DISITAMAB VEDOTIN, AN INVESTIGATIONAL HER2-DIRECTED ANTIBODY-DRUG CONJUGATE, SHOWS POTENT ANTITUMOR ACTIVITY AS A MONOTHERAPY AND IN COMBINATION WITH TUCATINIB IN PRECLINICAL CANCER MODELS

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Background

- Disitamab vedotin (DV, RC48-ADC) is an antibody-drug conjugate (ADC) that targets cancer cells expressing human epidermal growth factor receptor 2 (HER2), an oncogenic growth factor receptor that promotes cell proliferation and survival.
- DV consists of a clinically validated anti-HER2 monoclonal antibody, disitamab, conjugated with the clinically validated microtubule-disrupting agent monomethyl auristatin E (MMAE) via a cleavable linker.
- DV has multimodal antitumor mechanisms of action [1-5]:
  - Direct cytotoxicity of HER2-expressing cancer cells by target-mediated delivery of MMAE
  - Bystander effect based-cytotoxicity of neighboring cells, mediated by release of MMAE from within the targeted cell
  - Released MMAE can induce immunogenic cell death (ICD), which promotes immune cell recruitment to the tumor
  - Fc-gamma receptor mediated antibody-dependent cellular cytotoxicity (ADCC)
  - Inhibition of HER2-activated downstream signaling pathways
- DV is clinically active as a monotherapy and in combination with PD-1 inhibitors. DV was granted FDA Breakthrough Therapy designation for previously treated HER2-positive locally advanced or metastatic urothelial cancer (la/mUC); it is approved in China for la/mUC and metastatic gastric cancer.
- Tucatinib is a HER2-selective oral tyrosine kinase inhibitor approved in combination with trastuzumab and capecitabine for patients with HER2+ metastatic breast cancer and with trastuzumab in colorectal cancer. In vitro, tucatinib has been shown to increase ADC delivery by elevating HER2 protein levels at the cell surface [6].
- In this preclinical study, we investigated the following:
  - The antitumor activity of DV in breast and gastric cancer models across a range of HER2 expression levels, both as a monotherapy and in combination with tucatinib.
  - The mechanism by which dual HER2 targeting with DV in combination with tucatinib improved antitumor outcomes.

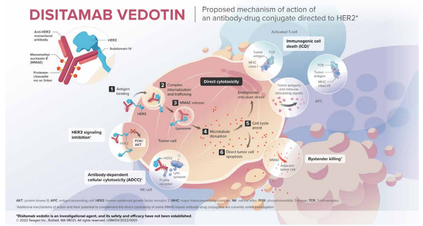

Figure 1. Proposed mechanism of action of disitamab vedotin (DV).

DV has potent cytotoxic activity against breast cancer cells of varying HER2 expression levels

Table 1. In vitro cytotoxicity of DV

Breast Cancer Cell Line	HER2 RPCs	IC50 (ng/mL)[a]		
		T-DM1	T-DXd	DV
SK-BR-3	1,384,000	13	16	**1**
BT-474	864,000	191	>10,000	**3**
UACC-893	776,000	>10,000	>5,000	**9**
HCC-202	304,000	40	>10,000	**2**
JIMIT-1	124,000	1395	>10,000	**238**

DV=disitamab vedotin; RPC=receptor copy number; T-DM1=trastuzumab emtansine; T-DXd=trastuzumab deruxtecan

a DV, as well as T-DM1 and T-DXd (two HER2-targeting ADCs with trastuzumab as a backbone), were screened in a panel of breast cancer cell lines spanning a range of HER2 surface expression (calculated by quantitative flow cytometry). Drugs were titrated across a range of 0.01 – 10,000 ng/mL in 96-hour CellTiter-Glo cytotoxicity assays. Data were analyzed in Prism (GraphPad), and IC50 values (concentration required for 50% inhibition) were generated from best-fit curves.

DV internalization is enhanced compared to trastuzumab and ADCs trastuzumab-emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd)

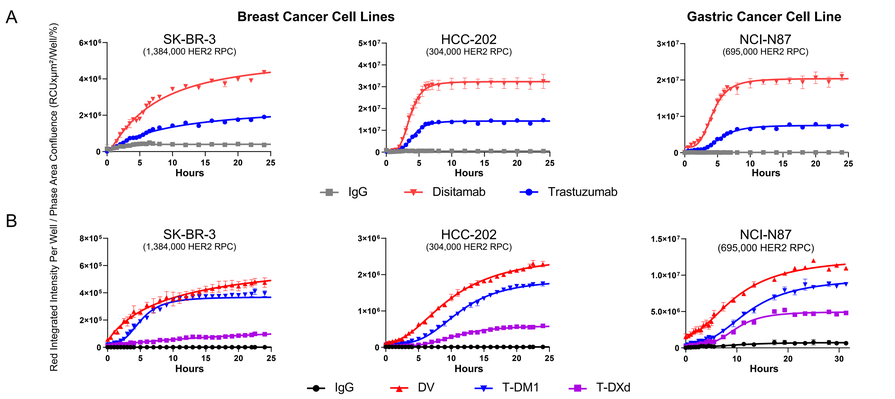

Figure 2. Internalization of HER2 monoclonal antibodies and ADCs in breast and gastric cancer cell lines with varying HER2 expression levels. Internalization assays were conducted using time-lapse microscopy (Incucyte platform) to image breast cancer cells continuously exposed to Fabfluor-labeled (A) mAb or (B) ADCs at a concentration of 1 µg/mL. Fabfluor dye fluoresces only upon exposure to an acidified compartment, namely endocytic vesicles and lysosomes, signifying internalization and intracellular processing of the ADC. Fluorescence intensity was analyzed using Incucyte software; the total fluorescence intensity of the well was divided by the area of confluence, as determined by simultaneous phase contrast imaging.

Tucatinib increases cell surface levels of HER2 and mediates increased internalization of DV across a panel of breast and gastric cancer cell lines

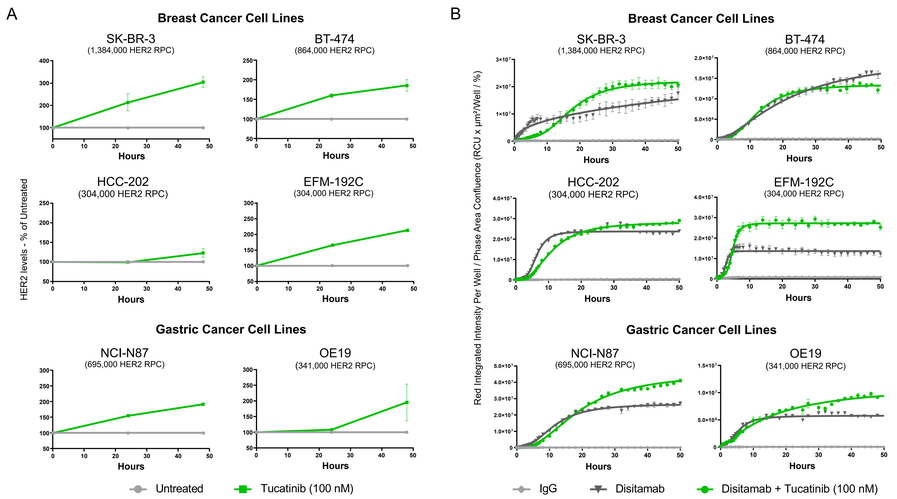

Figure 3. Evaluating mechanisms by which tucatinib may increase the efficacy of DV. (A) HER2 levels of breast and gastric cancer cells lines were measured by qFACS over the course of 48 hours after treatment with tucatinib (100 nM). **(B)** Internalization assays were conducted using time-lapse microscopy (Incucyte platform) to image cancer cells continuously exposed to Fabfluor-labeled disitamab (1 µg/mL) either in the absence or presence of tucatinib (100 nM). Fluorescence intensity was analyzed using Incucyte software; the total fluorescence intensity of the well was divided by the area of confluence, as determined by simultaneous phase contrast imaging.

The combination of DV and tucatinib has enhanced cytotoxicity compared to single agents

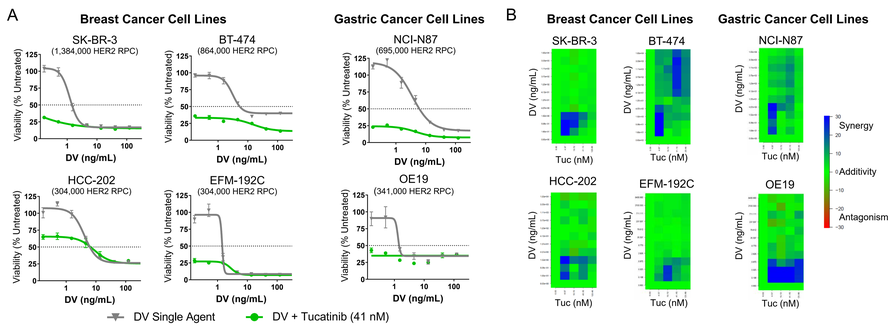

Figure 4. In vitro cytotoxicity assays and isobologram analysis testing the combination of DV with tucatinib. Combinatorial activity was evaluated for DV and tucatinib against breast and gastric cancer cell lines spanning a range of HER2 expression. A drug concentration range spanning 0.1 ng/mL – 6.4 µg/mL for DV and 4.6 – 120 nM for tucatinib was tested across a matrix in 96-hour CellTiterGlo cytotoxicity assays. **(A)** A subset of datapoints showing the titration of DV either in the absence or presence of tucatinib at 41 nM (physiological concentrations of clinical dose) were plotted into best-fit viability curves. **(B)** Isobologram analysis was performed on the readout of cytotoxicity assays by comparing to the highest single agent (HSA) additivity model. Results from the 96-hour timepoint (or 144 hours for EFM-192 cells) were analyzed for synergy (blue) or antagonism (red) and depicted as heatmaps.

The combination of DV and tucatinib shows improved antitumor effects in a subset of HER2-low breast cancer xenograft models

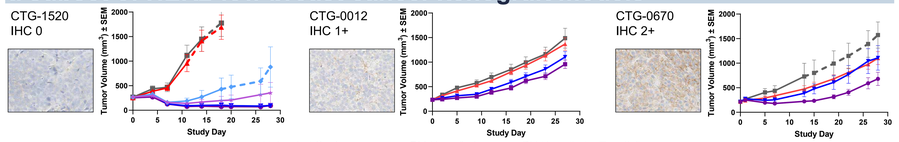

Figure 5. Antitumor activity of DV in combination with tucatinib in HER2-low breast cancer patient-derived xenograft (PDX) models. DV and tucatinib were tested as monotherapies and in combination in three PDX models with varying HER2 expression levels. Mice (n=10/group) were treated with the indicated concentrations of tucatinib (oral, twice daily), DV (intravenous, single dose), or a combination of tucatinib and DV, and tumor volume was measured over time. At sub-maximal doses of DV (1.0 - 2.0 mg/kg), the combination of DV and tucatinib improved tumor growth inhibition in 2 out of 3 models. Dotted lines represent carry-over of tumor volumes from individual animals that were removed from study when their tumors reached maximum allowable size (less than 3/10 animals per group).

Conclusions

- We investigated the differentiating features and the antitumor activity of DV, either as a monotherapy or in combination with tucatinib, in breast and gastric cancer cell lines with a wide range of HER2 expression levels. We demonstrate:
  1. Greater internalization and in vitro activity of DV compared to T-DM1 or T-DXd.
  2. Enhanced cytotoxicity driven by the combination of DV with tucatinib, supporting dual HER2-targeting modalities.
  3. Mechanistically, increased DV internalization in the presence of tucatinib, likely attributed to elevated HER2 levels at the cell surface. This may account for the enhanced direct cytotoxicity observed by the combination of DV with tucatinib.
- These findings provide scientific rationale to continue exploring the clinical activity of DV in HER2-positive and HER2-low breast and gastric cancer settings as a monotherapy, and to investigate the combination with tucatinib.

References

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