San Antonio Breast Cancer Symposium® - December 5-9, 2023

Disitamab vedotin, an investigational HER2-directed antibody-drug conjugate, shows potent antitumor activity as a monotherapy and in combination with tucatinib in preclinical breast cancer models

Kelsi Willis, Katie Snead, Robert Thurman, Renee Hein, Anita Kulukian

Seagen Inc., Bothell, WA USA

Introduction

- 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 microtubule-disrupting agent monomethyl auristatin E (MMAE) via a cleavable linker.
- DV is proposed to have multimodal antitumor mechanisms of action [1-4]:
  1. Direct cytotoxicity of HER2-expressing cancer cells by target-mediated delivery of MMAE
  2. Bystander effect based-cytotoxicity of neighboring cells, mediated by release of MMAE from within the targeted cell
  3. MMAE-induced immunogenic cell death (ICD), which promotes immune cell recruitment to the tumor
  4. 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 conditionally approved in China for HER2-positive (HER2 IHC3+ and 2+) 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. Tucatinib has been shown to increase the delivery of the HER2-targeted ADC trastuzumab emtansine (T-DM1) by elevating HER2 protein levels at the cell surface [5].
- In this preclinical study, we investigated the following:
  - The antitumor activity of DV in 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 and tucatinib improved antitumor outcomes.

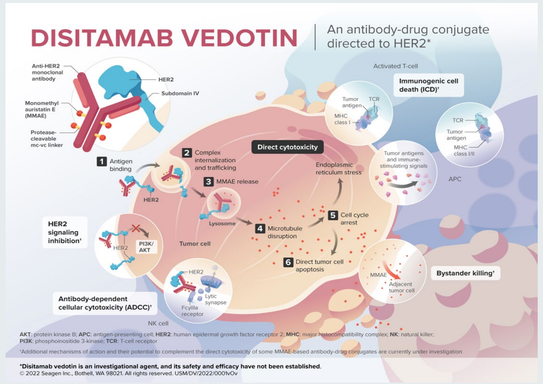

Figure 1. Proposed mechanism of action for disitamab vedotin.

Disclosures
Seagen Inc. provided research funding. KW, KS, RT, RH, and AK are employees of and have equity ownership in Seagen Inc

Disitamab differentiates from trastuzumab by enhanced internalization

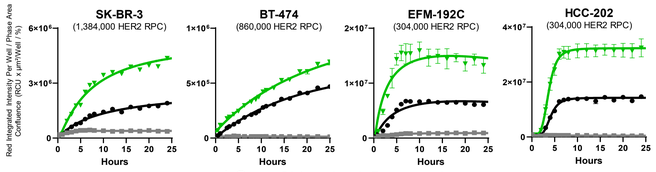

Figure 2. Evaluating differences in internalization between disitamab and trastuzumab. Internalization assays were conducted using time-lapse microscopy (Incucyte platform) to image breast cancer cells continuously exposed to Fabfluor-labeled monoclonal antibody (mAb) at a concentration of 1 µg/mL. Fabfluor dye fluoresces only upon exposure to an acidified compartment, namely endocytic vesicles and lysosomes, signifying internalization and intracellular processing of the mAb. 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. RPC = Receptor Per Cell, determined by quantitative flow cytometry.

DV has potent cytotoxic activity as a monotherapy in 2D breast cancer cell lines and in patient-derived tumor organoids with varying HER2 expression levels, including HER2-low tumors

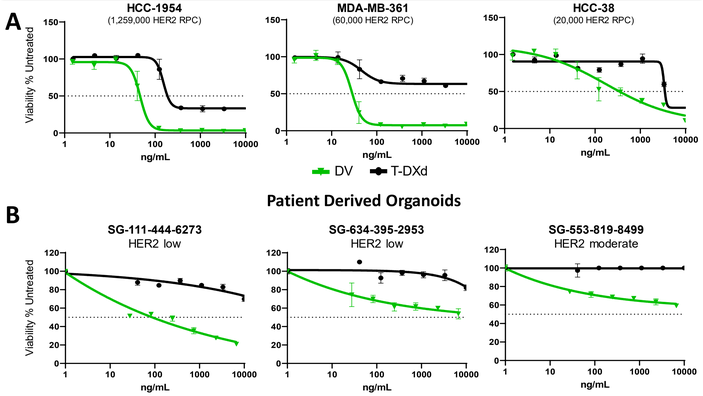

Figure 3. In vitro cytotoxicity assays in 2D breast cancer cell lines and in patient-derived organoids (PDOs) testing DV and other trastuzumab-based ADCs. (A) DV and trastuzumab deruxtecan (T-DXd) were evaluated against breast cancer cell lines spanning a range of HER2 expression levels (calculated by quantitative flow cytometry). Drugs were titrated (1 – 10,000 ng/mL), and viability was tested in a 144-hour CellTiter-Glo cytotoxicity assay. **(B)** DV and T-DXd were tested against a panel of PDOs. DV was titrated across a range of 27 - 6,700 ng/mL, while T-DXd was titrated across a range of 41 – 10,000 ng/mL. Drug and media was exchanged every four days. Analysis was conducted with fluorescence imaging: PDOs were identified from clusters of Hoechst positive cells, caspase-3/7 was used for the identification of apoptotic cells. Mean caspase-3/7 intensity was measured per well and normalized to cell count. *T-DXd data from day 12; DV data from day 8. Data shown as mean ± SEM.

Table 1. In vitro cytotoxicity of DV in breast cancer cell lines

Breast Cancer Cell Line	HER2 RPCs	IC50 (ng/mL)		
		T-DM1	T-DXd	DV
SK-BR-3	1,384,000	13	16	1
BT-474	864,000	191	>10000	3
UACC-893	776,000	>10000	>5000	9
HCC-202	304,000	40	>10000	2
JIMT-1	124,000	1395	>10000	238

DV, T-DM1, and T-DXd were screened in a panel of breast cancer cell lines spanning a range of HER2 surface expression. 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.

A single dose of DV shows antitumor effects in a dose-dependent manner in a subset of HER2-low breast cancer xenograft models

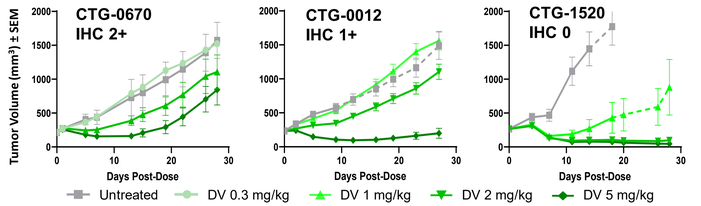

Figure 4. Antitumor activity of DV as a monotherapy in HER2-low breast cancer patient-derived xenograft (PDX) models. DV was tested as a monotherapy in 3 PDX models with varying HER2 expression levels (IHC images in Figure 7). Mice (n=10/group) were treated with the indicated concentrations DV (intravenous, single dose) and tumor volume was measured over time. Dashed 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).

Tucatinib increases cell surface levels of HER2 and mediates increased internalization of DV in a subset of breast cell lines

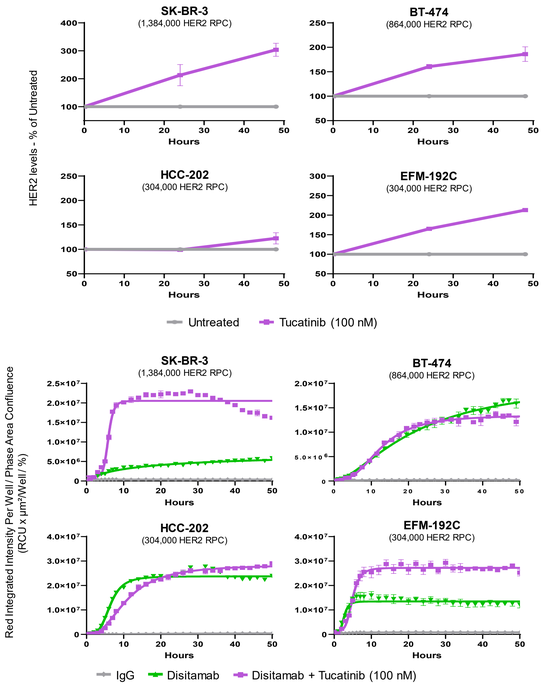

Figure 5. Evaluating mechanisms by which tucatinib may increase the efficacy of DV. (A) HER2 levels of breast cells lines were measured by quantitative flow cytometry at 24 and 48 hours after treatment with tucatinib (100 nM). **(B)** Internalization assays were conducted using time-lapse microscopy (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. Data shown as mean ± SEM.

The combination of DV and tucatinib shows synergistic cytotoxicity in a panel of breast cancer cell lines

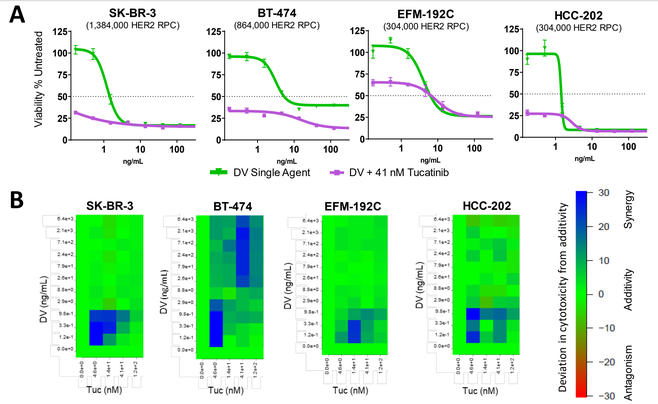

Figure 6. In vitro cytotoxicity assays and isobologram analysis testing the combination of DV with tucatinib. Combinatorial activity was evaluated for DV and tucatinib against breast cancer cell lines spanning a range of HER2 expression. A drug concentration range spanning 0.1 ng/mL – 6.4 µg/mL for DV and 4.6 – 120 nM for tucatinib was tested across a matrix of dose combinations 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 matrix of cytotoxicity values 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 activity with subtherapeutic doses of DV in a subset of HER2-low breast cancer xenograft models

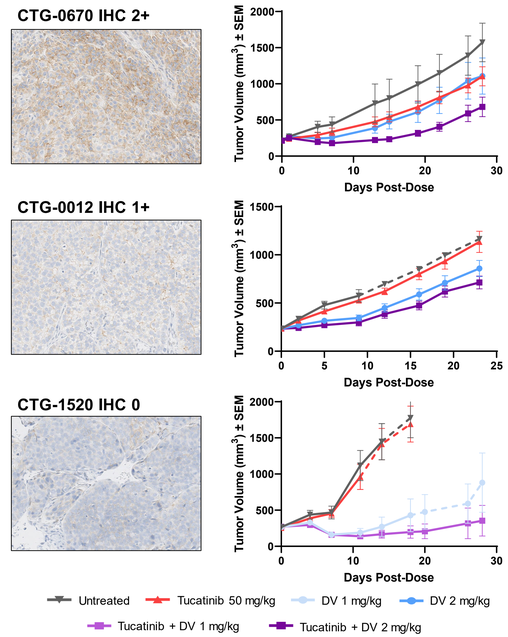

Figure 7. Antitumor activity of DV in combination with tucatinib in HER2-low breast cancer PDX models. DV and tucatinib were tested as monotherapies and in combination in 3 PDX models with varying HER2 expression levels. Mice (n=10/group) were treated with the indicated concentrations of tucatinib (oral, 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. Dashed 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 cancer cell lines, patient-derived organoids and patien-derived xenograft models with a wide range of HER2 expression levels. We demonstrate the following:

1. Disitamab has greater internalization compared to trastuzumab.
2. DV is potently cytotoxic in breast cancer cell lines and PDOs spanning a range of HER2 expression levels. In all PDOs DV had greater activity than T-DXd, and in all 2D cell culture models DV was comparable to, if not more potent than, T-DM1 and T-DXd.
3. DV has potent dose-dependent activity in breast cancer xenograft models with HER2-low expression.
4. Mechanistically, increased DV internalization in the presence of tucatinib is 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 cancer settings as a monotherapy and to investigate the combination with tucatinib.

References

1. Jiang J, Dong L, Wang L, et al. (2016). Eur J Pharm Sci 93:274–86.
2. Li L, Xu MZ, Wang L, et al. (2020). Eur Rev Med Pharmacol Sci 24:12929–37.
3. Yao X, Jiang J, Wang X, et al. (2015). Breast Cancer Res Treat.153(1):123-33.
4. Li F, Emmerton KK, Jonas M, et al. (2016). Cancer Res. 76(9):2710-2719.
5. Olson D, Taylor J, Willis K, et al. (2023). Cancer Res Commun. 3(9):1927-1939