Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

Molecular Pharmacodynamics of an ADC: DS-8201a

July 10, 2023 FNLAC Meeting

Ralph E Parchment, PhD

Managing Director, Pharmacodynamic Biomarkers Program (a R&D program supporting DCTD)

Leidos Biomedical Research, Inc

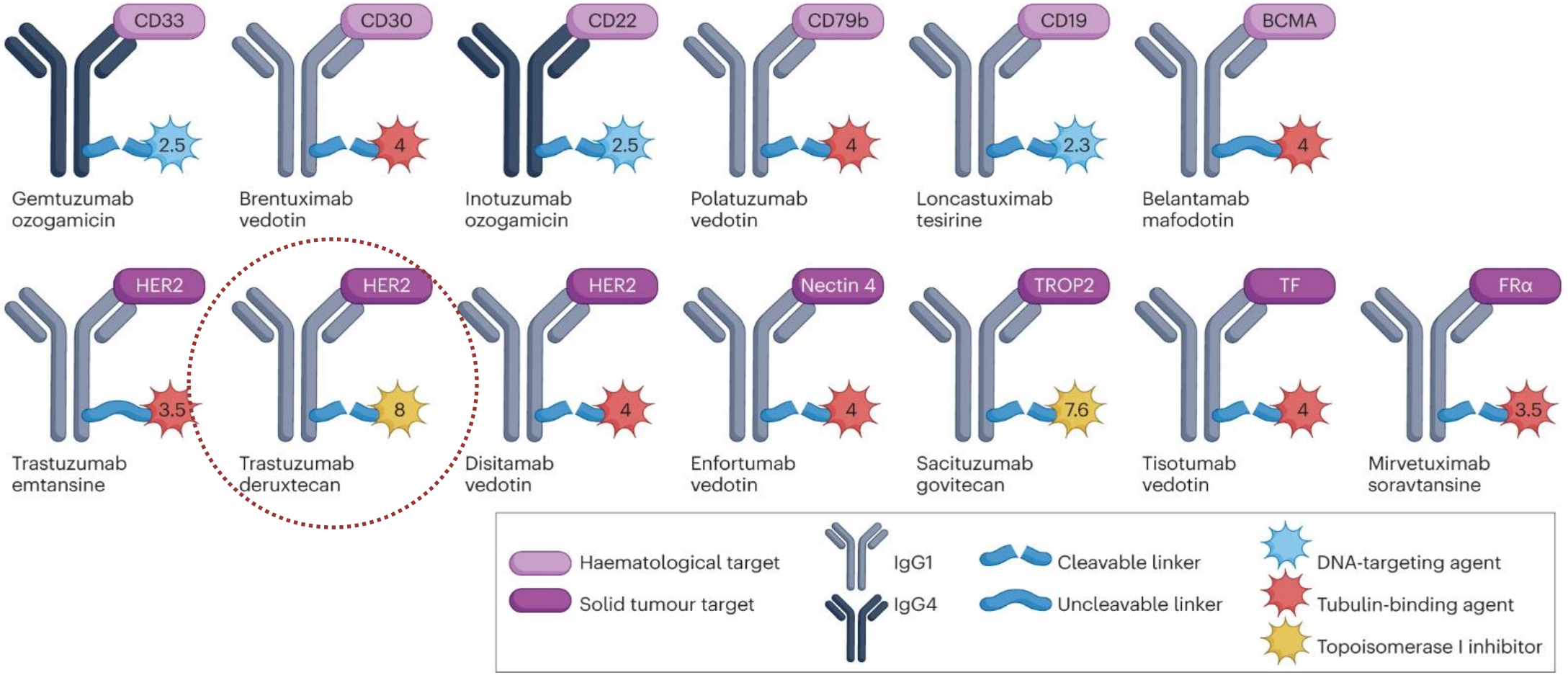
DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

Antibody-Drug Conjugates (aka “ADCs”)

Fig. 2: Main characteristics of approved ADCs. Dumontet et al (2023) *Nat Rev Drug Discov* https://doi.org/10.1038/s41573-023-00709-2

From: Antibody–drug conjugates come of age in oncology



trastuzumab deruxtecan, the API in Enhertu® (aka DS-8201a, T-DXd, NSC 807708)

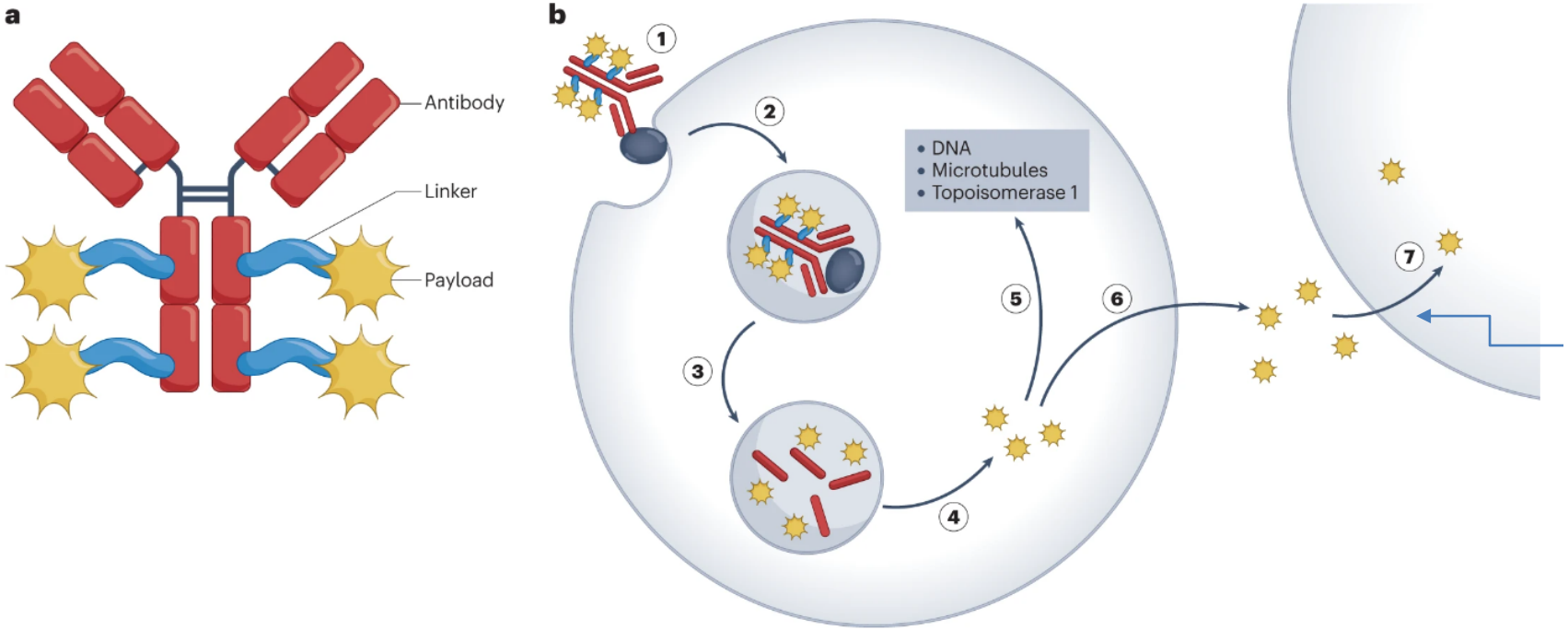
FDA-approved indications (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761139s021lbl.pdf, 8/2022; accessed July 2023)

- adult patients with unresectable or metastatic HER2-positive breast cancer who have received a prior anti-HER2-based regimen either in the metastatic setting or in the neoadjuvant or adjuvant setting with disease recurrence during/within 6 mos of completing therapy (DESTINY-Breast01, -Breast03 trials, NCT03248492, NCT03529110, randomized vs ado-trastuzumab emtansine)
  - HER2 expression: HER2 IHC 3+ or ISH-positive archival tissue tested at central laboratories
- adult patients with unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer who have received a prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy (DESTINY-Breast04 trial, NCT03734029, randomized vs physician's choice of chemotherapy, NEJM 2022)
  - HER2 expression: HER2 IHC 1+ or IHC 2+/ISH-negative archival tissue tested at a central laboratories
- adult patients with unresectable or metastatic non-small cell lung cancer (NSCLC) whose tumors have activating HER2 (ERBB2) mutations (local labs), as detected by an FDA approved test, and who have received a prior systemic therapy (accelerated approval based on ORR and DR) (DESTINY-Lung01 and –Lung02, NCT03505710, NCT04644237))
- adult patients with locally advanced or metastatic HER2-positive (IHC 3+ or IHC 2+/ISH-central lab) gastric or gastroesophageal junction adenocarcinoma who have received a prior trastuzumab-based regimen (DESTINY-Gastric01, NCT03329690)

trastuzumab deruxtecan, the API in Enhertu® (aka DS-8201a, T-DXd, NSC 807708)

Fig. 1: Structure and mechanism of action of conventional ADCs.

From: Antibody–drug conjugates come of age in oncology



Dumontet et al (2023) *Nat Rev Drug Discov* https://doi.org/10.1038/s41573-023-00709-2

ADC Target
human EGFR-2 (HER2)

Antibody
trastuzumab mAb to target HER2

Payload
deruxtecan, a TOP1 inhibitor

Linker
GGFC peptide

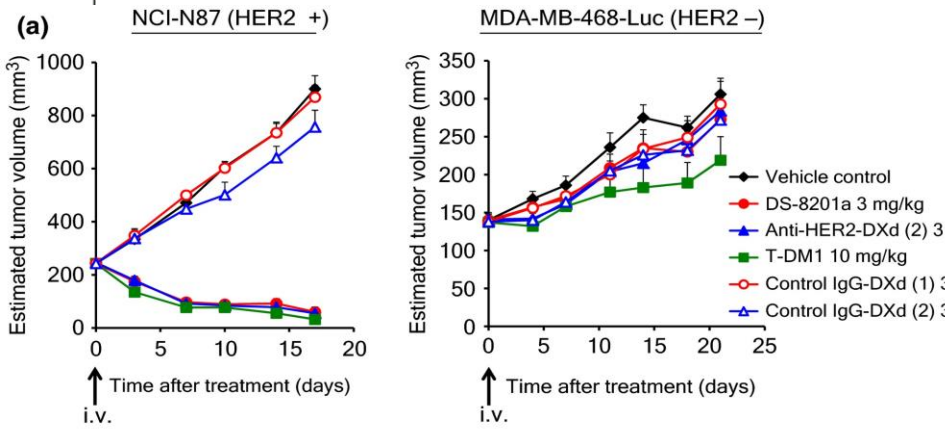
Membrane Permeable Payload?
YES (drug lactone)

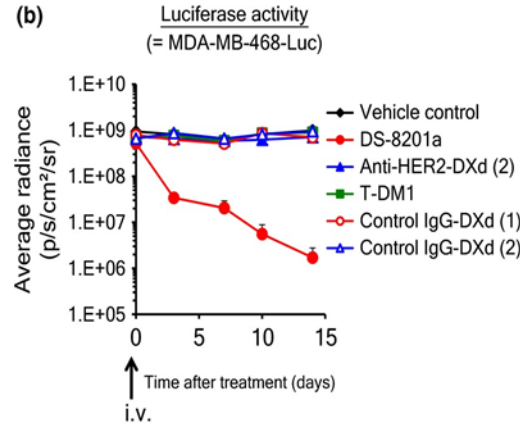
Mechanism of Action (MOA)
TOP1i → DNA damage → cell death?
MOA in HER2-high vs -low tumors?
similar MOA to topotecan?

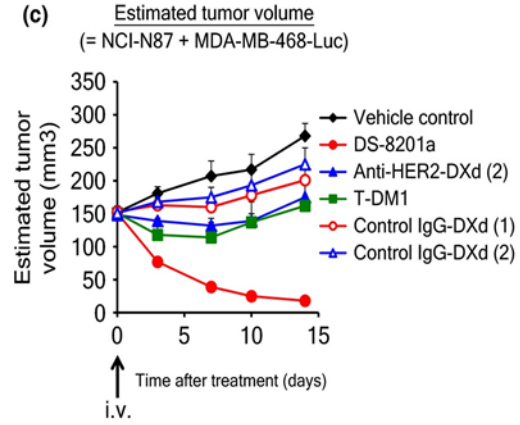
trastuzumab deruxtecan (DS-8201a, T-DXd) by-stander effect and membrane permeability

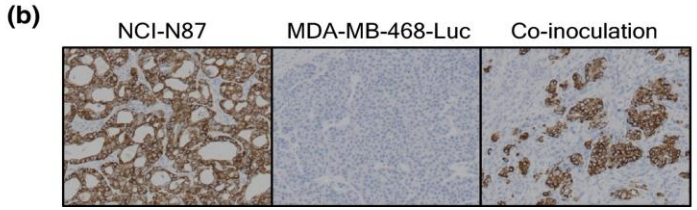
ADCs	Released payloads
DS-8201a [Anti-HER2–DXd (**1**)]	DXd (**1**)
Anti-HER2–DXd (**2**)	DXd (**2**)

Ogitani et al (2016) Cancer Science (DOI: 10.1111/cas.12966)





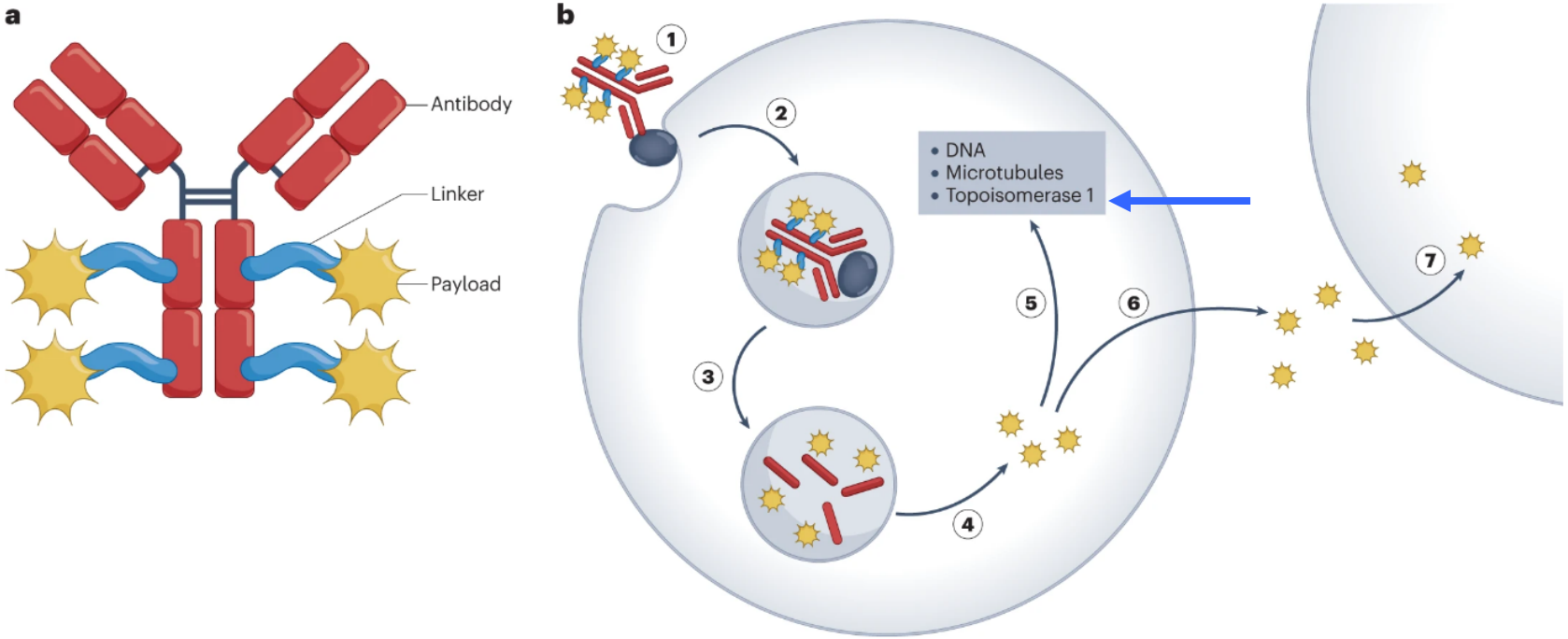




trastuzumab deruxtecan, the API in Enhertu® (aka DS-8201a, T-DXd, NSC 807708)

Fig. 1: Structure and mechanism of action of conventional ADCs.

From: Antibody–drug conjugates come of age in oncology



Dumontet et al (2023) *Nat Rev Drug Discov* https://doi.org/10.1038/s41573-023-00709-2

ADC Target
human EGFR-2 (HER2)

Antibody
trastuzumab mAb to target HER2

Payload
deruxtecan, a TOP1 inhibitor

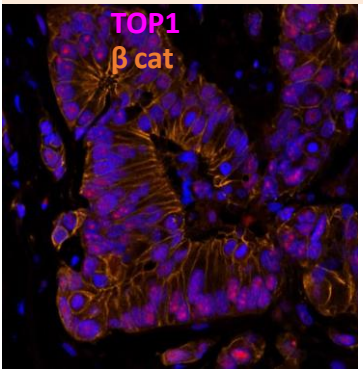
Linker
GGFC peptide

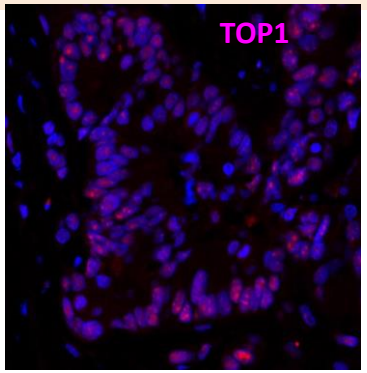
Membrane Permeable Payload
YES (drug lactone)

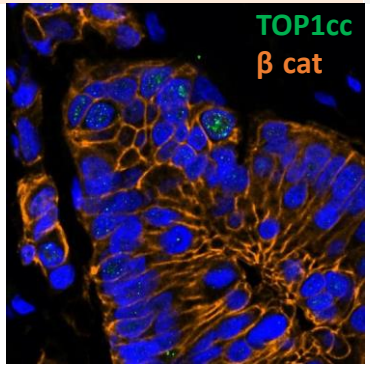
Mechanism of Action (MOA)
TOP1i → DNA damage → cell death?
MOA in HER2-high vs -low tumors?
similar MOA to topotecan?

trastuzumab deruxtecan (DS-8201a, T-DXd) - studying MOA using PD biomarker tools

nuclear PD Biomarkers of the TOP1 covalent complex: loss of nuclear TOP1, ccTOP1-pY^{723} (mAb from S. Kaufmann)

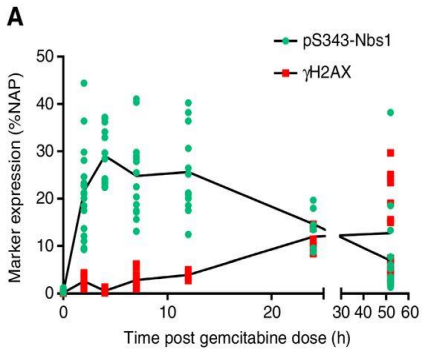




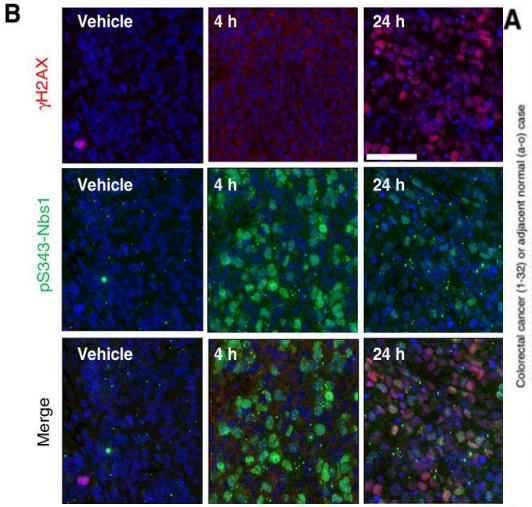


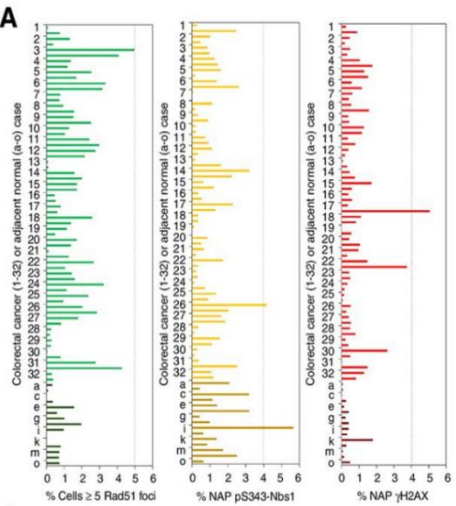
unpublished

nuclear PD Biomarkers of DNA Damage Response (DDR): RAD51, NBS1-pS^{343}, (γ)H2Ax-pS^{139}



Wilsker et al (2019)



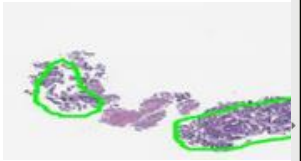


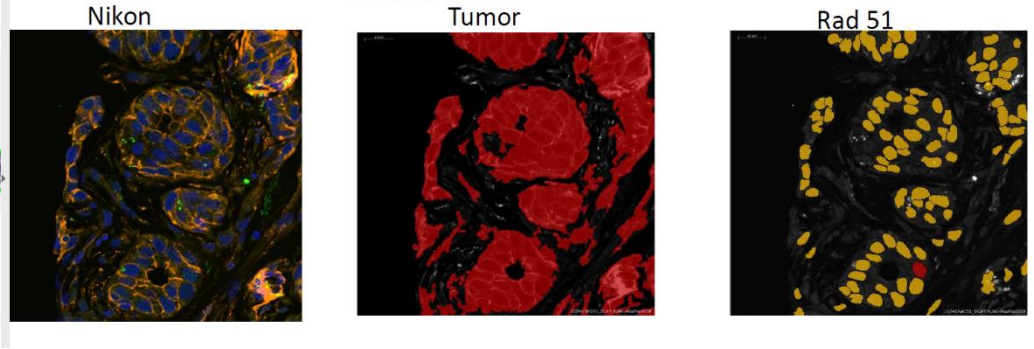
translational SOPs to preserve labile phosphorylation sites in tumor specimens (from DCTD "Research Resources" website)

γH2AX, pNBS1 IFA with β-CATN Segmentation, IFA	tumor	SOP340507 Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipping for PADIS, Frederick National Laboratory for Cancer Research (FNLCR) SOP340567 Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipment to EET Biobank	SOP340550 Tumor Frozen Needle Biopsy Preparation for Pharmacodynamic Immunofluorescence Assays Utilizing Murine Testis and/or Jejunum Control Tissues	SOP340543 γH2AX, pNBS1 IFA Staining with β-Catenin Segmentation for Tumor Biopsy Slides SOP340544 Whole Slide Image Capture of Tumor Biopsy Slides for γH2AX, pNBS1 Immunofluorescence Assay with β-Catenin Segmentation	SOP340545 Image Extraction and Analysis of Tumor Biopsy Slides from γH2AX, pNBS1 IFA with β-Catenin Segmentation

Kinders et al (2008), Srivastava et al (2016)
Parchment and Doroshow (2016)
Ferry-Galow et al (2018)
Ferry-Galow and Chen (2019), Levy et al (2020)

image analysis algorithms to restrict biomarker analysis to tumor cells (segmentation)





DS-8201a engagement of the TOP1 drug target in HER2+ human tumor xenografts

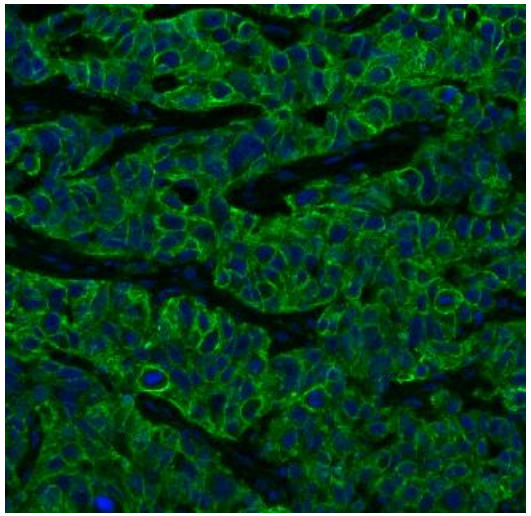
HER2 protein in tumor xenografts (CST cat #2242)

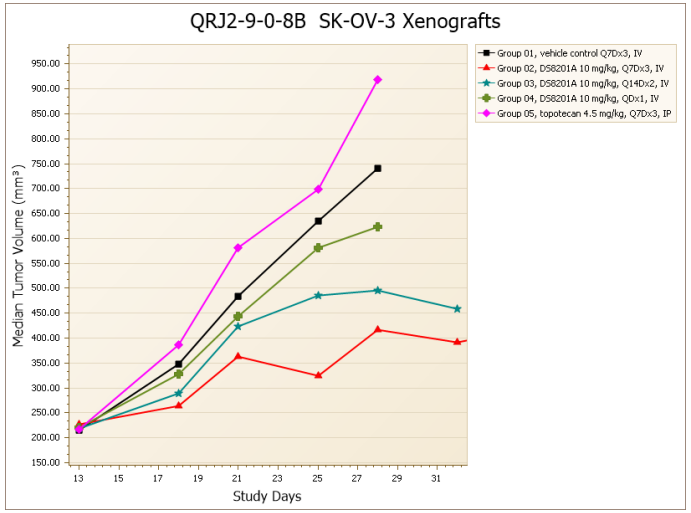
efficacy

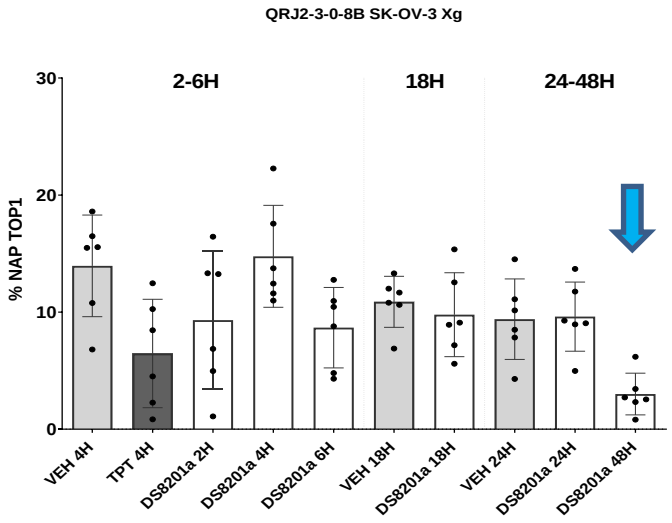
nuclear TOP1

nuclear (p)TOP1cc

SK-OV-3

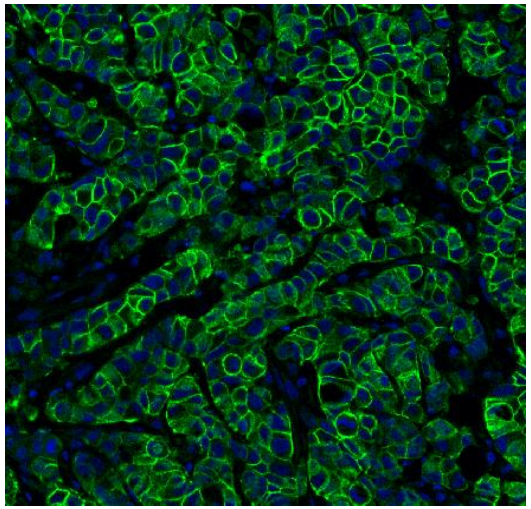


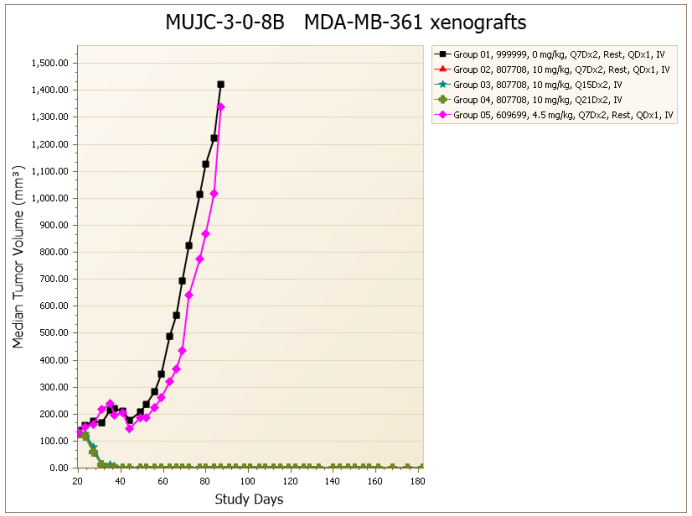


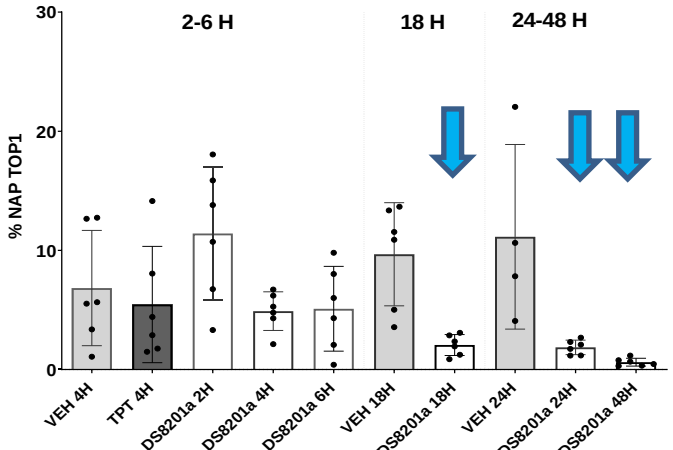


not detectable *in vivo*

MDA-MB-361

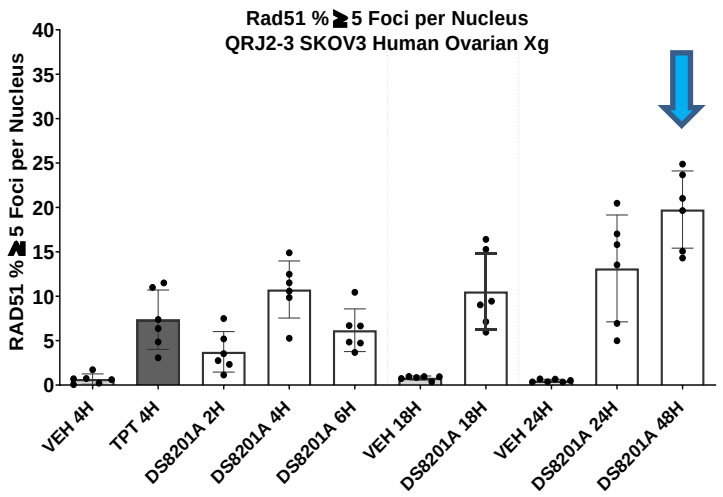
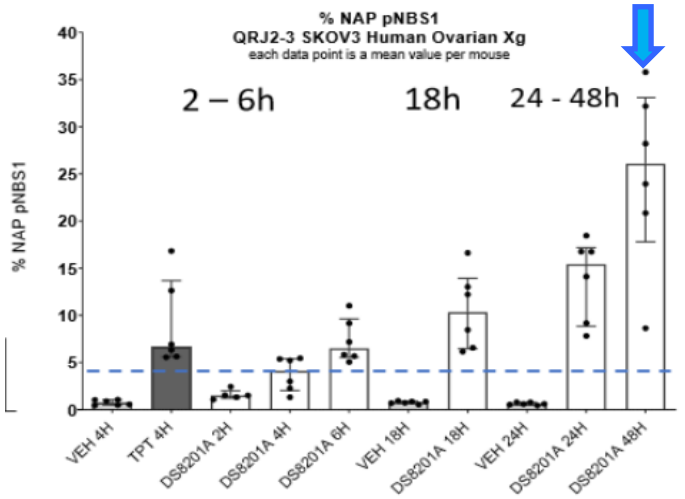
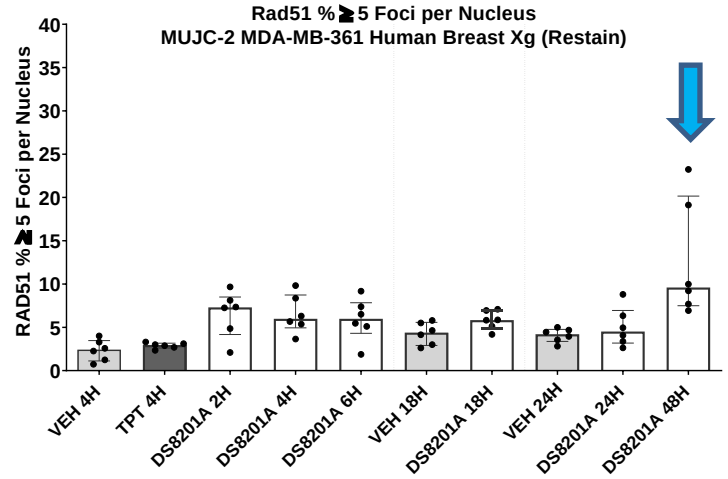
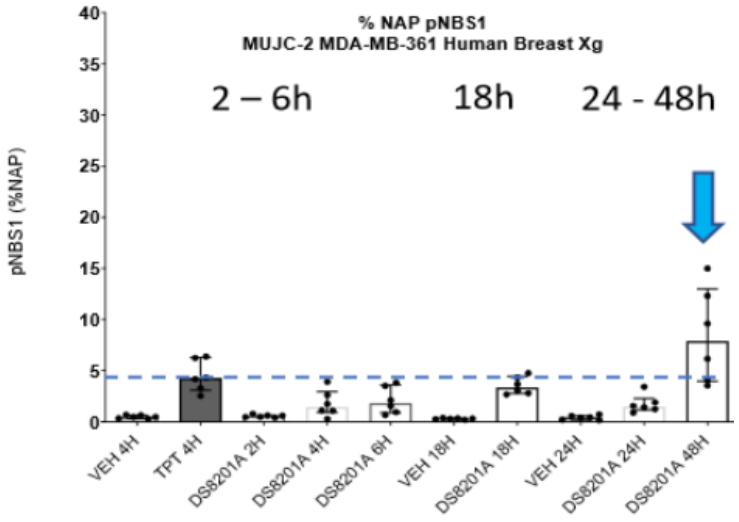




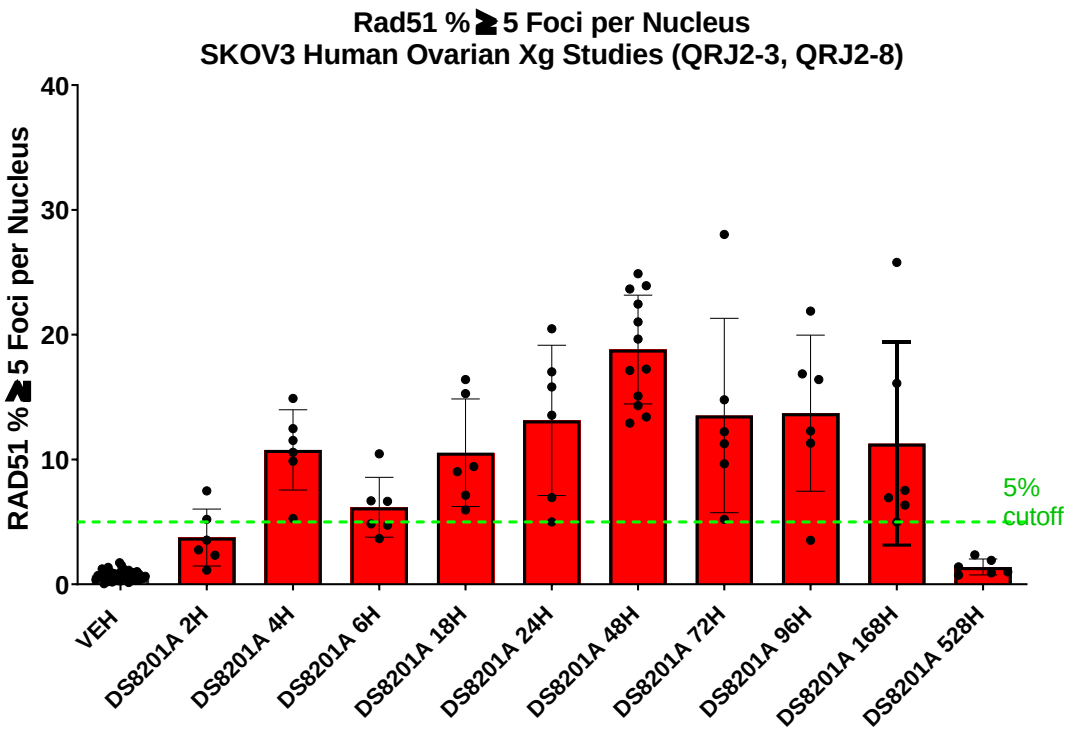


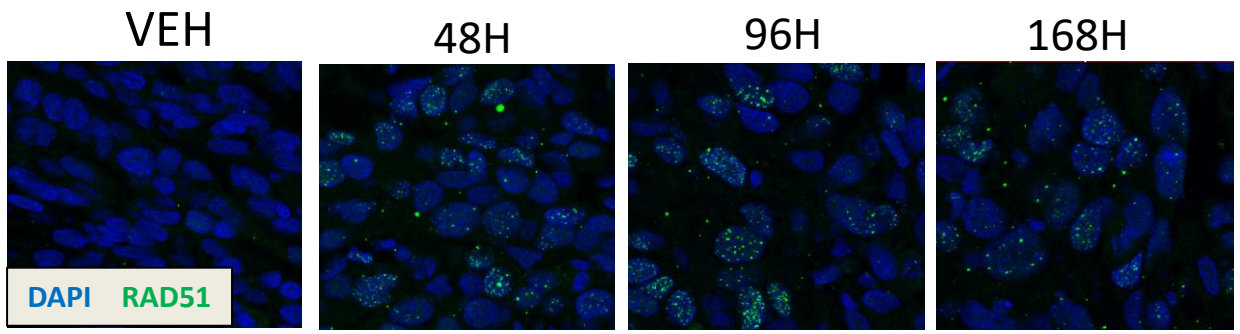
not detectable *in vivo*

DS-8201a-induced DNA damage responses in HER2+ human tumor xenografts

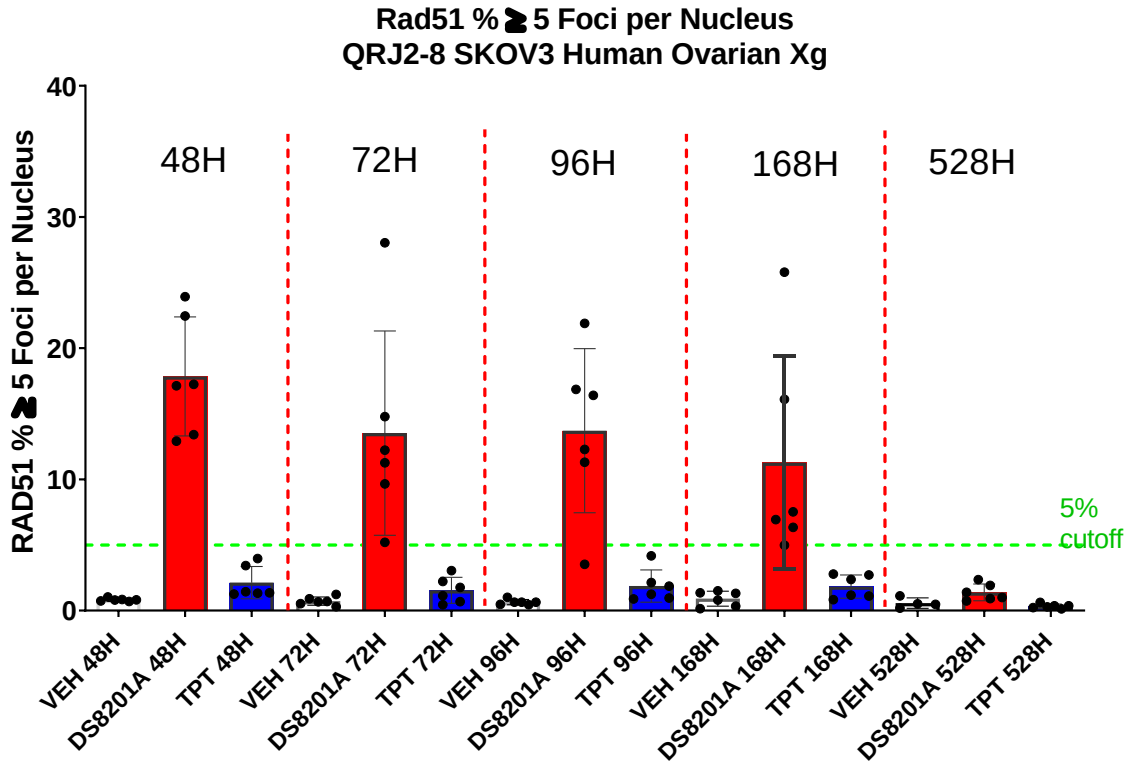
	nuclear RAD51 foci (ssDNA)	nuclear pNBS1 (in MRN complex)	nuclear γH2Ax DSB, DNA laddering
SK-OV-3			not detected
MDA-MB-361			not detected

Fit-for-Purpose Modeling of PD Biomarkers of DDR in a HER2+ human tumor model





Late Time Points (48h - 528h)

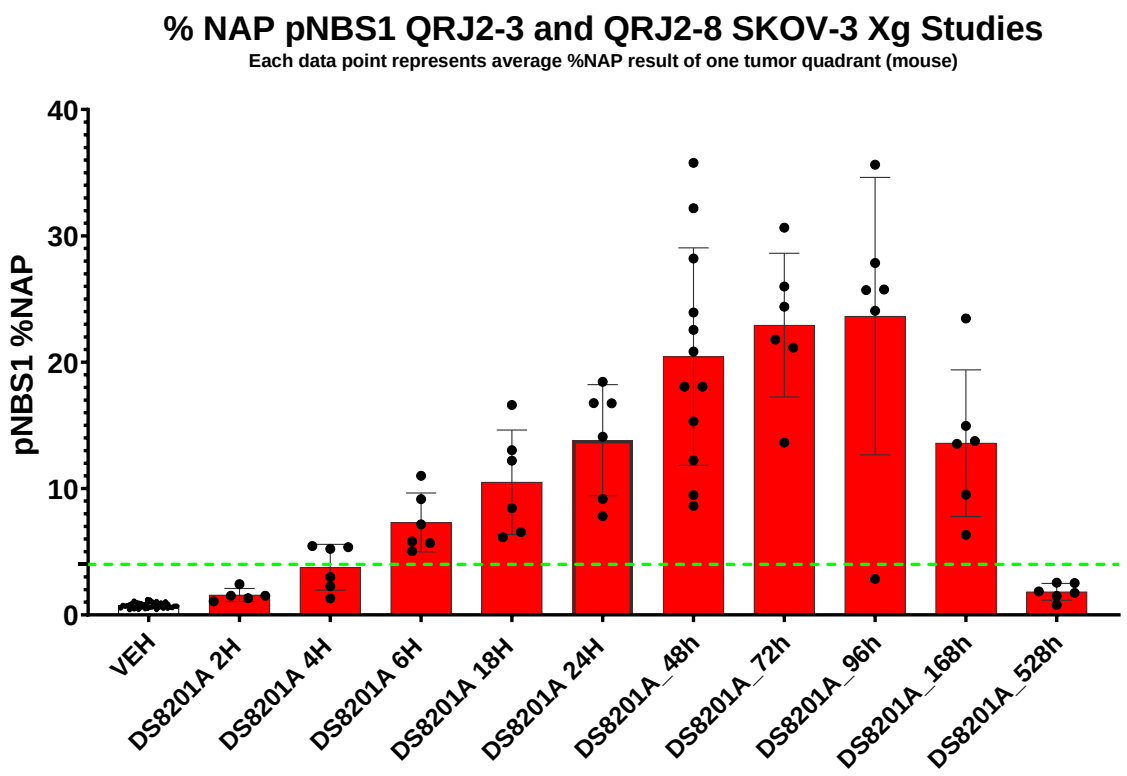


DS-8201a treatment

Topotecan treatment

Fit-for-Purpose Modeling of PD Biomarkers of DDR in a HER2+ human tumor model

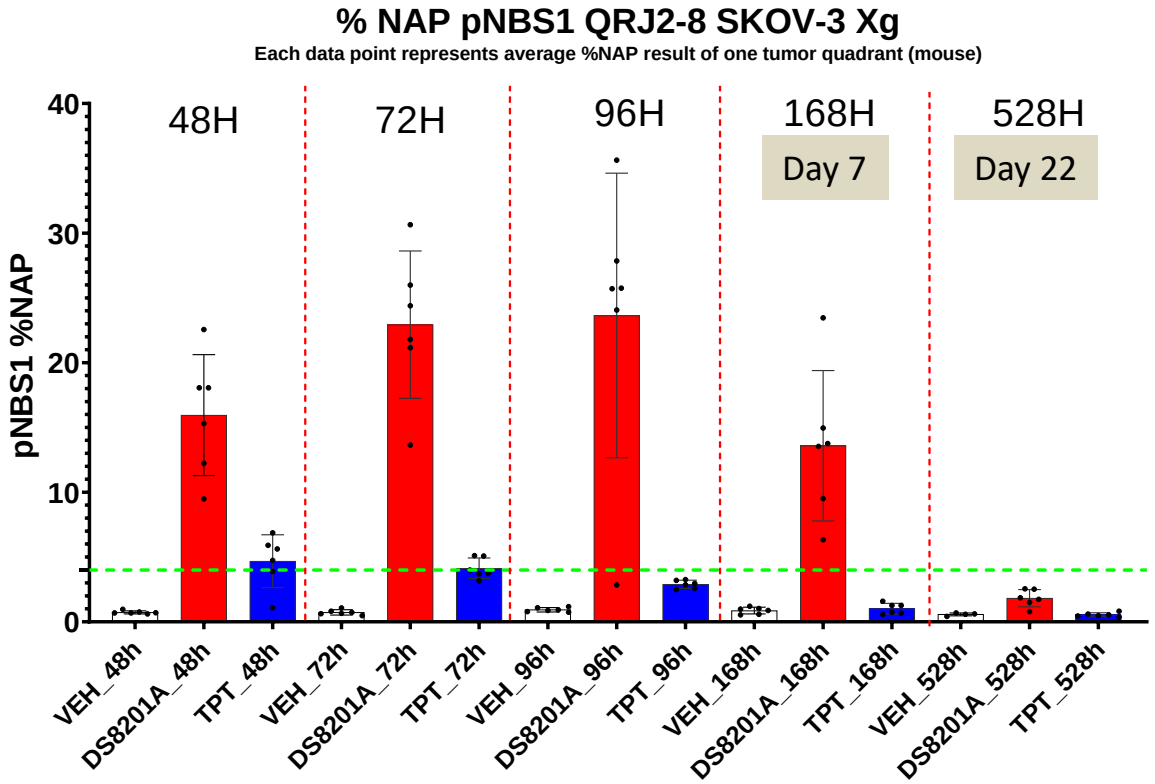
Early & Late Time Points plotted together (2h -528h):



Late Time Points (48h - 528h)

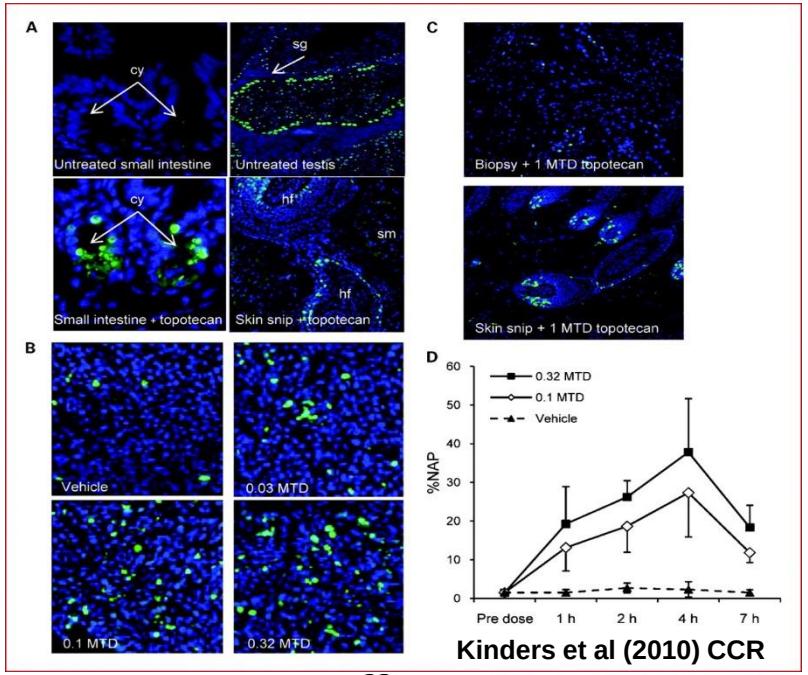
DS8201 treatment

TPT treatment

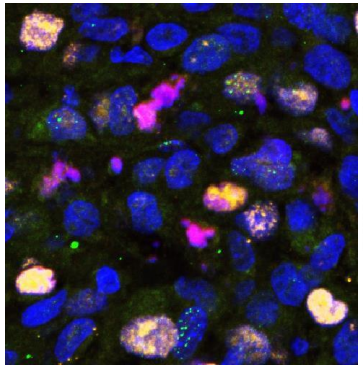


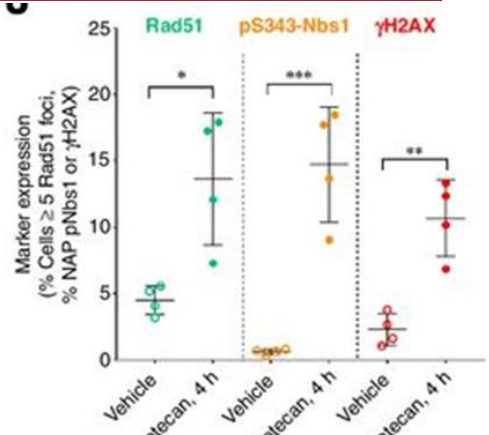
Translational Pharmacodynamics of DS-8201a - informing clinical trial design

- rapid reduction in nuclear pTOP1cc precludes analysis in patients
- PD biomarkers of DDR are fit-for-purpose for clinical use:
  - biopsy sampling window is 48-96 hours after drug administration
  - multiple PD biomarkers of DDR response provide opportunity to corroborate MOA
  - protein phosphorylation is stabilized by lab-developed SOPs for point-of-collection specimen preservation
- lack of γH2Ax response is informative, but surprising, given its robust response to topotecan in xenograft models
  - does release and diffusion of drug payload behave more like a depot dosage form, with a low C x prolonged t ?
  - not classical apoptotic cell death? (Dull et al (2018) Oncotarget)
- robust DDR response indicates the need to trace subsequent DDR events, their cell cycle context, and their connection to cell death (or not)



Kinders et al (2010) CCR





Wilsker et al (2019) CCR

Translational Pharmacodynamics of DS-8201a - confirmed MOA in patients

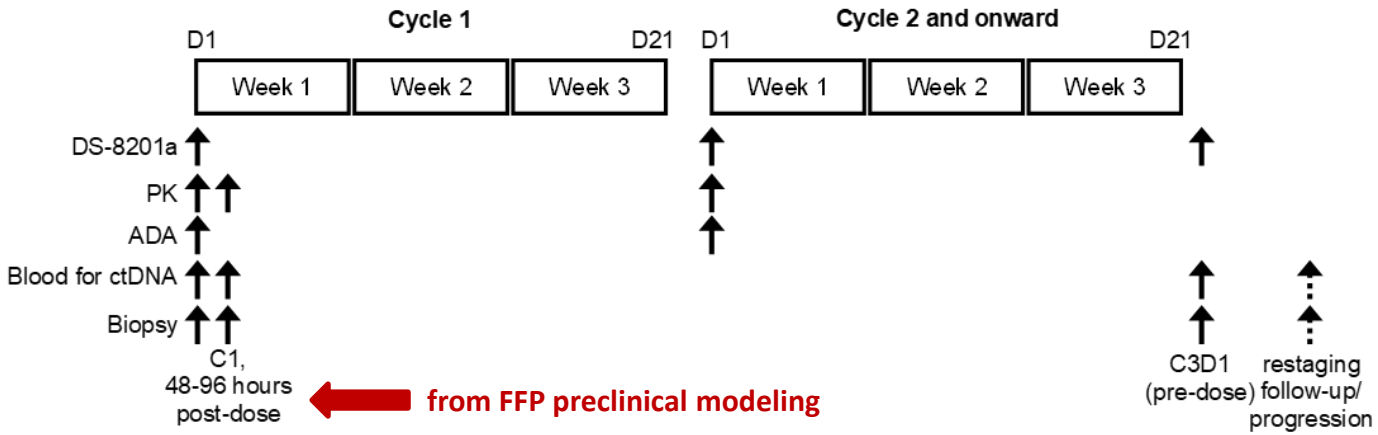
"Pilot Study of DS-8201a Pharmacodynamics in Patients with HER2-expressing Advanced Solid Tumors"

NCT Number: 04294628

LAO: NCI – Developmental Therapeutics Clinic (NIH Clin Ctr)

Principle Investigator: Alice P Chen

SCHEMA



Primary Objective:

- To assess the effects of DS-8201a on total TOP1 levels in biopsy specimens from patients with HER2-expressing advanced solid tumors, at early and late post-treatment time points, thereby establishing the degree and duration of DS-8201a target engagement

Eligibility:

- Patients ≥ 18 years of age, solid tumors who progressed on standard therapy, or no available standard therapy
- Biopsiable tumor
- HER2 IHC score of ≥1+ (CLIA-certified IHC) or *HER2* amplification (CLIA-certified NGS or ISH)

Study Design:

- Mandatory tumor biopsies at baseline and at 2 post-treatment time points, including one between 48-96 hours after dose 1, to assess TOP1 and DDR responses and their duration

Translational Pharmacodynamics of DS-8201a - confirmed MOA in patients (NCT04294628)

Molecular Pharmacodynamic Response in Tumor Biopsy (48-96 hrs after Dose 1)								
PT ID#	HER2	Dx	TOP1	TOP1cc	RAD51	pNBS1	γH2Ax	Response (Rx cycles)
19	2	OvCa	↓	nmc*	↑	↑	nmc	PR (17)
12	2	uterine (serous)	nmc	nmc	nmc	nmc	nmc	PD (3)
16	erbB2 amp	CRC	↓	nmc	↑	↑	nmc	SD (6)
33	erbB2 amp	bladder	↓	↓	↑	↑	nmc	PD (3)
36	**1**	**parotid**	**nmc**	**nmc**	**↑**	**↑**	**nmc**	**SD (5)**

***nmc, no molecular change**

Antibody Drug Conjugates - conclusions and planned studies

Conclusions

- fit-for-purpose studies in human tumor xenograft models accurately identified a crucial timeframe for tumor biopsy
- clinical PD biomarker responses to drug action occurred in HER2-low tumors (IHC 1+, 2+)
- PD study confirmed the intended MOA of DS-8201a in multiple cancer histologies

  TOP1i ✓ → DNA damage ✓ → repairable vs catastrophic damage **?** → cell fates **?**
- unexpected result: nuclear γH2Ax foci have *not* been detected during the DDR response to DS-8201a
- there are multiple, independent determinants of tumor response to HER2-targeting ADCs:
  - cell surface HER2 expression → delivered dose of TOP1i and resulting intratumor concentration (as a depot form?)
  - higher susceptibility to TOP1cc-mediated, catastrophic DNA damage → needs lower delivered dose of TOP1i → lower HER2 level
  - membrane permeability of the released payload → cytotoxic effect on HER2-negative by-stander cells (tumor heterogeneity)

Planned Studies of DS-8201a

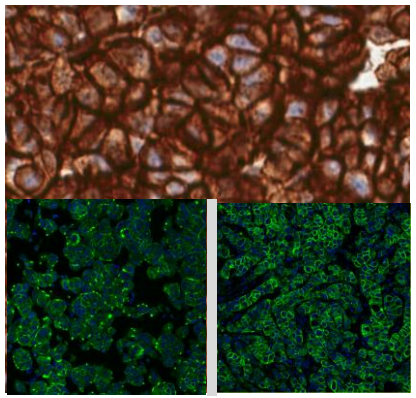
- extend studies to PD biomarkers of later events in DDR and their association with catastrophic DNA damage
- use PD biomarkers of DDR to compare MOA in HER2-high, -low and “-0+” human tumor models
- determine the PD biomarker profile of the minimum effective dose of DS-8201a in TOP1i-susceptible, HER2 1+ and HER2 0+ tumor models (Q: what is the HER2/DDR profile of a DS-8201a responsive tumor?)

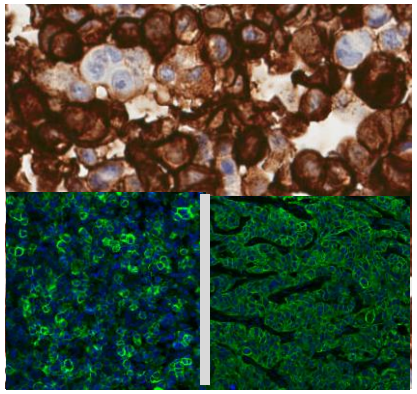
Laboratory Models Differing in HER2 Levels and Deruxtecan Sensitivities

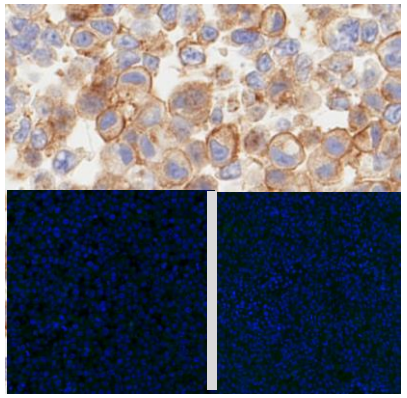
HER2 level

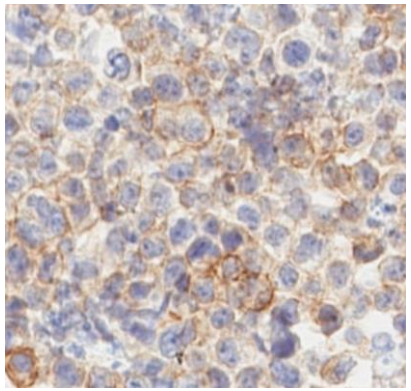
IHC of cell line

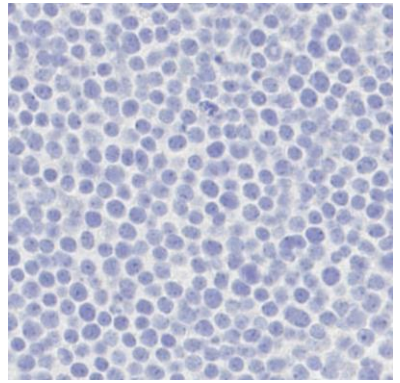
inserts: IFA of cell line and tumor XG











MDA-MB-361 **SKOV3** — HER2 amplified

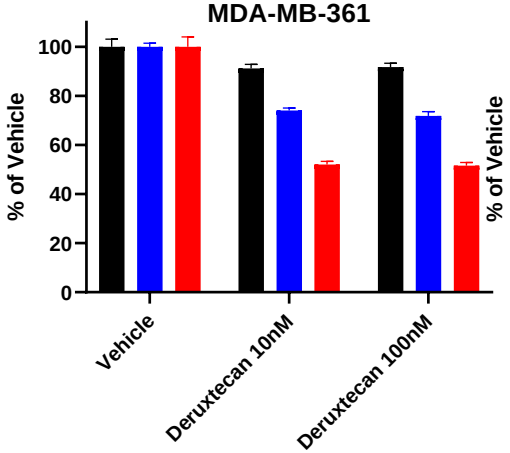
MKN45 **A2780** — *ERBB2* diploid

Jurkat — ERBB2-neg by RT-PCR (2 probe sets)

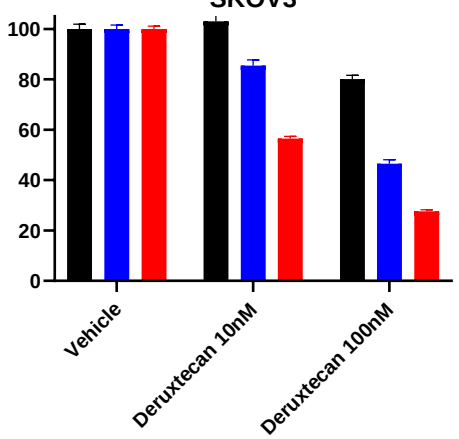
deruxtecan cytotoxicity

24 hr
48 hr
72 hr

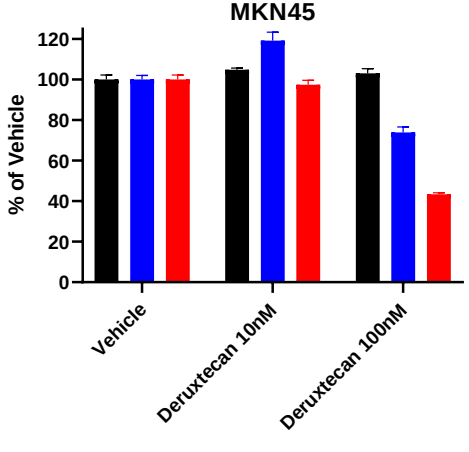
Xg was most sensitive

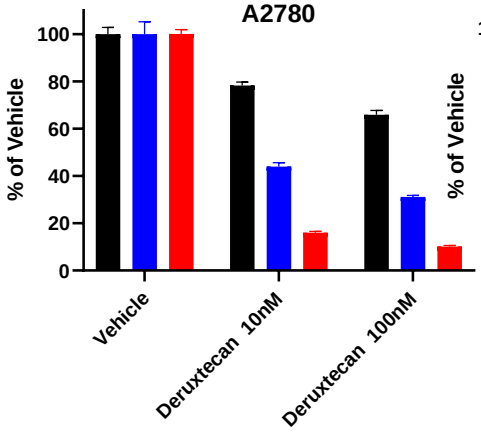


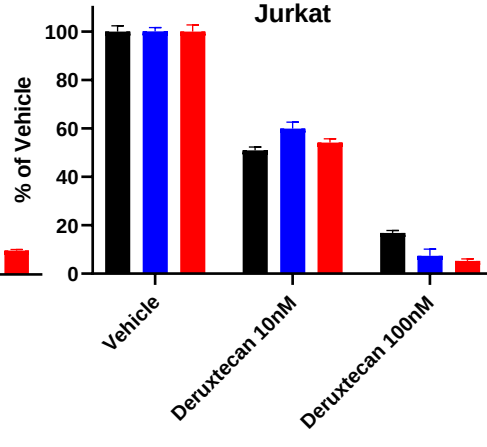
Xg was sensitive



Xg was resistant







DCTD Pharmacodynamic Biomarkers Program

NCI/DCTD

James Doroshow

Alice Chen

Geraldine O'Sullivan Coyne

Naoko Takebe

DT Clinic staff

NIH CC Interventional Radiology

CTEP Investigational Drug Branch

Melinda Hollingshead (DTP/BTB)

DT Clinic patients & families

FNLCR/LBRI

Managing Director - Ralph Parchment

ChangSoo Kim, Kay Gray

IQC Team Lead – Kate Ferry-Galow

Gabe Benton, Victor Lonsberry, Rachel Andrews, Amy Pantella

DDR Team Lead - Deb Wilsker

Angie Dull, Asma Begum, Hima Gali, Huanlian Chen, Alan Brooks, Weimin Zhu

CTC Team Leader-Lihua Wang

Brandon Miller, Sonny Khin, Francis Owusu

In Vivo Models – Dianne Newton

the entire BTB support program

Protein Expression Laboratory - Dominic Esposito

Clinical Specimen Lab (NIH Bldg 10) - Allyson Parr, Emily Lu

Clinical Protocol/Medical Writing – Melanie Simpson

Laura Kuhlmann, Sarah Miller

Lab Animal Sciences Program

Pathology/Histopathology

Lab Animal Husbandry

Questions?