared to DM1 and DM4.

In Vivo Immunomodulatory Changes of Clinical ADC Payloads

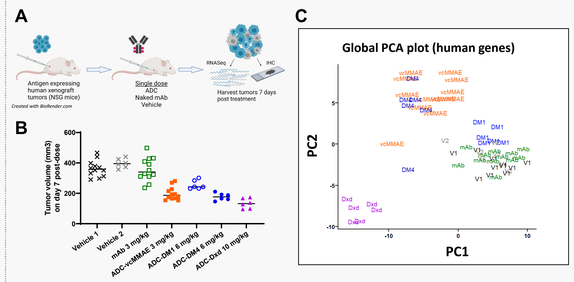

Figure 3. Preclinical in vivo model to evaluate immunomodulatory changes. (A) MDA-MB-468 tumor-bearing NSG mice (N=6) were treated with a single dose of unconjugated antibody (mAb) or ADCs with MMAE, DM1, DM4, or Dxd payloads. Tumors were harvested 7 days post-treatment and processed for RNAseq and immunohistochemistry (IHC). **(B)** Mice were treated with doses of ADC that drove similar anti-tumor activity. **(C)** Global principal component analysis (PCA) of 200 random **human** genes reveals ADC-Dxd clusters away from the microtubule-disrupting payloads MMAE, DM1, and DM4.

MMAE elicits unique immunomodulatory changes to human tumor cells in vivo

MMAE elicits distinct changes to interferon response, cytokine, and MHC class I antigen presentation genes

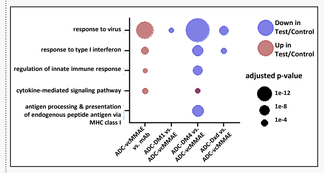

Figure 4. Gene ontology analysis reveals distinct immune changes in response to MMAE. Human gene ontology enrichment analysis revealed an upregulation of immune-related biological processes following ADC-vcMMAE treatment.

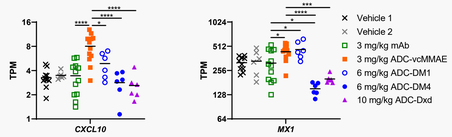

Figure 5. MMAE induces superior upregulation of IFN response genes compared to DM4 and Dxd. Human transcripts encoding IFN response genes in tumor cells were increased following treatment with ADC-vcMMAE, but decreased or unchanged following treatment with ADC-DM4 and ADC-Dxd.

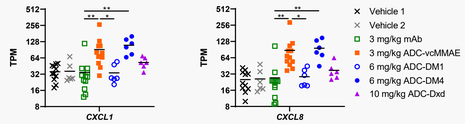

Figure 6. MMAE induces superior upregulation of cytokines compared to DM1 and Dxd. Human transcripts encoding cytokines in tumor cells were increased following treatment with ADC-vcMMAE & ADC-DM4, but not ADC-DM1 or ADC-Dxd.

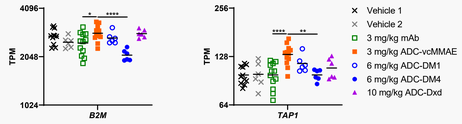

Figure 7. MMAE induces superior upregulation of antigen presentation genes compared to DM1 and Dxd. Human transcripts encoding the antigen processing and presentation genes *B2M* and *TAP1* in tumor cells were increased following treatment with ADC-vcMMAE but unchanged following treatment with ADC-DM1, DM4, or Dxd.

MMAE increases PDL1 expression on tumor cells

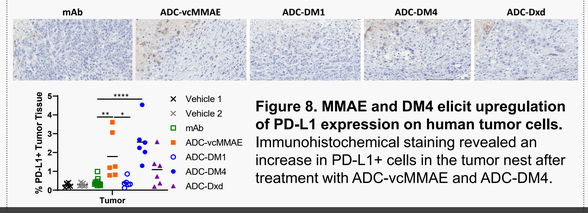

Figure 8. MMAE and DM4 elicit upregulation of PD-L1 expression on human tumor cells. Immunohistochemical staining revealed an increase in PD-L1+ cells in the tumor nest after treatment with ADC-vcMMAE and ADC-DM4.

MMAE elicits immunomodulatory changes to murine immune TME in vivo

MMAE and Dxd elicit superior recruitment of macrophages

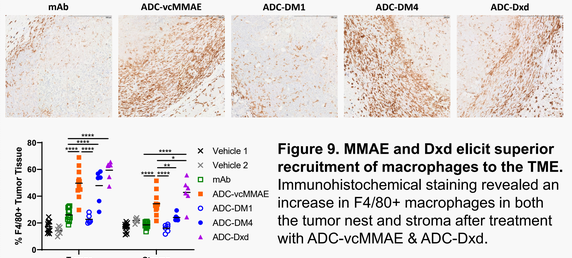

Figure 9. MMAE and Dxd elicit superior recruitment of macrophages to the TME. Immunohistochemical staining revealed an increase in F4/80+ macrophages in both the tumor nest and stroma after treatment with ADC-vcMMAE & ADC-Dxd.

MMAE elicits unique murine immune gene signatures

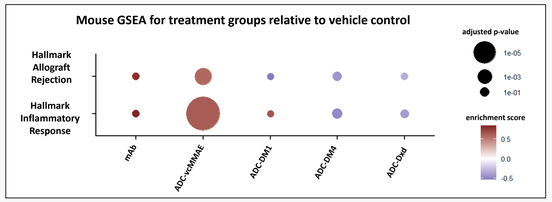

Figure 10. MMAE, but not DM1, DM4, or Dxd, elicits upregulation of murine immune-related gene signatures in the TME. Gene signature enrichment analysis (GSEA) revealed an increase in the hallmark gene signatures "allograft rejection" and "inflammatory response" following treatment with ADC-vcMMAE.

Conclusions

- Altogether, these data demonstrate that treatment with vedotin (MMAE) ADCs results in robust immunomodulatory changes, both in the tumor cells themselves as well as immune cells within the TME, that are distinct from other clinical ADC payloads.

	ADC-vcMMAE	ADC-DM1	ADC-DM4	ADC-Dxd
ER Stress induction	+++	++	++	+
ATP Release	+++	++	+++	+
HMGB1 Release	++	++	++	+
IFN response and cytokines (human)	+++	++ / -	- / +++	-
Antigen presentation (human)	++	+	-	+
Macrophages recruitment (mouse)	+++	-	++	+++
Proinflammatory immune gene signatures (mouse)	+++	-	-	-

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DISCLOSURES: All authors are employees of and/or hold stock in Seagen, Inc.

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