Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

NCI Experimental Therapeutics Program (NExT):
Molecular Pharmacodynamics In Drug Discovery And Development

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Leidos Biomedical Research, Inc

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

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OUTLINE

- Pharmacodynamics (PD) for proof of mechanism (POM) and proof of concept (POC) in anticancer drug development: Example of cMET TKIs
- Lability and preservation of biomarkers at the point of collection and point of processing
- DNA damage response biomarkers and NCI early phase clinical trials
- Apoptosis biomarkers in support of CBC drug discovery
- FNLCR PD efforts in support of the extramural community
- What is ahead on the PD biomarker horizon?

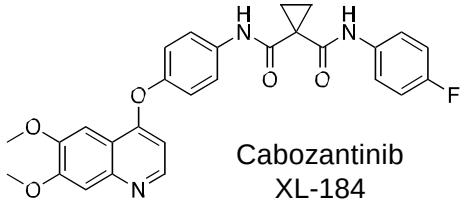
Pharmacodynamic Biomarkers

- Pharmacodynamics (PD) is broadly defined as “what a drug does to the body”
- At the molecular level, it’s the intended biochemical response of the target and its downstream pathways to drug action

Pharmacological Principles in PD:

- Proof of Mechanism of Action (POM): confirming that a drug acts as designed upon its intended target in a living tumor
- Proof of Concept (POC): confirming that a drug’s MOA leads to a therapeutic effect

Pharmacodynamic Biomarkers – POC and POM of MET-TKIs



Cabozantinib
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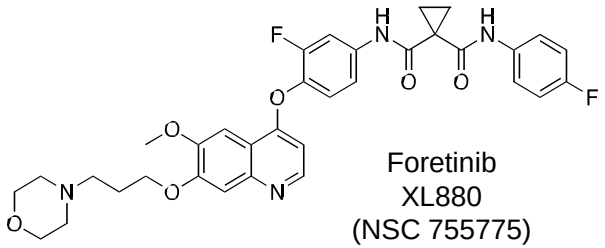
NSC 761068



Foretinib
XL880
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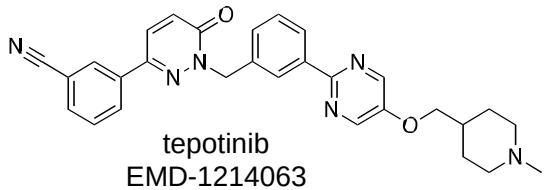

tepotinib
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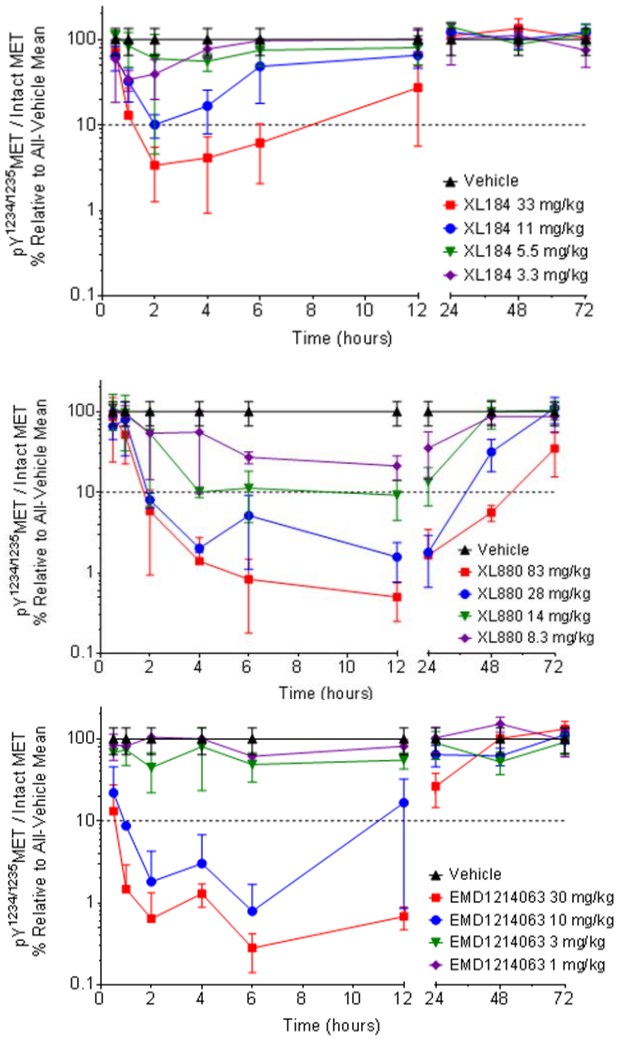
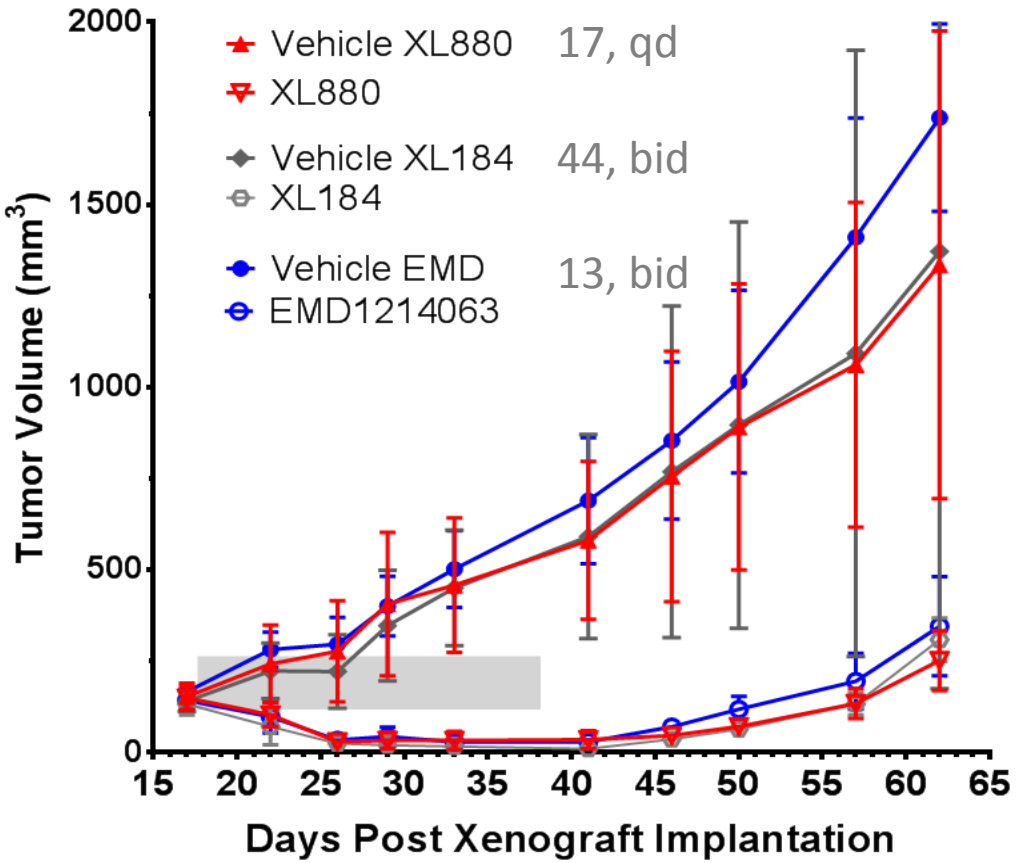
(NSC 758244)

Single Dose POM Studies



POC using PD-Guided Dose Scheduling



Srivastava et al. Mol Cancer Ther 2018;17:698-709

PD-guided Clinical Development of MET-TKIs?? Made Possible by Generation of a Specific mAb

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Product Type +

Development Stage +

Therapeutic Area +

ANTI-PY1235-MET IMMUNOLOGICAL BINDING REAGENT AS CANCER DIAGNOSTIC

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SUMMARY

This technology consists of highly specific rabbit monoclonal antibodies reactive with phosphorylated tyrosine located at amino acid 1235 in the human MET sequence. Binding to this pYI235 residue is independent of the phosphorylation of other tyrosines in the vicinity (1230 and 1234), does not cross-react with these nearby phosphotyrosine residues, and does not occur when Y1235 is unphosphorylated. Researchers at the NCI seek licensing and/or co-development research collaborations to commercialize and develop a companion diagnostic for selective MET inhibitors.

REFERENCE NUMBER

E-130-2016

PRODUCT TYPE

Diagnostics

KEYWORDS

Antibody, MET, Diagnostic, Companion Diagnostic, Rabbit Monoclonal Antibody, Tyrosine Kinase Inhibitor

COLLABORATION OPPORTUNITY

This invention is available for licensing and co-development.

CONTACT

John D. Hewes
NCI - National Cancer Institute

PCT Application
No. PCT/US17/22783
filed March 16, 2017

Time for PD-guided Development of MET-TKIs??

Trends in Cancer

CellPress
REVIEWS

Have Clinical Trials Properly Assessed c-Met Inhibitors?

Veronica S. Hughes[1,*] and Dietmar W. Siemann[1]

The c-Met/HGF pathway is implicated in cancer progression and dissemination. Many inhibitors have been developed to target this pathway. Unfortunately, most trials have failed to demonstrate efficacy. However, clinical trials have not

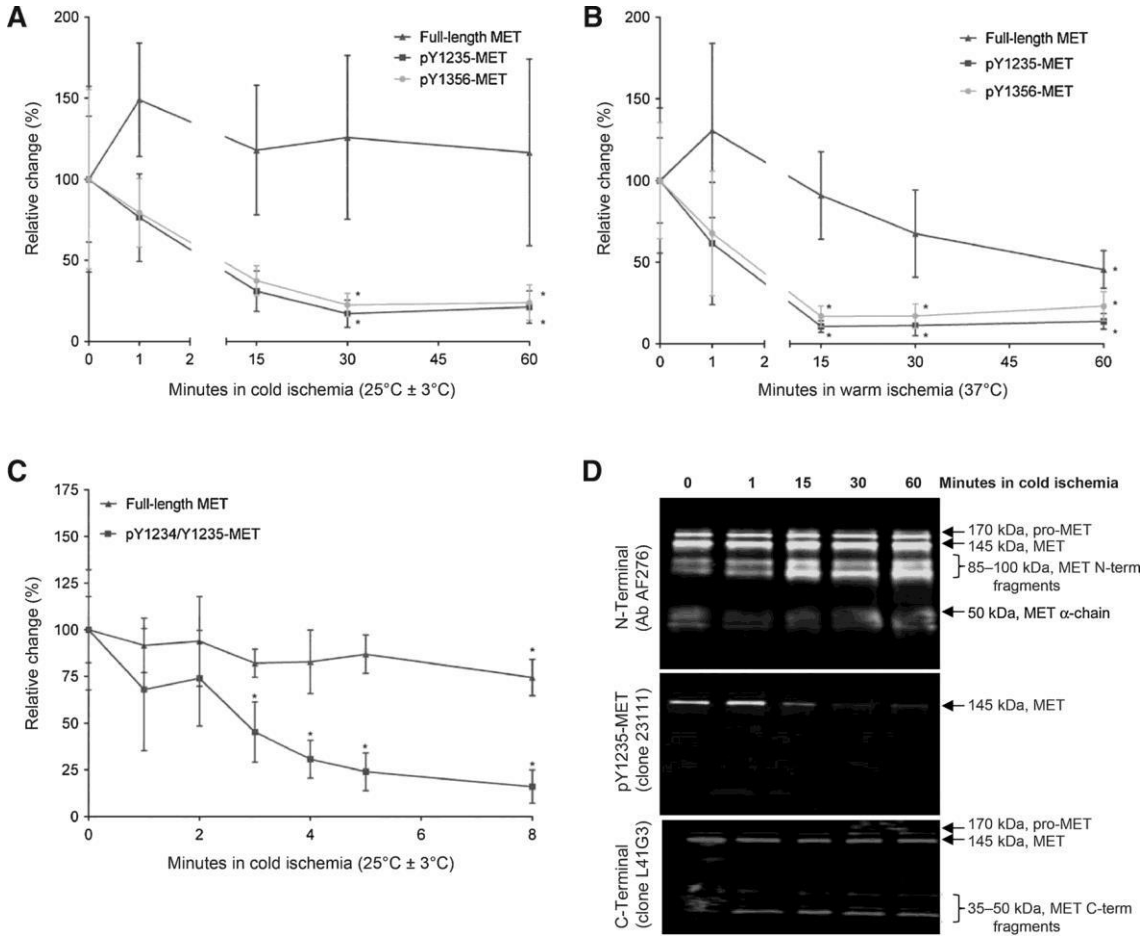
Trends in Cancer 2018; 4:94-97

Pathway activity should be verified in patients using an appropriate biomarker, yet biomarkers are rarely validated. Although a validated phospho-Met immunoassay has been developed, it is not currently used in clinical trials [12].

Assays such as this must be utilized if we are to advance therapeutics. Enrolling patients whose tumors do not express phospho-Met in a clinical trial of c-Met inhibition is unlikely to have a positive outcome, and is also unjust to the patients. Ultimately, potentially beneficial drugs may be discarded.

Highly Labile Post-translational Phosphorylation of Tyrosines: METpY1235 and pY1356 Are Examples

Stability vs Ischemia Time in core needle biopsies full-length MET and pMET (SNU5 xenografts)



Srivastava et al. Clin Cancer Res 2016;22:3683-3694

Demonstrating Fitness-for-Purpose 18-gauge core needle biopsy of xenograft tumor models

Dr Hollingshead, from Kinders et al. Clin Cancer Res 2008;14:6877-6885

SOP: 340507

Title: "Tumor Frozen Needle Biopsy Specimen Collection and Handling"

Purpose: point-of-care specimen handling for PD biomarker preservation

Method: snap-freeze within 2 minutes of collection

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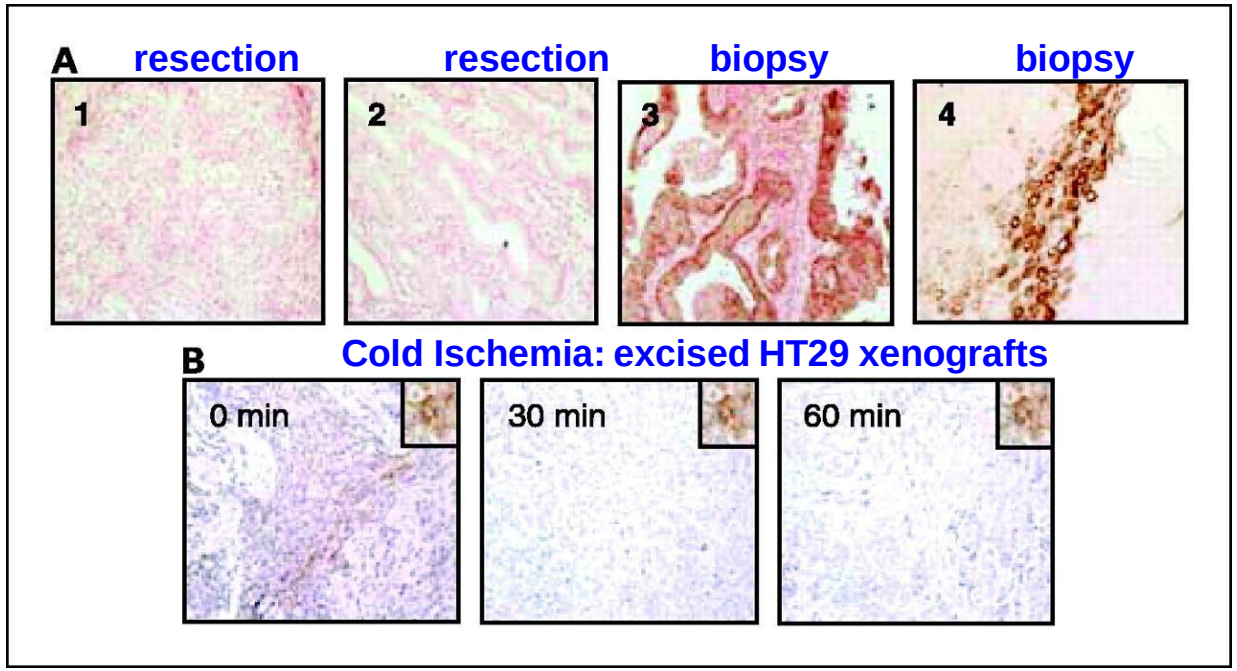
research community access via the

DCTD Website for Validated Biomarker Assays

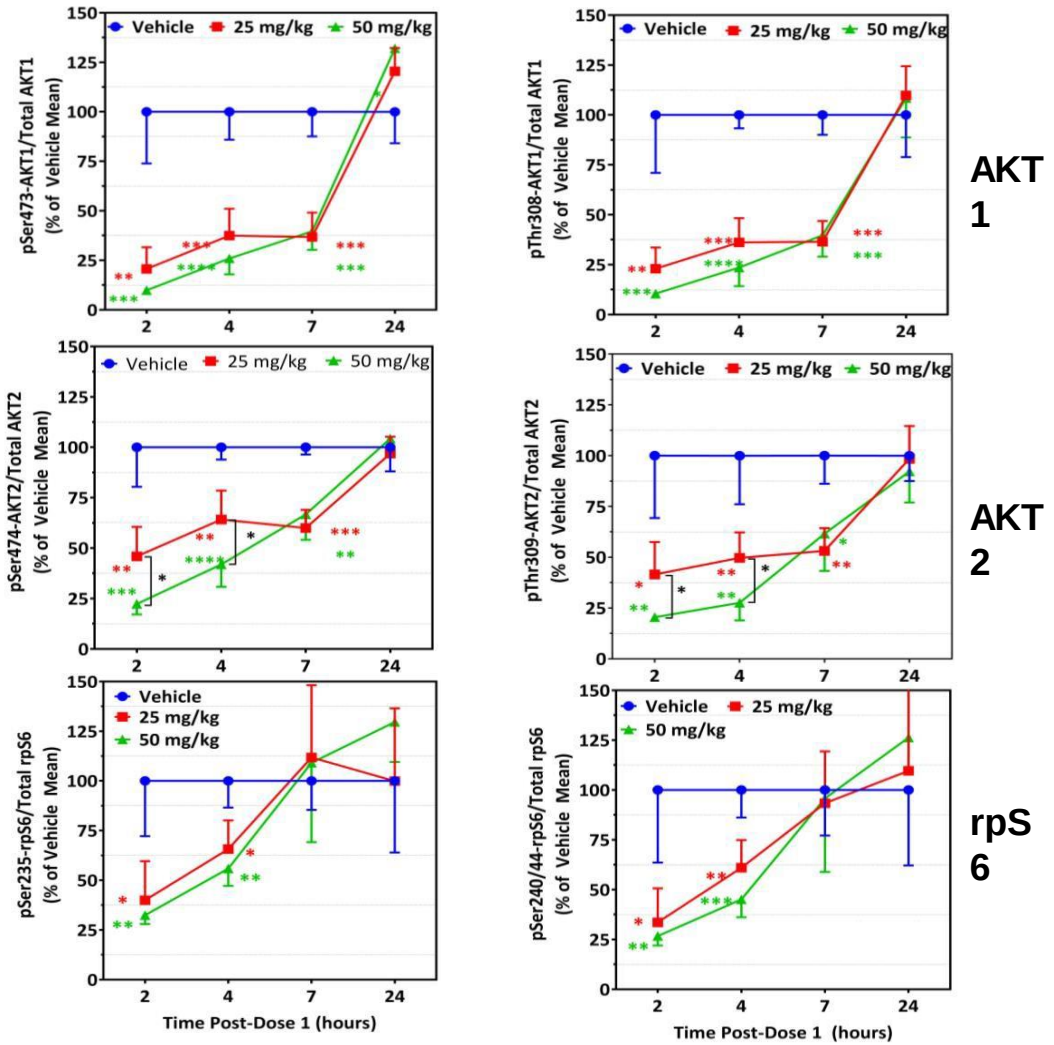
https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

PD Biomarkers are Dynamic and Highly Labile but are Evaluable if Pre-analytical Variables Are Controlled

phospho-Ser^{473}-AKT in human GE tumors and HT-29 colon cancer xenografts



Baker et al. Clin Cancer Res 2005;11:4338-4340



Srivastava et al, 2018 (unpublished)

Preservation of phosphoProtein Biomarkers at Point of Collection and at Point of Processing (Lab)

DCTD Website for Validated Biomarker Assays

https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

SOP 340507

Title:
"Tumor Frozen Needle Biopsy Specimen Collection and Handling"

Purpose:
Point-of-care core biopsy handling for PD biomarker preservation

Method:
Snap-freeze within 2 min of collection

SOP 341401

Title:
"Tumor Biopsy Lysate Preparation and Fractionation for IA"

Purpose:
Lab core biopsy processing for analysis of PD phosphobiomarkers

Method:
Thaw specimen under 4°C extraction buffer containing Roche PhosphoStop and Roche cOmplete™ Mini Protease Inhibitor Cocktail

SOP 340522

Title:
"Tumor Frozen Needle Biopsy Preparation for IFA"

Purpose:
Lab core biopsy processing for analysis of PD phosphobiomarkers

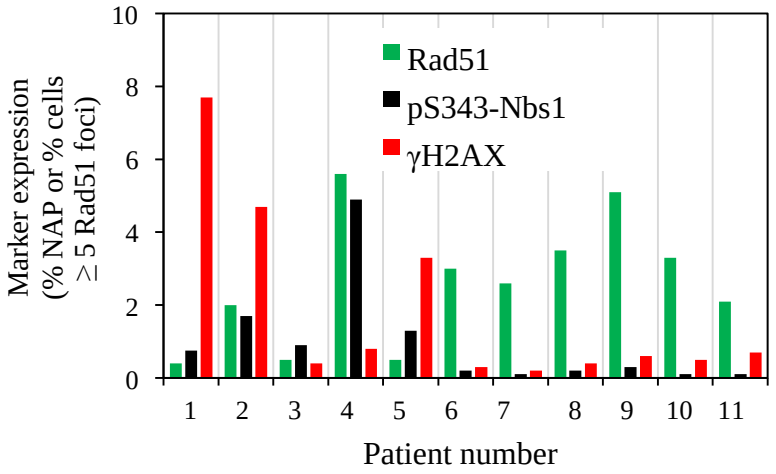
Method:
Thaw specimen under 10% neutral buffered formalin

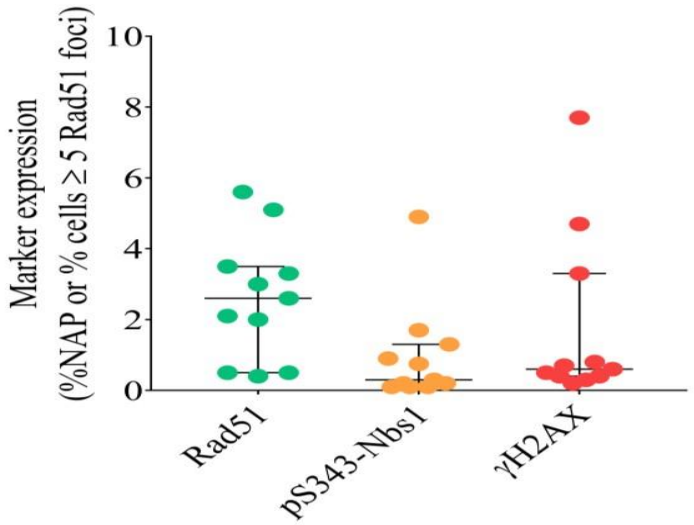
ETCTN Portfolio:
DNA Damage Response Modifiers

ETCTN Early Clinical Development of DDR modulators			
Pathway Target	Molecular Target	Agents	DDR PD Biomarker(s)
Single/Double Strand Break Induction	mulitple TOP1i	any chemoRx agents irinotecan, topotecan, indenoisoquinolines	pNBS1, γH2Ax, RAD51, ERCC1 γH2Ax w/cCasp3 (apoptosis)
Single Strand Break Response: BER	PARP1/2i	veliparib, olaparib, talazoparib	PAR polymer
	APE blockade w/TOP2 sensitivity	TRC102 (methoxyamine)	Late DNA damage (pNBS1, γH2Ax, RAD51)
Single Strand Break Response: TMB, MSI	PD1/L1 blockade	pembrolizumab nivolumab, durvalumab, atezolizumab	MLH1, MSH2, MSH6, PMS2
DDR Sensors	ATRi	M6620 (formerly VX-970)	pS1989 autophosphorylation
	DNA-PKi	M3814 M9831 (aka VX-984)	γH2Ax, pKAP1 (recent project plan)

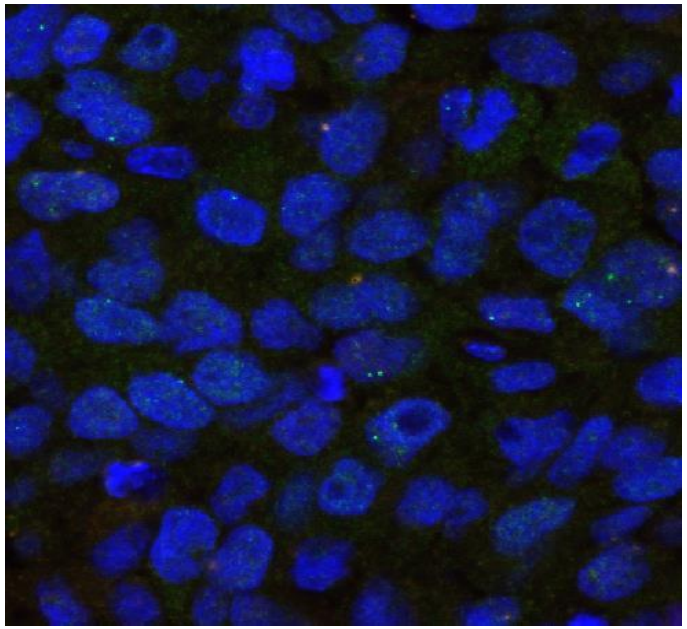
Multiplex Evaluation of DDR Biomarkers to Support ETCTN Portfolio of DNA Damage Response Modifiers

Baseline Biomarker levels DT Clinic – CRC Series

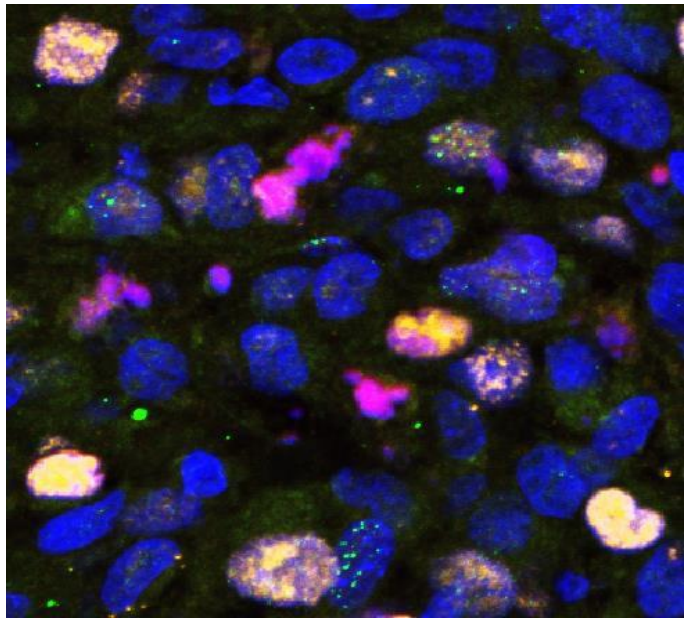




Vehicle-treated



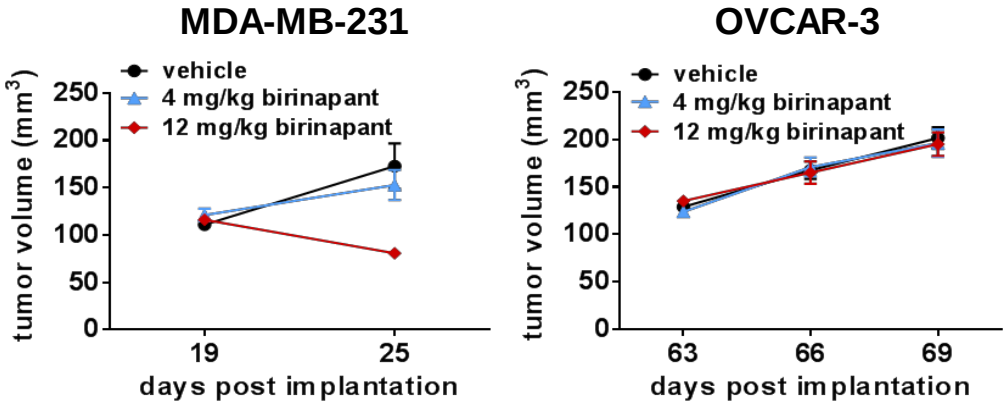
Topotecan-treated
(4.7 mg/kg; 4 hours post-treatment)

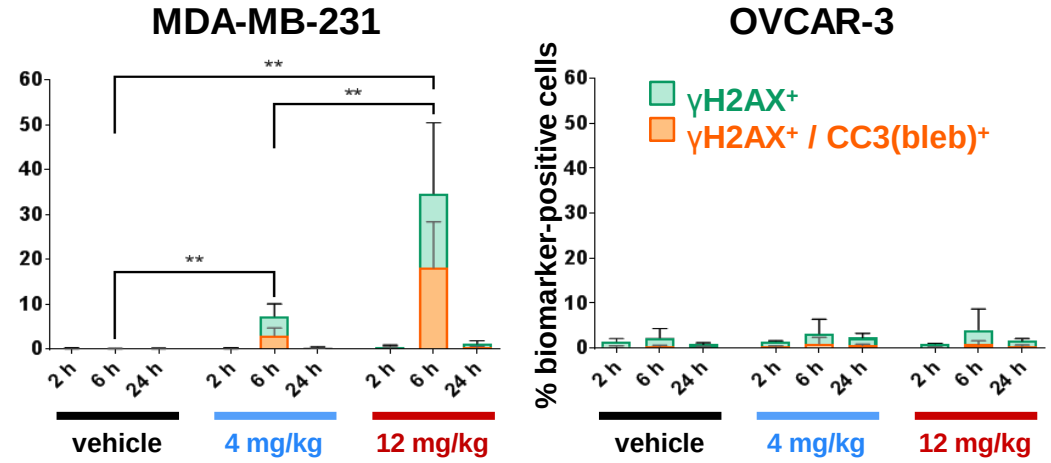


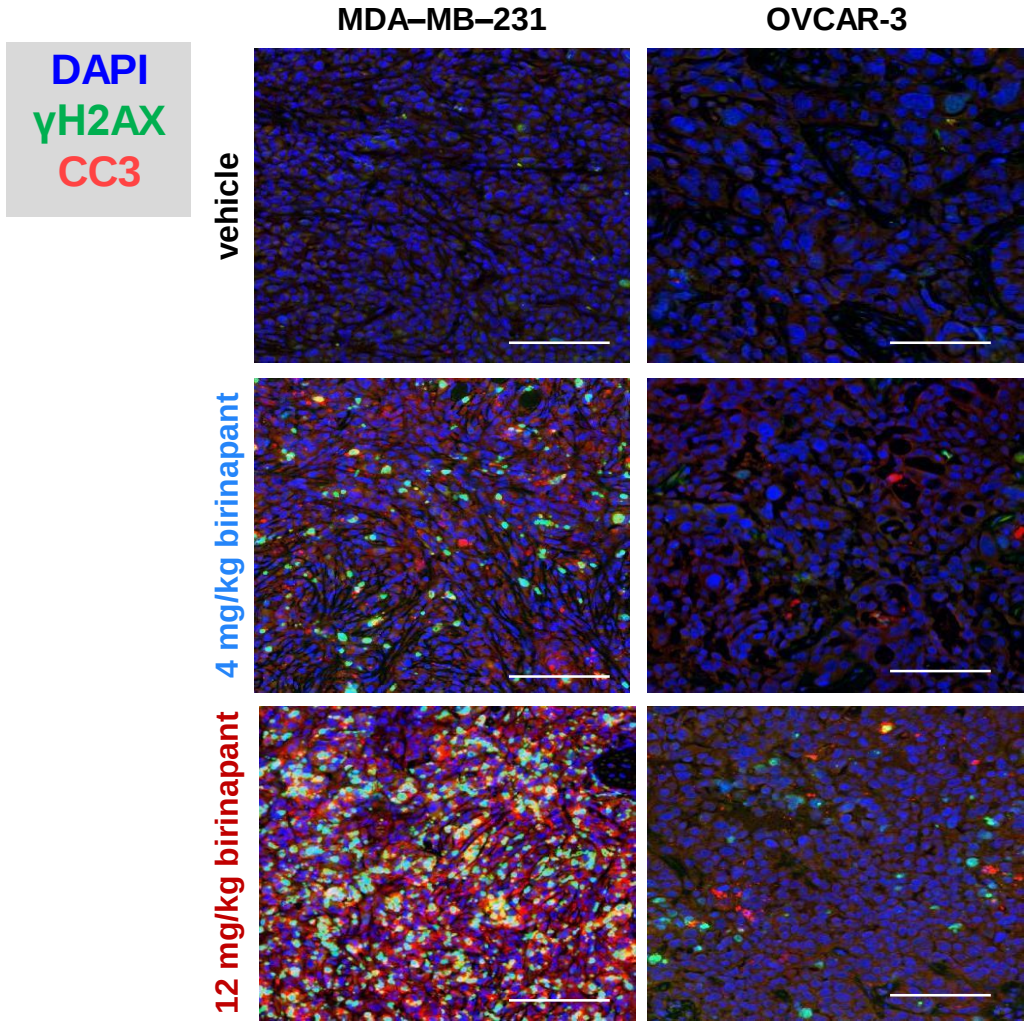
DDR Biomarkers: Rad51, pS343-Nbs1, γH2AX, DAPI

Wilsker, Dull and Kinders, submitted 2018

What Does a γH2AX Response Mean?

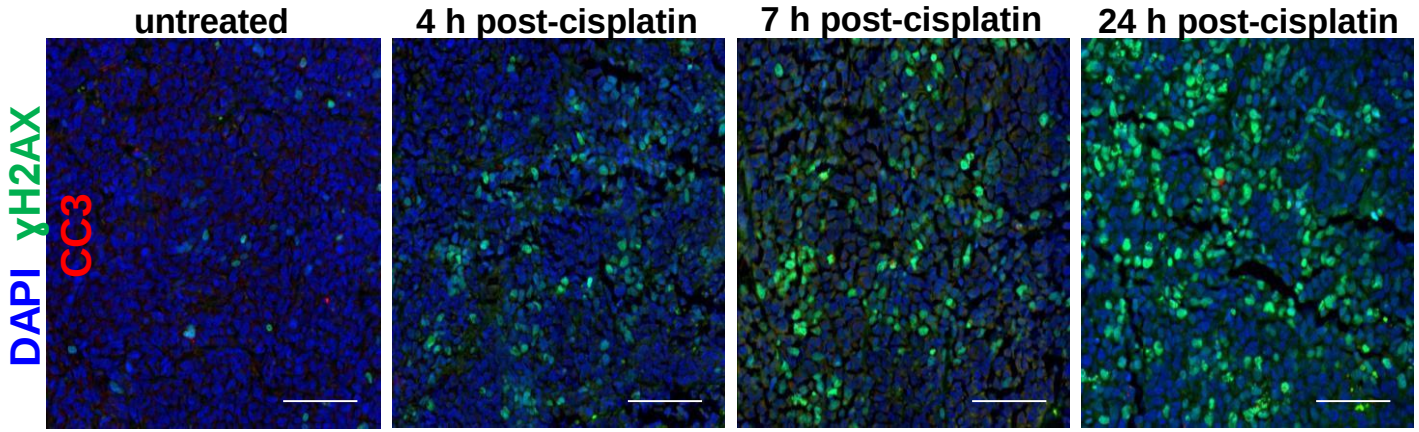


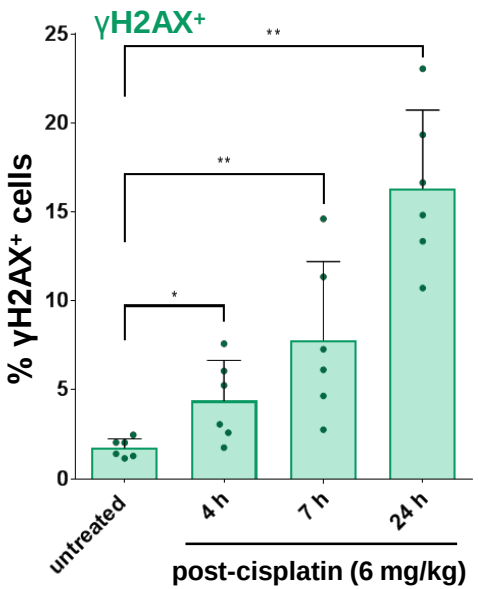


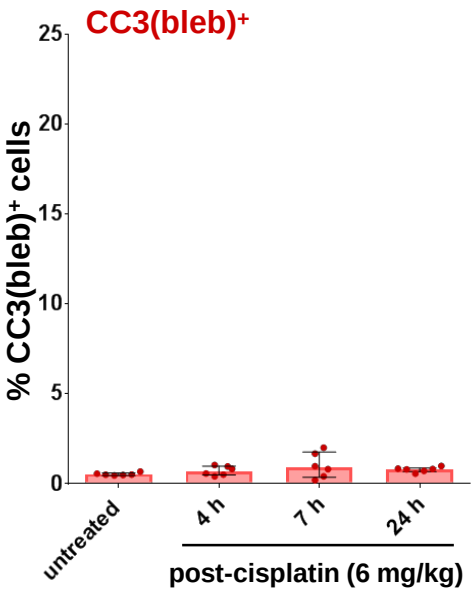


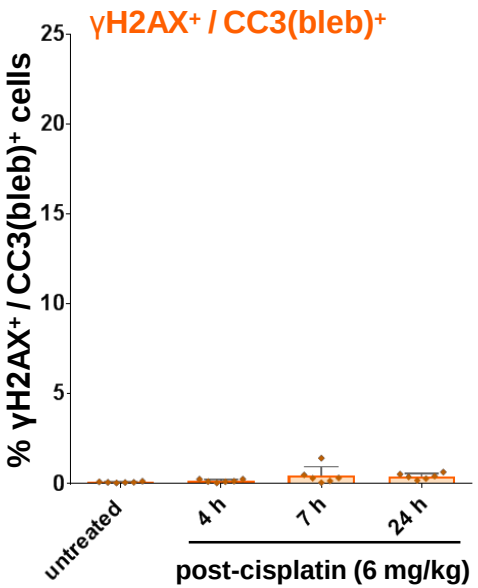
Dull et al. Oncotarget 2018; 9:17104-17116

What Does a γH2AX Response Mean?

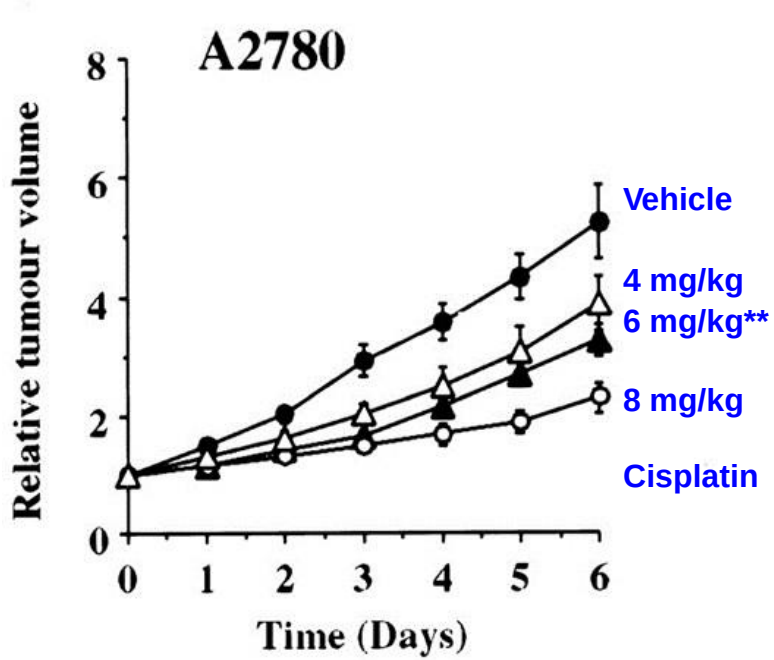








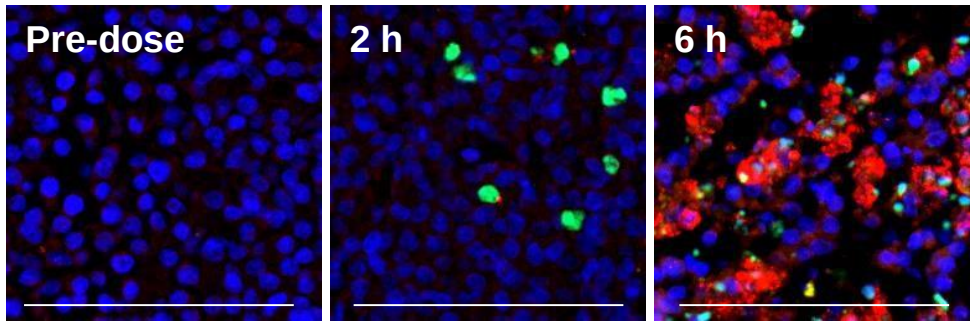
Dull et al. Oncotarget 2018; 9:17104-17116

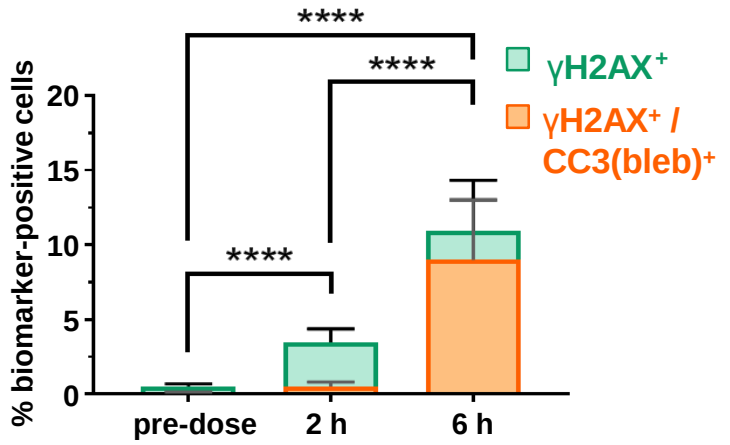


Plumb et al. Cancer Res 2000;60:6039-6044

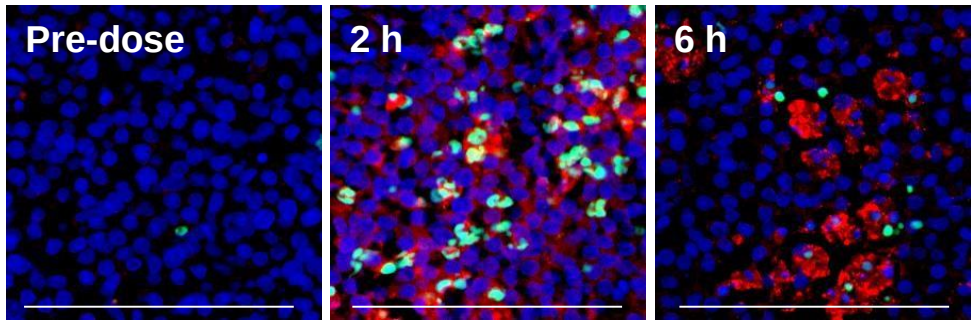
DNA Damage Response in Spontaneous Cancer Model (Canine Lymphoma Pts Treated with Topo 1 Inhibitors)

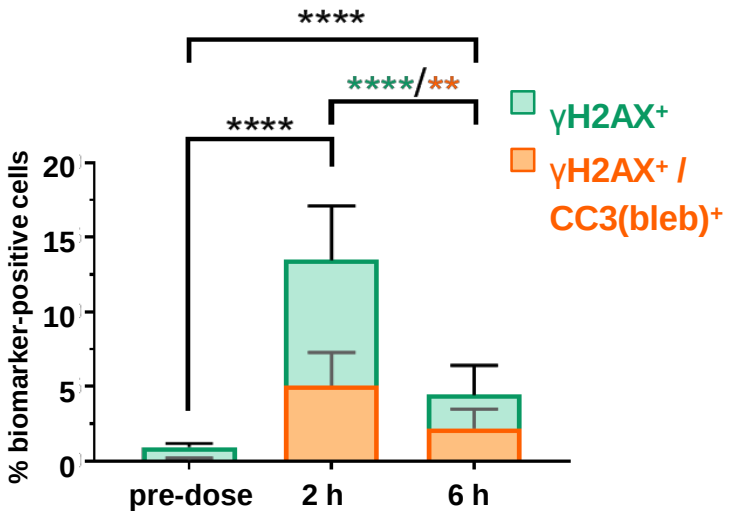
Patient A (indimitecan (LMP776), >60% tumor reduction)





Patient B (indotecan (LMP400),>60% tumor reduction)





reported assay values*

max % cells in apoptosis

Responders
- **Patient A: 9%**
- **Patient B: 5%**

Non-Responders
- **7 pts: <1.5%**

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max % of apoptotic cells in the γH2Ax population–

Responders
- **Patient A: 82%**
- **Patient B: 38%**

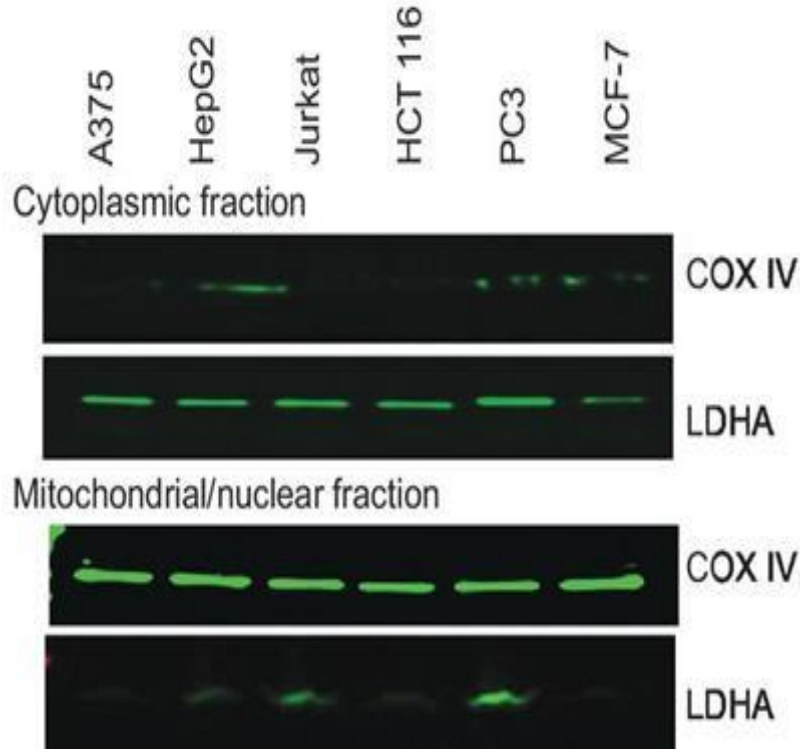
***15,000 cells evaluated per Bx**

Dull et al. Oncotarget 2018; 9:17104-17116

Apoptosis Biomarkers (Multiplex Immunoassay Elisa)

Intrinsic Apoptosis Sandwich Immunoassay v1.0

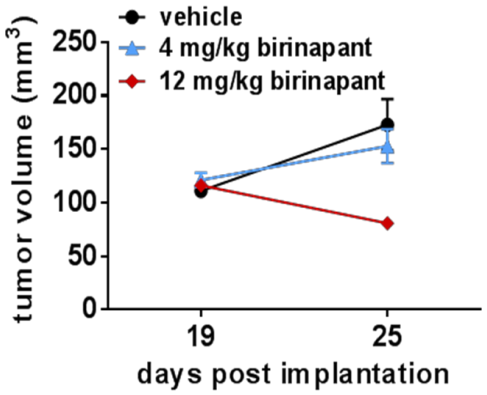
- **Panel 1: Bak, Bax, total Casp-3, total Lamin-B, SMAC**
- **Panel 2: Bad, Bax::Bcl2, Bcl-XL, Bim, Mcl1**
- **Panel 3: cCasp-3, Bcl-XL::Bak, Mcl1::Bak, pS^{99}Bad, survivin**

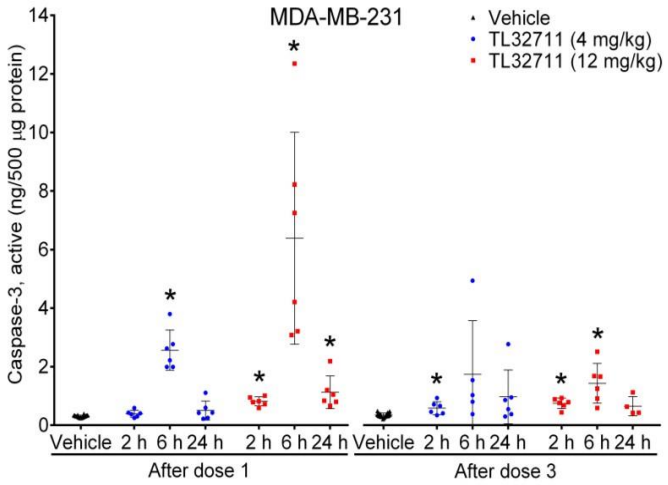


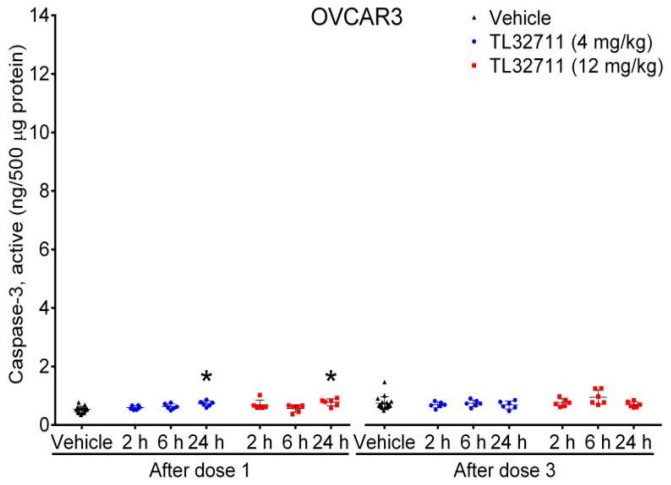
Srivastava et al. Clin Cancer Res 2016;22:1000-1010

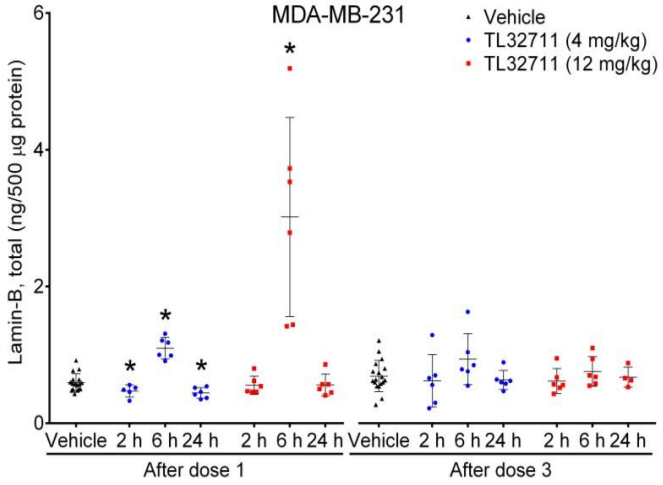
Apoptosis Biomarkers (Multiplex Immunoassay Elisa)

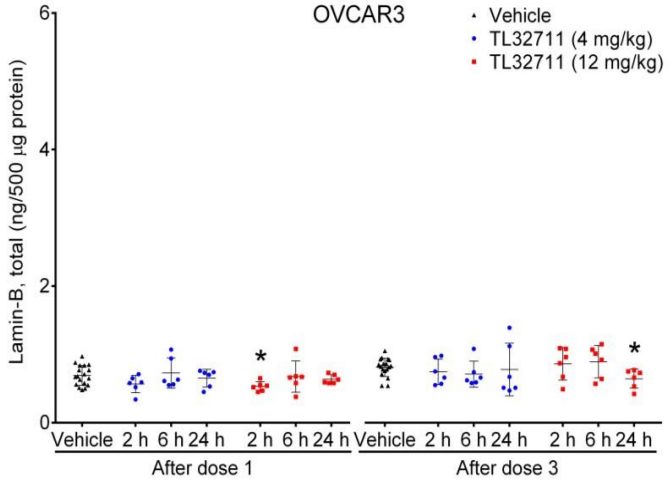
Responsive Model
MDA-MB-231



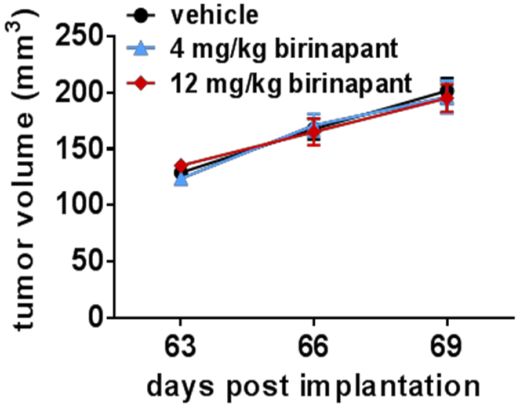






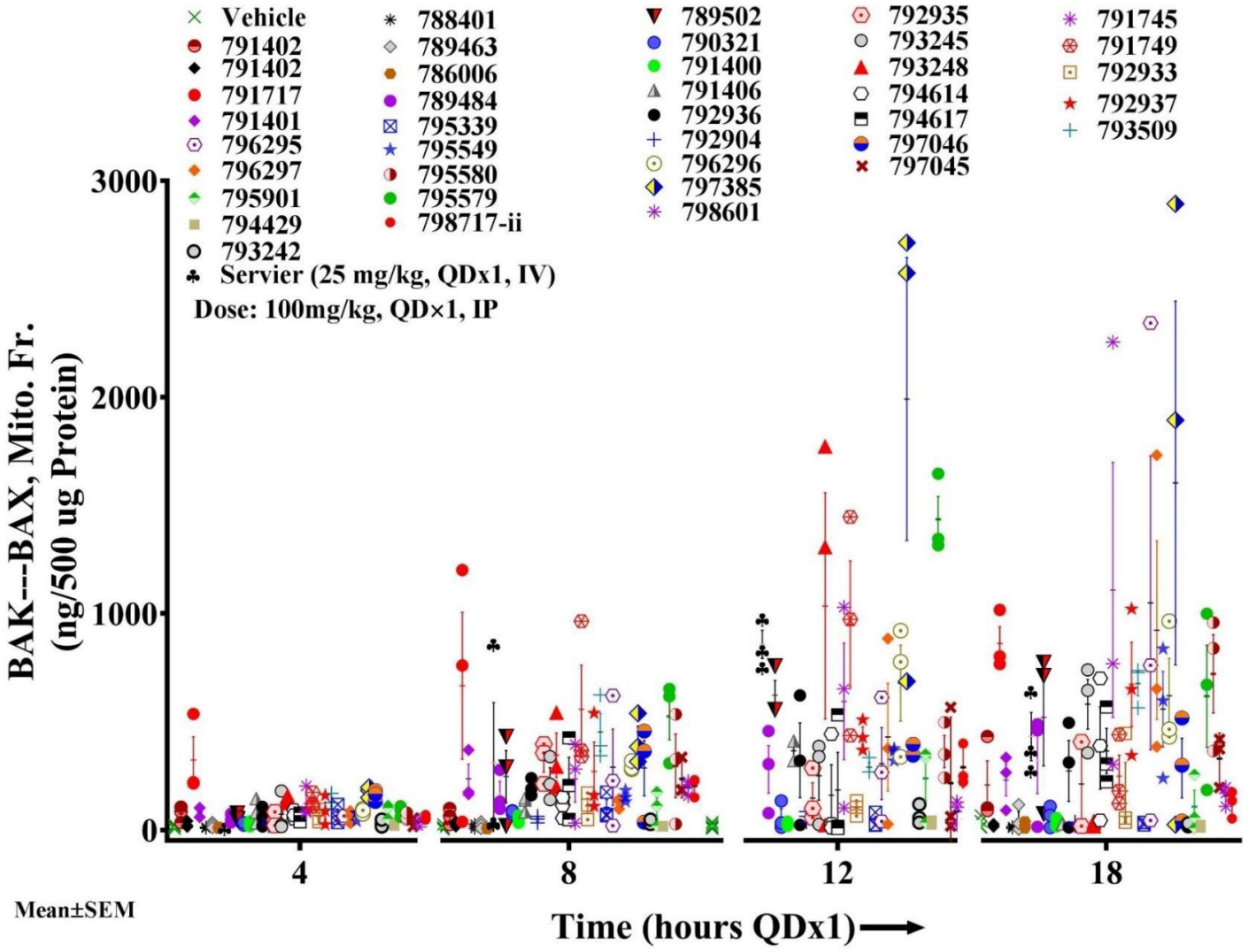


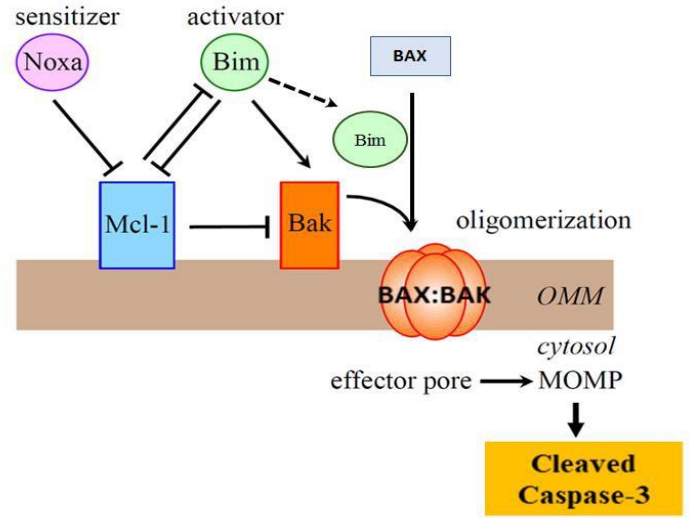
Non-Responsive Model
OVCAR-3



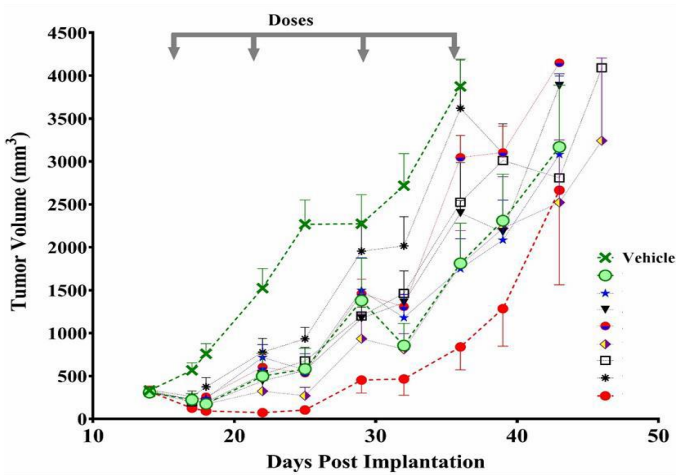
Srivastava et al. Clin Cancer Res 2016;22:1000-1010

PD Biomarker-Informed Drug Discovery by CBC Project Teams: MCL1 Inhibitors





CD4+ Plasmacytoma Model



Confidential Unpublished Data

Srivastava AK et al, AACR 2018, ASCO 2018

Apoptosis Biomarker Immunoassay – Community Access

Intrinsic Apoptosis Panel v1.0

- **Panel 1: Bak, Bax, total Casp-3, total Lamin-B, SMAC**
- **Panel 2: Bad, Bax::Bcl2, Bcl-XL, Bim, Mcl1**
- **Panel 3: cCasp-3, Bcl-XL::Bak, Mcl1::Bak, pS^{99}Bad, survivin**

Commercialization - Bio-Rad Inc w/Myriad RBM

- **Panel 1: Bak, Bax, ~~total Casp-3~~, total Lamin-B, SMAC**
- **Panel 2: Bad, Bax::Bcl2, Bcl-XL, Bim, Mcl1**
- **Panel 3: cCasp-3, Bcl-XL::Bak, Mcl1::Bak, ~~pS^{99}Bad~~, survivin**

http://www.biorad.com/webroot/web/pdf/lsr/literature/Bulletin_6474.pdf

Community Access

***User Model* –**
Direct purchase of assay kits from BioRad, coupled with NCI-Frederick assay training program
(see https://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm)

***Fee-for-Service Model* –**
Ampersand Biosciences Inc (NY), GLP but not CLIA uses

***Contract Model* –**
RBM Myriad Lab (TX), CLIA-certified lab for diagnostic uses

Contributions to the Extramural Community

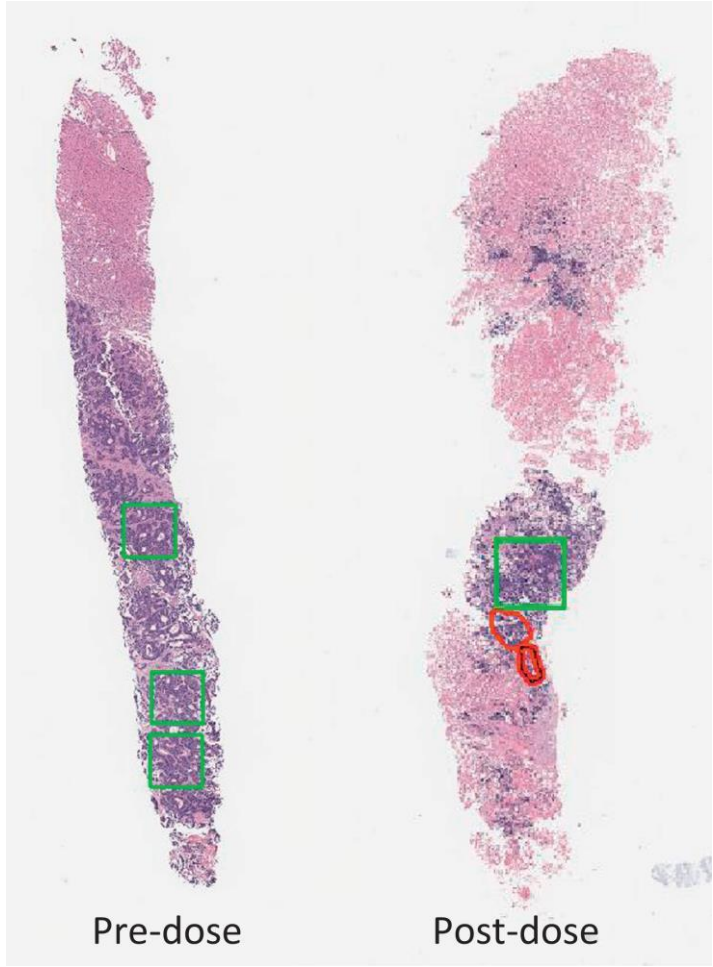
Assay Support of NExT/CBC Discovery Teams and NExT/ETCTN Development Teams

- Generating new Mabs to measure precise MOAs (MET-pY^{1235}, MET-pY^{1356}, ATR-pT^{1989})
- Qualifying commercial Mabs for intended use, and QC of supply chain
- Creating recombinant protein calibrators (e.g., heterodimeric proteins, such as Mcl1::Bak for the apoptosis panel)
- Distributing assay kits
- Transferring Validated, Fit-for-Purpose Assays to End-User Laboratories
  - lab-based training of certified assay operators at the NCI-F campus
  - enable formal, SOP-based assay transfer by providing qualified key reagents and proficiency panels
  - monitor assay performance as a lab network
    - root cause analysis in case of an assay failure
    - assessment of the proposed solution
    - amend the SOP to the next version, if necessary
    - issue a recommended hold on clinical specimen analysis during the correction process
- Providing centralized lab assay support to NCI-sponsored clinical trials in the ETCTN

Contributions to the Extramural Community (2)

Initiative to improve core biopsy quality and suitability <u>for pharmacodynamic and other biomarker analyses</u>

- Recognized biopsy quality as a major issue to solve for the success of POM and POC studies
- Created tumor “biopsy board” at NCI – regular <u>communication</u> between clinical trial oncologists, pathologists, radiologists, and PD biomarker laboratory to connect clinical and lab findings
- May 2017 NCI conference on re-thinking biopsy collection for non-diagnostic purposes
- Developed or changed laboratory methods to increase biopsy evaluability (tumor cell segmentation, lab practices/procedures)



Parchment and Doroshow. Semin Oncol 2016;43:427-435

What's Ahead on the PD Biomarker Horizon?

CBC ETCTN

- Signaling Pathways (MEK/ERK/RP6, PI3K/AKT) → PI3Kβ/δi
- Cell Death mechanisms → multiple agents
  - apoptosis-intrinsic v2.0, apoptosis-extrinsic, necroptosis, ferroptosis, anaphase catastrophe, pyroptosis, oxeiptosis, perforin/granzymes, autophagy
- DNA Damage Response and "Cell Cycle Catastrophe"
  - enhance chemotherapy-induced DNA damage → PARPi, BERi, ATRi, DNA-PKi
  - exploit intrinsic tumor defects in DNA repair (HRD, mutATM, MMR status) - PARPi, ATRi
  - exploit intrinsic tumor defects in cell cycle control during DNA repair (CDKi) → Wee1i
- Protein Homeostasis, Oxidative ER Stress (ubiquitination, proteasome inhibitors) → p97i
- Methylation of protein and DNA (p16/p21 induction, methylome patterns) → WDR5i, KDM5i, DNMT1,3i
- Immune Checkpoint Inhibitors and CTL function (CTLA4i, integration of PD1 and TCR signaling) → SHP2i
- Mapping targets to EMT and CSC subpopulations to treat biological tolerance of therapy → multiple
- Adaptation of biopsy-based assays for hematologic malignancies and for CTCs → apoptosis

DCTD Pharmacodynamic Biomarkers Program

NCI/DCTD

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Alice Chen

Geraldine O'Sullivan Coyne

Naoko Takebe

Melinda Hollingshead

Yves Pommier

DT Clinic staff

NIH CC Interventional Radiology

Investigational Drug Branch

DT Clinic patients

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- Will Herrick
- Casey Kilpatrick

IQC Team Lead - Kate Ferry-Galow

- Victor Lonsberry
- Rachel Andrews
- Manisha Mohandoss

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- Ravi Putvatana
- Lan Tran

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- Sharon Allison

Project Manager – Kay Gray

Clinical Protocol/Medical Writing – Melanie Simpson

- Andrea Voth, Sarah Miller, Laura Fogli, Miriam Konaté

In Vivo Models – Dianne Newton

- the entire BTB support program

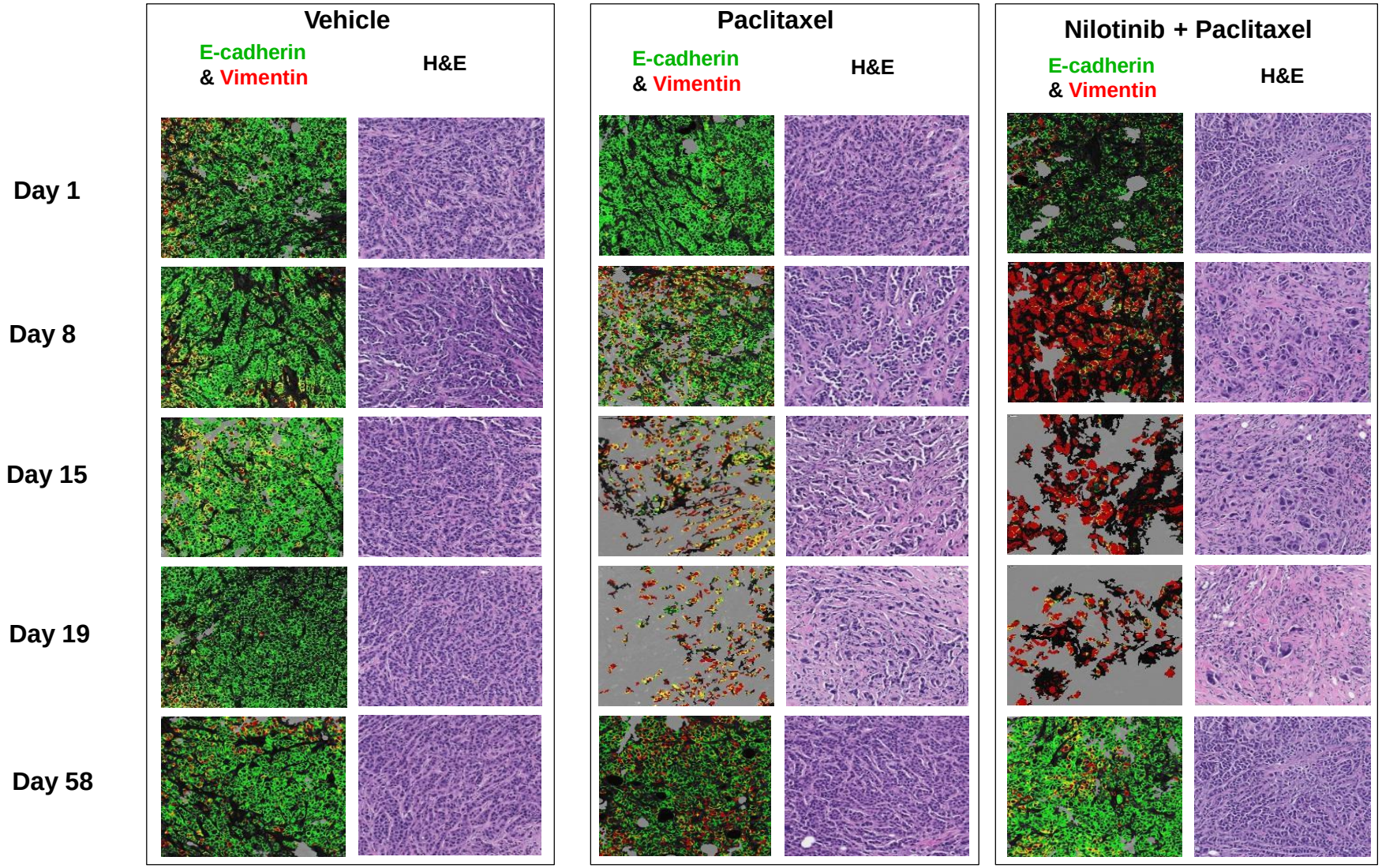
Protein Expression Laboratory - Dominic Esposito

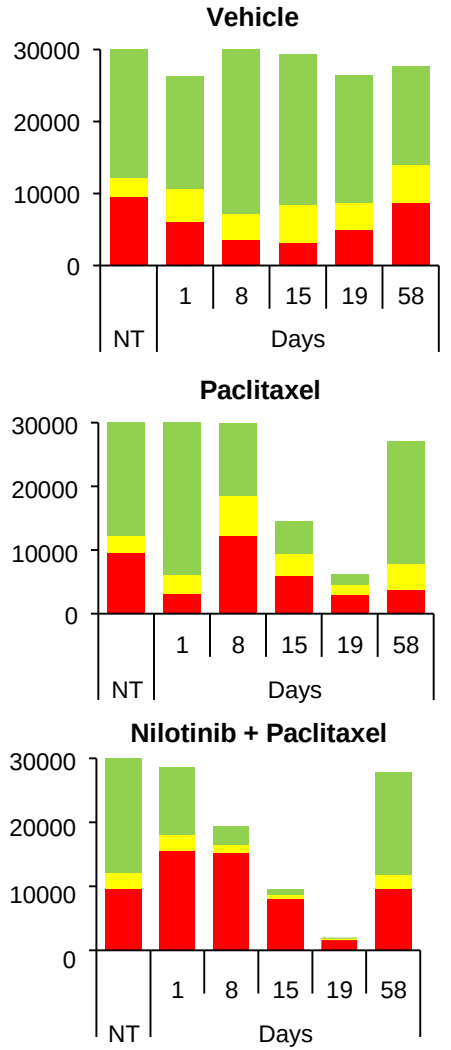
Lab Animal Sciences Program

- Pathology/Histopathology Lab
- Animal Husbandry

Supplemental slides

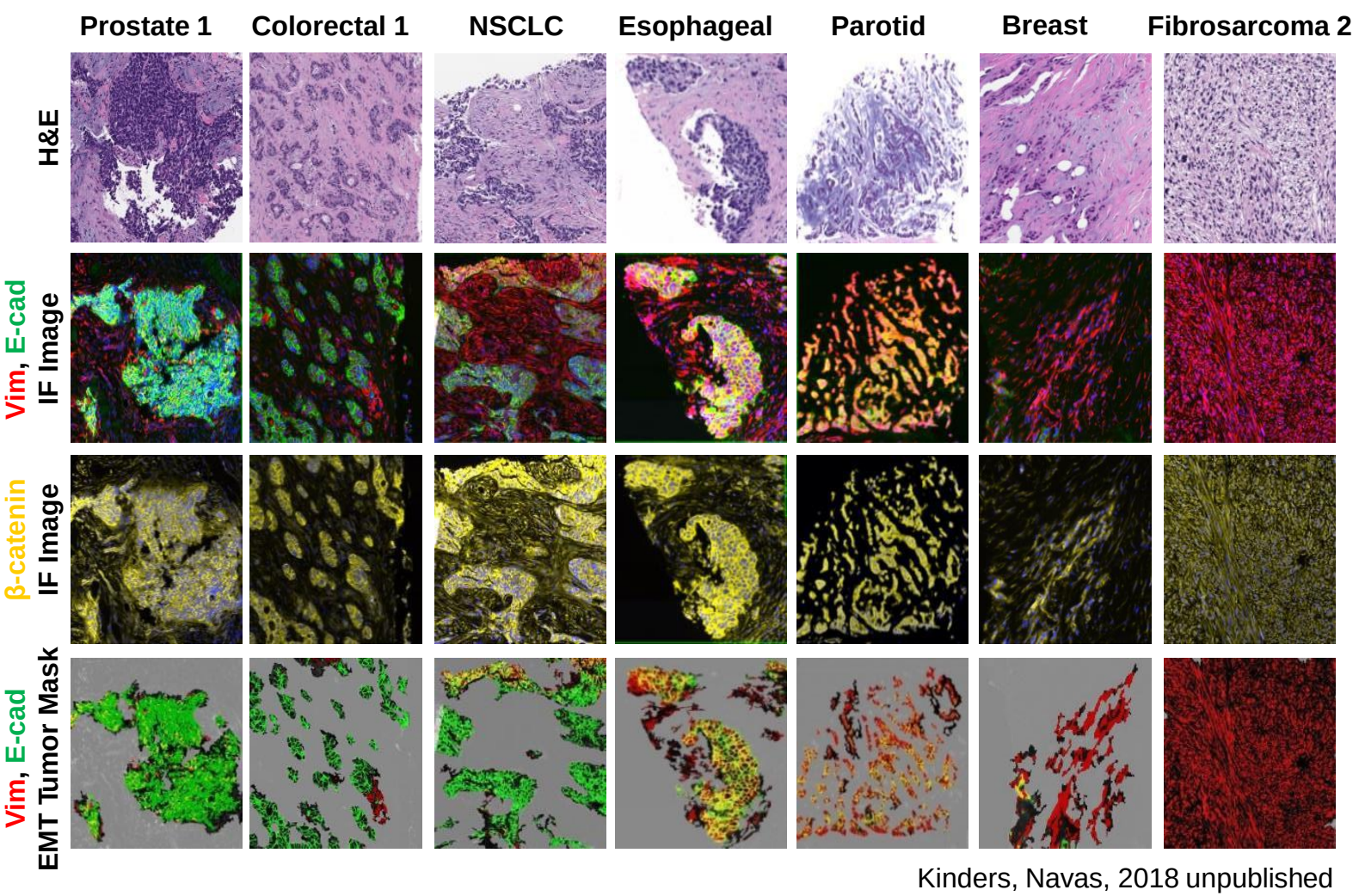
PD Biomarkers in Clinical Translation of Novel Drug Combinations: EMT-based Survival of Nilotinib + Paclitaxel



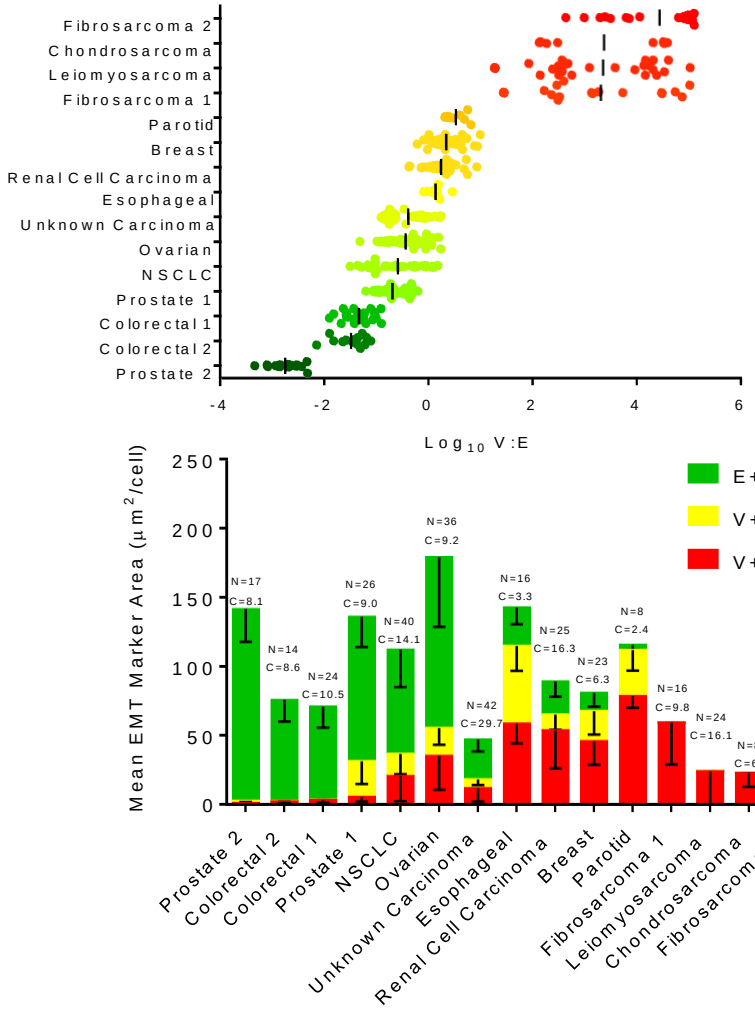


Kinders, Wilsker, Navas, 2018 unpublished

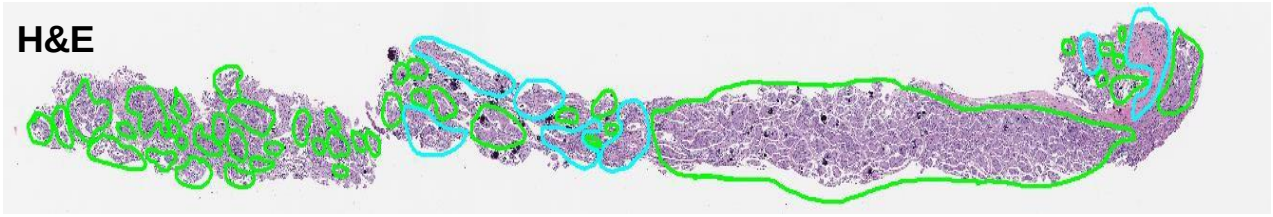
PD Biomarkers in Clinical Translation of Novel Drug Combinations: EMT in the Clinic

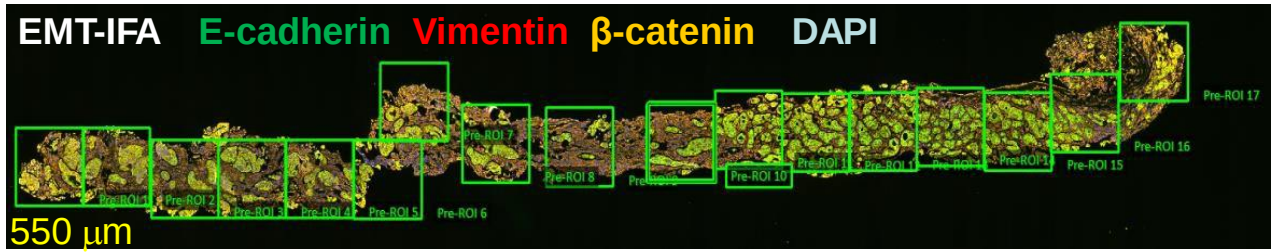


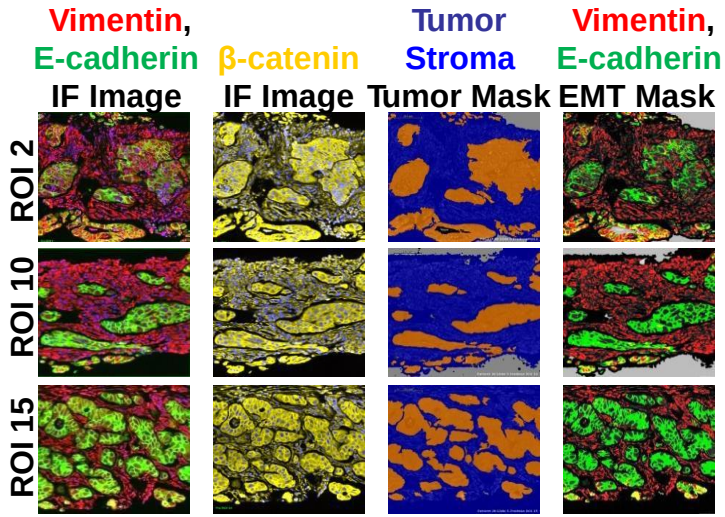
Kinders, Navas, 2018 unpublished

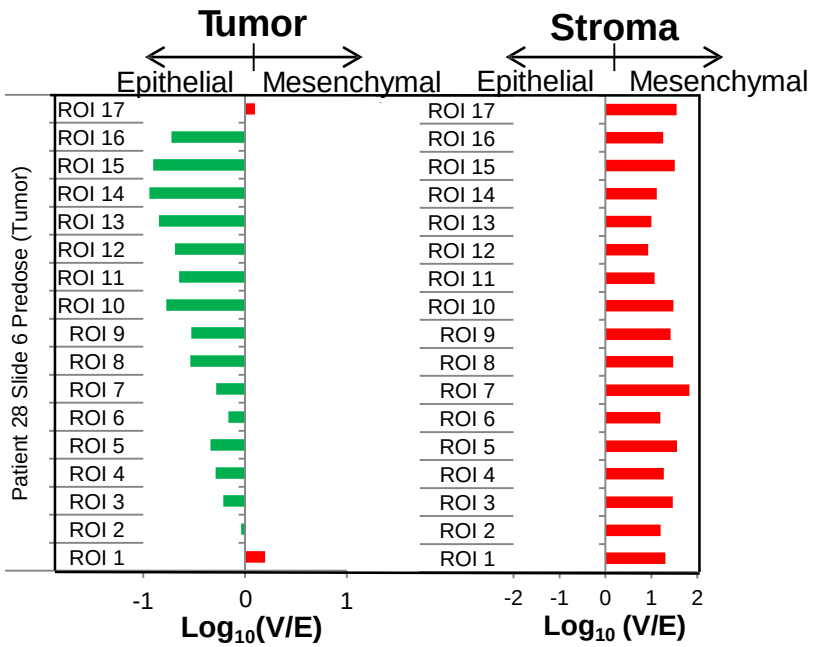


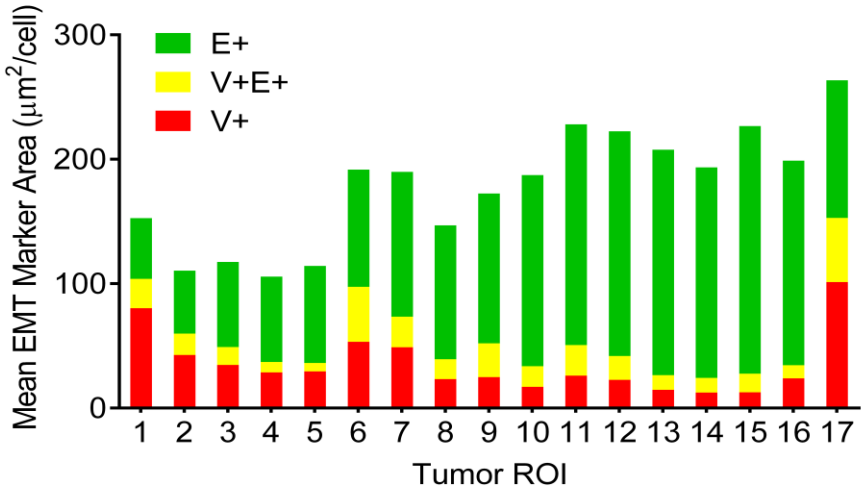
Epithelial-Mesenchymal Transition (EMT): Image Analysis Pipeline to Quantify EMT in Tumor Bx





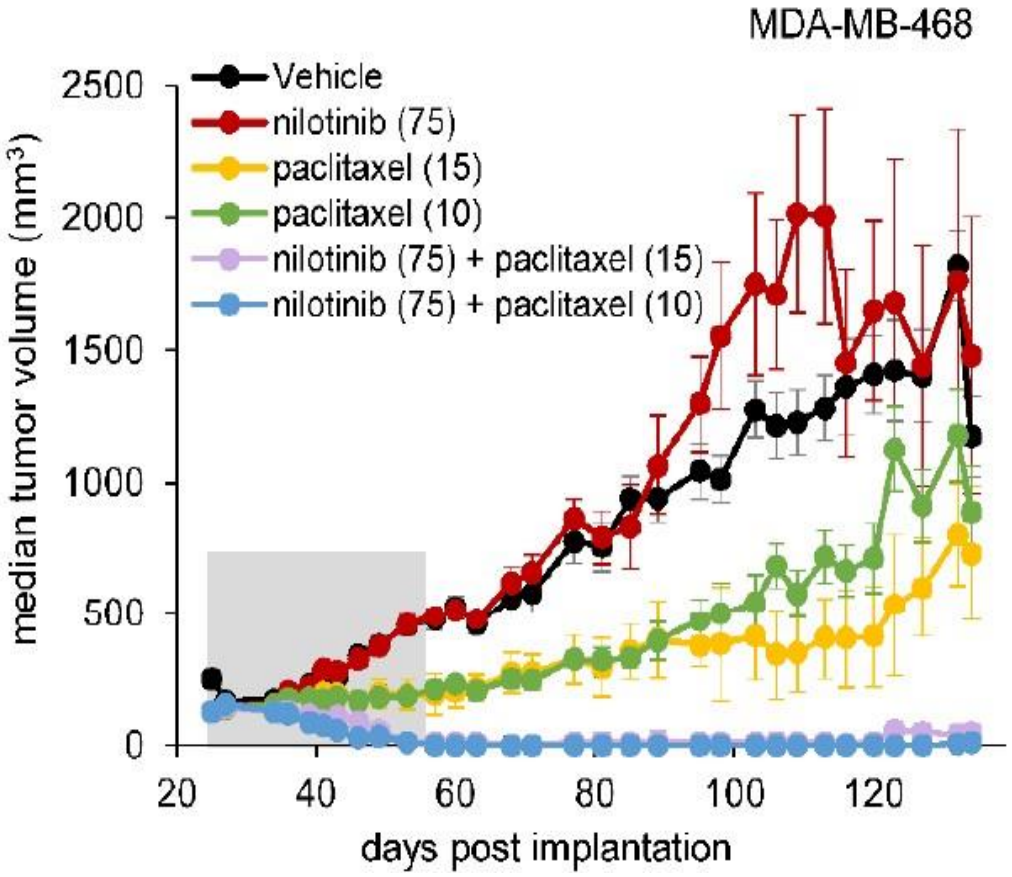




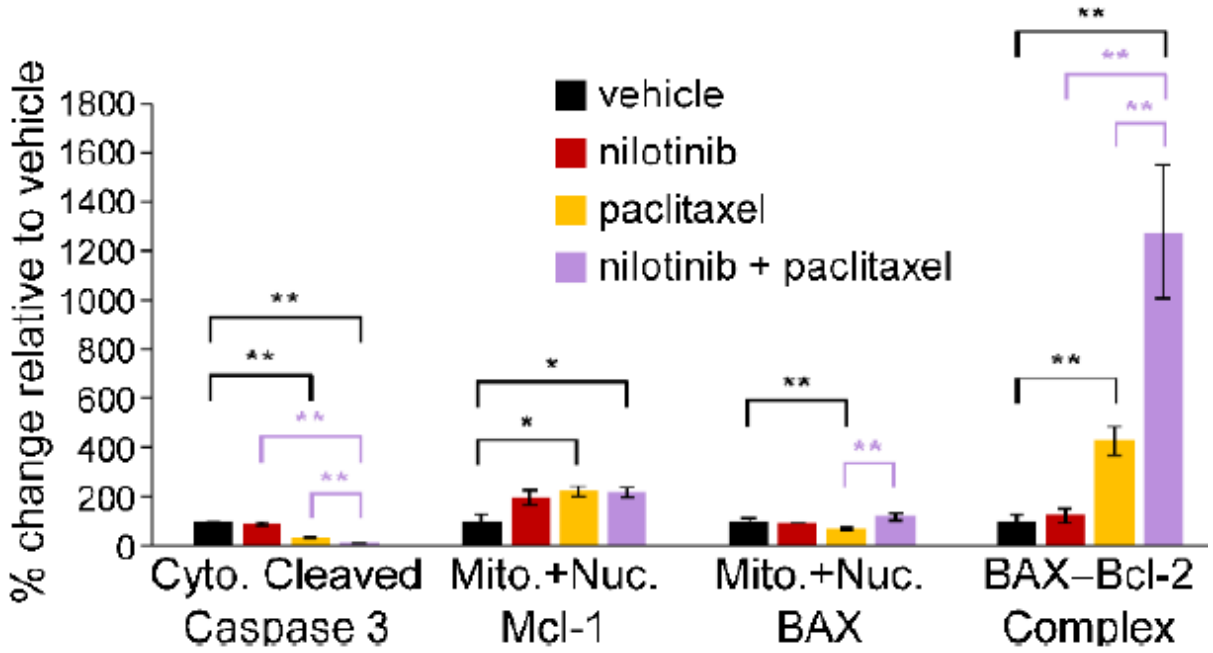


Kinders, Navas unpublished

PD Biomarkers in Clinical Translation of Novel Drug Combinations: Nilotinib + Paclitaxel



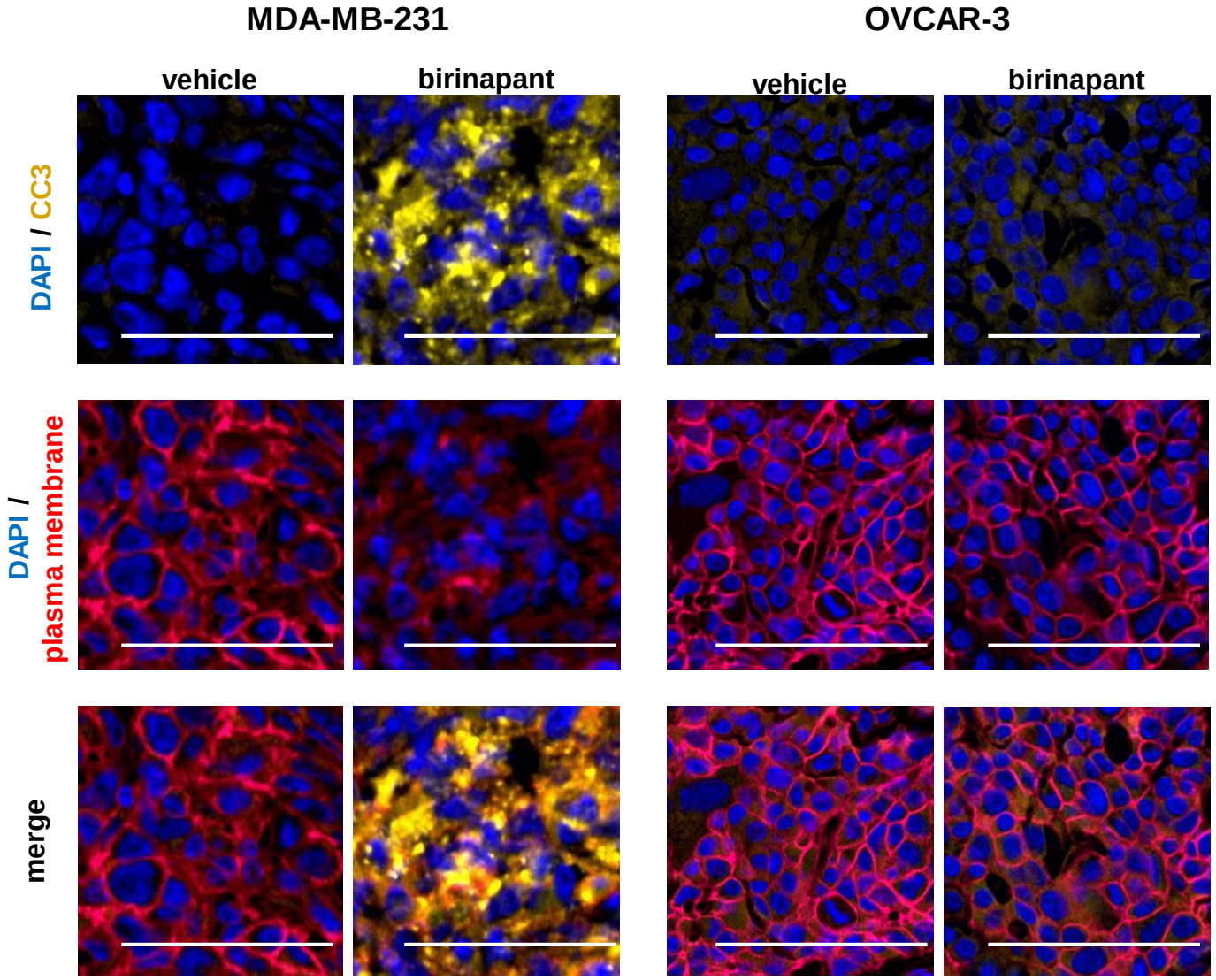
Holbeck S et al., *Cancer Research*, 2017



Intrinsic apoptosis pathway markers suggest cells remaining after two cycles of treatment appears to be resistance to apoptosis

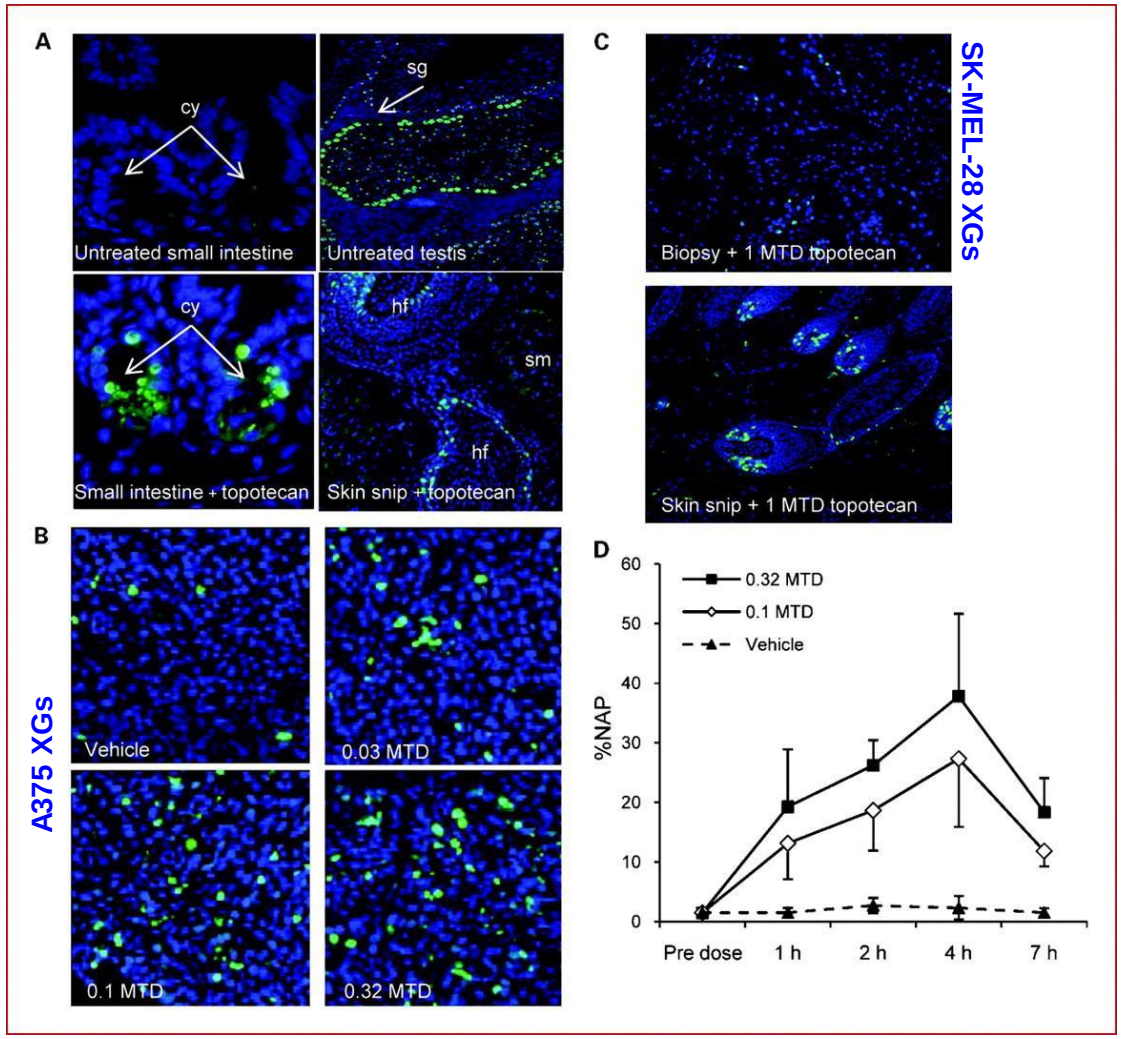
Unpublished data show modulation of a necroptosis regulator

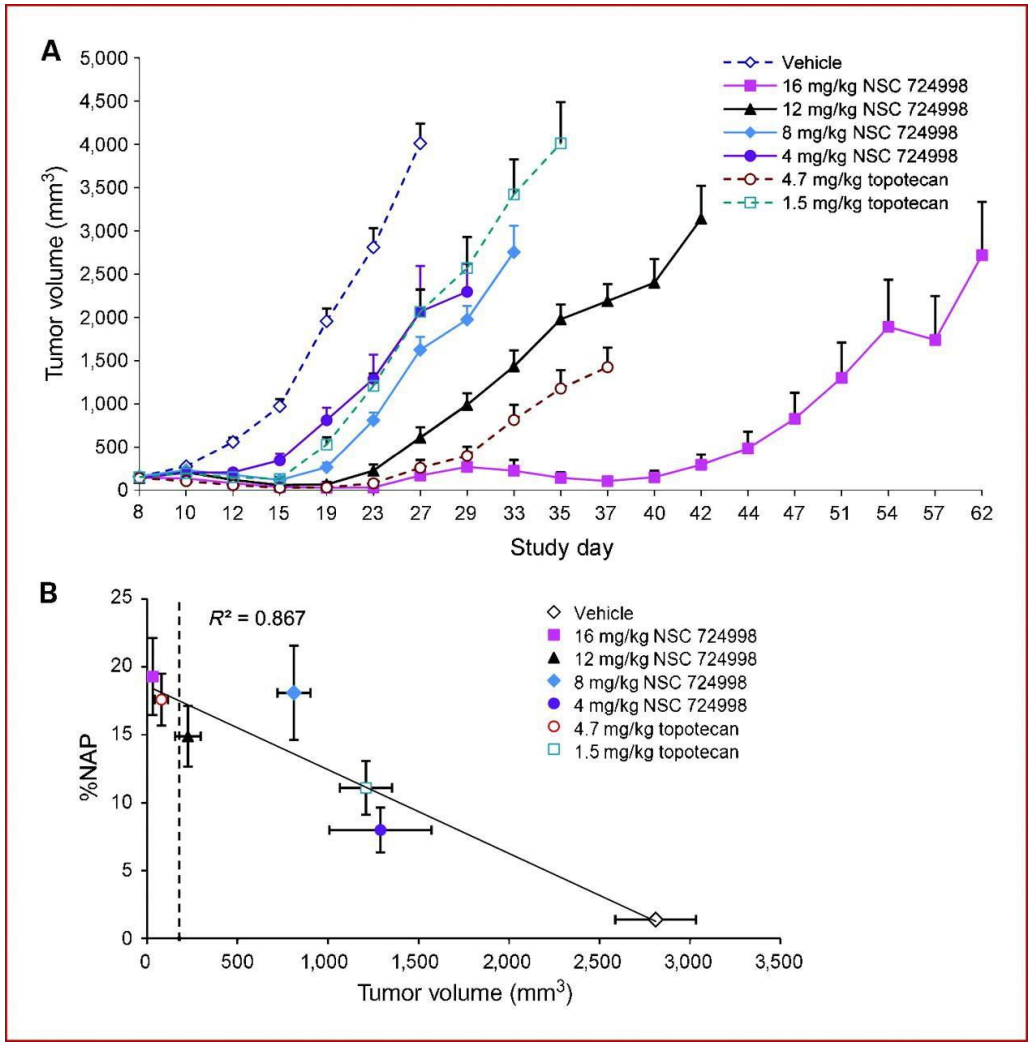
Supplementary Figure S1. CC3 puncta are associated with plasma membrane blebbing in tumor tissue from drug-treated xenograft models



Dull et al. Oncotarget 2018; 9:17104-17116

Disambiguating the Meaning of γH2Ax Response





Kinders et al. Clin Cancer Res 2010;16:5447-5457

Comprehensive PD Biomarker Portfolio with Frequent Use in NExT

- **PD biomarker assays that visualize drug responses (multiplex fluorescence microscopy)**
  - **chemotherapy damage → nuclear γH2Ax[3,4,5,7,8,9,15]**
    - **add nuclear pNBS1, key in BRCA deficiency[15]**
    - **add nuclear RAD51 and pATR foci[16]**
    - **add cytoplasmic cCasp3+ "blebs"—key to distinguishing DDR from apoptosis[14]**
      - **add induction of LC-3 cytoplasmic "puncta" for autophagy[16]**
  - **cell cycle alterations → CDK1/2-pY^{15}, pHH3[9]**
  - **immunoPD → CD8, CD3-pY^{142}zeta, ZAP70-pY^{493}, βCATN[11]**
  - **autophagy → LC3[16]**
  - **plasma membrane pY^{1235}-MET (clone 7334), GLUT1 and NaKATPase-alpha[12]**
- **PD biomarker assays that survey drug responses in tissue extracts (sandwich immunoassays)**
  - **PARP1/2 signaling → PARylated protein (*bid* schedule used in >50 clinical trials)[1,2,5,6,7,11,15]**
  - **enzymatic MET signaling → full-length MET- pY^{1235}, pY$^{1234/1235}$, pY^{1356} (clones 7334, 23111)[12,13]**
  - **intrinsic apoptosis with recombinant heterodimer standards[10]**
  - **isoform-specific signaling → AKT$_{1/2/3}$-pT^{308}/pS^{473}, rpS6-pS$^{235/236/240/244}$ (16)**

[1]Kinders et al CCR 2008; [2]Kummar et al JCO 2009; [3]Wang et al CCR 2010; [4]Kinders et al CCR 2010; [5]Kummar et al Can Res 2011; [6]Ji et al PLoSOne 2011; [7]Kummar et al 2012; [8]Reiss et al CCR 2015; [9]Do et al JCO 2015; [10]Srivastava et al Clin Can Res 2016; [11]Parchment and Doroshow Sem Oncol 2016; [12]Srivastava et al Ann Trans Med 2017; [13]Srivastava et al MCT 2018; [14]Dull et al Oncotarget in press; [15]LoRusso et al CCR 2016;[16]unpublished data

Pharmacodynamic Biomarkers

- Pharmacodynamics broadly defined as “what a drug does to the body”
- At the molecular level, a biochemical response of the intended target to drug action and its planned downstream biochemical and cellular consequences
- The sequence of events provides a framework for PD biomarker study design:

1º drug effect
“Target Engagement”
post-translational modification
inhibition of activity
conformational change
↪change in stability

➡

2º drug effect
“Pathway Changes”
signaling alterations
stressor signals
DNA damage
cell death signals
gene de-silencing

➡

3º drug effect
“Cellular Changes”
tumor cell death
reduced proliferation
CTL lysis of tumor

Clinical surrogates
medical imaging
physical findings
survival surrogates

➡

Therapeutic effect
increased OS
increased PFS