# Tisotumab Vedotin Shows Immunomodulatory Activity Through Induction of Immunogenic Cell Death

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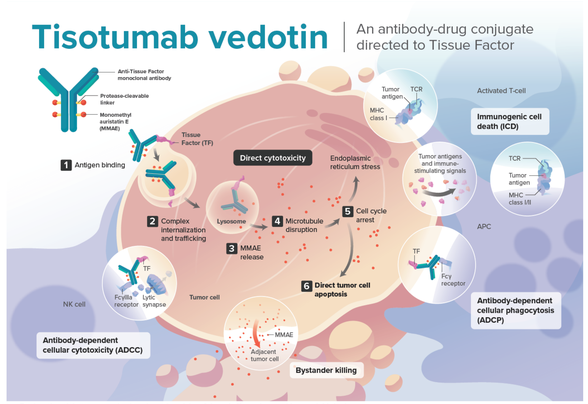
## BACKGROUND

- Tisotumab vedotin (TV) is an investigational antibody-drug conjugate composed of a tissue factor (TF)-directed human monoclonal antibody covalently linked to the microtubule-disrupting agent monomethyl auristatin E (MMAE) via a protease-cleavable linker.
- TV demonstrated single agent activity (24% objective response rate [ORR] and 8.3 month median duration of response [DOR]) in recurrent or metastatic cervical cancer previously treated with doublet chemotherapy and bevacizumab, if eligible (NCT03438396) [1]. Currently, in this population there is no standard of care and ORRs are typically less than 15% and often of limited duration [2-9].
- TV is currently being evaluated in combination with pembrolizumab (PD-1 inhibitor), bevacizumab, or carboplatin in cervical cancer (NCT03786081), or as a monotherapy in multiple other solid tumors (NCT03913741, NCT03485209, NCT03657043).
- The anti-tumor activity of TV may be multimodal as TV can induce tumor cell death through several mechanisms, including direct and bystander MMAE-mediated cytotoxicity, as well as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and immunogenic cell death (ICD) [10,11].
- To better characterize immune-mediated tumor cell killing by TV and further the rationale for combination with pembrolizumab, we set out to refine our understanding of TV-mediated ICD and subsequent immunomodulatory effects.

## PROPOSED MECHANISM OF ACTION




**Tisotumab vedotin is an investigational agent, and its safety and efficacy have not been established.**



References

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## RESULTS

### Tisotumab vedotin induces hallmarks of early ICD

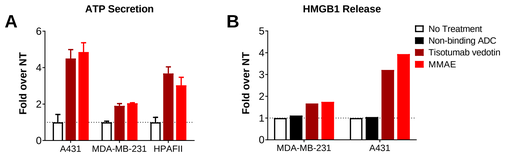


**Figure 1. TV induces secretion of ATP and HMGB1.** TV and MMAE drove ATP secretion **(A)** and high mobility group box 1 (HMGB1) release **(B)**. TF-expressing A431, MDA-MB-231, and/or HPAFII tumor cells were incubated with 100nM MMAE free drug or 1μg/mL TV or non-binding ADC for 48 hours [10].

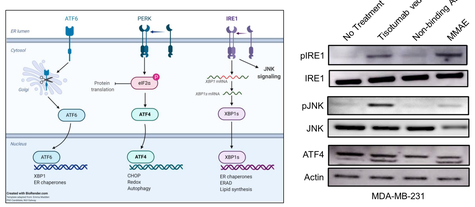


**Figure 2. TV induces ER stress signaling.** TV and MMAE triggered multiple ER stress pathways. TF-expressing MDA-MB-231 tumor cells were incubated with 100nM MMAE free drug or 1μg/mL TV or non-binding ADC for 16 hours [10].

### Tisotumab vedotin-treated tumor cells activate immune cells in vitro

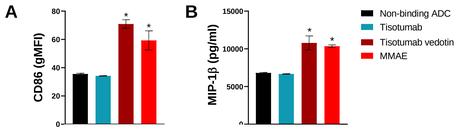


**Figure 3. TV-killed tumor cells activate innate immune cells.** Upregulation of the activation marker CD86 on CD14+ monocytes **(A)** and release of the MIP-1β chemokine **(B)** was observed when PBMCs were co-cultured for 24 hours with TF-expressing MDA-MB-231 tumor cells treated with TV or MMAE free drug.

Significance was determined using non-binding ADC as the comparison with the Holm-Sidak method, with alpha = 0.05. Each row was analyzed individually, without assuming a consistent SD. P-value: * < 0.05.

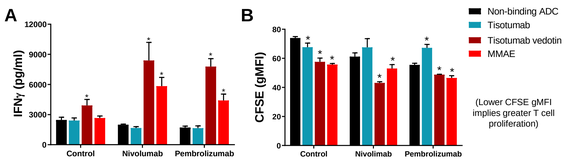


**Figure 4. TV-killed tumor cells promote T cell activation that is amplified by PD-1 blockade**. Increased release of IFNγ **(A)** was observed following co-culture of PBMCs with tumor cells treated with TV or MMAE free drug in the presence of nivolumab or pembrolizumab. T cell proliferation, measured by CFSE dilution, was observed following co-culture of PBMCs for 48 hours with tumor cells treated with TV or MMAE free drug **(B)**.

Significance was determined using non-binding ADC as the comparison with the Holm-Sidak method (alpha = 0.05). Each row was analyzed individually, without assuming a consistent SD. P-value: * < 0.05.

### Tisotumab vedotin has anti-tumor activity in vivo

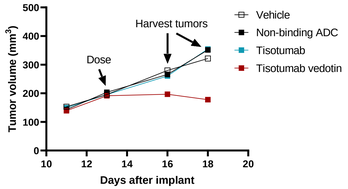


**Figure 5. TV has anti-tumor activity in vivo in a TF-expressing xenograft tumor model.** HPAFII pancreatic tumors in nude mice were treated with a single 3 mg/kg dose of TV, non-binding ADC, or naked tisotumab antibody. Tumors were harvested at days 3 and 5 post-treatment, cut in half, and processed for either RNAseq (Illumina HiSeq platform) or IHC.

### Tisotumab vedotin stimulates expression of chemokine and type I IFN response genes by xenograft tumor cells

A

| Comparison | Direction | ID | Description | Day | p.adjust | geneID |
|---|---|---|---|---|---|---|
| TV vs. Vehicle | UP | GO:0097529 | myeloid leukocyte migration | 3 | 1.82E-05 | **CCL20**, CSF1, **CXCL1**, **CXCL10**, CXCL11, CXCL2, CXCL3, CXCL5 **CXCL8,** DUSP1, EDN1, IL23A, PIK3CD, SERPINE1 |
| | | | | 5 | 2.42E-06 | **CCL20**, CSF1, **CXCL1**, **CXCL10**, CXCL11, CXCL2, CXCL3, CXCL5, **CXCL8**, DUSP1, EDN1, IL23A, IL6R, PIK3CD, SAA1, VEGFC |
| TV vs. Vehicle | UP | GO:0034340 | response to type I interferon | 3 | 1.28E-05 | EGR1, IFI6, **IFIT1**, IFIT2, **IFIT3**, **MX1**, MX2, **OAS2**, RSAD2, USP18, ZBP1 |
| | | | | 5 | 1.21E-04 | EGR1, IFI6, **IFIT1**, **IFIT3**, ISG15, **MX1**, MX2, RSAD2, USP18, ZBP1 |

B

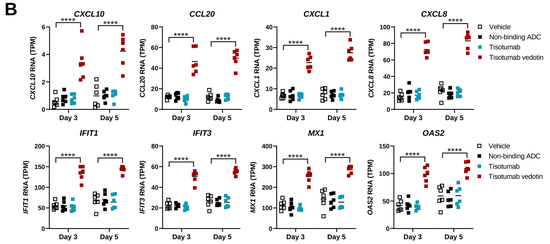


**Figure 6. TV induces upregulation of human chemokine and type I IFN response genes by xenograft tumor cells.** Gene ontology (GO) analysis of human transcripts from tumors treated as in **Figure 5** demonstrated that immune-related gene categories were upregulated in tumor cells following treatment with TV. Two examples of GO gene categories were selected from the top 40 most significantly altered categories **(A)**. Transcripts encoding human chemokines **(B, top)** as well as type I interferon (IFN) response genes **(B, bottom)** were significantly upregulated in tumor cells following treatment with TV**.**

Statistical analysis was performed using a one-way ANOVA with Sidak's multiple comparison test. P-value: **** <0.0001.

### Tisotumab vedotin induces HLA and PD-L1 gene expression by xenograft tumor cells

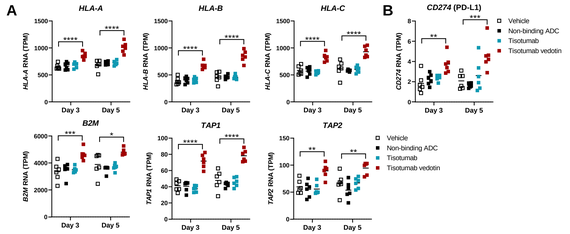


**Figure 7. TV induces upregulation of human genes encoding HLA Class I and PD-L1 by tumor cells.** Human transcripts encoding MHC-Class I, B2M, and the transporter associated with antigen processing genes (TAP-1, -2) **(A)** as well as PD-L1 **(B)** were upregulated by tumor cells following treatment with TV. Upregulation of MHC-Class I genes may allow tumor cells to present tumor antigens to CD8 T cells and drive anti-tumor adaptive immune responses.

Statistical analysis was performed using a one-way ANOVA with Sidak's multiple comparison test. P-value: * <0.05, ** <0.01, ***< 0.001, **** <0.0001.

### Tisotumab vedotin promotes recruitment of mouse immune cells to xenograft tumors

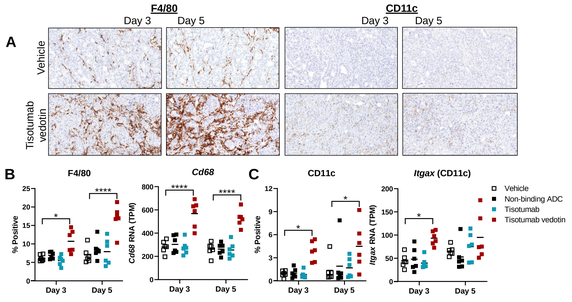


**Figure 8. TV recruits mouse F4/80+ macrophages and CD11c+ APCs to tumors.** IHC staining of TF-expressing xenograft tumors treated as above demonstrated an increase in F4/80+ macrophages and CD11c+ antigen-presenting cells (APCs) at the tumor site following treatment with TV **(A** and **B/C, left panels)**. RNAseq analysis of mouse transcripts corroborated these findings, showing a significant increase in *Cd68* transcripts (which encode the macrophage marker CD68) and an increase in *Itgax* transcripts (which encode CD11c) in tumors following treatment with TV **(B/C, right panels)**.

Statistical analysis was performed using a one-way ANOVA with Sidak's multiple comparison test. P-value: * <0.05, **** <0.0001. Digital image analysis was performed using Halo image analysis.

## CONCLUSIONS

- Data from these preclinical models show that TV induces immunogenic tumor cell death, which can promote activation and recruitment of immune cells to the tumor.
- The totality of data provides evidence for the immunomodulatory effects of TV and bolsters rationale for combining TV with immune checkpoint agents.
- Ongoing analyses aim at further characterizing the immune response induced by TV in preclinical models and patients.

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