Poster 2160

Trastuzumab deruxtecan induces immunogenic cell death, immune cell activation and migration in viable human breast cancer slices

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Objective

- To elucidate the mechanism of action (MoA) of the antibody-drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) in ex vivo culture of primary breast cancer patient tissue

Conclusions

- T-DXd was internalized into tumor cells, induced immunogenic tumor cell death, activation of immune cells (T cells and macrophages), pro-inflammatory cytokine release and immune cell migration towards the tumor cells in HER2-positive and HER2-low breast cancer tissue slices.
- Viable human tumor slice culture of breast cancer patient samples proved to be a suitable model system for elucidating the MoA of T-DXd and potentially other ADCs.

Plain language summary

Why did we perform this research?

We wanted to verify how T-DXd works in human breast cancer patient samples including whether it can activate the immune system.

How did we perform this research?

Fresh human breast cancer tissue obtained from surgeries was cut into thin slices, cultured in the presence of T-DXd, and analyzed to find out whether T-DXd caused tumor cells to die in a way that activates the immune system.

What were the findings of this research?

T-DXd was taken up into cancer cells that express the T-DXd target protein HER2, causing these cancer cells to die in a way that triggers an immune response. T-DXd also activated immune cells already present in the tumor, like T cells and macrophages, and attracted more immune cells into the tumor tissue.

What are the implications of this research?

The results show that T-DXd can activate the immune system in human tumor samples, similar to what was seen in studies with mice[1]. This suggests that it is worth exploring whether patients would benefit even more from combination treatment of T-DXd with immunomodulatory drugs.

Where can I access more information?

Information about T-DXd can be found here: www.enhertu.com

[1]Iwata et al., Mol Cancer Ther 2018 17 (7): 1494–1503]

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Introduction

- Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate composed of a humanized immunoglobulin G1 monoclonal antibody specifically targeting HER2, a tetrapeptide-based cleavable linker, and a potent topoisomerase I inhibitor payload (DXd, an exatecan derivative)[1].
- T-DXd is approved for several indications including adult patients with HER2-positive, HER2-low, and HER2-ultralow breast cancers[2].
- Immune activation by T-DXd was reported in mouse models[3-5] and combination treatment of T-DXd and immune checkpoint inhibitors is under investigation in clinical studies[6-8]. Still, immunostimulatory effects of T-DXd have not been confirmed in breast cancer patient samples.
- We hypothesize that T-DXd can induce an anti-tumor immune response in patients' tumors.

Methods

- Viable human tumor slices (VHTS) were prepared from surgically removed human breast cancer tissue with a vibratome and cultured for up to five days with substances or left untreated. After culture, slices were dissociated enzymatically and subjected to flow cytometry (FCM) analysis, while supernatants were analyzed by LEGENDplex™ cytokine assays, HMGB1 ELISA or transwell migration assay (Figure 1).
- For each readout, we analyzed three to seven samples from an overall pool of five HER2-positive and 16 HER2-low patient samples (three pretreated with chemotherapy). HER2 classification was performed in-house via IHC and FISH analysis following ASCO-CAP guidelines.
- To analyze internalization, VHTS were cultured overnight in presence of fluorescently labeled T-DXd or control IgG (10-15 µg/ml).
- For immunogenic cell death and immune modulation analysis, VHTS were cultured for 3-5 days in the presence of T-DXd (15 or 150 µg/ml), DXd (30 or 100 nM) or left untreated and analyzed by multi-color FCM (Table 1).
- Induction of monocytic cell migration was analyzed by subjecting supernatants from treated versus untreated HER2-expressing SK-BR-3 cells or VHTS to transwell migration assays with the monocytic cell line THP-1.
- Immune cell infiltration into VHTS was analyzed by co-culturing VHTS with autologous, CellTracker™ Green-labeled PBMCs for three days followed by quantification of CellTracker™-positive infiltrated immune cell populations in dissociated VHTS by FCM.
- Statistical significance was calculated by two-sided, unpaired t-test. *p<0.05, **p<0.01, ***p<0.001

Figure 1: VHTS culture and analysis

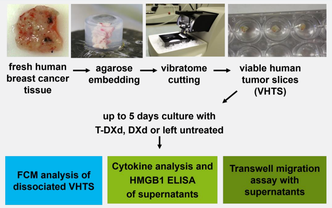
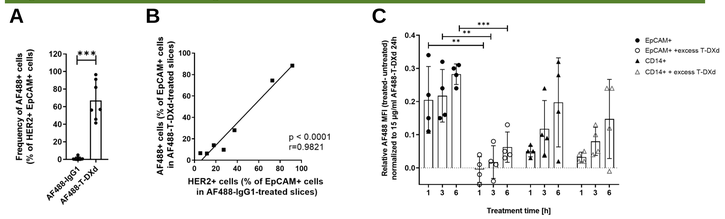


Table 1: FCM antibody panel

marker	function	marker (continued)	function (continued)
EpCAM	Tumor cell marker	HLA-DR	MHC class II
CD45	Immune cell marker	CD69	T cell activation marker
CD3	T cell lineage marker	PD-1	T cell exhaustion marker
CD4	T cell lineage marker	LAMP-1 (CD107a)	T cell activation marker
CD8	T cell lineage marker	Granzyme B	T cell activation marker
CD19	B cell lineage marker	CD137	T cell activation marker
CD14	Macrophage lineage marker	PD-L1	Immune checkpoint
CD56 + NKP46	NK cell lineage markers	CD86	Myeloid cell activation marker
CD11c	DC lineage marker	Active caspase-3	Apoptosis marker
HLA-ABC	MHC class I	HER2	T-DXd target/ growth factor receptor

Results

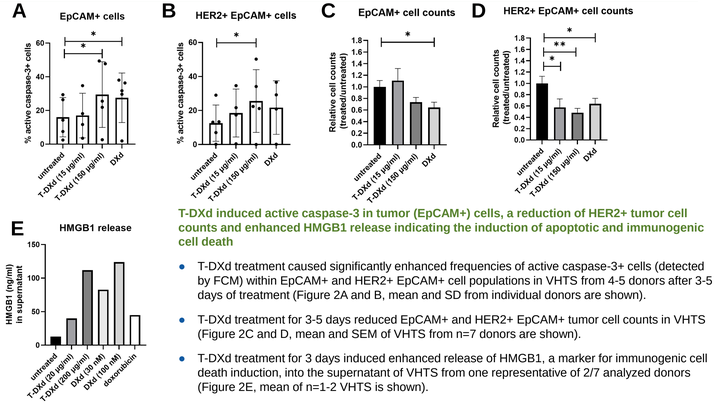
Figure 1. T-DXd showed target-dependent internalization into tumor cells



T-DXd was internalized target-dependently into tumor cells

- AF488-labeled T-DXd was significantly internalized into HER2+ EpCAM+ tumor cells in VHTS from n=7 donors overnight compared to control-IgG1 (Figure 1A, mean and SD from individual donors are shown).
- AF488-T-DXd uptake into EpCAM+ tumor cells showed significant positive correlation with the frequency of HER2+ cells among EpCAM+ cells in VHTS from n=7 donors (Figure 1B).
- AF488-T-DXd internalization into tumor cells was significantly reduced when VHTS were pre-incubated with excess unlabeled T-DXd for 30 min, while internalization into macrophages was only slightly affected (Figure 1C, mean and SD of n=4 donors are shown).

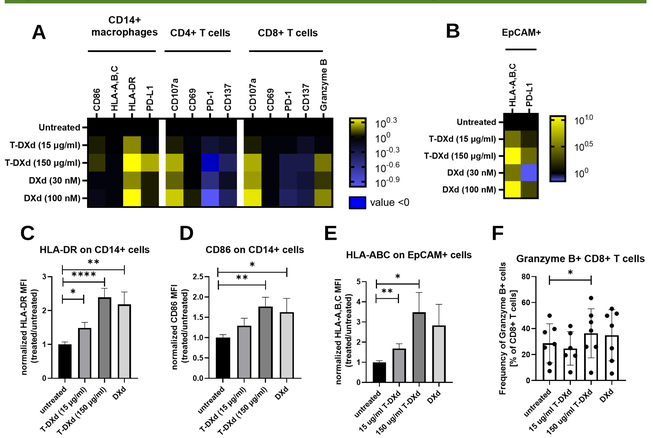
Figure 2. T-DXd induced apoptotic and immunogenic cell death of tumor cells



T-DXd induced active caspase-3 in tumor (EpCAM+) cells, a reduction of HER2+ tumor cell counts and enhanced HMGB1 release indicating the induction of apoptotic and immunogenic cell death

- T-DXd treatment caused significantly enhanced frequencies of active caspase-3+ cells (detected by FCM) within EpCAM+ and HER2+ EpCAM+ cell populations in VHTS from 4-5 donors after 3-5 days of treatment (Figure 2A and B, mean and SD from individual donors are shown).
- T-DXd treatment for 3-5 days reduced EpCAM+ and HER2+ EpCAM+ tumor cell counts in VHTS (Figure 2C and D, mean and SEM of VHTS from n=7 donors are shown).
- T-DXd treatment for 3 days induced enhanced release of HMGB1, a marker for immunogenic cell death induction, into the supernatant of VHTS from one representative of 2/7 analyzed donors (Figure 2E, mean of n=1-2 VHTS is shown).

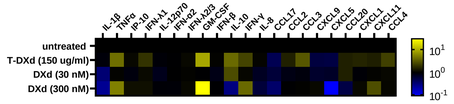
Figure 3. T-DXd enhanced immune cell activation and antigen presentation



T-DXd induced upregulation of activation markers on immune cells (T cells, CD14+ macrophages) and enhanced expression of antigen-presenting molecules on macrophages and tumor cells (EpCAM+)

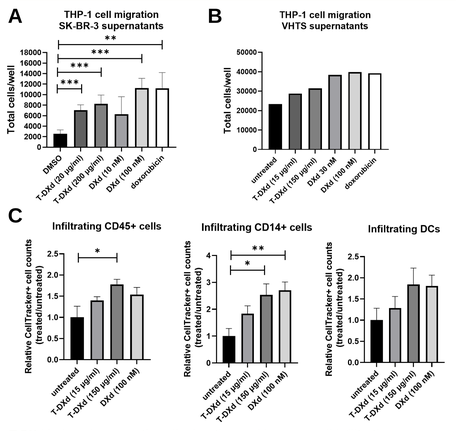
- T-DXd treatment for 5 days increased expression of activation/exhaustion markers and antigen-presenting molecules on immune cells (Figure 3A) and of HLA-ABC and PD-L1 on tumor cells (Figure 3B) in VHTS from one representative of n=7 donors.
- T-DXd treatment for 3-5 days significantly and concentration-dependently enhanced HLA-DR and CD86 expression on CD14+ macrophages (Figure 3C and D) and HLA-ABC on tumor cells (Figure 3E) (mean and SEM of VHTS from n=6-7 donors are shown) and significantly increased the frequency of Granzyme B+ CD8+ tumor-resident T cells (Figure 3F, mean and SD of n=6-7 donors are shown).

Figure 4. T-DXd induced pro-inflammatory cytokine release



T-DXd and DXd caused enhanced release of pro-inflammatory cytokines such as GM-CSF, TNFα, and IFN-γ as fold change of untreated VHTS in one representative of eight individual donors (5 days of treatment).

Figure 5. T-DXd induced immune cell migration



T-DXd induced monocytic cell migration and immune cell infiltration

- Monocytic THP-1 cells were significantly attracted towards supernatants of HER2-expressing SK-BR-3 cells treated with T-DXd, DXd and doxorubicin for 48h compared to untreated cells (Figure 5A, n=4) and towards supernatants from 3-5 days treated VHTS from one representative of 3/7 analyzed donors (Figure 5B, mean of n=1-2 VHTS).
- T-DXd significantly enhanced infiltration of VHTS by CellTracker Green™-labeled CD45+ and CD14+ cells and slightly by DCs in VHTS co-cultured with labeled PBMCs in presence of T cell TransAct™ for 3 days (Figure 5C, mean and SD of n=3 donors).

Conclusions

- T-DXd was target-dependently internalized into tumor cells.
- T-DXd induced immunogenic tumor cell death as seen by caspase-3 activation and release of HMGB1.
- T-DXd led to activation of immune cells (T cells and macrophages), release of pro-inflammatory cytokines and immune cell migration towards the treated tumor cells in HER2-positive and HER2-low breast cancer slices.
- These findings support further investigation of T-DXd in combination with immuno-oncology drugs in patients.

Abbreviations

antibody-drug conjugate (ADC), dendritic cells (DCs), exatecan derivative (DXd), enzyme-linked immunosorbent assay (ELISA), flow cytometry (FCM), fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), mechanism of action (MoA), NK (natural killer cells), PBMC (peripheral blood mononuclear cells), Trastuzumab deruxtecan (T-DXd), viable human tumor slices (VHTS)

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Disclosures

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M. Ikuta and T. Deguchi are employees of Daiichi Sankyo Co., Ltd..

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