Preclinical Assessment of Valemetostat, a Dual Inhibitor of EZH2 and EZH1, Combined With Trastuzumab Deruxtecan and Datopotamab Deruxtecan for Multiple Solid Tumors

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Disclosure Information

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Daisuke Honma

I have the following relevant financial relationships to disclose:

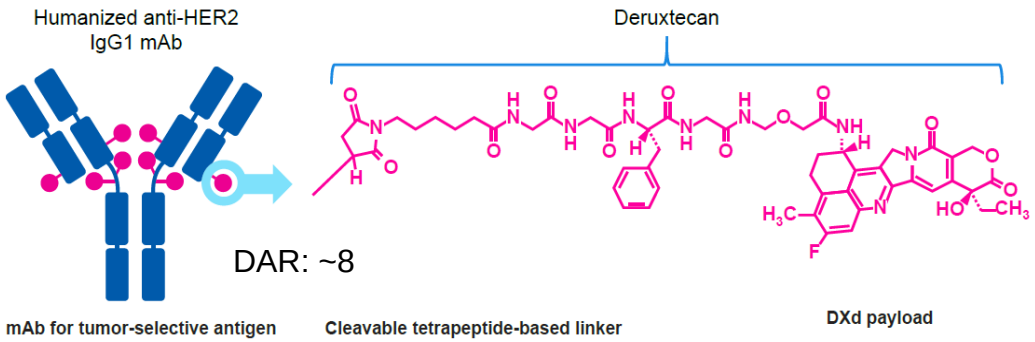
Employee of: Daiichi Sankyo

My additional financial relationship disclosures are:

Patent title: Combination of antibody drug–conjugate with EZH1 and/or EZH2 inhibitor (WO 2023/209591)

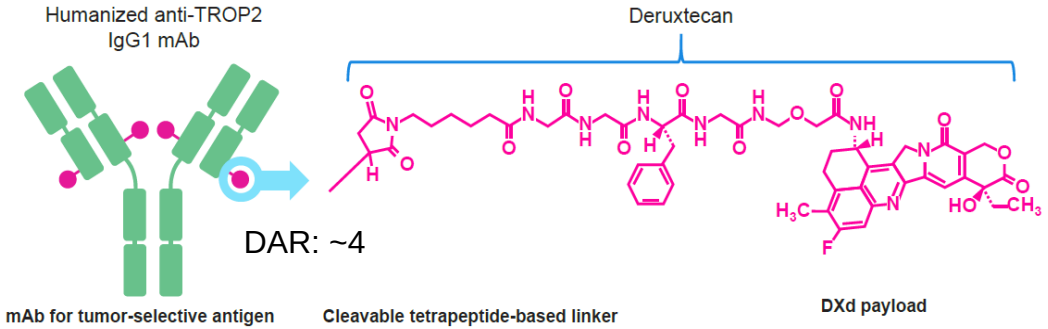
T-DXd and Dato-DXd in solid tumors

T-DXd



- **T-DXd:** HER2-directed ADC with a humanized anti-HER2 mAb, cleavable tetrapeptide-based linker, and DXd payload[1–3]
  - Approved as tumor-agnostic HER2-directed therapy for patients with metastatic HER2+ solid tumors, including BC, GC, and NSCLC[4]
  - Approved for treatment of patients with HER2-low or HER2-ultralow metastatic BC[5]

Dato-DXd

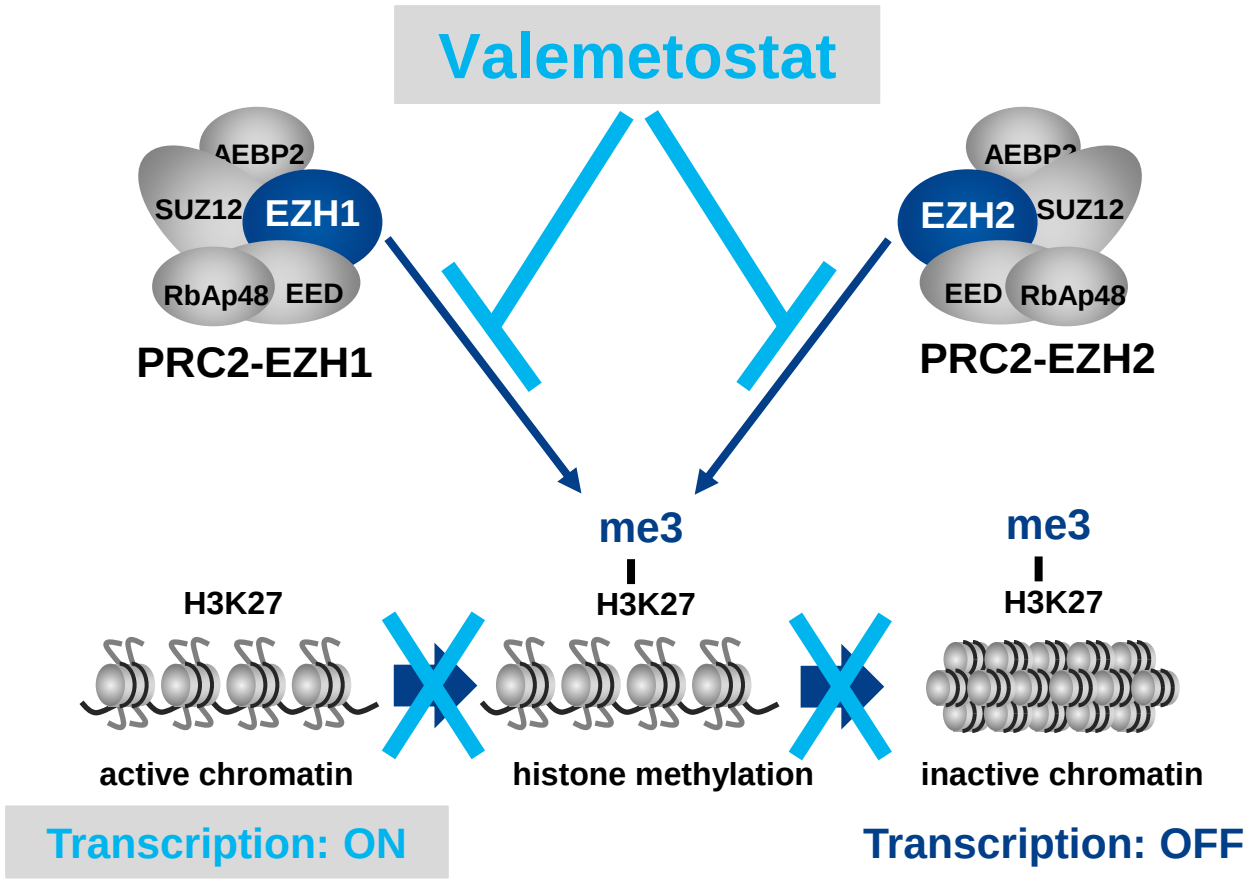


- **Dato-DXd:** TROP2-directed ADC composed of a humanized anti-TROP2 mAb, plasma-stable tetrapeptide-based cleavable linker, and DXd payload[6]
  - Significantly prolonged PFS vs docetaxel in previously treated advanced/metastatic NSCLC, driven by nonsquamous histology[7]
  - Approved treatment of for patients with previously treated, metastatic, HR+/HER2– BC[8]

ADC, antibody–drug conjugate; BC, breast cancer; DAR, drug-to-antibody ratio; Dato-DXd, datopotamab deruxtecan; DXd, topoisomerase I inhibitor payload; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IgG1, immunoglobulin 1; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; PFS, progression-free survival; T-DXd, trastuzumab deruxtecan; TROP2, trophoblast cell surface antigen 2.

1. Nakada T, et al. *Chem Pharm Bull (Tokyo)* 2019;67:173–185. 2. Ogitani Y, et al. *Clin Cancer Res* 2016;22:5097–5108. 3. Ogitani Y, et al. *Cancer Sci* 2016;107:1039–1046. 4. Daiichi-Sankyo. Press release April 5, 2024. ENHERTU® Approved in the U.S. as first tumor agnostic HER2 directed therapy for previously treated patients with metastatic HER2 positive solid tumors. https://daiichisankyo.us/documents/364091/14382402/PP-US-EN-2479_ENHERTU+Tumor+Agnostic+FDA+Approval_DS+Press+Release.pdf/a0929569-7bb0-75ff-afb5-5ac3588a394b. Accessed March 25, 2025. 5. Daiichi-Sankyo. Press release January 27, 2025. ENHERTU® approved in the US as first HER2-directed therapy for patients with HER2-low or HER2-ultralow metastatic breast cancer following disease progression after one or more endocrine therapies. https://www.daiichisankyo.com/files/news/pressrelease/pdf/202501/20250127_E.pdf. Accessed March 25, 2025. 6. Okajima D, et al. *Mol Cancer Ther* 2021;20:2329–2340. 7. Ahn MJ, et al. *J Clin Oncol* 2025;43:260–272. 8. Daiichi-Sankyo. Press release January 17, 2025. DATROWAY® approved in the US for patients with previously treated metastatic HR-positive, HER2-negative breast cancer. https://www.daiichisankyo.com/files/news/pressrelease/pdf/202501/20250117_E.pdf. Accessed March 25, 2025.

Valemetostat

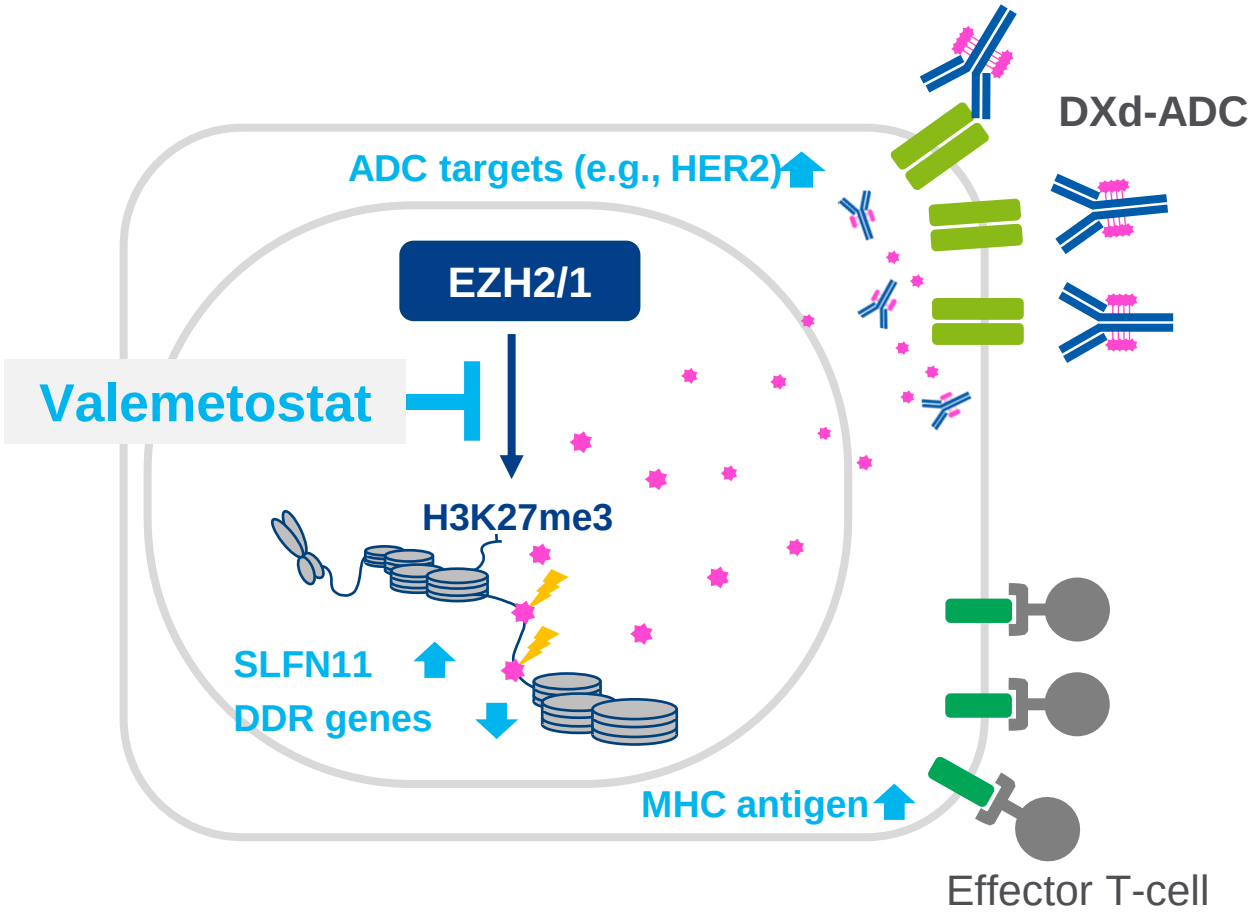


- Valemetostat is an oral, potent, and selective dual inhibitor of histone methyltransferases EZH2 and EZH1,[1–5] approved in Japan for treatment of R/R PTCL and ATLL[6]
- EZH1 and EZH2 are key subunits of the PRC2, that catalyze the attachment of 3 methyl groups to H3K27[7–11]
- H3K27me3 is a repressive transcriptional mark that plays a key role in tumor cell proliferation and migration, tumor stem cell maintenance, and cellular differentiation in multiple tumor types[7,9]
- Valemetostat prevents H3K27me3, altering gene expression patterns that attenuate the proliferation of EZH1/EZH2-dependent cancer cells

AEBP2, adipocyte enhancer-binding protein 2; ATLL, adult T-cell leukemia/lymphoma; EED, embryonic ectoderm development; EZH, enhancer of zeste homolog; H3K27, histone H3 lysine 27; H3K27me3, trimethylation of H3K27; me3, trimethylation; PRC2, polycomb repressive complex 2; PTCL, peripheral T-cell lymphoma; R/R, relapsed/refractory; RbAp48, histone-binding protein RBBP4; SUZ12, suppressor of zeste 12.

1. Honma D, et al. *Cancer Sci* 2017;108:2069–2078. 2. Nakagawa M, et al. *Cancer Sci* 2019;110:194–208. 3. Yamagishi M, et al. *Cell Rep* 2019;19;29:2321–2337. 4. Kagiyama Y, et al. *Cancer Sci* 2021;112:2314–2324. 5. Wu G, et al. *Sci Transl Med* 2023;15:eadi7244. 6. EZHARMIA® (valemetostat tosilate) [package insert]. Tokyo, Japan: Daiichi Sankyo; 2024. 7. Herviou L, et al. *Oncotarget* 2016;7:2284–2296. 8. Xu B, et al. *Exp Hematol* 2015;43:698–712. 9. Juan AH, et al. *Cell Rep* 2016;17:1369–1382. 10. Peirs S. *Immunol Rev* 2015;263:50–67. 11. Shen X, et al. *Mol Cell* 2008;32:491–502.

Rationale for combining valemetostat with DXd-ADCs



Proposed mechanisms

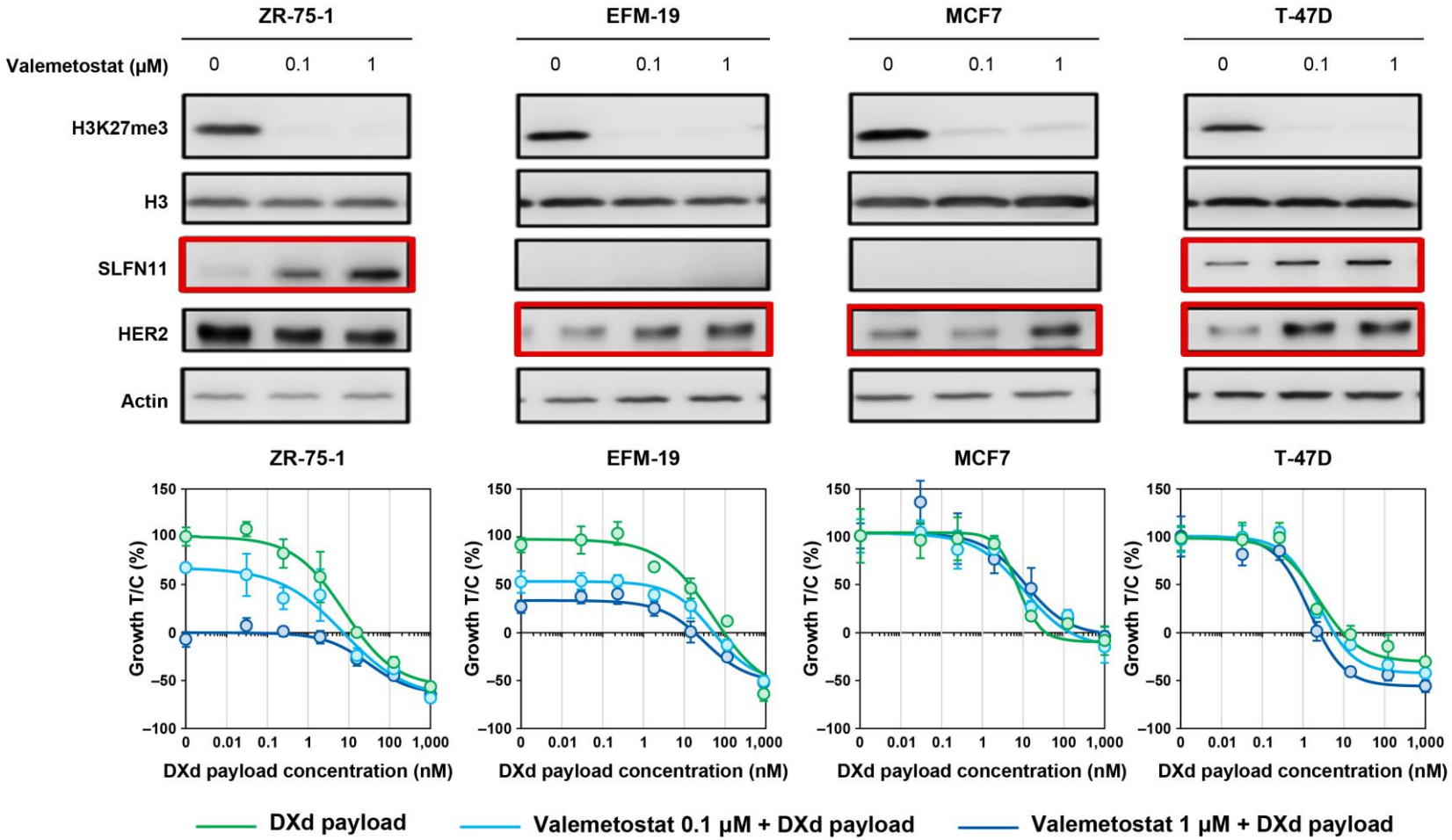
- **Increased ADC Antitumor Activity**
  - **Increased target expression:** enhancing ADC accessibility to cancer cells
  - **Induction of SLFN11:** causing a persistent replication block and inducing apoptosis[1]
  - **DDR genes downregulation:** inhibiting DNA damage repair activity
- **Enhanced Immune Response**
  - **Increased neoantigen presentation**: activating anti-tumor immune response

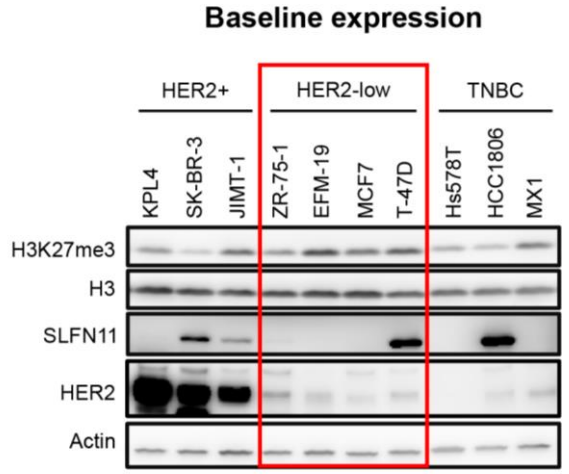
Purpose: Investigate the activity of valemetostat in combination with T-DXd or Dato-DXd in non-clinical models of BC, GC, and NSCLC

ADC, antibody–drug conjugate; BC, breast cancer; Dato-DXd, datopotamab deruxtecan; DDR, DNA damage repair; DXd, topoisomerase I inhibitor payload; EZH, enhancer of zeste homolog; GC, gastric cancer; H3K27me3, trimethylation of histone H3 at lysine 27; HER2, human epidermal growth factor receptor 2; MHC, major histocompatibility complex; NSCLC, non-small-cell lung cancer; SLFN11, DNA/RNA helicase Schlafen 11; T-DXd, trastuzumab deruxtecan. 1. Gardner EE, et al. *Cancer Cell* 2017;31:286–299.

H3K27me3 inhibition and expression of SLFN11 and HER2 in HER2-low BC cell lines

- Levels of H3K27me3, histone H3, SLFN11, HER2, and beta-actin were assessed by western blot analysis using specific antibodies in HER2-low BC cell lines treated with valemetostat for 7 days





Valemetostat decreased H3K27me3 and upregulated the expression of SLFN11 and HER2 in a cell-line-specific manner

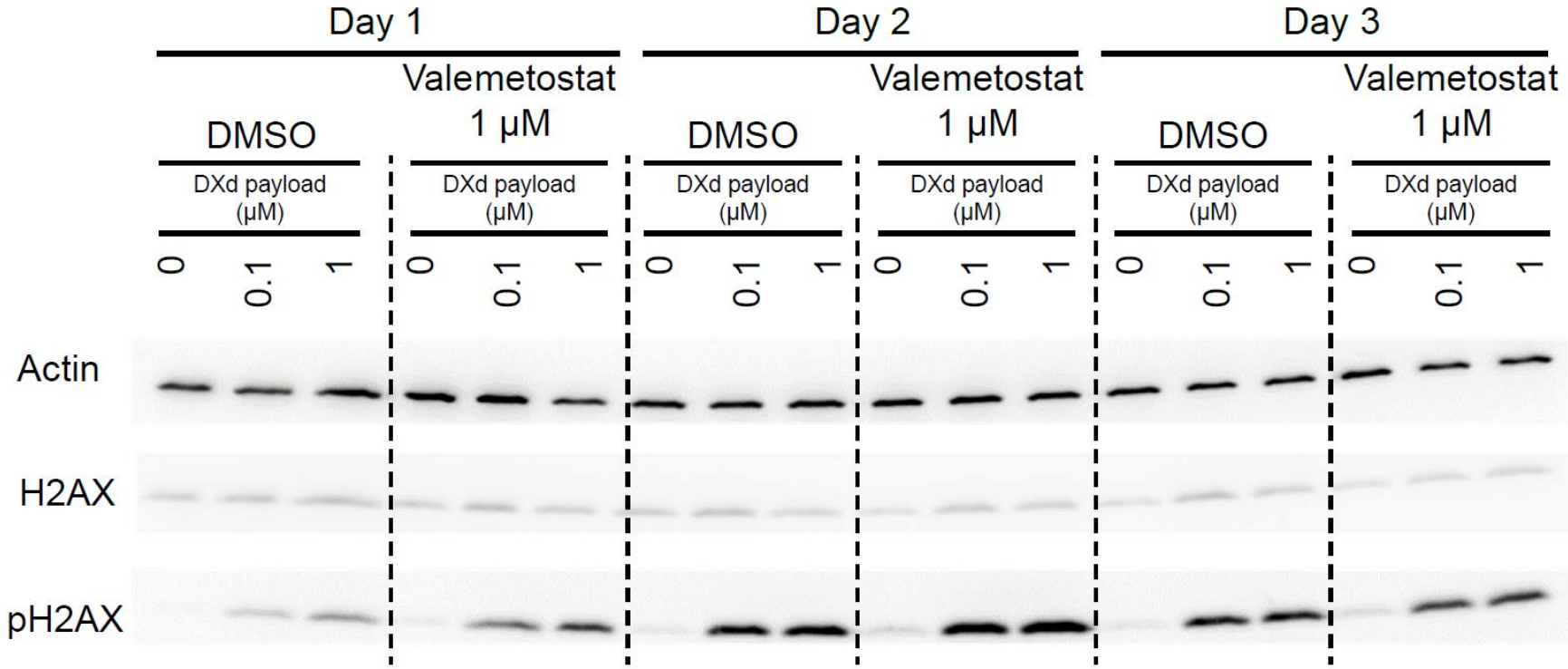
Tumor growth inhibition with valemetostat plus DXd increased with DXd payload concentration

Cells were pre-treated with valemetostat 0.1 μM or 1 μM, or DMSO for 7 days, then re-seeded on day 7 and re-treated with valemetostat 0.1 μM or 1 μM, or DMSO; on day 8 all cells were treated with increasing concentration of DXd payload. Concentration-dependent growth inhibitory activity was assessed on day 11.

BC, breast cancer; DSMO, dimethyl sulfoxide; DXd payload, topoisomerase I inhibitor payload; H3, histone 3; H3K27me3, trimethylation of histone H3 at lysine 27; HER2, human epidermal growth factor receptor 2; SLFN11, DNA/RNA helicase Schlafen 11; T/C, treatment-to-control ratio; TNBC, triple negative breast cancer.

In vitro assessment of H2AX phosphorylation in GC cell lines

- The expressions of beta-actin, H2AX, and pH2AX after treatment with DXd payload +/- valemetostat were assessed by western blot analysis using specific antibodies in GC cell lines on 3 consecutive days



Valemetostat plus DXd payload promoted a transient increased phosphorylation of H2AX (a marker of DNA damage) vs DXd alone, activating the DDR pathway

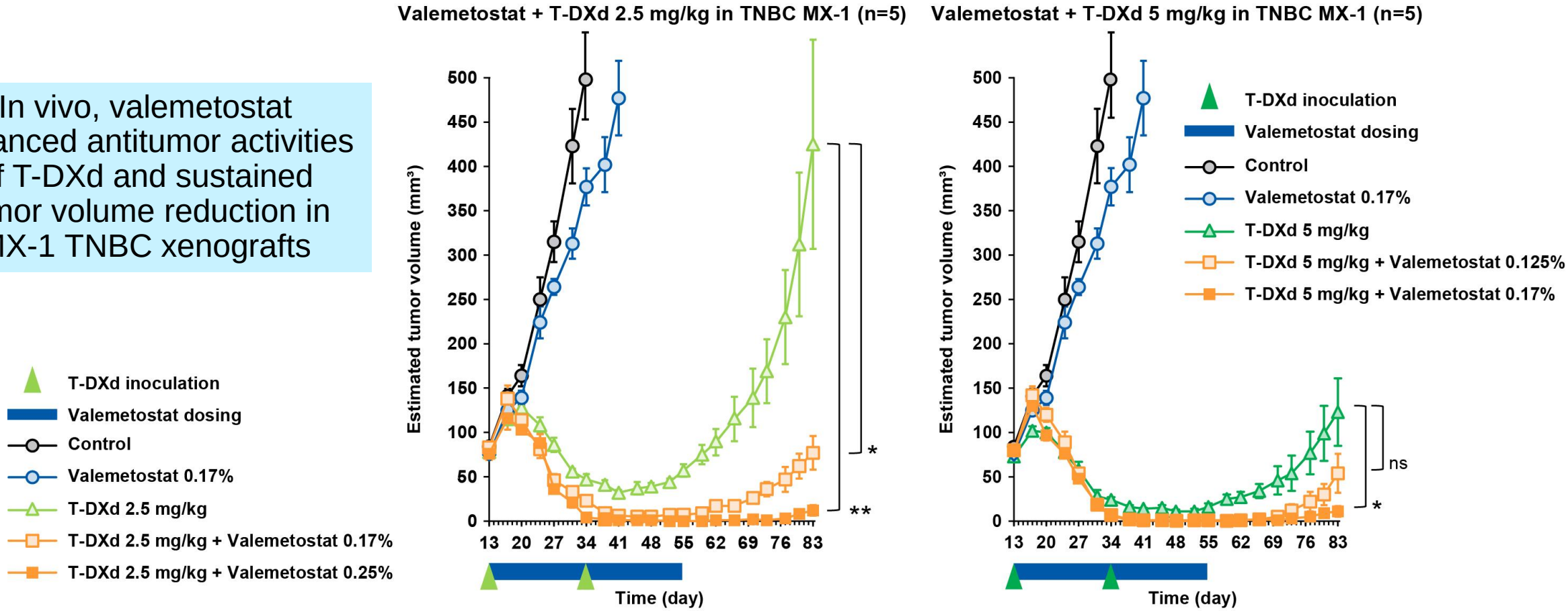
NCI-N87 cells were pre-treated with valemetostat 1 μM or DMSO for 7 days (day –8 to –1), re-seeded on day –1, and re-treated with valemetostat 1 μM or DMSO; on day 0 cells were treated with 0.1 μM or 1 μM of DXd payload, or DMSO. Protein expression was assessed on day 1, 2, and 3.

DDR, DNA damage repair; DMSO, dimethyl sulfoxide; DXd payload, topoisomerase I inhibitor payload; GC, gastric cancer; H2AX, H2A histone family member X; pH2AX, phosphorylated H2AX.

Antitumor activity of valemetostat plus T-DXd in TNBC xenograft models

- The in vivo combined effect of valemetostat and T-DXd was assessed in subcutaneously xenografted mice bearing TNBC MX-1 cell lines

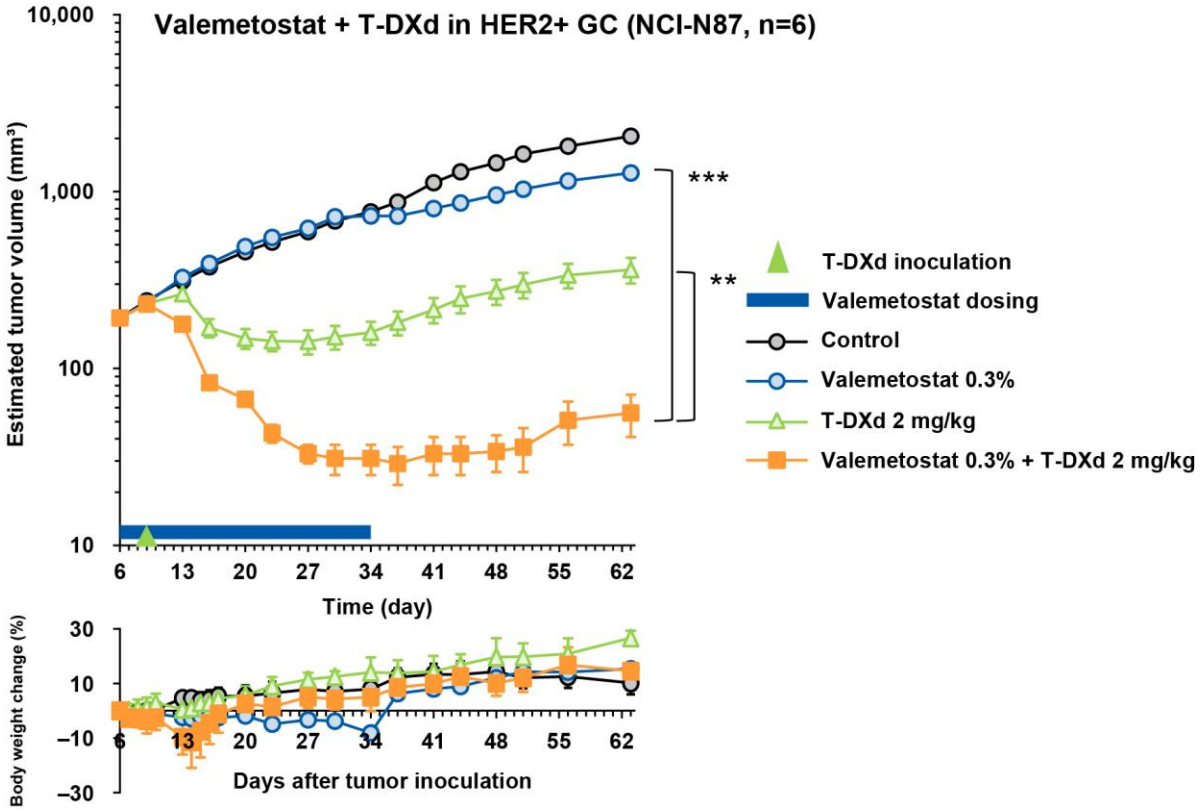
In vivo, valemetostat enhanced antitumor activities of T-DXd and sustained tumor volume reduction in MX-1 TNBC xenografts

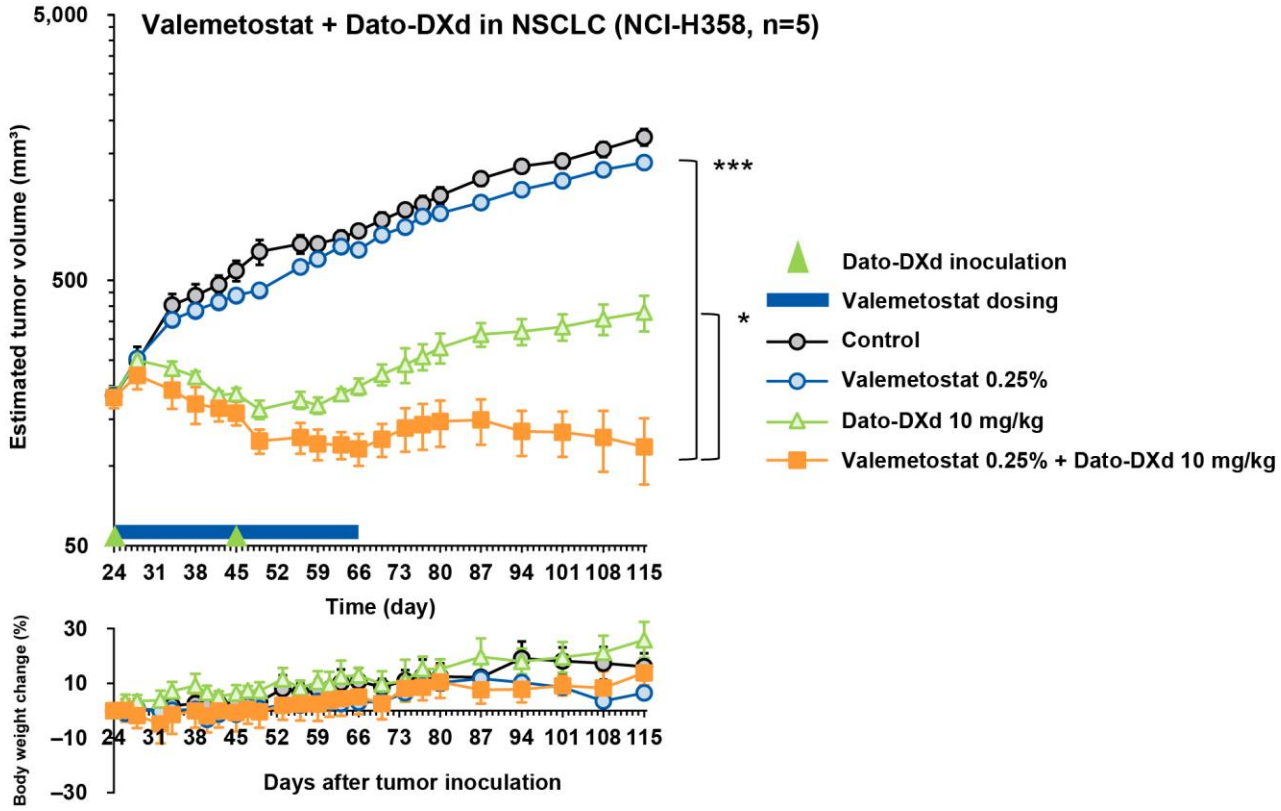


Valemetostat (0.125%, 0.17%, or 0.25%) was administered daily with food (ad libitum) for 6 weeks; T-DXd (2.5 mg/kg or 5 mg/kg) was concurrently administered intravenously twice, Q3W. Combination activities were assessed by Dunnett's test: *P < .05; **P < .01. Statistical significances between valemetostat monotherapy and other treatment groups are not shown in this figure but all data were significant (P < .0001). All treatment groups showed less than 5% of body weight loss rate.
ns, not significant; Q3W, every 3 weeks; T-DXd, trastuzumab deruxtecan; TNBC, triple negative breast cancer; valemetostat, valemetostat tosylate.

Valemetostat plus T-DXd in HER2+ GC and Dato-DXd in NSCLC xenograft models

- Valemetostat plus either T-DXd or Dato-DXd was assessed in subcutaneously xenografted mice bearing HER2+ GC or NSCLC adenocarcinoma cells, respectively





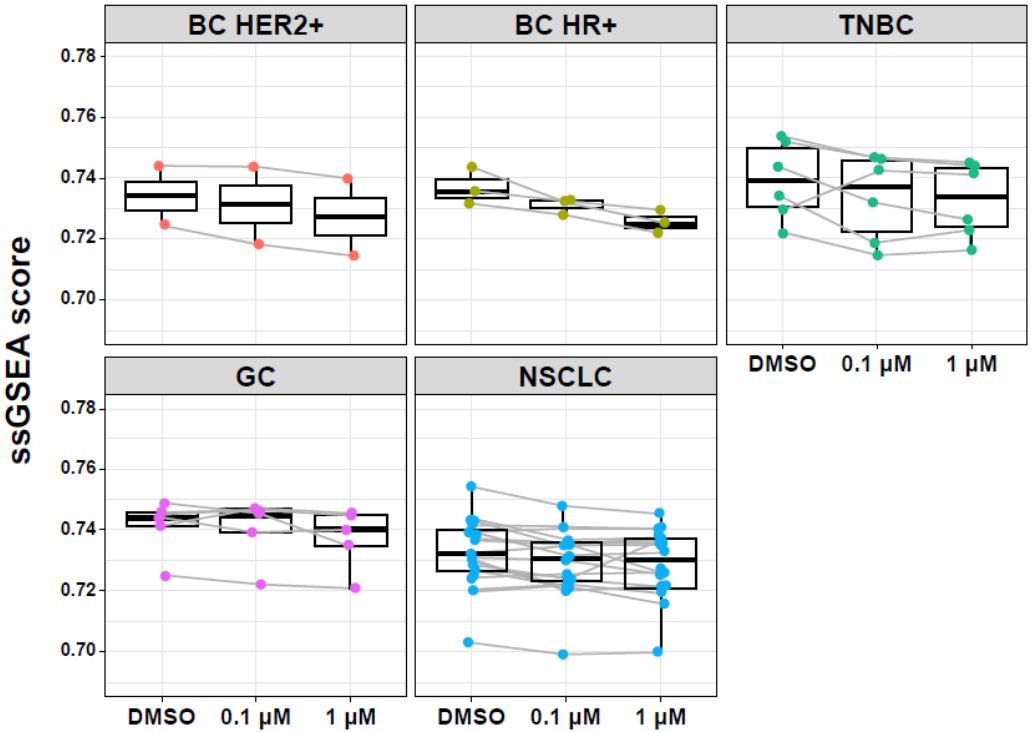
Valemetostat enhanced the in vivo antitumor activities of T-DXd against HER2+ GC and of Dato-DXd against NSCLC xenografted tumors

Valemetostat (0.3% or 0.25%) was administered with food (ad libitum) daily for 4 weeks to GC models or 6 weeks to NSCLC models; T-DXd (2 mg/kg) was administered once intravenously 4 days after the start of the treatment with valemetostat; Dato-DXd (10 mg/kg) was concurrently administered intravenously twice Q3W. Combination activities were assessed by Dunnett's test: **P* < .05; ***P* < .01; ****P* < .001.
Dato-DXd, datopotamab deruxtecan; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; NSCLC, non-small-cell lung cancer; Q3W, every 3 weeks; T-DXd, trastuzumab deruxtecan; valemetostat, valemetostat tosylate.

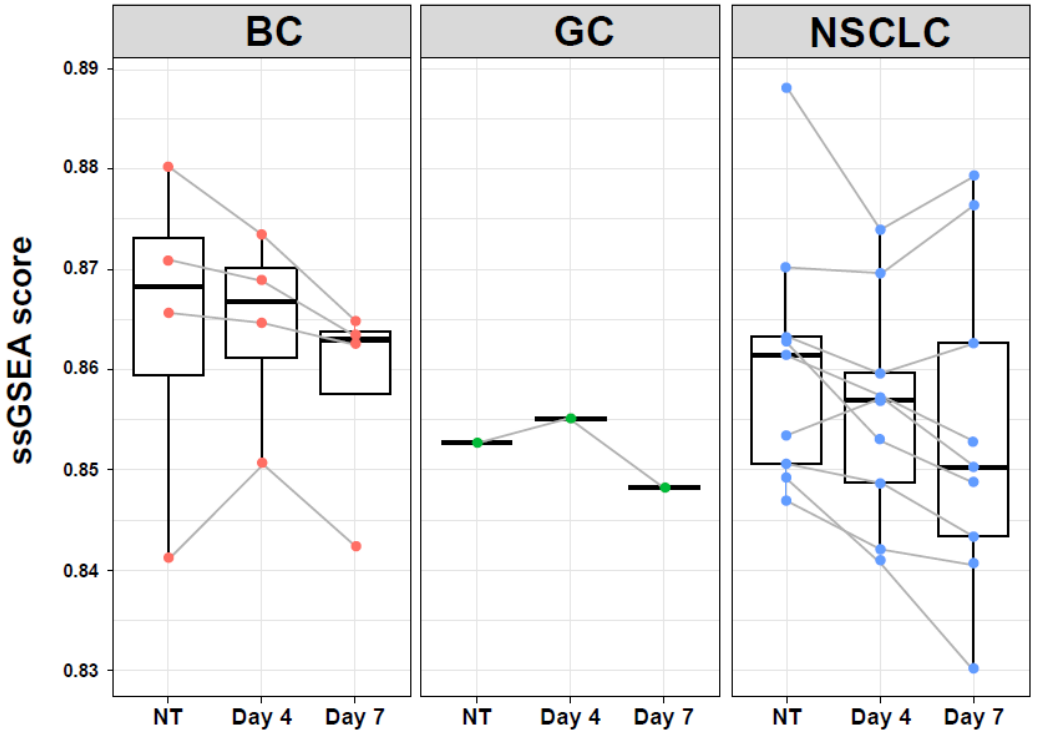
DDR gene expression changes after valemetostat treatment

- DDR gene expression changes were analyzed via ssGSEA in BC, GC, and NSCLC cells collected from in vitro cultures and from xenografted mouse models

In vitro expression of DDR genes after valemetostat treatment



In vivo expression of DDR genes after valemetostat treatment



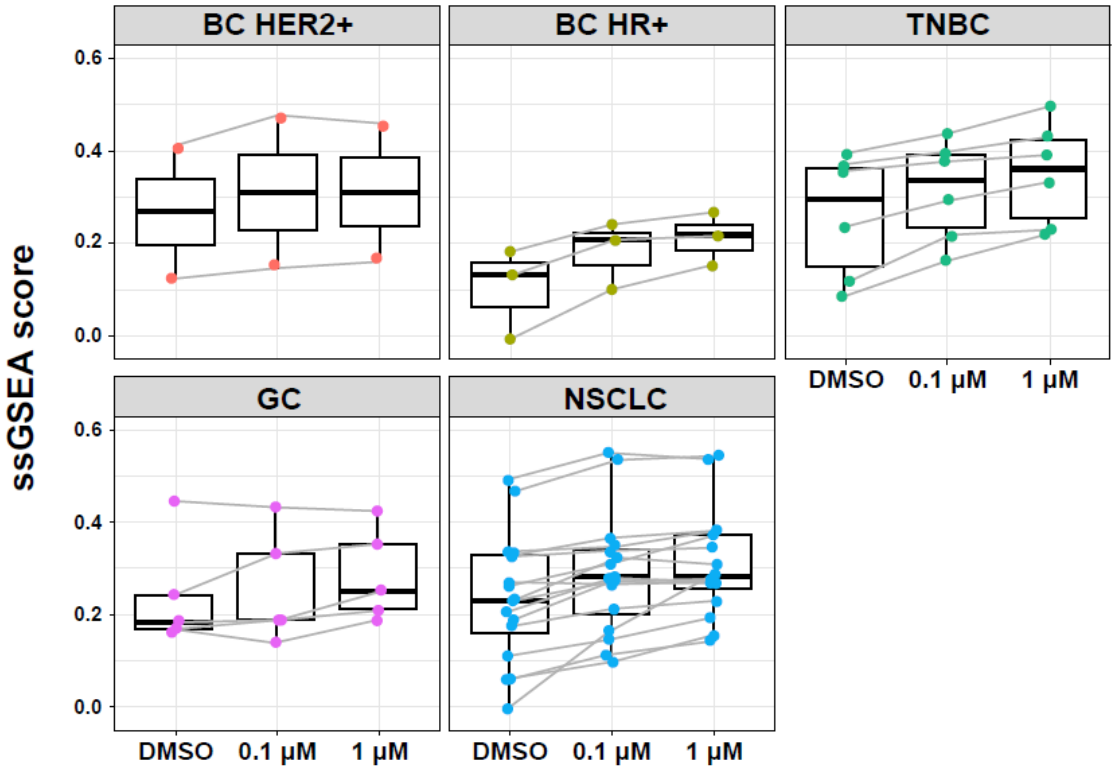
There was a trend for reduced DDR signatures in BC, GC, and NSCLC cells after valemetostat treatment in vitro and in vivo

Expression changes of mRNAs of genes involved in DDR genes (147 genes included in HALLMARK_DNA_REPAIR17) were analyzed in BC (N=11), GC (N=5), and NSCLC (N=16) cell lines collected from in vitro cultures treated with DMSO or valemetostat (0.1 μM or 1 μM) for 7 days and in BC (N=4), GC (N=1), and NSCLC (N=9) cell lines collected from xenografted mouse models treated with valemetostat (0.25% or 0.3%) that was administered daily with food (ad libitum) for 4 or 7 days.
BC, breast cancer; DDR, DNA damage repair; DMSO, dimethyl sulfoxide; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; NSCLC, non-small-cell lung cancer; NT, not treated; ssGSEA, single sample gene set enrichment analysis; TNBC, triple negative breast cancer; valemetostat, valemetostat tosylate.

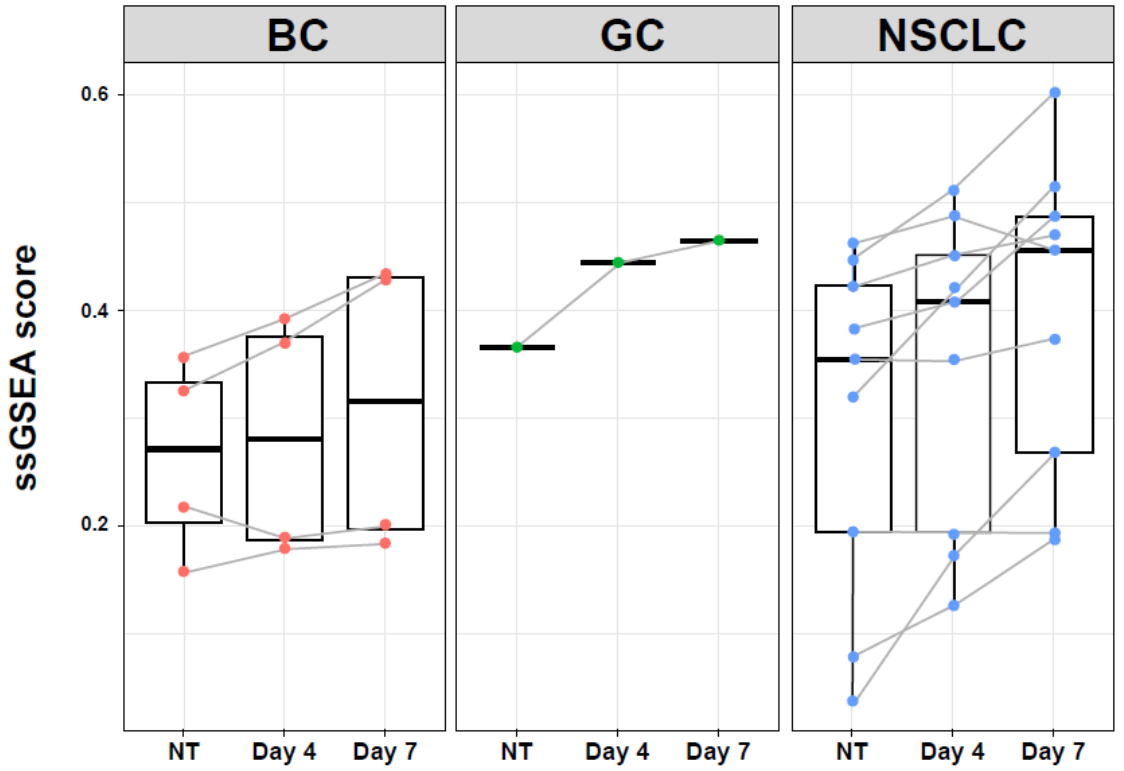
MHC gene expression changes after valemetostat treatment

- Expression changes of mRNAs of MHC genes were analyzed via ssGSEA in BC, GC, and NSCLC cells collected from in vitro cultures and from xenografted mouse models

In vitro expression of MHC genes after valemetostat treatment



In vivo expression of MHC genes after valemetostat treatment



Valemetostat increased MHC signatures in BC, GC, and NSCLC cells from in vitro and in vivo models

Expression changes of mRNAs of MHC genes (15 genes: B2M, HLA-A, HLA-B, HLA-C, TAP1, TAP2, CIITA, HLA-DRA, HLA-DQA1, HLA-DQA2, HLA-DQAB1, HLA-DQB2, HLA-DPA1, HLA-DPB1, HLA-DPB2) were analyzed in BC (N=11), GC (N=5), and NSCLC (N=16) cell lines collected from in vitro cultures treated with DMSO or valemetostat (0.1 μM or 1 μM) for 7 days and in BC (N=4), GC (N=1), and NSCLC (N=9) cell lines collected from xenografted mouse models treated with valemetostat (0.25% or 0.3%) that was administered daily with food (ad libitum) for 4 or 7 days.
BC, breast cancer; DMSO, dimethyl sulfoxide; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; MHC, major histocompatibility complex; NSCLC, non-small-cell lung cancer; NT, not treated; ssGSEA, single sample gene set enrichment analysis; TNBC, triple negative breast cancer; valemetostat, valemetostat tosylate.

Conclusions

- Valemetostat appears to improve the antitumor effect of T-DXd and Dato-DXd via multiple cellular mechanisms, including increased expression of SLFN11, neoantigen presenting molecules (eg, MHC), and ADC targets (eg, HER2), with a trend for reduced expression of genes involved in DDR
- Valemetostat potentiates the cell-killing activity of the DXd payload of T-DXd and Dato-DXd in different solid tumor cell lines in vitro, in a dose-dependent manner
- Combination treatment of valemetostat with T-DXd or with Dato-DXd showed enhanced antitumor response in xenograft models of different solid tumors compared with each as a single agent
- A phase 1b, open-label, Master Protocol trial is currently ongoing to investigate the clinical safety, tolerability, and preliminary efficacy of valemetostat in combination with T-DXd in patients with HER2-low BC or HER2+ GC/GEJ cancer, and in combination with Dato-DXd in patients with nonsquamous NSCLC (NCT06244485)
  - Additional combinations are under investigation, including a phase1b trial of valemetostat + pembrolizumab in first-line NSCLC with PD-L1 TPS ≥50% and no AGAs

ADC, antibody–drug conjugate; AGA, actionable genomic alteration; BC, breast cancer; Dato-DXd, datopotamab deruxtecan; DDR, DNA damage repair; DXd, topoisomerase I inhibitor payload; GC, gastric cancer; GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; MHC, major histocompatibility complex; NSCLC, non-small-cell lung cancer; PD-L1, programmed death ligand 1; SLFN11, DNA/RNA helicase Schlafen 11; T-DXd, trastuzumab deruxtecan; TPS, tumor proportion score; valemetostat, valemetostat tosylate.

Acknowledgments

- This study is sponsored by Daiichi Sankyo
- Daiichi Sankyo Company, Limited (referred to as Daiichi Sankyo) and AstraZeneca entered into a global collaboration to jointly develop and commercialize T-DXd in March 2019 and Dato-DXd in July 2020, except in Japan where Daiichi Sankyo maintains exclusive rights for each ADC. Daiichi Sankyo is responsible for the manufacturing and supply of T-DXd and Dato-DXd
- All authors contributed to and approved the presentation
- Medical writing and editorial support was provided, under the direction of the authors, by Luca Scrivano, PhD, of Excerpta Medica, and was funded by Daiichi Sankyo in accordance with Good Publication Practice guidelines

ADC, antibody–drug conjugate; Dato-DXd, datopotamab deruxtecan; T-DXd, trastuzumab deruxtecan.