Clinical Assay Development, Validation & Training

Pharmacodynamic Assay Support of DCTD-Sponsored Early Clinical Trials

May 30, 2012

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Division of Cancer Treatment & Diagnosis

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SAIC-Frederick

Should Early Phase Trials Be Designed to Generate Evidence Supporting Proof-of-Mechanism?

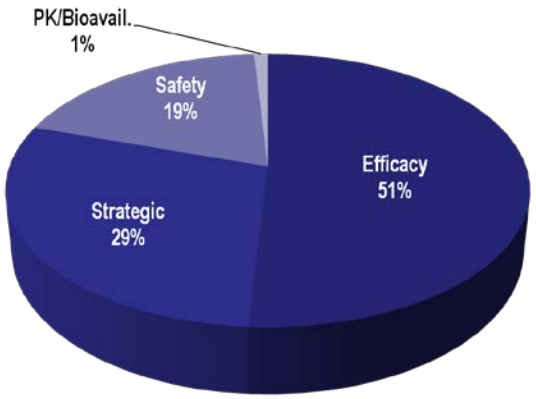
- **Background:** Recent cancer small molecule development has been characterized by both high profile successes (crizotinib; vemurafenib) and failures (BSI-201). Successes were rapid and resulted from molecular stratification; failure associated with lack of P-of-M.

- **Feasibility:** Should we only develop agents that can be brought to the clinic under conditions that demonstrate P-of-M? Should resources be refocused around this paradigm with a consequent decrease in the number of trials performed and drugs evaluated?

- **Implications for success:** Fewer costly, late stage development failures; improved understanding of actual mechanism of action or resistance in the clinic; improved rationale for the selection of combination therapies for development.

Should Early Phase Trials Be Designed to Generate Evidence Supporting Proof-of-Mechanism?

- **Demonstrate drug action on intended tumor target (proof of mechanism) in a human malignancy early in development**
  - evaluate hypotheses surrounding mechanism of action *per se*
  - evidence of target modulation in the clinic assists decision to move agent forward, or not . . .
  - evaluate relationship of drug schedule and systemic exposure to target effects
  - examine relevance of marker chosen to represent target modulation
  - prior to expectation of efficacy



Overall Phase II Success Rate 18% (2008-2009)

Nature Rev. Drug Discov. 10: 1, 2011

- **NOT predictive of clinical benefit**
  - only later stage (larger) trials can define relevance of target modulation to tumor growth inhibition
  - only consequent changes in cell biology (and perhaps biochemistry) would be expected to predict clinical benefit

Clin. Cancer Res. 14: 3658-3663, 2008

Modern Drug Development Needs PoM/PoC-Based Trials

DCTD tasked FNLCR/SAIC-F to provide pharmacodynamic (PD) assay support

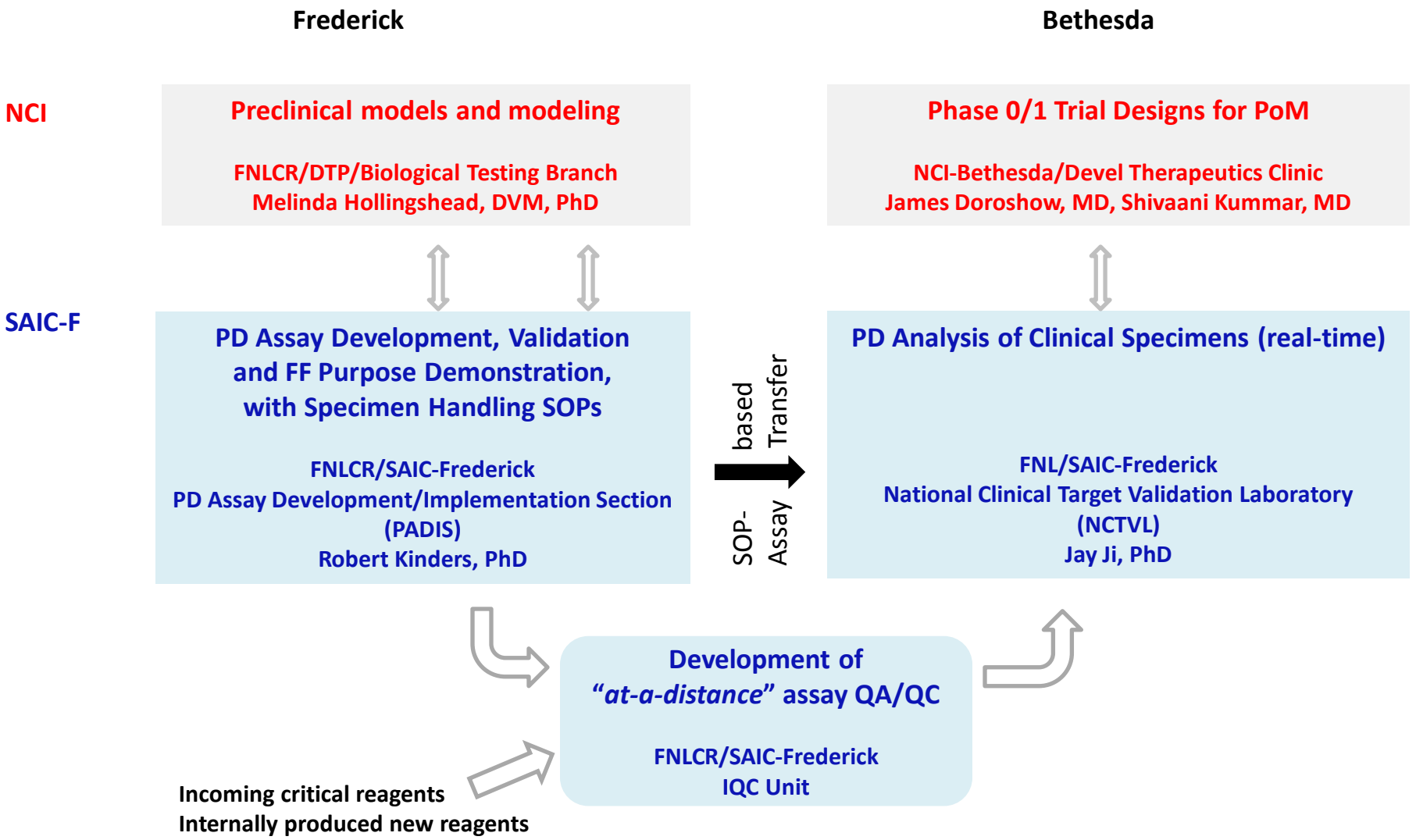
- PD assay development lab (PADIS) - develop, validate and prove assay fitness-for-purpose
- Clinical PD lab (NCTVL) – real time PD analysis of internal and CTEP trial specimens
- Long-term, open access to clinically proven assays, while maintaining assay quality

Portfolio of PD assays for high value molecular responses, based on expert input

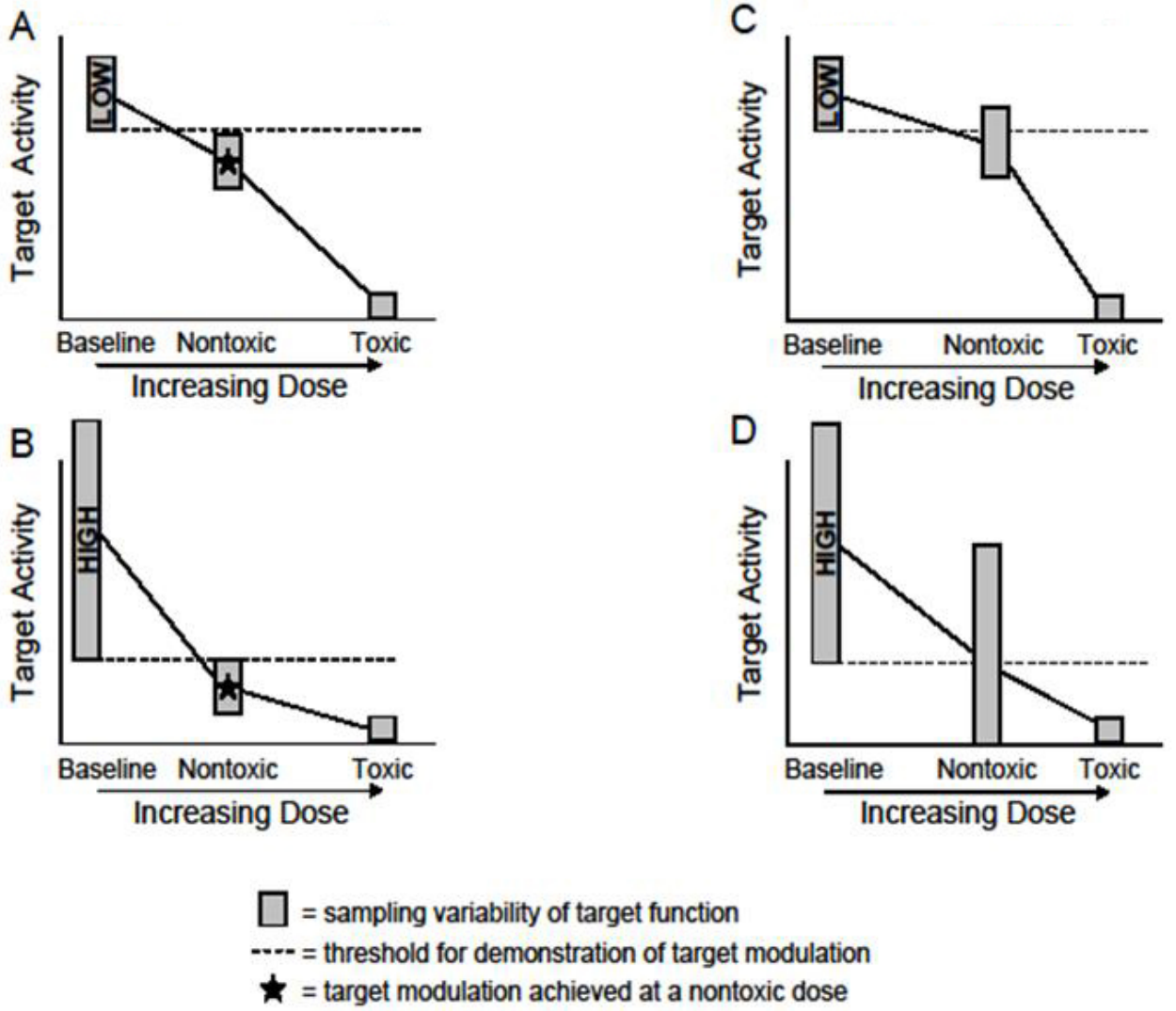
Developmental Therapeutics Clinic to explore trial designs incorporating tumor PD

Mandatory target assessment during CTEP Phase 1 trials (“no assay, no trial”)

Integrated PD Assay Support of the DCTD Program



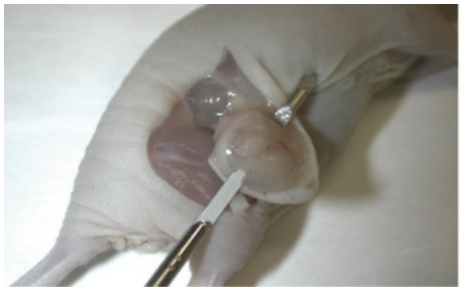
Both Target Variability and Drug Drive PD Success

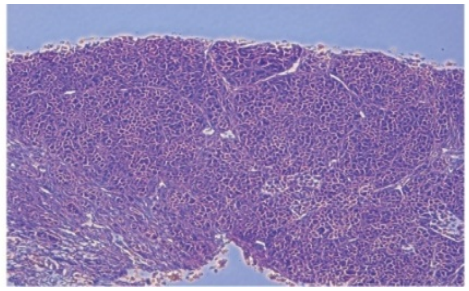


Clin. Cancer Res. 14: 3658-3663, 2008

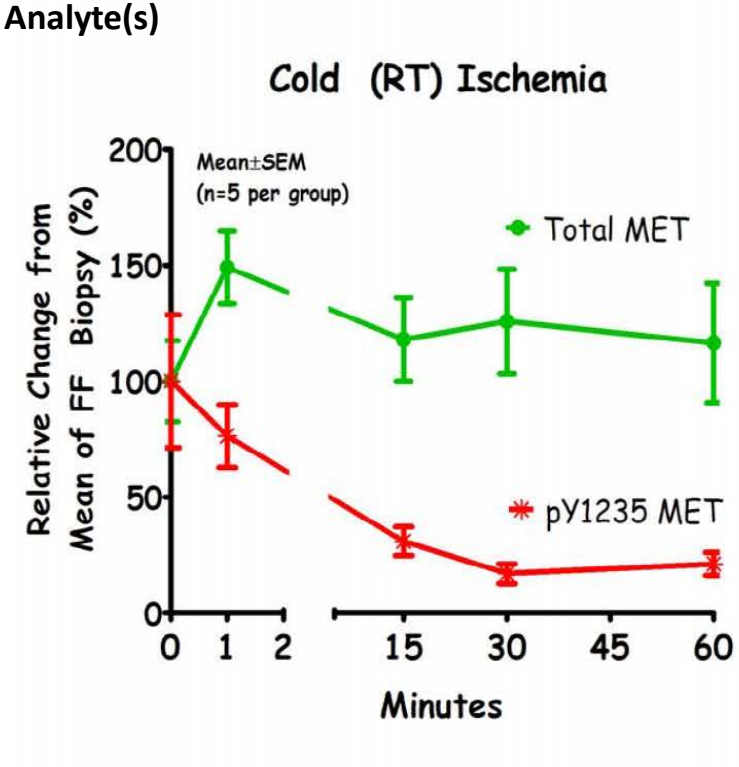
Specimen Handling SOPs – a Key Fit-for-Purpose Issue

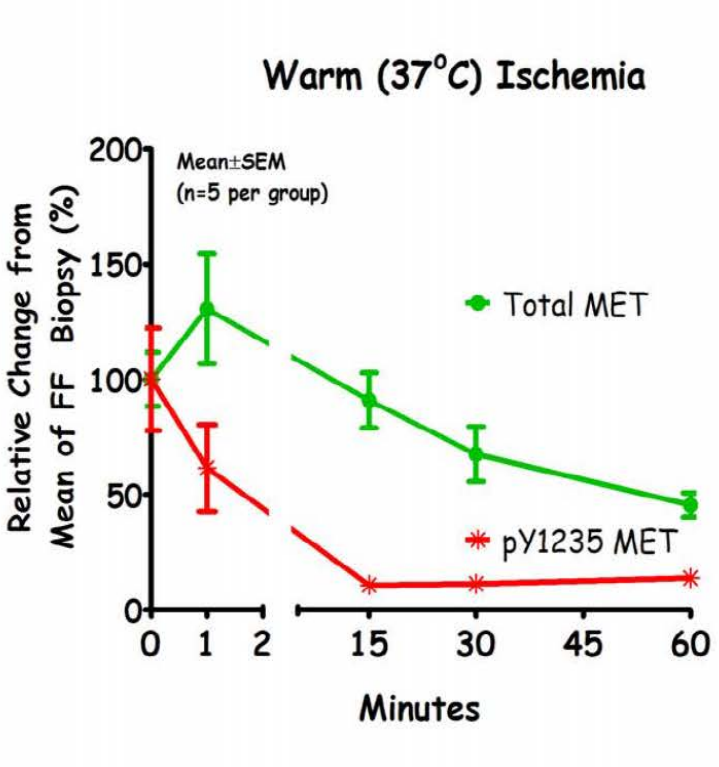
Develop with Clinically-Relevant Samples





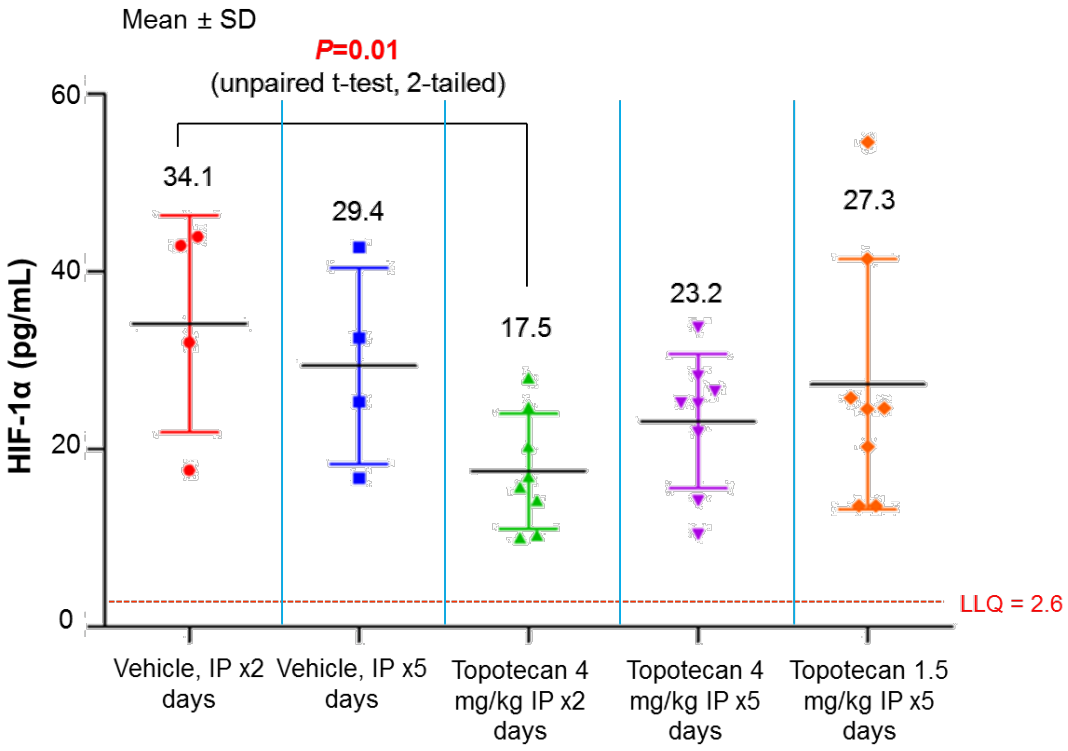
Stabilize the Analyte(s)

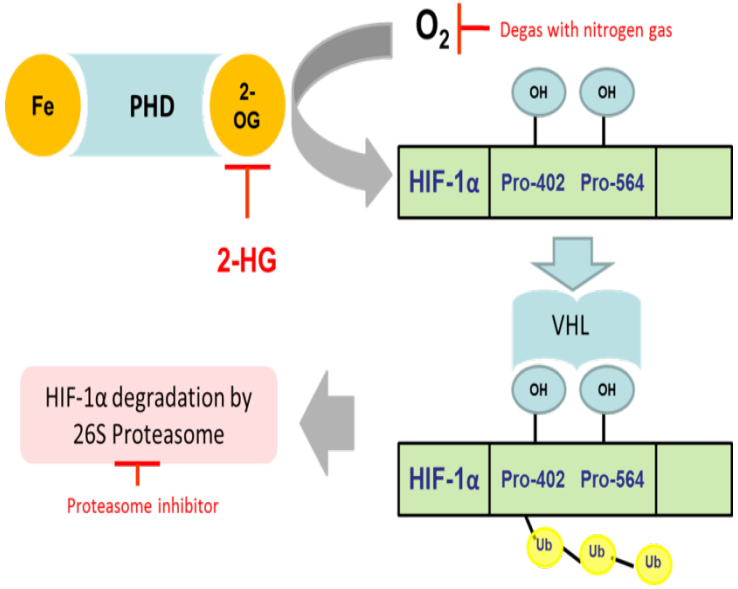




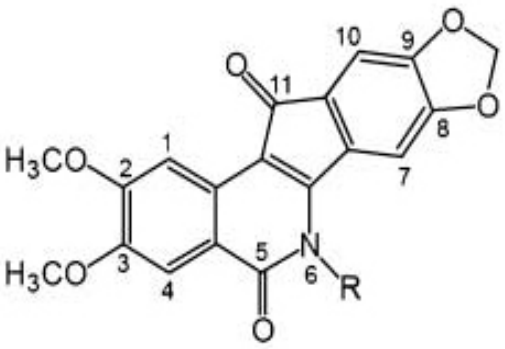
Minimize the Influence of Specimen Processing - Key for Immunoassay of Hif-1α (Hif1α-IA)

Stabilize the Analyte(s)





Development of Indenoisoquinolines with Clinical PD Assays



R =

—CH_3	**NSC 314622**
—$(CH_2)_3$—NH—$(CH_2)_2OH$	**NSC 706744 (MJ-III-65)**
—$(CH_2)_3$—N⟨ ⟩N	**NSC 725776**
—$(CH_2)_3$—N⟨ ⟩O	**NSC 724998**

Unique, non-camptothecin Topo I inhibitors

- chemically stable
- low cross-resistance with camptothecin analogs (irinotecan; topotecan)
- not substrates for ABCG2 efflux pump
- prolonged stability of complex
- unique patterns of DNA cleavage
- produce dose- and time-dependent DNA double strand breaks → γH2Ax

Discovery/Development Path

- discovered by Yves Pommier (NCI intramural program)
- developed by DCTD
- FIH Randomized NCI Phase I trial of NSC 724998 vs 725776

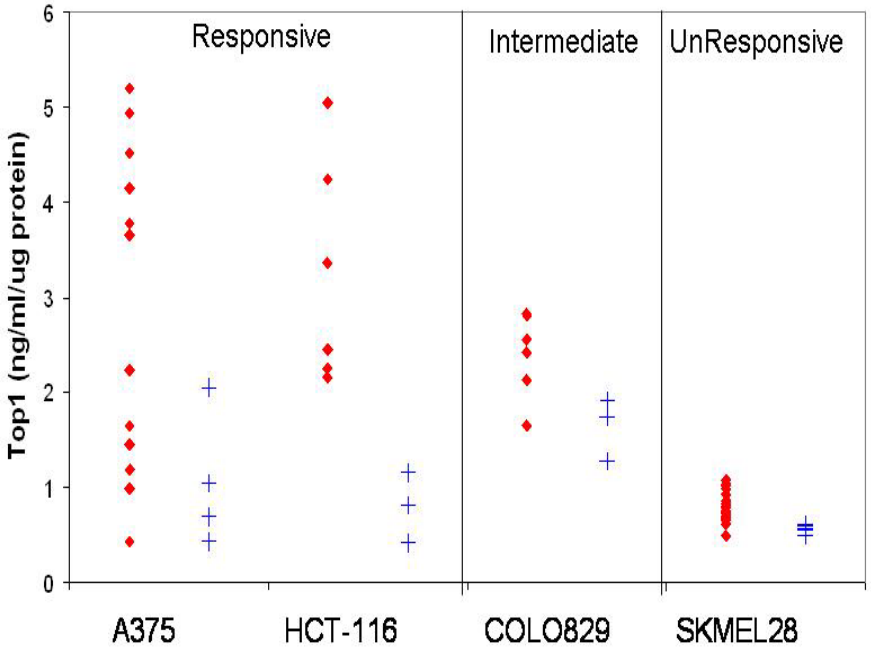
Develop comprehensive PD package for proof of mechanism evaluation PRIOR to FIH studies:

1º level PD: TOP1-immunoassay (new)
2º level PD: γH2Ax-qIFA
2º level PD: γH2Ax-CTC

Immunoassay for total Topoisomerase 1 (TOP1-IA) - Preclinical

Effect of Topotecan on TOP1 Levels in Xenograft Bx Specimens

4h Topotecan (15 mg/kg) vs Vehicle Control

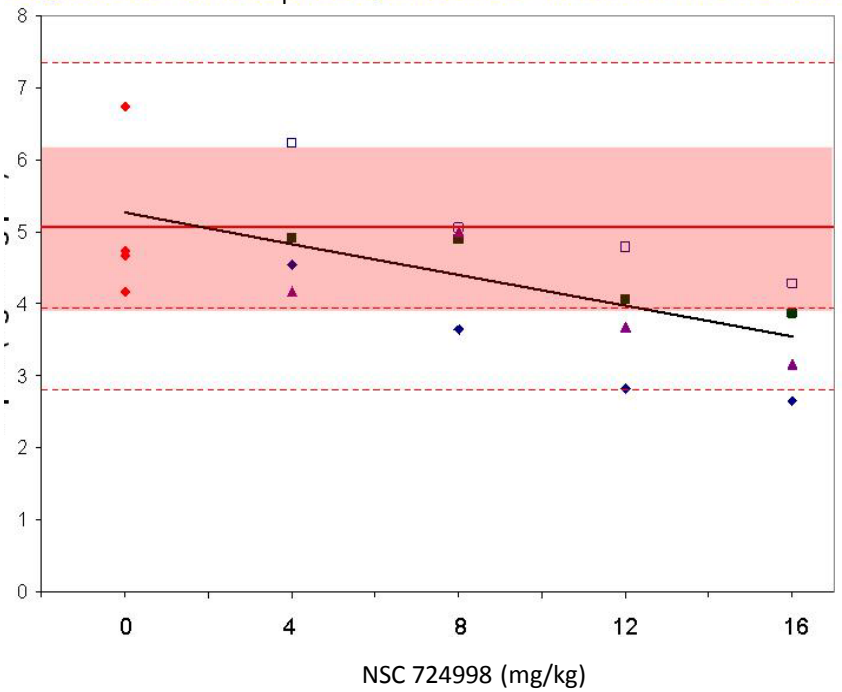


Effect of NSC 724998 on TOP1 Levels in A375 Xenografts

Vehicle Range:
Solid red line = Avg

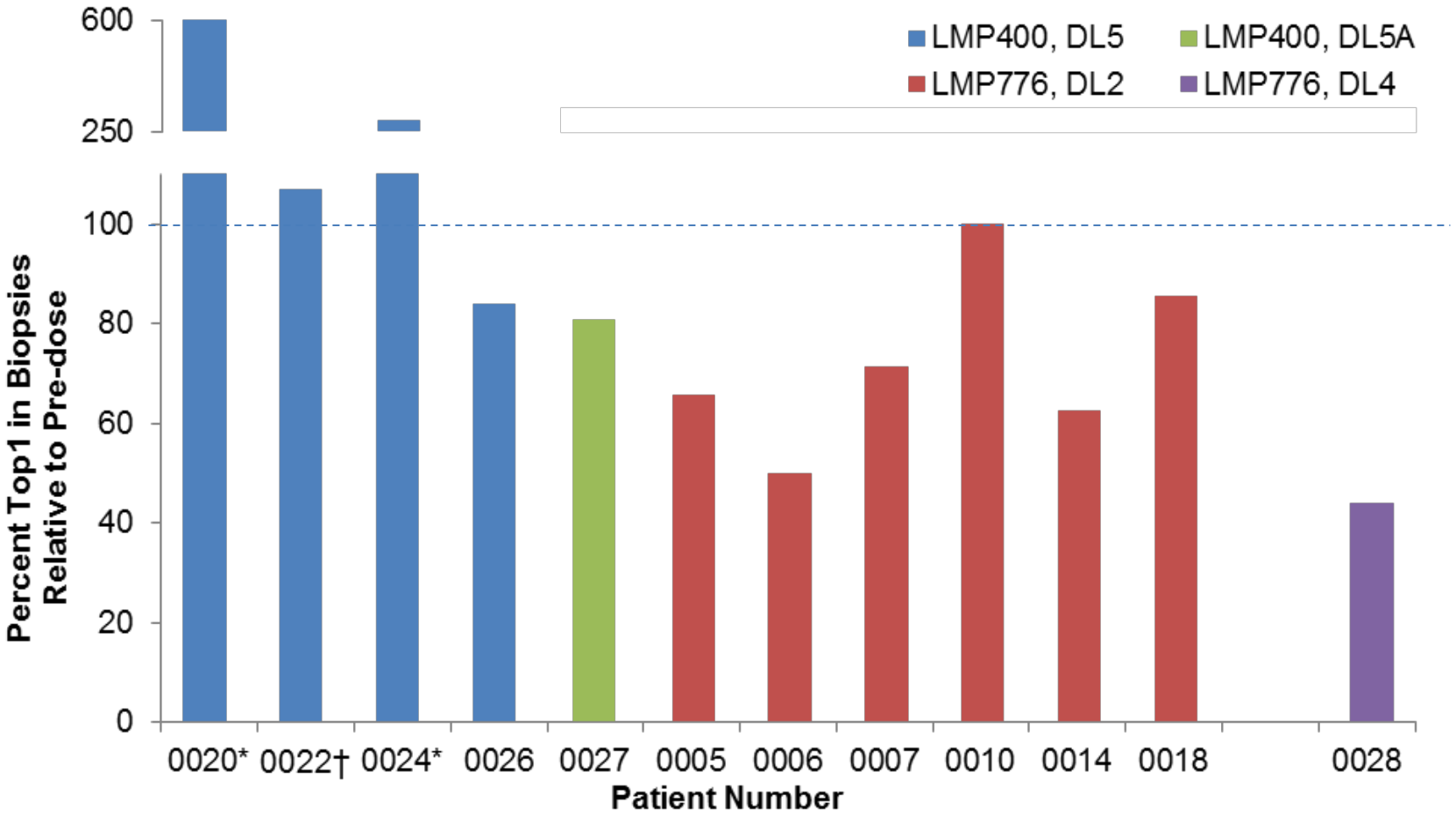
Dashed red line = Avg ± 1 and 2 SD

Black line = Dose Response of indeno NSC 724998



PlosOne, submitted

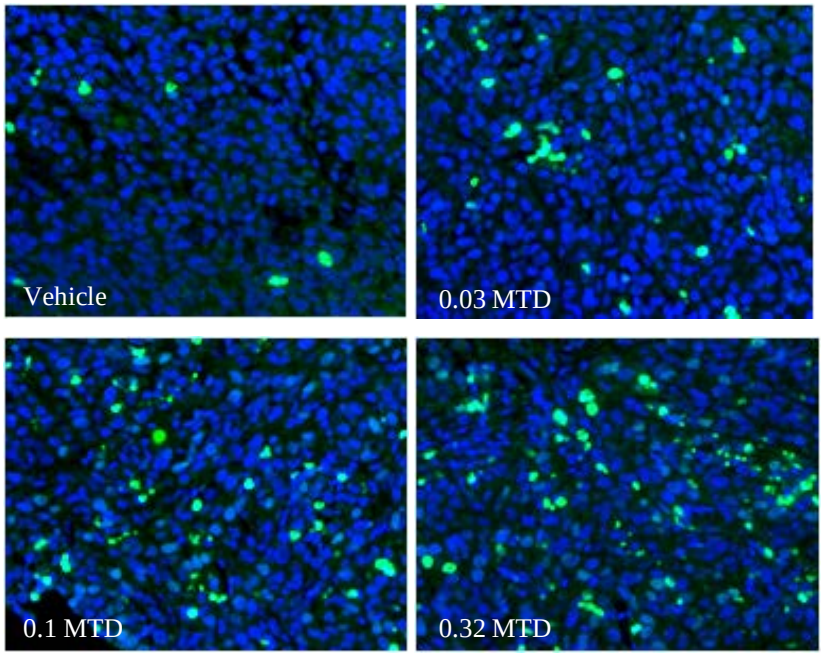
Immunoassay for total Topoisomerase 1 (TOP1-IA) - Clinical



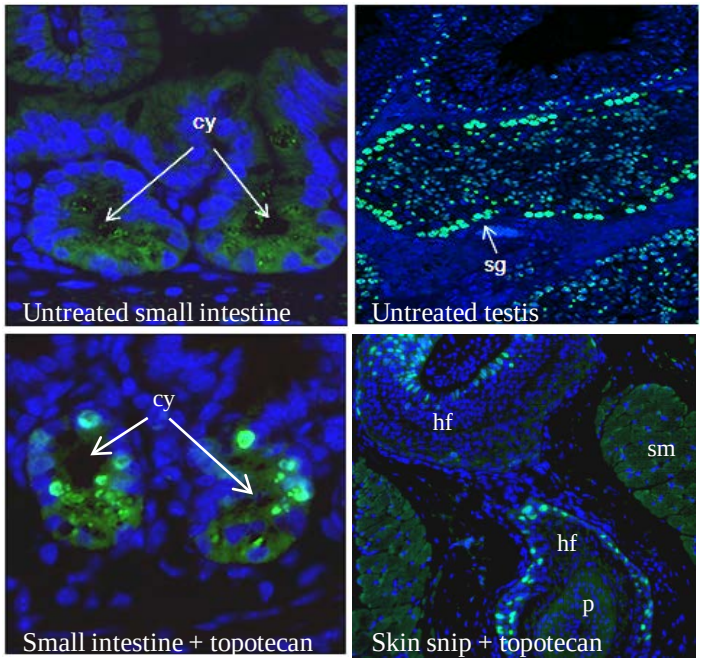
Unpublished, ASCO 2012

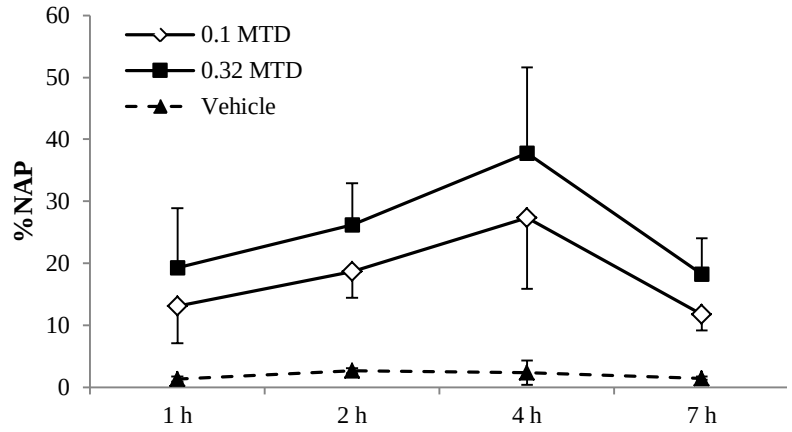
Quantitative Immunofluorescence Assay for γH2Ax (γH2Ax-qIFA)

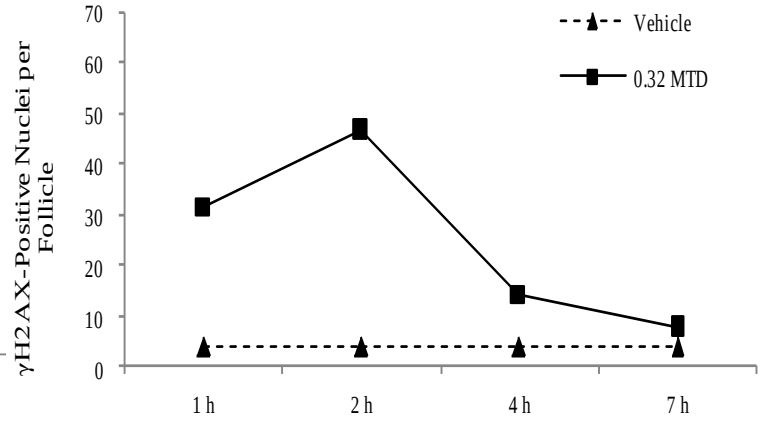
Topotecan-Treated Mice; A375 Xenografts



Topotecan-Treated Non-Tumor Bearing Mice

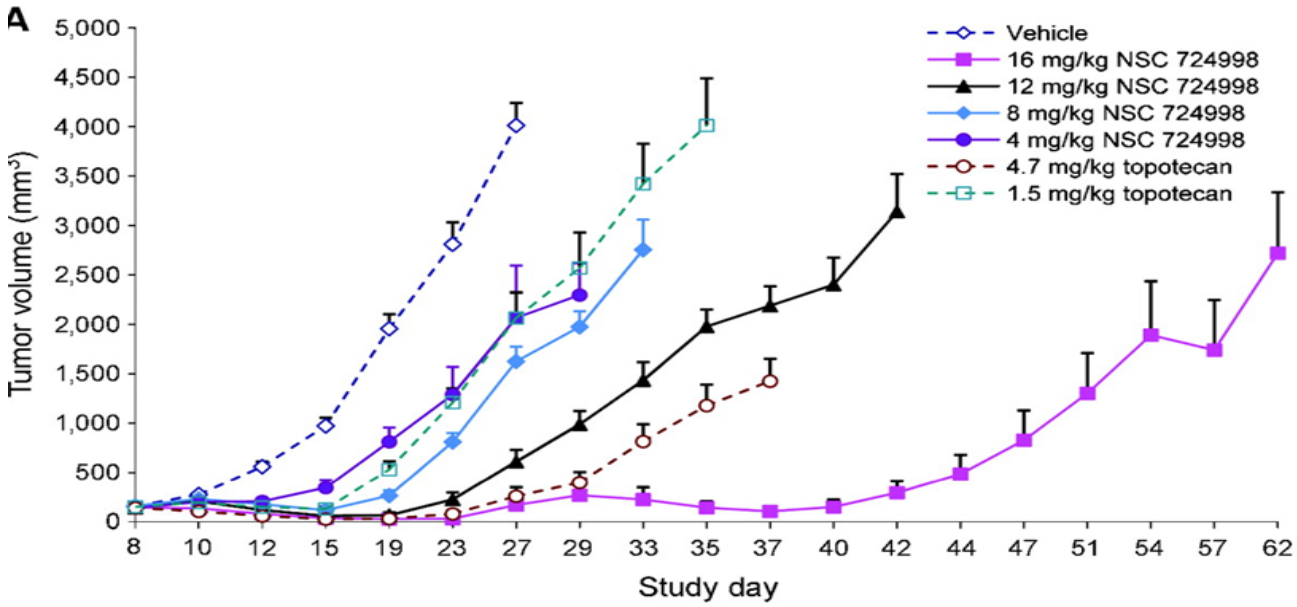


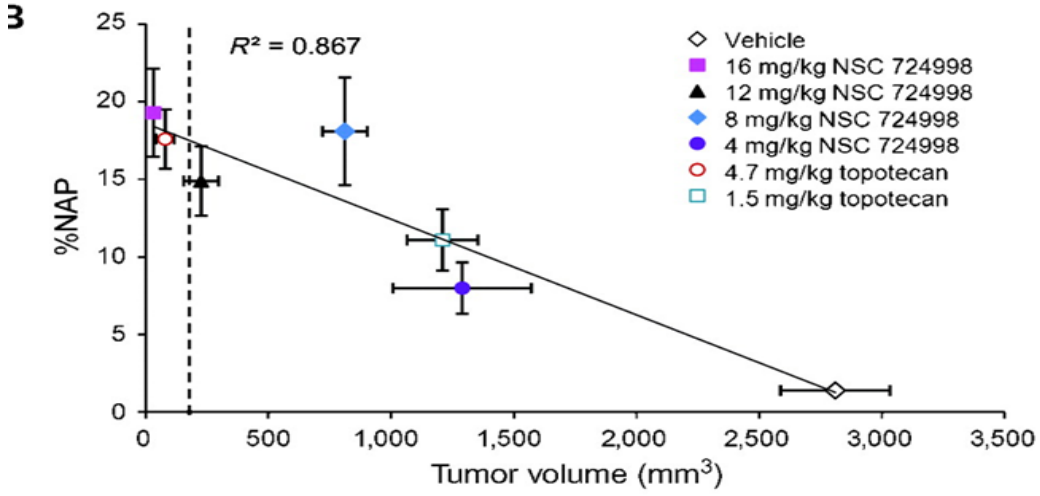




Clin. Cancer Res 16: 5447-57, 2010

Quantitative Immunofluorescence Assay for γH2Ax (γH2Ax-qIFA)





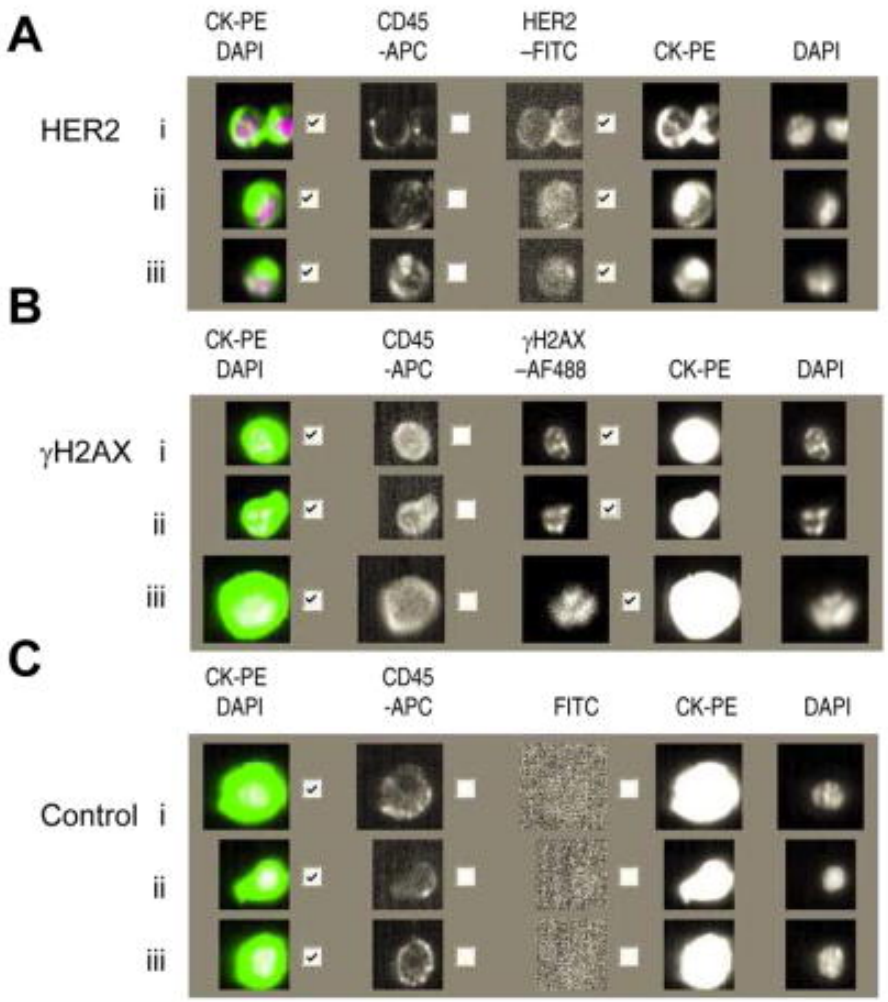
in vitro tolerance of TOP1 inhibitors by human and murine hematopoietic stem cells (CFU-GM)

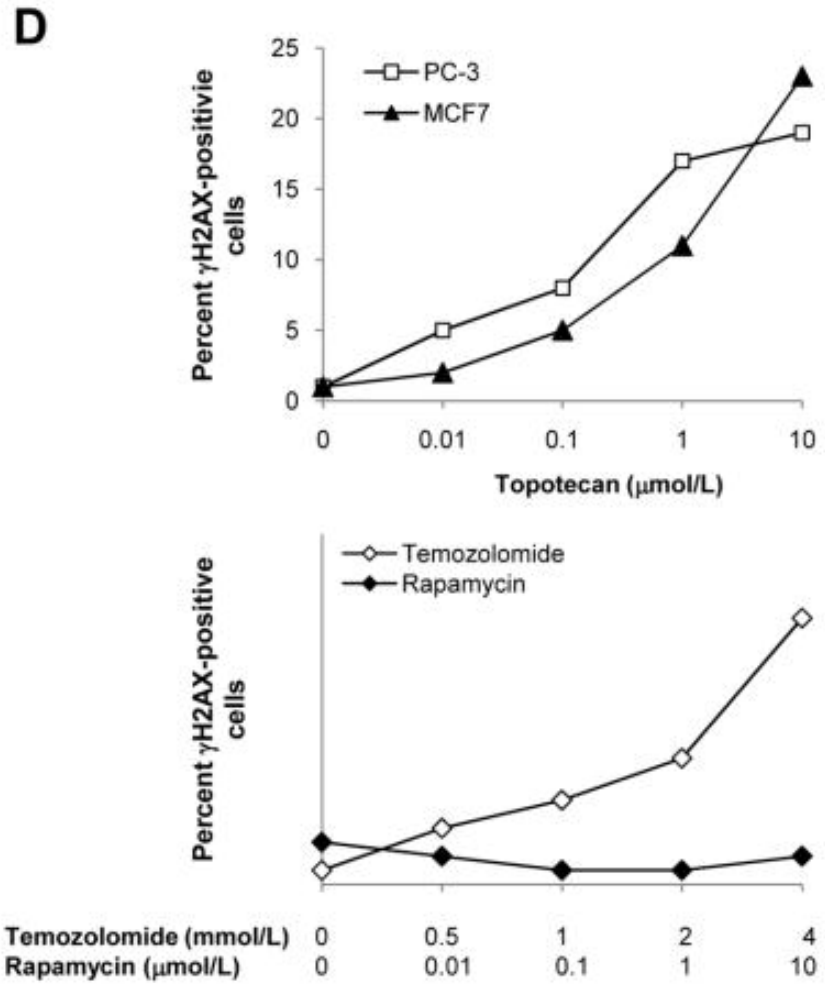
Investigational Agent	Mouse IC90 (nM) μ ± SD (range)	Human IC90 (nM) μ ± SD (range)
Topotecan Hydrochloride (Hycamtin)	120 ± 50 (64 - 160)	5.9 ± 5.1 (1.7 - 15)
NSC 724998	29 ± 12 (18 - 41)	27 ± 14 (7.1 - 45)
NSC 725776	26 ± 12 (12 – 35)	6.6 ± 2.6 (4.1 – 10)
Direct comparison study conducted on 3 mouse, 6 human marrow specimens		

Clin. Cancer Res. 16: 5447-57, 2010

Unpublished

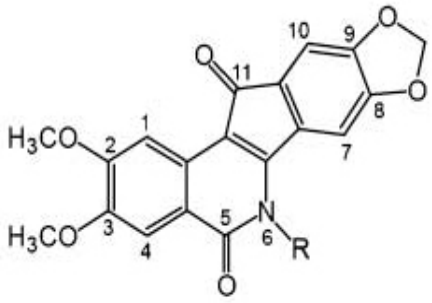
Adapting the γH2Ax Assay to Circulating Tumor Cells (γH2Ax-CTC)





Clin Cancer Res 16: 1073-1084, 2010

CTC γH2Ax Response to Indenoisoquinolines



R =

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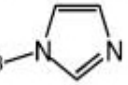
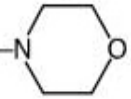
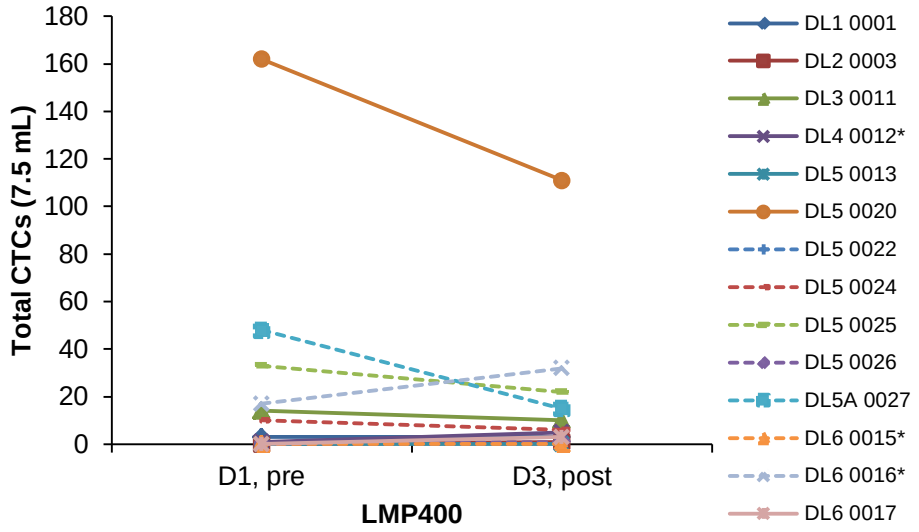
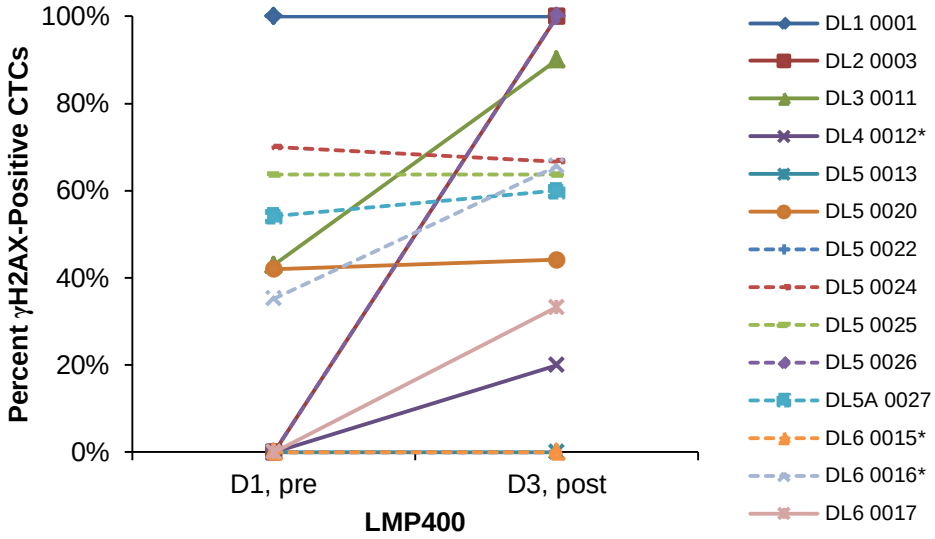
—$(CH_2)_3$— 	**NSC 725776**
—$(CH_2)_3$— 	**NSC 724998**





Pushing CTC Technology toward Universal Analysis

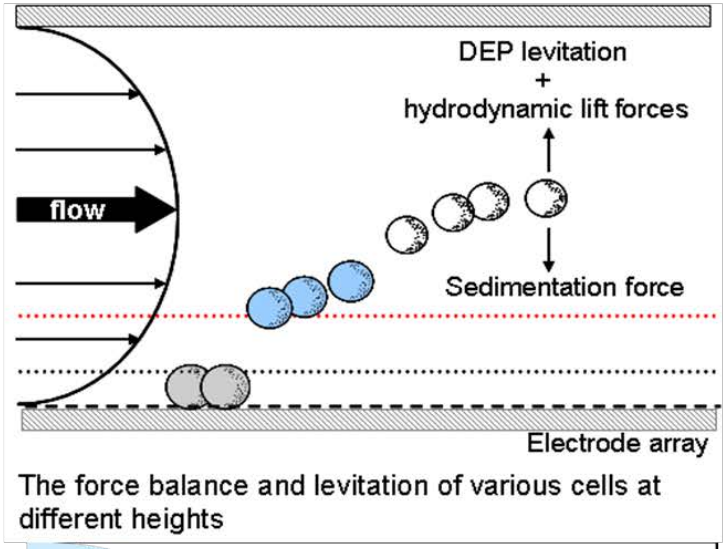
DESIGN FEATURES from SAIC-F to Meet Expected Needs in CTC Analysis

- Cell surface antigen-independent separation of CTCs from blood (EpCAM-neg CTCs)
- Capable of evaluating carcinomas, sarcomas and lymphomas
- Clinically validated with small volume samples (0.1 – 1.0 cc)
- Interfaces directly with down-stream molecular analyses – both PD and Dx
- Capable of evaluating non-clinical cancer models

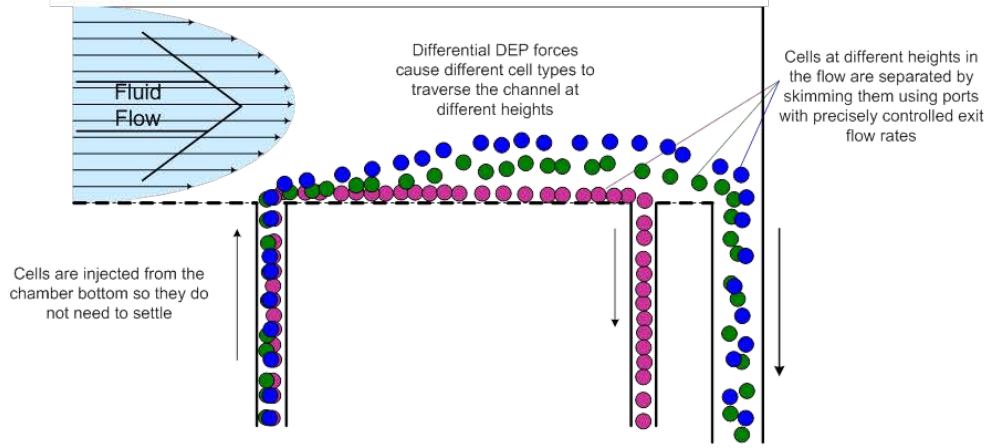
These Design Features were incorporated into a SAIC-F RFP to develop instrumentation that moves past limitations of the Veridex Cell Search and other marker-based systems

Selection of ApoCell, Inc to Deliver a Universal CTC Device

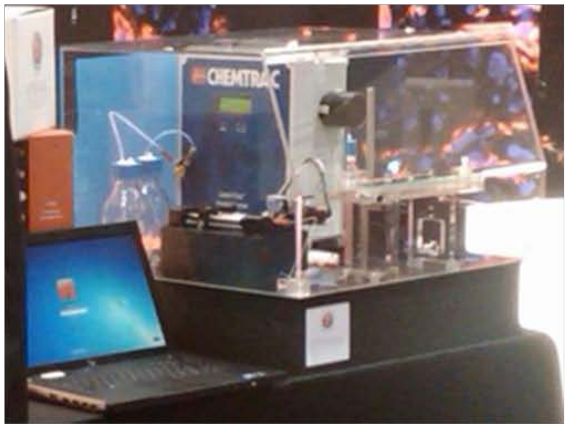
Dielectrophoretic Field-Flow Fractionation (DEP-FFF)



The force balance and levitation of various cells at different heights



- DEP-FFF utilizes balance of physical forces in a laminar flow chamber to isolate CTCs from blood cells
- Throughput is high compared to other systems; 1 ml of blood can be processed in <30 minutes



APOCELL
molecular profiling & diagnostics

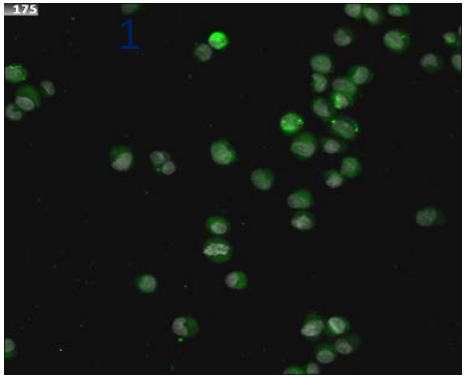
Universal CTC Isolation Technology with PD Evaluation

ApoStream prototype isolated viable EpCAM negative tumor cells:
spiked human ovarian carcinoma and canine osteosarcoma cell lines

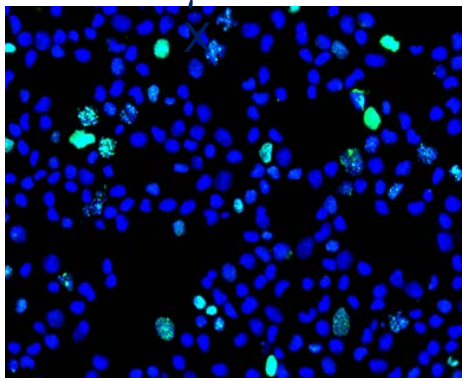
	Recovery	Viability	Enrichment
preoptimization	%	%	Fold
	33+/-1.3	>95	1326
IGROV-1 (EpCam Neg)	83+/-6.0	>95	1446
BW.KOSA (Canine)	78.5+/-0.5	>95	4440
IGROV-1 cells (EpCAM neg) spiked into human PBMCs			
.KOSA cells spiked into dog blood			

PD response (γH2AX) of a canine OS cell line to indenoisoquinoline ex vivo:

Fibronectin-



γH2A



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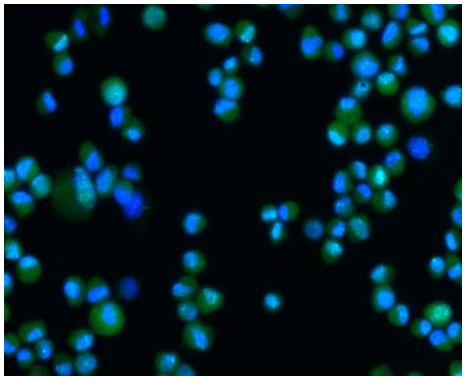
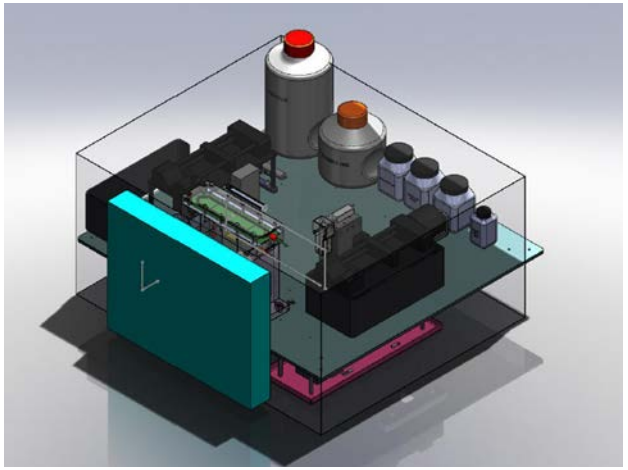


Alpha Prototype Delivery and Use at the FNLCR

- Fit-for-Purpose demonstration using blood specimens from a canine clinical trial of the indenoisoquinolines
  - Ongoing
  - Uses Breadboard Prototype
  - Dog Lymphoma phenotyping
  - Use γH2Ax as the PD marker

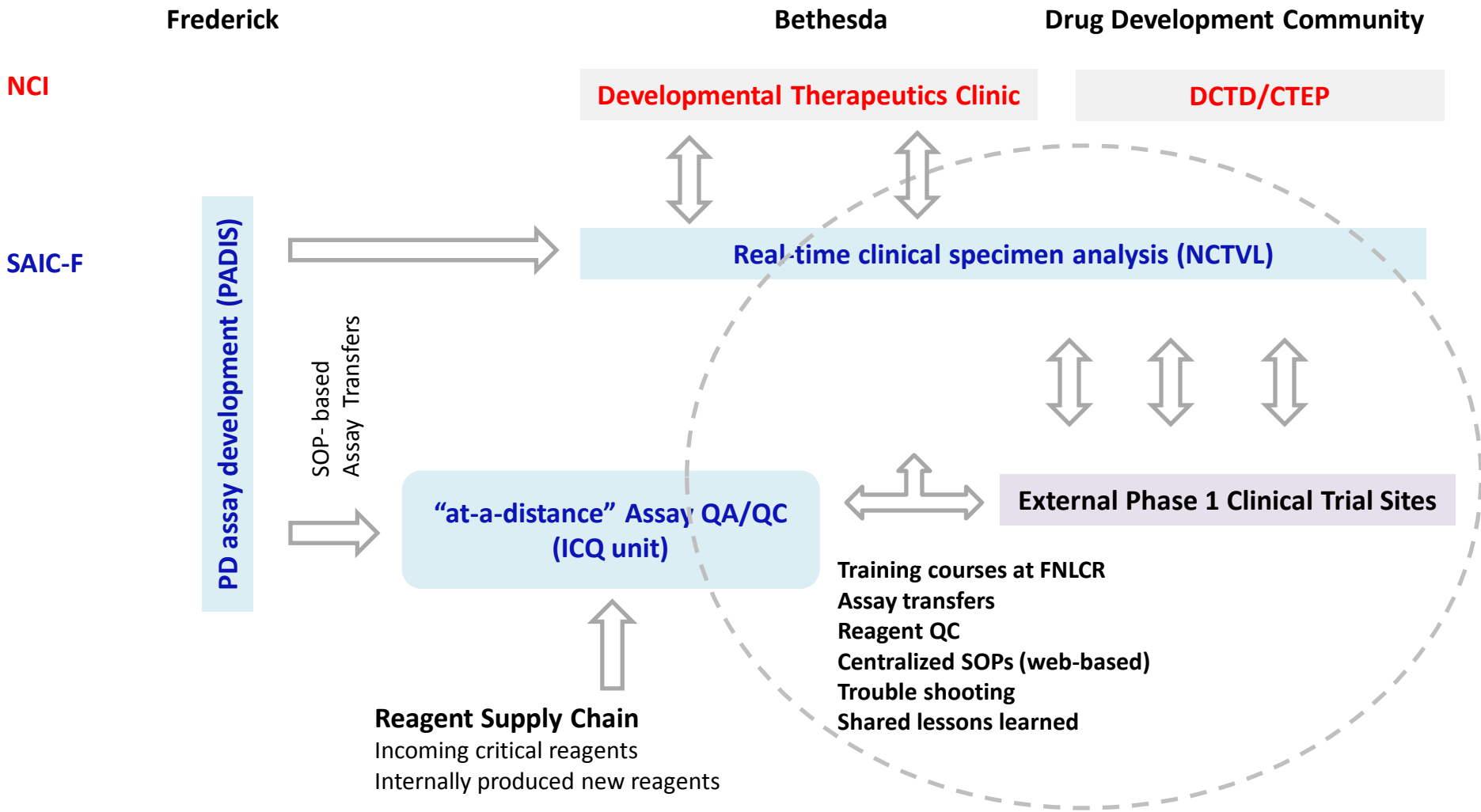


- Alpha prototype delivery:
  - ApoCell in August 2012
  - FNLCR/PADIS in October 2012

- SOP-based Methods Transfer
  - December 2012

- Initiate clinical trial support
  - March-May 2013

Creating User Groups for Validated, Proven PD Assay



Quality Assurance/Quality Control at a Distance - Shared Clinical PD Assays with Robust Performance

A) Onsite, laboratory-based training classes at the FNLCR

PD Assay Certification Courses at FNLCR					
Assay	# of classes	# of attendees	universities & research institutions	NIH programs	pharma/ Biotech/CRO
PAR-IA	9	29	16	9	4
γH2Ax-qIFA	5	18	9	7	2
γH2Ax-CTC	3	8	5	1	2
TOP1-IA	preparation/scheduling				

as of May 2012

B) Quality controlled supply chain for key reagents

- Assays faced with using R&D grade, rather than Dx grade, reagents and suppliers
- Custom orders of reagents/subcontracts to specifications (Epitomics, Argonne Natl Lab)
- Acceptance criteria applied to incoming batches before distribution to clinical labs
  - ❖ batches both of PcAb and MoAb have been rejected (fate of these in the community is unknown)

C) Web accessible current SOPs, training dates, and forms to request key reagents

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

D) Assay "User Groups"

- Centralized change control of SOPs
- Assay troubleshooting results shared with all assay sites
- Recalls of key reagent batches are possible via a distribution tracking system

Selection of Molecular Targets in Early Assay Development

	Selection of Targets	Scientific Foundation	Feasibility
NCI	NExT Special Emphasis Panel prioritized drug discovery and development projects • Includes early clinical trials		
	PD Functional Working Group of the Therapeutic Subcomm, NCAB	PD FWG	
SAIC-F		ad hoc experts and consults weekly PD program/assay meetings	

PD assay development lab

PD Assay Development Portfolio – Emphasis on Multiplex

	PD POM (MOA)	Pathway Consequences	Cell Stasis/Loss (POC)
Concept (scientific foundation)	pGSK3α/β-IA‡‡ Mer Kinase-IA‡‡	**Energy Control-IA‡‡** AXIN, β-CATNN, PKA, LKB1, AMPK, PKCβ, AKT2, ULK1, GYS1, PDH-A1, PDP-1, BIM1 **DDR2-qIFAx** pATR and FANCD2/--/--/--/-- (DAPI) *BRCA1, ATM, XRCC1, DNA-PK, XPA	Cell Cycle Status Necrosis-qIFA Hydropic Degeneration-qIFA Caspase-independent Death-qIFA Oncosis-qIFA
Feasibility	JAK/pSTAT3-qIFA‡‡ pATR-qIFA	**Rx2-qIFA4** Rx1-qIFA3 + vim/ker (DAPI) **Signal Transduction-IA‡‡** PIK3CA, pS^{473}Akt, Akt isoforms, pT^{308}Akt (covered by SBIR), mTORC1/2, pS6K, p4EBP1, PTEN, pERK	Autophagy-IA LC3-II-qIFA
Development and Validation (PADIS)	ccTOP1-IA pMET-IA ver 2.0 (denaturing) pY^{1235}/ pY^{1356}MET-IA cIAP-qIFA‡‡ HSP70 RT-qPCR‡‡	**Glycolysis-IA‡‡** HK2, pPDHE1α, PKM2, LDH-A **DDR1-qIFA4** **HR/BER/NHEJ/NER/MMR** pNBS1, RAD51/--/--/ERCC1/γH2Ax (DAPI) **Angiogenesis** ESM1, CD68, CD31, CD163 GSTπ or RASSF1-CTC	**EMT1-qIFA4** β-CATN, E/N-Cad, Vim or Ker (DAPI) **Apoptosis (intrinsic)-IA** Dimerized BAX-Bcl-2, BAX-BAX, BAK-BAX, BAK-BAK, Bak-Bcl-2, SMAC-SMAC Total pS^{99}BAD, cleaved-Lamin-B, BAD, BAX, BAK, BIM, 17/19 Kd neoantigen cCasp-3, Mcl-1, Bcl-xl, survivin
SOP-based Transfer (PADIS→IQC, NCTVL)	pMET-IA pY^{1235}/ pY^{1356}MET-IA	**Rx1-qIFA3** γH2A/cCasp-3/Ki67 (DAPI) HIF1α-IA	

DCTD Clinical Pharmacodynamics Team

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