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ENFORTUMAB VEDOTIN INDUCES IMMUNOGENIC CELL DEATH, ELICITS ANTITUMOR IMMUNE MEMORY, AND SHOWS ENHANCED PRECLINICAL ACTIVITY IN COMBINATION WITH A PD-1 INHIBITOR

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Background

- Enfortumab vedotin (EV) is an antibody-drug conjugate (ADC) comprised of a fully human Nectin-4-directed monoclonal antibody conjugated to the microtubule-disrupting agent monomethyl auristatin E (MMAE) by a protease-cleavable maleimidocaproyl-valine-citrulline linker.
- EV has demonstrated single-agent activity with improved overall survival in patients with previously treated advanced-stage urothelial carcinoma [1]. EV is approved in this setting in multiple countries after progression on platinum-containing chemotherapy and PD-1 or PD-L1 inhibitor therapy.
- The ability of EV to demonstrate clinical activity when combined with an anti-PD-1 immune-checkpoint inhibitor (pembrolizumab) has been observed in the EV-103 study (NCT03288545) [1, 2], and a pivotal trial to confirm this activity is ongoing.
- EV has a multifaceted mechanism of action that includes direct cytotoxicity on Nectin-4-expressing malignant cells and indirect bystander effect on neighboring cells, both of which are mediated by intracellular release of MMAE [3].
- Here, we expand upon the mechanism of action and show that EV induces tumor cell killing in a manner leading to immunogenic cell death (ICD) and improves antitumor responses when combined with PD-1 inhibitors.

EV induces early hallmarks of ICD in vitro

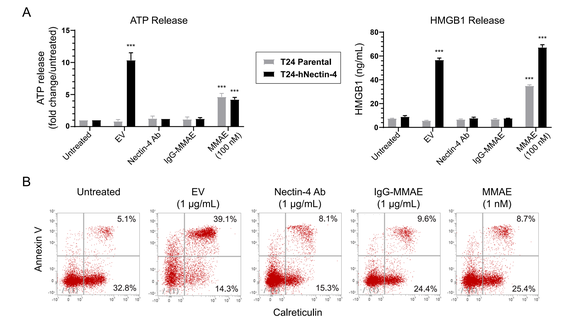

Figure 1. EV induces early hallmarks of ICD, namely secretion of ATP and HMGB1 and surface exposure of calreticulin in T24 urothelial cancer cells expressing human Nectin-4 (T24-hNectin-4). (A) ATP and HMGB1 release was measured in the cell-free supernatants of tumor cells treated with 1 µg/mL EV, 1 µg/mL unconjugated Nectin-4-specific antibody lacking an MMAE payload (Nectin-4 Ab), 1 µg/mL non-targeted control ADC (IgG-MMAE), or 100 nM MMAE for 48 hours. ***P<0.001 shows significant difference from untreated determined using multiple t-tests with Bonferroni correction. (B) T24-hNectin-4 cells were treated with 1 µg/mL EV, Nectin-4 Ab, or hIgG1-MMAE or 1 nM MMAE for 40 hours and stained for Annexin V (marker of apoptosis) and calreticulin.

EV-treated tumor cells activate monocytes in coculture

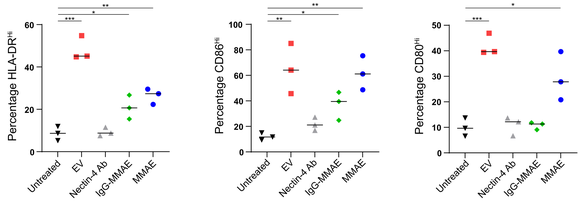

Figure 2. EV-treated T24-hNectin-4 urothelial carcinoma cells induce monocyte activation in coculture with monocytes isolated from PBMCs. T24-hNectin-4 cells were treated with 1 µg/mL EV, Nectin-4 Ab, or hIgG1-MMAE or 1 nM MMAE for 24 hours and then cocultured with purified monocytes for 24 hours. Next, cells were stained with a live/dead reagent and incubated with antibodies specific for CD14, HLA-DR, CD86 and CD80. CD14+ gated monocytes/macrophages were then analyzed for the following activation markers: HLA-DR[hi], CD86[hi], and CD80[hi]. *P<0.05, **P<0.01, and ***P<0.001; unpaired t-test.

References

1. T. Powles et al., N Engl J Med, vol. 384, no. 12, pp. 1125-1135, Mar 25 2021, doi: 10.1056/NEJMoa2035807.
2. T. W. Friedlander et al., Journal of Clinical Oncology, vol. 39, no. 15_suppl, pp. 4528-4528, 2021, doi: 10.1200/JCO.2021.39.15_suppl.4528.
3. B. A. Liu et al., Abstract 5581, Cancer Research, vol. 80, no. 16_Supplement, pp. 5581-5581, 2020, doi: 10.1158/1538-7445.Am2020-5581.
4. J. Humeau et al., Methods Mol Biol, vol. 1884, pp. 297-315, 2019, doi: 10.1007/978-1-4939-8885-3_21.
5. J. Zhou et al., J Cell Mol Med, vol. 23, no. 8, pp. 4854-4865, Aug 2019, doi: 10.1111/jcmm.14356.

EV induces immunomodulatory changes in mouse xenografts

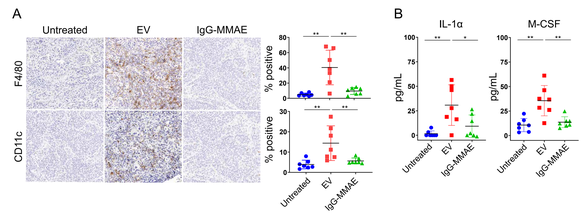

Figure 3. EV-treated tumors increase the levels of macrophage-stimulating cytokines and the proportion of F4/80+ macrophages and CD11c+ APCs, indicating the recruitment of mouse innate immune cells. T24-hNectin-4 cells were implanted into athymic nude mice, and 7 mice/group were treated with a single IP injection of 3 mg/kg EV or IgG-MMAE. Tumors were collected on Day 5 post treatment for IHC (A), cytokine (B), and RNA-seq (Table 1) analyses. (A) IHC staining of T24-hNectin-4 xenograft tumors shows enriched immune cell infiltration by F4/80 and CD11c staining in the EV group compared the control groups. Percent positive staining of IHC images was quantified with Halo image analysis software. (B) Concentrations of the indicated macrophage-stimulating mouse cytokines were measured by Luminex assay. *P<0.05, **P<0.01; unpaired t-test.

Table 1. EV induces gene expression patterns associated with ICD

GO Term[a]	Description	Q-Value	Gene Count
GO:0006914	Autophagy	1.29E-08	170
GO:0032479	Regulation of type I interferon production	1.09E-05	54
GO:0034340	Response to type I interferon	2.82E-05	45
GO:0000422	Autophagy of mitochondrion	7.56E-04	32
GO:0034976	Response to endoplasmic reticulum stress	2.13E-03	89
GO:0060333	Interferon-gamma-mediated signaling pathway	4.10E-03	35
GO:1903533	Regulation of protein targeting	4.82E-03	32
GO:0070059	Intrinsic apoptotic signaling pathway in response to ER stress	6.72E-03	25

[a]List of relevant unique Gene Ontology (GO) biological processes significantly upregulated in the human component of EV-treated tumor cells compared to untreated tumor cells. Q-value is the Storey family-wise error corrected P-value. Gene Count is the number of genes out of 3,846 genes meeting the selection criteria associated with each GO term.

Tumor vaccination results suggest that EV induces ICD in vivo

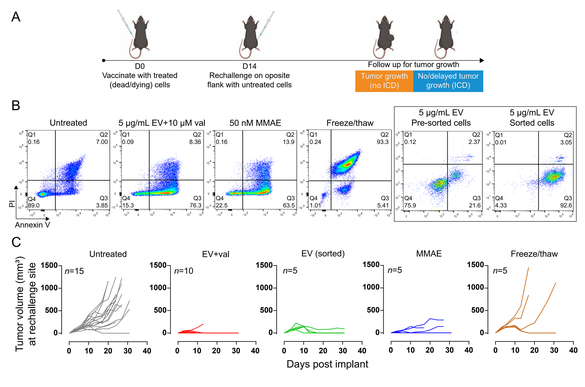

Figure 4. Vaccination with EV-treated hNectin-4-expressing mouse urothelial carcinoma (MB49-hNectin-4) cells shows improved protection from rechallenge with untreated tumor cells, indicative of the development of antitumor immunity and consistent with ICD induction. Valspodar (val), a P-glycoprotein inhibitor, was included to prevent energy dependent efflux of MMAE and increase the proportion of cells in the process of dying (Annexin V+/PI-, Q3). As another way to enrich for dying cells, EV-treated cells were positively sorted with Annexin V-conjugated microbeads. Cells subjected to 5 freeze/thaw cycles were included as an apoptotic non-ICD control. (A) Experimental schematic (BioRender). (B) MB49-hNectin-4 tumor cells were treated as indicated for 96 hours. Apoptosis induction was measured by Annexin V/PI staining. In the far right 2 panels, cells were treated with EV and sorted with Annexin V-conjugated microbeads. The cells in Q3 (Annexin V high/PI low) were considered apoptotic. (C) Tumor growth curves in C57BL/6 mice implanted with 3×10[5] treated MB49-hNectin-4 cells and rechallenged 14 days later with 3×10[5] untreated MB49-hNectin-4 tumor cells in the contralateral flank (day 0 in graphs).

6. M. D. Galsky et al.,J Clin Oncol, vol. 39 (6_suppl), no. 6_suppl, p. TPS507, 2021, doi: 10.1200/JCO.2021.39.6_suppl.TPS507.
7. M. S. V. D. Heijden et al., J Clin Oncol, vol. 40 (6_suppl), no. 6_suppl, p. TPS589, 2022, doi: 10.1200/JCO.2022.40.6_suppl.TPS589.
8. C. J. Hoimes et al., J Clin Oncol, vol. 39 (15_suppl), no. 15_suppl, p. TPS4587, 2021, doi: 10.1200/JCO.2021.39.15_suppl.TPS4587.
9. "Treatment Combination of Durvalumab, Tremelimumab and Enfortumab Vedotin or Durvalumab and Enfortumab Vedotin in Patients With Muscle Invasive Bladder Cancer Ineligible to Cisplatin or Who Refuse Cisplatin," ed: https://ClinicalTrials.gov/show/NCT04960709.

EV+anti-mPD-1 changes the tumor microenvironment

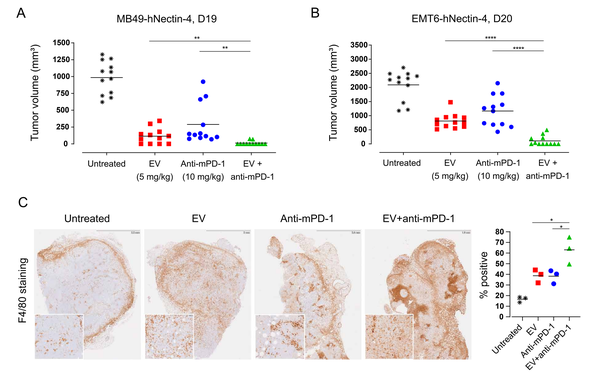

Figure 5. The combination of EV with PD-1 inhibition significantly improves the antitumor activity of each agent alone in syngeneic mouse models and increases macrophage recruitment (F4/80 staining) to the tumor. (A) Significantly lower tumor volume on Day 19 post treatment with EV+anti-mPD-1 antibody in MB49-hNectin-4 murine bladder carcinoma model. C57BL/6 mice (*n*=12/group) were implanted with 5×10[5] MB49-hNectin-4 cells and 4 days later were treated with 5 mg/kg EV (weekly for 2 weeks, IV) alone or in combination with 10 mg/kg anti-mPD1 (twice a week for 2 weeks, IP). (B) Significantly lower tumor volume on Day 20 post treatment with EV+anti-mPD-1 antibody in EMT6-hNectin-4 syngeneic mouse model of breast cancer. BALB/c mice (*n*=12/group) were subcutaneously inoculated with 2×10[5] EMT6-hNectin-4 cells. When tumors reached ~45 mm³, mice were treated with 3 mg/kg EV (single dose, IV) alone or in combination with 10 mg/kg anti-mPD-1 (twice a week for 2 weeks, IP). (C) IHC images of F4/80 staining (brown) in MB49-hNectin-4 tumors show increased macrophage recruitment to the tumor 5 days after treatment with EV+anti-mPD-1 compared to single agents. F40/80 percent positive staining was quantified with Halo image analysis software. *P<0.05, **P<0.01, ****P<0.0001; unpaired t-test.

Table 2. Tumor growth inhibition in MB49-hNectin-4 syngeneic model

Treatment	Dose	Tumor Volume (mm³)[a] (Day 19)	TGI (%)[b] (Day 19)	CTR[c] (Day 19)	dCTR[d] (Day 52)
Untreated	-	985.21 ± 70.41	-	0/12	0/20
EV	5 mg/kg	114.40 ± 33.30	96.2	4/12	0/20
Anti-mPD-1	10 mg/kg	289.68 ± 86.09	77.1	0/12	6/20
EV Anti-mPD-1	5 mg/kg 10 mg/kg	12.02 ± 8.11	108.0	10/12	11/20

[a]Mean ± SEM
[b]TGI: tumor growth inhibition; TGI (%) = 100 × [1 − (V_{treat} − $V_{treat-1}$) / ($V_{control}$ − $V_{control-1}$)], where V_{treat} and $V_{treat-1}$ are mean tumor volumes of the treated and control groups on grouping day, and V_{treat} and $V_{control}$ 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 (≤63 mm³)
[d]dCTR: durable complete tumor regression; number of animals with no measurable tumors (≤63 mm³) at the end of the study (day 52)

Table 3. Tumor growth inhibition in EMT6-hNectin-4 syngeneic model

Treatment	Dose	Tumor Volume (mm³)[a] (Day 20)	TGI (%)[b] (Day 20)	CTR[c] (Day 20)
5% dextrose Control rat IgG2a	- 10 mg/kg	2092.70 ± 149.63	-	0/12
EV Control rat IgG2a	3 mg/kg 10 mg/kg	809.88 ± 75.32	62.6	0/12
5% dextrose Anti-mPD-1	- 10 mg/kg	1166.46 ± 158.49	45.2	0/12
EV Anti-mPD-1	3 mg/kg 10 mg/kg	108.59 ± 49.10	96.9	7/12

[a]Mean ± SEM
[b]TGI: tumor growth inhibition; TGI (%) = 100 × [1 − (V_{treat} − $V_{treat-1}$) / ($V_{control}$ − $V_{control-1}$)], where V_{treat} and $V_{treat-1}$ are mean tumor volumes of the treated and control groups on grouping day, and V_{treat} and $V_{control}$ are mean tumor volumes of the treated and control groups on a given day
[c]CTR: complete tumor regression; tumor volume shrinkage to 0 mm³

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EV+anti-mPD-1 induces robust antitumor activity

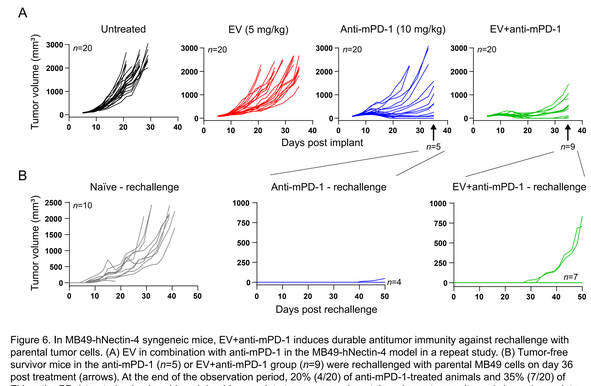

Figure 6. In MB49-hNectin-4 syngeneic mice, EV+anti-mPD-1 induces durable antitumor immunity against rechallenge with parental tumor cells. (A) EV in combination with anti-mPD-1 in the MB49-hNectin-4 model in a repeat study. (B) Tumor-free survivor mice in the anti-mPD-1 (*n*=5) or EV+anti-mPD-1 group (*n*=9) were rechallenged with parental MB49 cells on day 36 post treatment (arrows). At the end of the observation period, 20% (4/20) of anti-mPD-1-treated animals and 35% (7/20) of EV+anti-mPD-1-treated animals achieved durable complete tumor regression at the primary tumor site and showed complete rejection of parental tumor cells, indicative of host immunity against tumor antigens.

Proposed mechanism of action

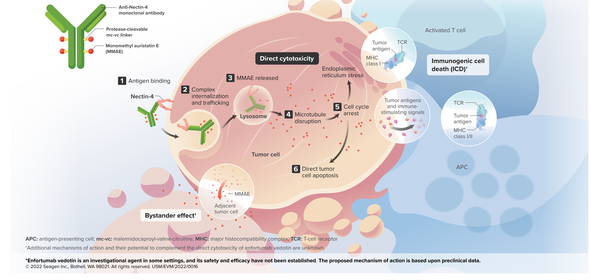

Figure 7. Schematic illustrating the proposed mechanisms of action for EV. EV induces antitumor activity via direct cytotoxicity through intracellular release of MMAE. Intracellular MMAE can also diffuse into neighboring tumor cells in a process known as the bystander effect. Furthermore, EV can induce ICD via MMAE-mediated microtubule disruption and concomitant ER stress.

Conclusions

- These preclinical results demonstrate that EV induces ICD, which results in immune cell activation in the tumor microenvironment in vitro.
- Vaccination with EV-treated Nectin-4-expressing tumor cells can drive antitumor immunity and protect against rechallenge - a gold standard experiment to verify an ICD inducer [4].
- ICD-inducing anti-cancer therapies, including vedotin ADCs, are thought to stimulate a dysfunctional tumor microenvironment [5] and result in combination antitumor activity with immunotherapies. Accordingly, we observe enhanced preclinical antitumor activity when we combine EV with a PD-1 inhibitor.
- Overall, these findings bolster the scientific rationale to explore combinations of EV with checkpoint inhibitors, which is currently an area of active clinical investigation across multiple studies [2, 6-9].

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