Poster # 16

Evaluating the combination of Datopotamab deruxtecan (Dato-DXd) with saruparib (AZD5305), a highly potent, PARP1-selective inhibitor, in preclinical models

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Objective

To investigate combination benefit of Dato-DXd and saruparib in preclinical models.

Conclusions

- Dato-DXd combination with AZD5305 increased cytotoxic activity, increased Top1CC accumulation and pharmacodynamic response *in vitro.*
- This combination led to superior tumor growth inhibition in a Gastric Cancer (GC) cell line xenograft model (CLX) and an Ovarian Cancer (OC) patient derived xenograft (PDX) model from a PARPi resistant patient *in vivo.*
- These pre-clinical findings support the ongoing clinical evaluation of Dato-DXd as a monotherapy and in combination with AZD5305 in patients with various advanced solid tumor types (NCT05489211, NCT04644068).

Plain language summary

Why did we perform this research?

Topoisomerase I inhibitors stabilize DNA-topoisomerase covalent complexes that lead to double-strand breaks and activation of DNA damage response (DDR). Poly(ADP-ribose) polymerase 1 (PARP1) mediates signal transduction and is an important regulator of DDR. AZD5305 is a highly potent and selective inhibitor of PARP1 (Illuzzi G et al. 2022). Since PARP1 is a key component driving the repair of trapped TOP1CC, we aimed to investigate if combinations of Dato-DXd with AZD5305 led to synergistic anti-tumor activity in preclinical models.

How did we perform this research?

We evaluated the cytotoxic effect of the combination of Dato-DXd with AZD5305 in a panel of six non-small cell lung cancer (NSCLC) and five triple negative breast cancer cell lines (TNBC) in a 7-day viability assay. We evaluated PARylation inhibition and γH2AX response by Western blotting. TOP1CC accumulation in response to combination treatment was evaluated by immunofluorescence. This combination was evaluated in vivo in a TROP2+, homologous recombination deficient (HRD)-negative GC cell line xenograft model at varying concentrations of AZD5305 and in a TROP2+, PARPi-resistant OC PDX model (CTG-3718).

What were the findings of this research?

- Enhanced cytotoxicity, TOP1CC accumulation and PD response were observed in response to the combination treatment *in vitro.*
- We observed dose-dependent increase in PARylation inhibition and enhanced induction of gH2AX in the combination treatment.
- Significantly higher tumor growth inhibition (TGI) was observed in comparison to either monotherapies.
  - In the N87 tumor model, while Dato-DXd and AZD5305 provided 74% and 15% TGI as monotherapies, respectively the addition of AZD5305 to Dato-DXd resulted in 93% TGI.
  - In a TROP2+, ovarian cancer PDX model (CTG-3718), while Dato-DXd and AZD5305 provided 70% and <10% TGI as monotherapies, respectively the addition of AZD5305 to Dato-DXd resulted robust combination benefit.

What are the implications of this research?

These findings provide the pre-clinical rationale for the combination of Dato-DXd + AZD5305 and support the ongoing clinical evaluation of Dato-DXd as a monotherapy and in combination with AZD5305 in patients with various advanced solid tumor types (NCT05489211, NCT04644068).

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Introduction

- Dato-DXd is a TROP2-directed antibody drug conjugate comprised of a topoisomerase I inhibitor (DXd) conjugated to datopotamab, a humanized anti-TROP2 IgG1 antibody, via a cleavable plasma-stable tetrapeptide-based linker.
- Topoisomerase I inhibitors stabilize DNA-topoisomerase cleavage complexes (Top1CC) and lead to double-strand breaks and activation of DDR. Topoisomerase 1 forms protein-DNA cleavage complexes and becomes covalently bound to the catalytic DNA strand break. TOP1 inhibitors intercalate into DNA at these TOP1 active sites to obstruct the religation step and stabilize the covalent topo1-DNA complexes. (Pommier Y, et al. 2016)
- PARP1 mediates signal transduction in the DDR as an important regulator. AZD5305 is a highly potent and selective inhibitor of PARP1.[3] PARP1 functions as a DNA damage sensor that can be activated by DNA lesions resulting in formation of PAR chains that serve as a docking platform for DNA repair factors. Poly ADP-ribosylation (PARylation) is a pivotal post-translational protein modification (PTM) that appears rapidly at DNA damage sites.[3] (Illuzzi G, et al. 2022),(Wei H, et al. 2016)

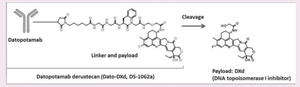


Figure 1. Schematic structure of Dato-DXd. (Okajima D, et al. 2021)

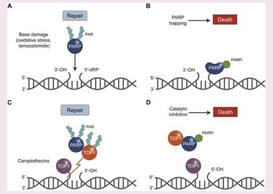


Figure 2. Base damage induced by oxidative stress is sensed by PARP1/2. (B) PARP inhibitors (PARPi) trap PARP1/2 on DNA and induce cell death. (C) TOP1 inhibitors (TOP1i) trap TOP1 on DNA at the 3' end of the break to form Top1cc. TDP1 is activated by PARP1 and cleaves the Top1cc to repair the lesion (D) PARPi block the activity of TDP1 and hence inhibit the repair of TOP1-DNA complexes and synergize with TOP1i, which eventually leads to cell death. (Das B, et al. 2014)

Methods

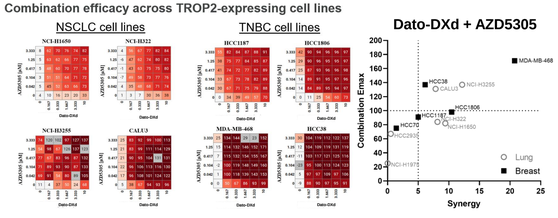
- **Cell Viability Assay:** Tested Cytotoxic effect of the combination of Dato-DXd with AZD5305. Cells were plated and treated the following day with Dato-DXd and AZD5305. Cell viability was assessed using Cell Titer Glo.
- **Western Blotting:** Evaluated PARylation inhibition and γH2AX response. Cell lysates were prepared from cells harvested by scrapping followed by deglycosylation. Samples were then run on SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane and probed with Anti-PARylation antibody and anti-gamma-H2AX antibody.
- **Immunofluorescence:** Evaluated target engagement and pharmacodynamic response to the combination treatment. Cells were treated with Dato-DXd for 24 hours followed by AZD5305 for two hours then fixed and permeabilized. TOP1CC were detected using Anti-Topoisomerase I-DNA Covalent Complex Antibody and gamma-H2AX was detected using anti-gamma-H2AX antibody.
- **In vivo Studies:** Dato-DXd and AZD5305 combination was evaluated in a TROP2+, HRD-, GC N87 CLX and in a TROP2+ OC PDX model derived from a PARPi resistant patient (CTG-3718). Cells were implanted subcutaneously in athymic nude mice. Dato-DXd was administered as a single dose intravenously at 10 mg/kg and AZD5305 was administered BID at 0.01, 0.1 and 1 mg/kg for 21 Days.
- **Immunohistochemistry**: TROP2 expression in the tumor tissue was detected using an anti-TROP2 antibody.

Results and interpretations

AZD5305 enhances Dato-DXd induced cytotoxicity *in vitro*

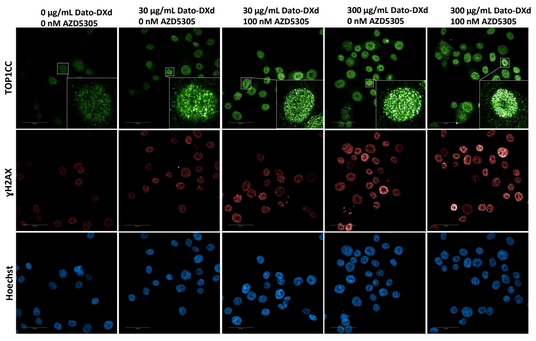
		HCC1975	H1650	H322	HCC2935	NCIH3255	Calu3	HCC70	HCC1187	HCC1860	MDA-MB468	HCC38	
TROP2 RNA		26	604	168	238	456	125	378	29	256	313	277	
Dato-DXd (Emax)		4	34	40	46	57	96	57	66	75	102	105	Monotherapy
Exatecan (Emax)		152	155	102	134	169	150	75	150	152	191	161	Combinations
Compound A Dato-DXd	Compound B AZD5305	0.01	9.43	8.14	0.52	12.23	7.84	1.42	5.70	11.60	20.75	6.19	HSA synergy Scores*

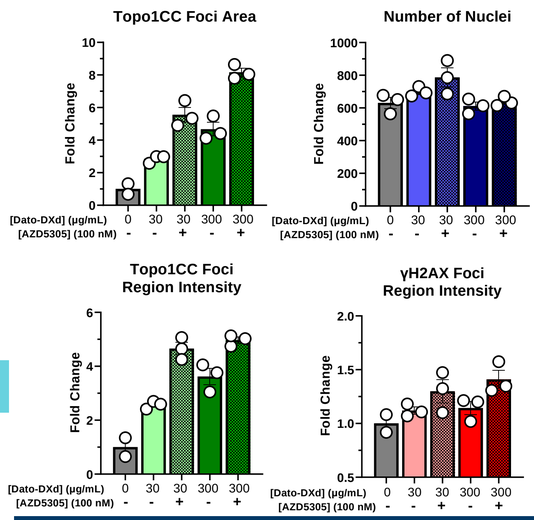
Combination efficacy across TROP2-expressing cell lines



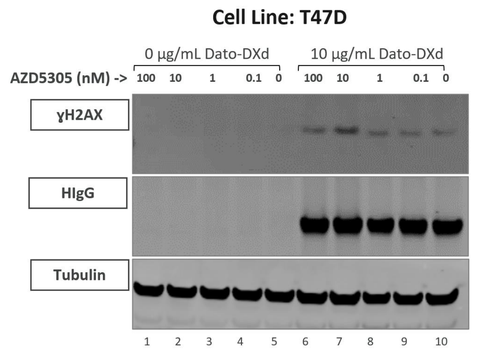
*Footnote 1 'HSA Synergy' scores (the average of HSA excess across dosing matrix; the higher the number, the more combination benefit)

AZD5305 enhances Dato-DXd induced TOP1CC and γH2AX foci in NSCLC cell line

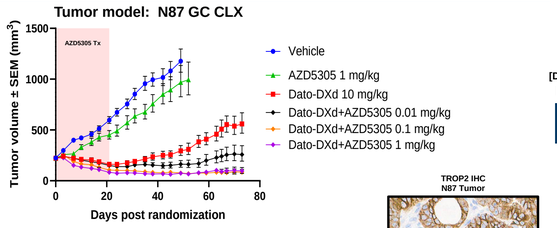




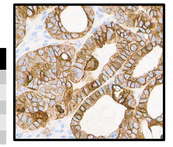
AZD5305 + Dato-DXd induced γH2AX in a breast cancer cell line

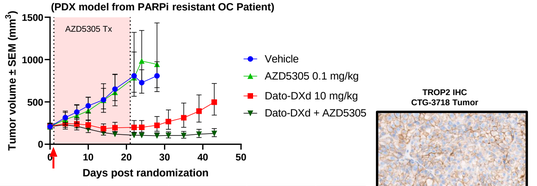


Addition of AZD5305 to Dato-DXd enhances anti-tumor activity in TROP2-expressing GC CLX and OC PDX models



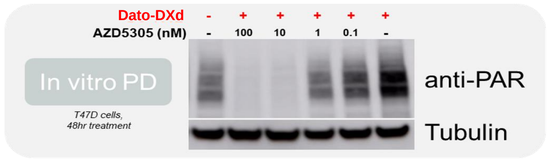
Treatment	Mean Tumor volume (mm³) ± SEM	% TGI compared to vehicle control	% TGI compared to Dato-DXd	p-value vs vehicle
Dato-DXd 10 mg/kg	319.5+44.18	74		0.0001
AZD5305 1 mg/kg	1034+136.35	15		0.0242
Dato-DXd + AZD5305 1 mg/kg	88.38+22.07	93	72	0.0001
Dato-DXd + AZD5305 0.1 mg/kg	80.75+8.93	93	74	0.0001
Dato-DXd + AZD5305 0.01 mg/kg	189.75+42.42	84	41	0.0001





Treatment	Mean Tumor volume (mm³) ± SEM	% TGI compared to vehicle control	% TGI compared to Dato-DXd	p-value vs vehicle
Dato-DXd 10 mg/kg	280.57+98.75	70		0.0001
AZD5305 1 mg/kg	979.14+194	-5		0.975
Dato-DXd + AZD5305 0.1mg/kg	129+30.6	86	54	0.0001

AZD5305 reduces Dato-DXd induced PARylation of intracellular proteins



Summary

- Dato-DXd combination with saruparib showed increased cytotoxic activity in NSCLC and TNBC cell panels. The HSA scores, increased TOP1CC accumulation and pharmacodynamic response *in vitro* indicate a combination benefit over either monotherapies.
- This combination led to superior tumor growth inhibition in a GC CLX model where Dato-DXd and AZD5305 provided 74% and 15% TGI as monotherapies, respectively. The addition of AZD5305 to Dato-DXd resulted in 93% TGI.
- Robust combination benefit was observed in an OC PDX model from a PARPi resistant patient where Dato-DXd and AZD5305 provided 70% and <10% TGI as monotherapies, respectively. The addition of AZD5305 to Dato-DXd resulted in 86% TGI.
- These pre-clinical findings support the ongoing clinical evaluation of Dato-DXd as a monotherapy and in combination with saruparib in patients with various advanced solid tumors (NCT05489211, NCT04644068).

Abbreviations

γH2AX: Phospho-gamma-Histone H2AX
BID: bis in die
CLX: Cell line xenograft
ds: double stranded
GC: Gastric cancer
HSA: highest single agent
HRD: Homologous recombination deficiency
NSCLC: Non small cell lung cancer
OC: Ovarian cancer
PD: Pharmacodynamic
PDX: Patient derived xenograft
ss: single stranded
SDS-PAGE: sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TDP1: Tyrosyl-DNA phosphodiesterase 1
TNBC: Triple negative breast cancer
TOP1: Topoisomerase 1
Top1CC:Top1 cleavage complex
TROP2: Trophoblast antigen 2
XRCC1: X-ray repair cross-complementing protein 1

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Disclosures

All authors are employees of AstraZeneca Pharmaceuticals.