Enfortumab vedotin (EV) is a first-in-class Nectin-4-directed antibody-drug conjugate (ADC) with demonstrated improved overall survival in patients with previously treated advanced-stage urothelial carcinoma. \(^1\) EV is 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-cit-rulline linker. EV has a multifaceted mechanism of action. Previously, we demonstrated that EV induces antitumor activity in vitro via direct cytotoxicity on Nectin-4-expressing malignant cells and indirect bystander activity on neighboring Nectin-4 negative cells, both of which are mediated by MMAE release within target cells. Here, we expand upon the mechanism of action and show EV induces tumor cell killing in a manner leading to immunogenic cell death (ICD) and improves antitumor responses when combined with checkpoint inhibitors.

**Methods**

The ability of EV to induce hallmarks of ICD was evaluated in vitro in Nectin-4-expressing human urothelial carcinoma cell lines. Immune activation associated with ICD was assessed in vitro in monocytes co-cultured with EV-treated tumor cells and in vivo by immunohistochemistry, RNA-seq, flow cytometry, and immune cytokine profiling. The effects of EV plus anti-PD-1 on tumor growth inhibition, the tumor microenvironment, and immune memory were evaluated in syngeneic mouse models engineered to express human Nectin-4. Antitumor immune memory was also assessed in mice vaccinated with EV-treated cells.

**Results**

In vitro, EV induced ICD via MMAE-mediated microtubule disruption and concomitant endoplasmic reticulum (ER) stress, as evidenced by increased phosphorylation of JNK, extracellular release of inflammatory mediators ATP and HMGB1, and cell surface exposure of calreticulin. Xenograft tumors treated with EV demonstrated upregulation of MHC genes as well as genes involved in ER stress, autophagy, and type I interferon response. Additionally, there were noted increases in both macrophages and dendritic cells along with cytokines involved in chemotraction and T-cell stimulation. Consistent with ICD induction, vaccination with EV-treated Nectin-4-expressing tumor cells promoted antitumor immunity and provided protection against tumor rechallenge. Lastly, the combination of EV with PD-1 inhibition improved antitumor activity and durable immunity in vivo, consistent with complementary modes of action of these two anticancer agents.

**Conclusions**

These data provide insight into the clinical activity observed with EV and bolster the scientific rationale to combine EV with checkpoint inhibitors, which is currently an area of active clinical investigation across multiple studies.\(^2\)\(^6\)

**REFERENCES**


**Ethics Approval**

All animal studies were conducted in accordance with protocols reviewed and approved by the Institutional Animal Care and Use Committee at Seagen, Astellas, or the external testing facilities that conducted the studies.