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MEDICAL CENTER

Proteogenomic Characterization of Muscle Invasive Bladder Cancer to Identify Mechanisms of Resistance and Targets for Therapy

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Disclosures

- Clinical trials
  - Endo, FKD, JBL (SWOG), Roche/Genentech (SWOG), UroGen, Viventia
- Advisory Board/Consultant
  - Anchiano Therapeutics, Ferring, Genentech, QED Therapeutics, UroGen, Vaxiion
- Honoraria
  - Dava Oncology, MSD Korea, Nucleix

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Innovation Award

- Bladder Cancer Research Network (BCRN)
- BCAN
- “Exceptionally novel and creative with great potential to produce breakthroughs in our understanding of the management of bladder cancer”
  - High-risk and high-reward
- Additional funding:
  - CPRIT PDX Pilot project
  - Philanthropy – WES, RNAseq

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The Team

- Proteomics Core
  - Anna Malovanaya, Hamssika Chandrasekaran, Sung Jung
- Ellis group
  - Mathew Ellis, Bing Zhang, Beom-Jun Kim (KiP)
  - David Wheeler (WES and RNAseq)
- Genome Center
  - Marie-Claude Gingras
- Mouse PDX
  - Keith Chan, Lacey Dobrolecki, Michael Lewis (PI Core)
- CAM PDX
  - Hugo Vilanueva, Mariana Vilanueva, Ravi Pathak, Andrew Sikora (PI Core)
- Pathology/HTAP
  - Mike Ittman, Patricia Castro
- Urology
  - Karoline Kremers (project mgr), Weiguo Jian, Amanda Watters
- Collaborators (Biospecimens)
  - Lars Dyrskjot (Aarhus, Denmark)
  - Kurshid Guru (Roswell Park)
  - John Taylor (Kansas U)
  - Joshua Meeks (Northwestern)
- Mouse PDX
  - Chong-Xian Pan (UC Davis)

Rationale – MIBC Integrated Therapy

- MIBC
  - L1 evidence for cisplatin-based NAC
  - No evidence of non-cisplatin-based NAC
- RR in cisplatin-based NAC
  - 50% path response
  - 40% pT0
  - Not a validated endpoint
  - OS absolute margin of benefit < 10%
- Unmet need
  - If 50% eligible and 50% of eligible respond then 75% of patients have no effective integrated treatment options supported by L1 evidence.
  - *Our treatments fail these patients (DZQ)*

Patients with residual muscle invasive cancer following NAC have poor outcomes and there is no standard of care for these patients

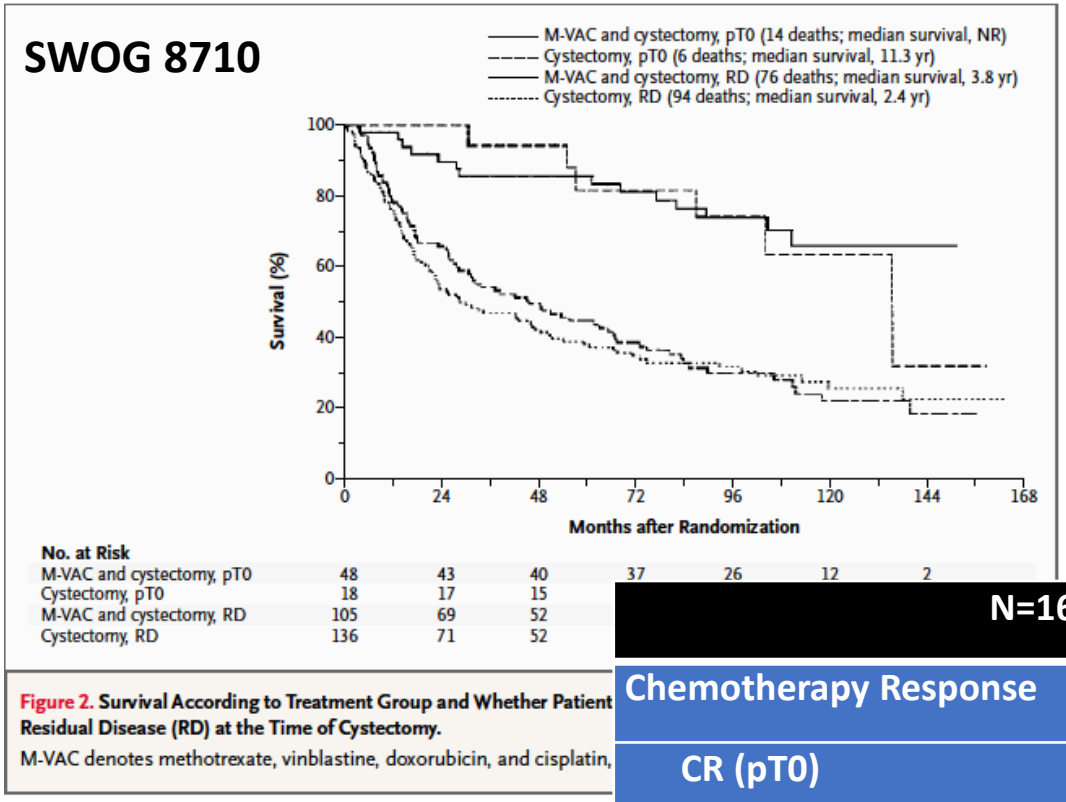


Figure 2. Survival According to Treatment Group and Whether Patient Residual Disease (RD) at the Time of Cystectomy.

M-VAC denotes methotrexate, vinblastine, doxorubicin, and cisplatin,

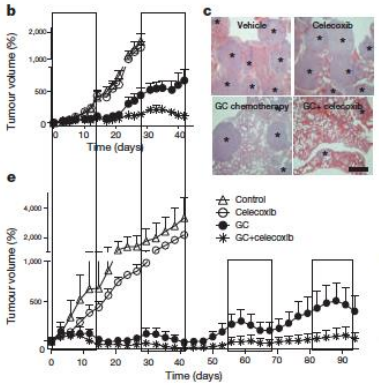
Grossman, et al NEJM 349:859, 2003

SWOG 1314 - COXEN

N=167	GC (N=82)	ddMVAC (N=85)
Chemotherapy Response		
CR (pT0)	28 (35%)	27 (32%)
PR (downstaged to ≤T1)	12 (15%)	20 (24%)
CR + PR	*40 (50%)*	*47 (56%)*
Non-responders	**42 (50%)**	**38 (44%)**

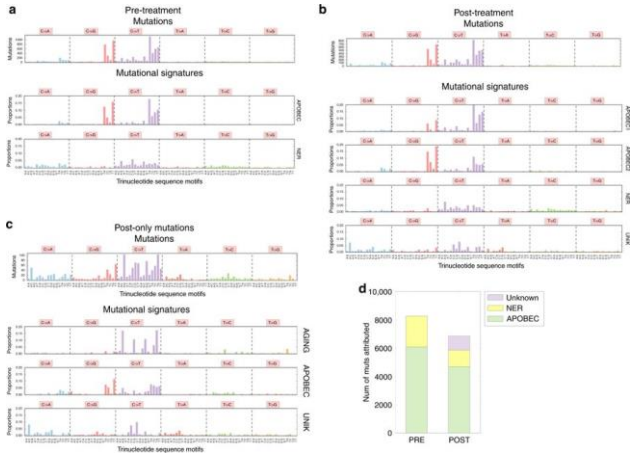
Resistance pathways

- Wound repair



Kurtova, et al Nature 517:209, 2015

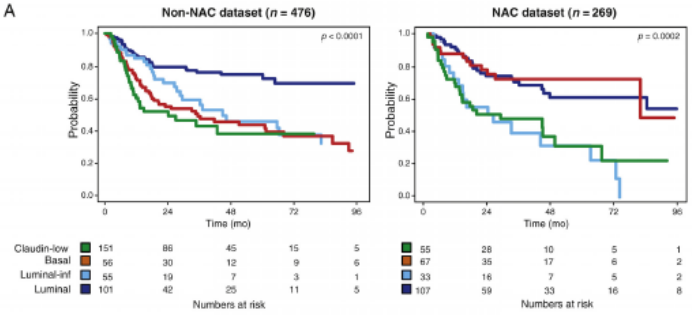
- Cisplatin resistance signature



Liu, et al Nature Comm 8:2193, 2017

- Expression subtype

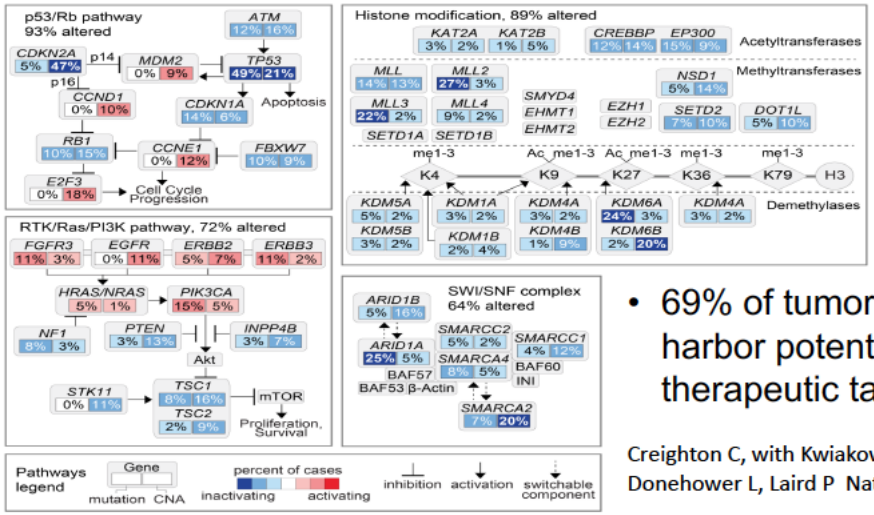
Seiler, et al Eur Urol 72:544, 2017



Alternative Integrated Treatment Options

- Immunotherapy
- Chemotherapy/Immunotx
- Targeted therapy/TKI

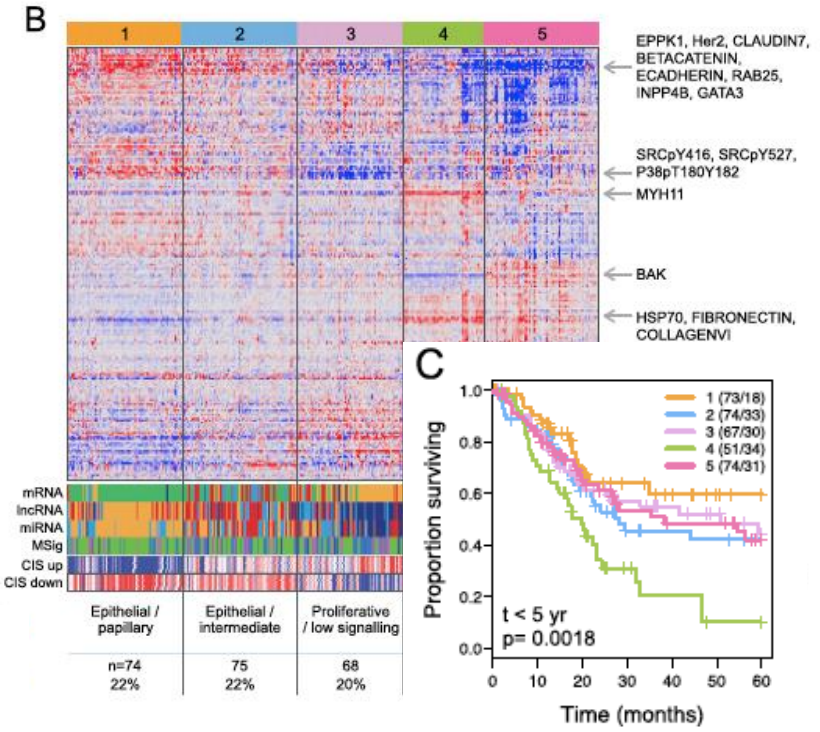
Altered Pathways in Bladder Cancer: Mutation/CNA



- 69% of tumors harbor potential therapeutic targets

Creighton C, with Kwiakowski D, Donehower L, Laird P Nature 2014

TCGA: RPPA 208 antibodies



Robertson, et al Cell 2018

Hypothesis

- Integrated analysis of proteomics and genomics of primary muscle invasive bladder cancer (MIBC) and their patient-derived xenografts (PDX) will define mechanisms responsible for chemotherapy resistance and identify candidate driver genes leading to the identification of specific targeted therapies for those resistant to the current standard of care.

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Biobanking

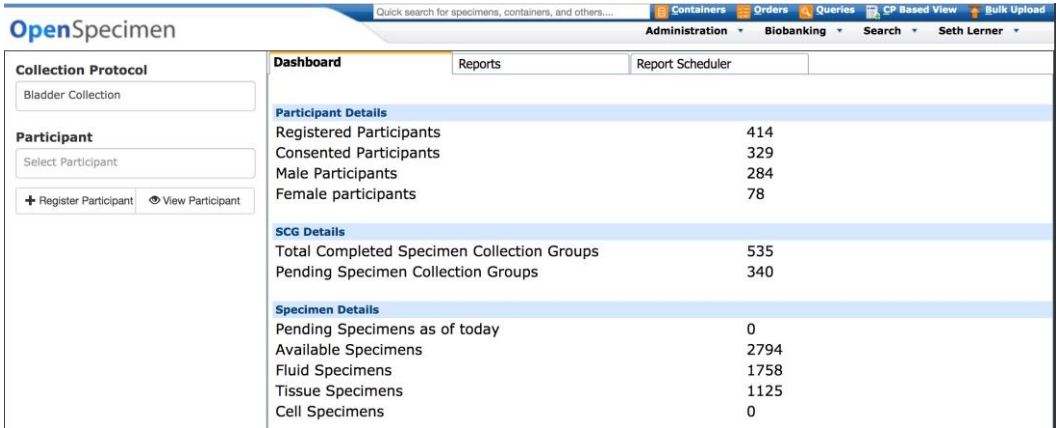
Clinical and banking workflow

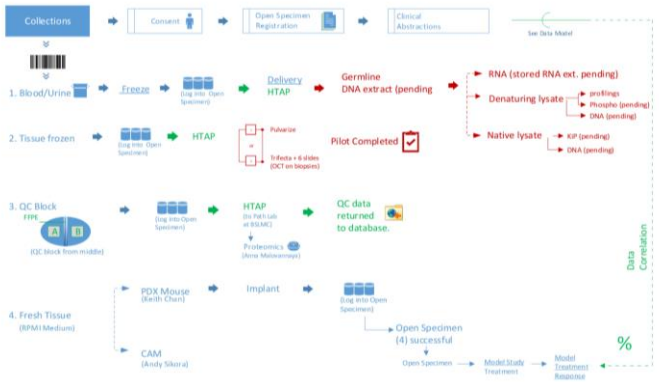
cT2-4

Referral → Restaging EAU/TURBT → NAC or Chemo(N+M+) → Restaging EAU/TURBT → RC/PLND

Restaging EAU/TURBT → Tumor Bank
Tissue
Blood
Urine

Restaging EAU/TURBT → Tumor Bank
Tissue
Blood
Urine





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Cohort Annotation

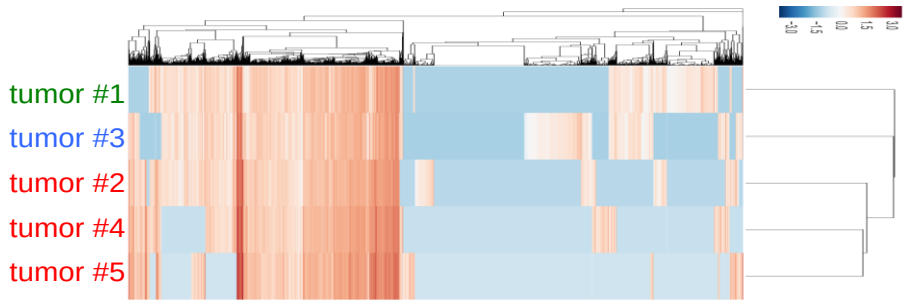
- Target n = 80
- Pre-NAC or pre-Chemotherapy N+/M+
- Fresh tissue from TURBT
  - Prior BCG allowed
  - No prior systemic therapy
- QC GU pathologist
- Minimum >50% tumor cellularity
- Urothelial, NOS
- Variants: Small cell, plasmacytoid, SCCa

Tissue Source Sites

- BCM
- Aarhus (Lars Dyrskjot)
- Buffalo (Khurshid Guru)
- University of Kansas (John Taylor)
- Northwestern (Josh Meeks)

Preliminary Data

(A) Proteomics Expression Clusters of 5 Bladder Cancer Samples.



(B) DNA and cell cycle biology overexpressed in Tumor #3.

Symbol	tumor #1 SRA	tumor #1 Pepts	tumor #1 PSMs abs	tumor #1 PSMs ratio	tumor #1 AUC7 abs	tumor #1 AUC7 ratio	tumor #2 SRA	tumor #2 Pepts	tumor #2 PSMs abs	tumor #2 PSMs ratio	tumor #2 AUC7 abs	tumor #2 AUC7 ratio	tumor #3 SRA	tumor #3 Pepts	tumor #3 PSMs abs	tumor #3 PSMs ratio	tumor #3 AUC7 abs	tumor #3 AUC7 ratio	tumor #4 SRA	tumor #4 Pepts	tumor #4 PSMs abs	tumor #4 PSMs ratio	tumor #4 AUC7 abs	tumor #4 AUC7 ratio	tumor #5 SRA	tumor #5 Pepts	tumor #5 PSMs abs	tumor #5 PSMs ratio	tumor #5 AUC7 abs	tumor #5 AUC7 ratio
HMGA2										1		1		5	22	1000	1035	1000				1		1				1		1
SMC2		1	1							0.001		1		44	69	69	430	1000				0.001		1				0.001		1
SMC4										1		1		46	72	1000	341	1000				1		1				1		1
TYMS										1		1		12	27	1000	335	1000				1		1				1		1
MDC1		4	4							0.001		1		37	56	14	216	1000				0.001		1				0.001		1
NCAPD2										1		1		37	55	1000	212	1000				1		1				1		1
CCNB1										1		1		7	8	1000	24.8	1000		1	1	1000	2.5	1000				1		1
RFC4		4	5		2.3			1	1	0.2	0.15	0.07		14	22	4.4	155	67.4		2	2	0.4	0.43	0.19		2	4	0.8	0.21	0.09
MSH6		8	8		12.6			1	1	0.13		0.001		24	39	4.9	252	20		1	1	0.13		0.001		2	2	0.25	0.95	0.08

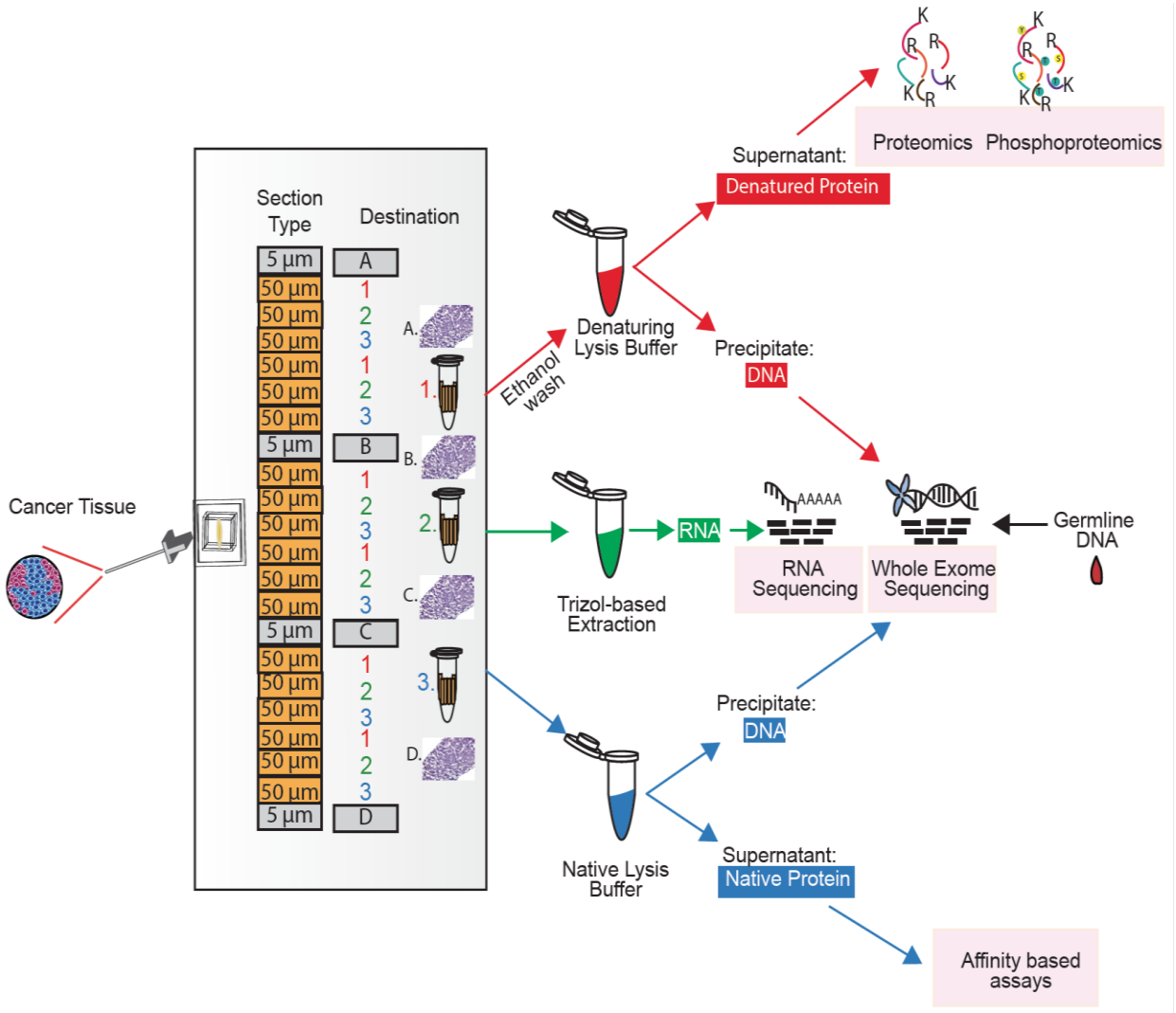
(C) Druggable kinases overexpressed in Tumor #3.

Symbol	tumor #1 SRA	tumor #1 Pepts	tumor #1 PSMs abs	tumor #1 PSMs ratio	tumor #1 AUC7 abs	tumor #1 AUC7 ratio	tumor #2 SRA	tumor #2 Pepts	tumor #2 PSMs abs	tumor #2 PSMs ratio	tumor #2 AUC7 abs	tumor #2 AUC7 ratio	tumor #3 SRA	tumor #3 Pepts	tumor #3 PSMs abs	tumor #3 PSMs ratio	tumor #3 AUC7 abs	tumor #3 AUC7 ratio	tumor #4 SRA	tumor #4 Pepts	tumor #4 PSMs abs	tumor #4 PSMs ratio	tumor #4 AUC7 abs	tumor #4 AUC7 ratio	tumor #5 SRA	tumor #5 Pepts	tumor #5 PSMs abs	tumor #5 PSMs ratio	tumor #5 AUC7 abs	tumor #5 AUC7 ratio
BTK		1	1							0.001		1		3	4	4	3.5	1000				0.001		1		2	2	2		1
ATR										1		1		2	2	1000	1.3	1000				1		1		1	1	1000		1
AURKA										1		1		1	1	1000	1.2	1000				1		1				1		1
CSF1R										1		1		1	1	1000	0.65	1000				1		1				1		1
CDK6		3	3		2.3			3	4	1.3	16.2	7		7	7	2.3	54.2	23.6		1	1	0.33		0.001		1	3	1		0.001
ABL1		2	2		0.06			1	1	0.5		0.001		4	4	2	1.4	23.3		1	1	0.5	0.56	9.3				0.001		0.001
CDK4		5	6		2.8			3	4	0.67	16	5.7		7	11	1.8	47.1	16.8		1	1	0.17		0.001		3	5	0.83	8.9	3.2
EGFR		7	8		20.8			13	16	2	29.6	1.4		15	23	2.9	88.5	4.3		5	6	0.75	3.1	0.15		4	4	0.5	3.9	0.19
MAP2K1		10	20		63			10	12	0.6	51.2	0.81		13	22	1.1	149	2.4		5	8	0.4	30.7	0.49		6	12	0.6	23.2	0.37
LYN		5	7		48.6			9	11	1.6	29.3	0.6		14	18	2.6	97.1	2		5	5	0.71	7	0.14		5	8	1.1	17.9	0.37

Anna Malovannaya
Hamssika Chandrasekaran
Sung Jung

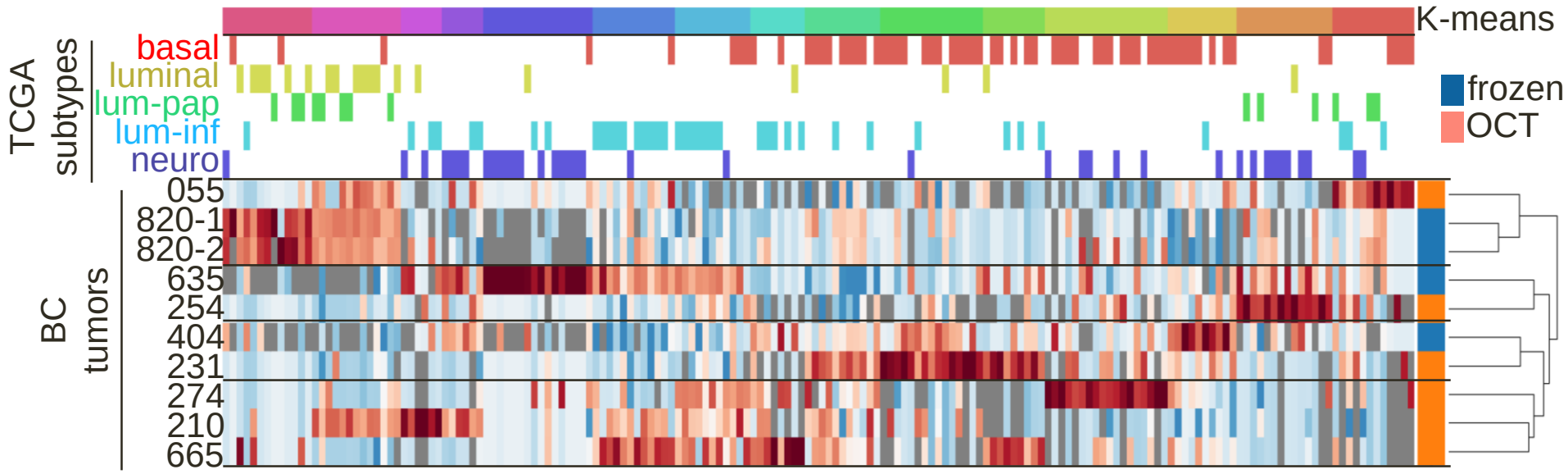
No photos
No social media

Biopsy Trifecta Extraction (BioTExt)



Ellis lab

Compared to TCGA subtype signatures

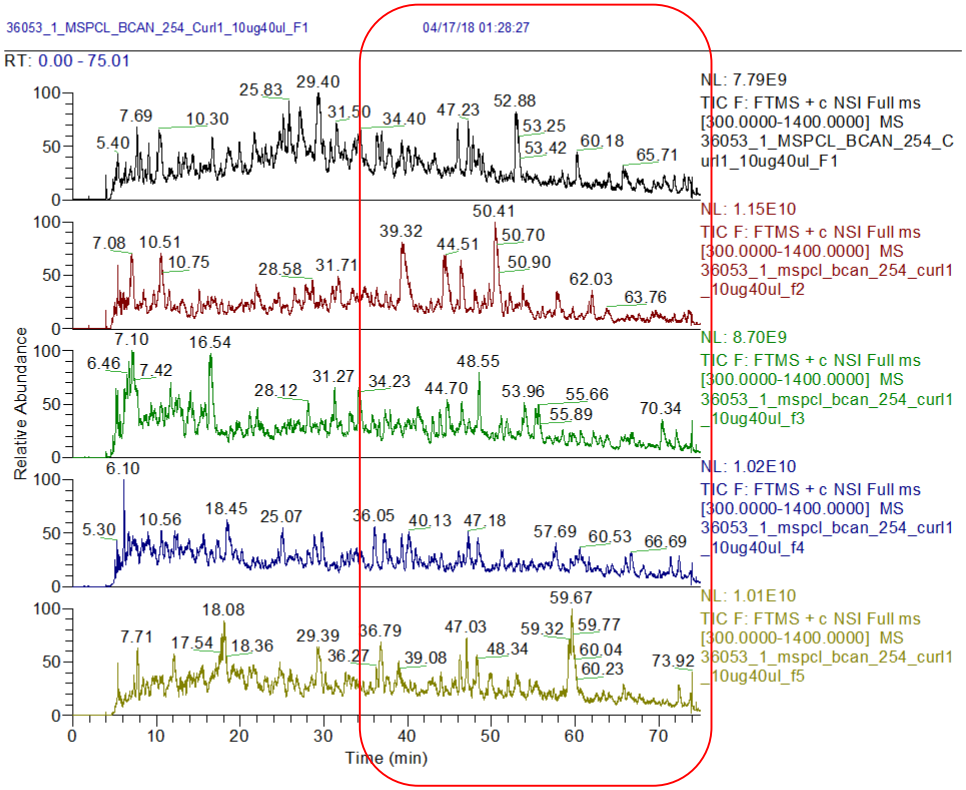


- by total profiles also largely along these lines
- finer heterogeneity evident in proteomics data

- about 50% of samples give much lower recovery numbers – why?

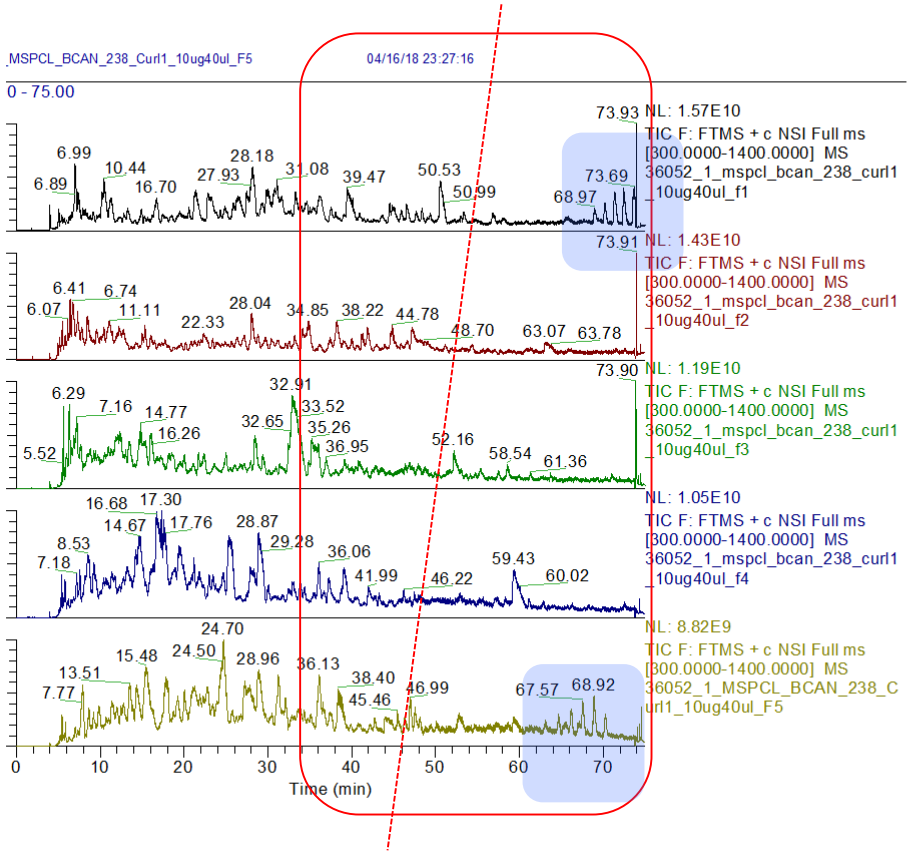
good data

Tumor #254



bad data

Tumor #238

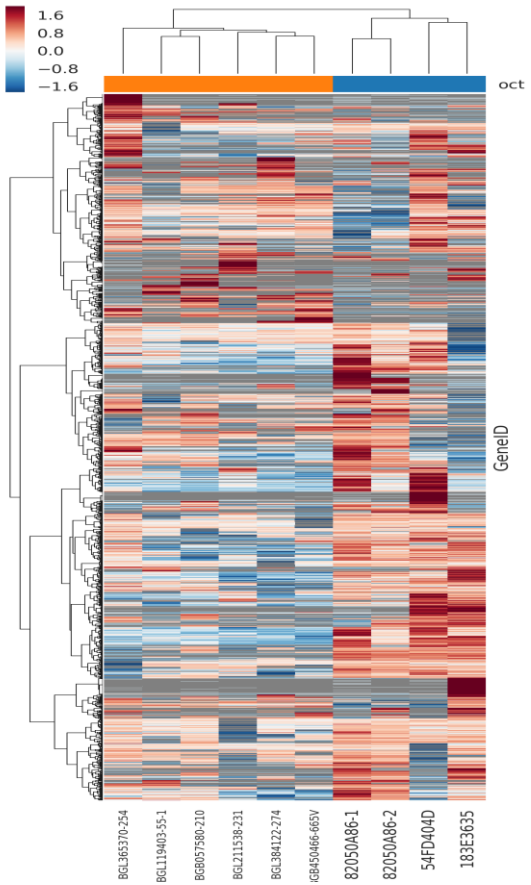


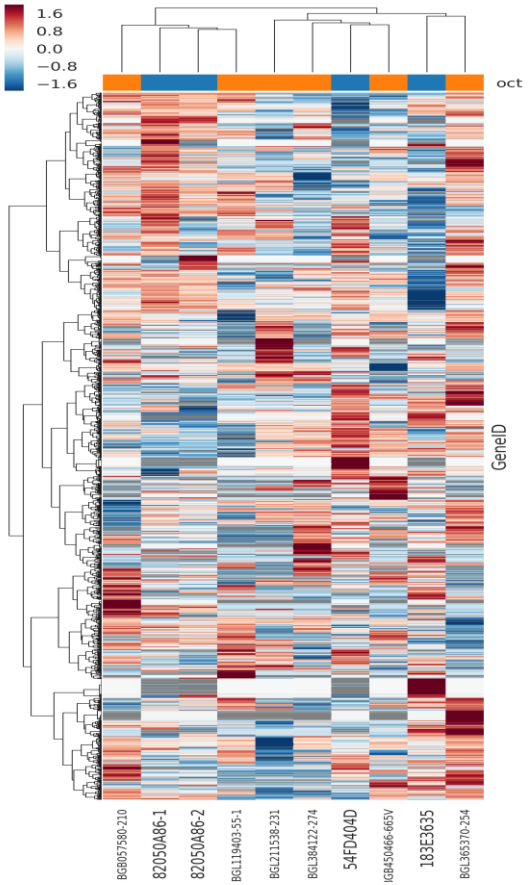
missing a whole class of peptides
not fully cleared of OCT

Proteomic Profiling

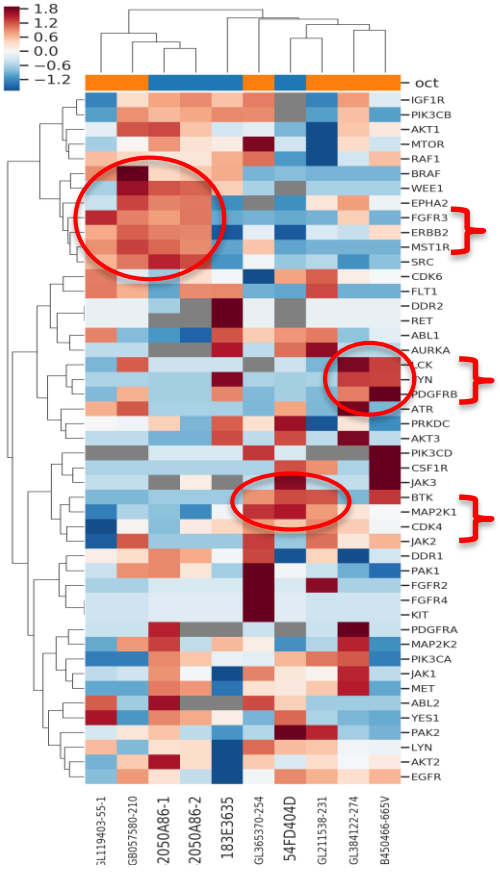
OCT batch effect

Left: OCT lower observed protein abundances vs frozen tumor
Right: After Bioinformatic correction





Druggable kinases



Multiplexed inhibitor bead Kinome Pull-down (KiP) profiling

Kinome Pull-down (KiP) with 9 kinase inhibitor-conjugated beads

abemaciclib*: *CDK4, CDK6*

afatinib*: *EGFR, ERBB2*

axitinib*: *VEGFR, PDGFR, KIT*

AZD4547: *FGFR, VEGFR*

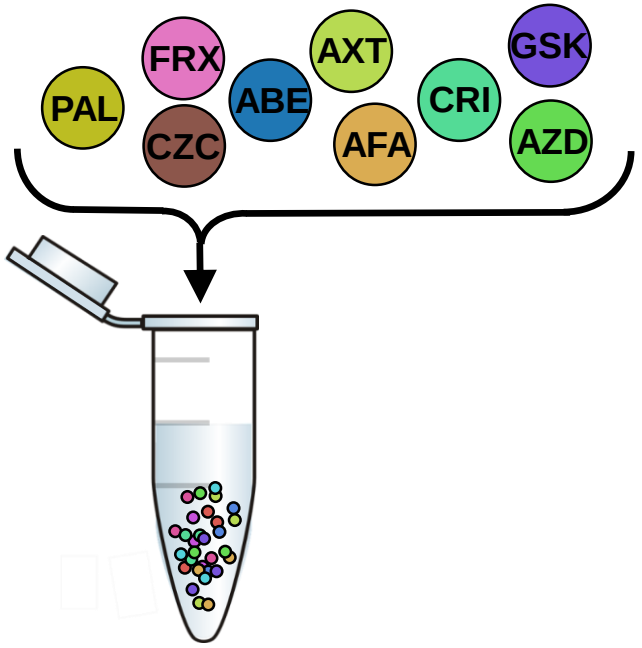
Crizotinib: *ALK, MET, AXL*

CZC-8004: *pan tyrosine kinase*

FRAX597*: *PAKs*

GSK690693: *AKTs*

Palbociclib: *CDK4, CDK6*



The mixture of 9 kinase inhibitor beads is designed to isolate and enrich the kinases with most activity

KiP Breast Cancer

Ellis, Kim unpublished
No photo/social media

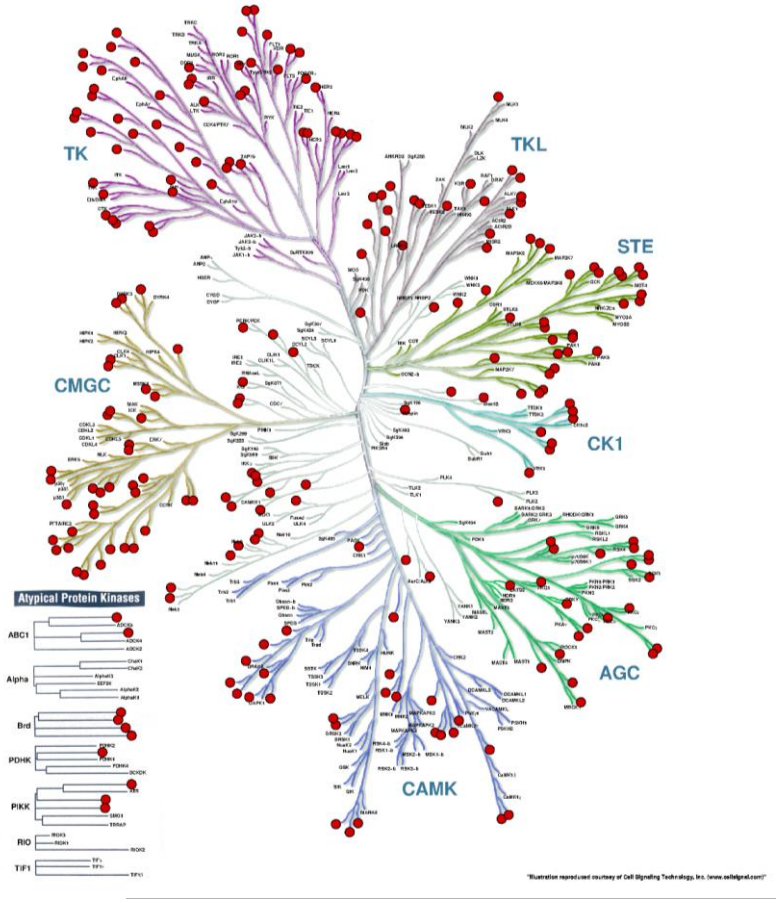


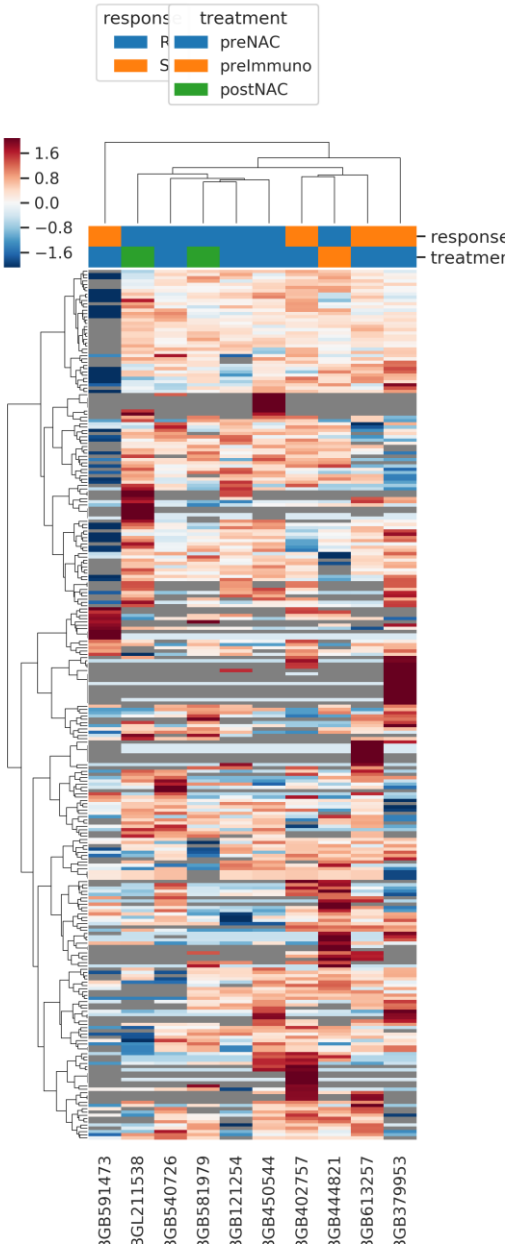
Table 2. Duplicate PDX (WHIM series) analysis comparing macroscale with microscale input showing high reproducibility and only modest reduction in kinase identification with 25 fold reduction in sample input.

Figure 2 Broad kinome coverage with a

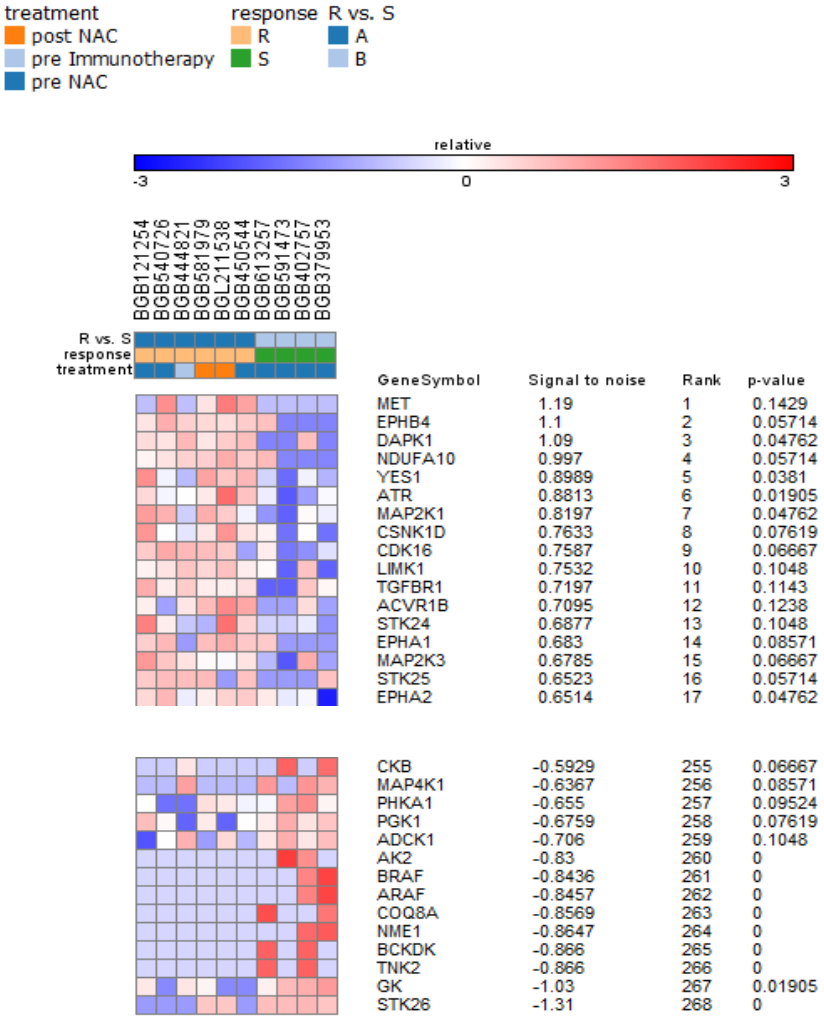
WHIM number	Macro-scale (500ug)	Micro-scale (20ug)
Numbers of experiments	47 duplicates from 24 PDX	44 duplicates 22 PDX
# total kinases (human & mouse)	723	524
# human kinases	388	285
# human kinase quantified	382	284
Average duplicate correlation (range)	0.983 (1.000 to 0.901)	0.975 (0.999 to 0.927)

KiP profiling

Beom-Jun Kim
Ellis lab (BCM)



- Pre NAC clusters together with similar kinase expression profile



- Kinase enrichment stratified by chemosensitivity
- DAPK1, YES1, ATR, MAP2K1, and EPHA2 higher in Res
- Sens higher in BRAF, ARAF

PDX - Principles

- Faithfully represent parental human tumor
- Maintain genomic and biologic fidelity and heterogeneity in passage
- Serially passaged x 3: P0 (initial engraftment), P1,P2
- Take rates may vary by subtype
  - Enhanced with Matrigel
- Gender and ethnicity may affect treatment response
- Does the PDX respond to treatment similar to tumor of origin in the patient?
  - Not affected by immunodeficiency of the host (PDX)

Dobroleki, et al Cancer Met Review 35:547, 2016

Mouse PDX

Mouse background

Table 1: Mouse PDX

July 2017-April 2019 (Chan)	
Attempts	77
Engraftment	16
Ongoing	4
Viably frozen	1
No engraftment	56
May-June 2019 (PDX Core)	
Attempts	10
Palpable tumors	3
Ongoing	3
Viably frozen	4

Gender	Histology	Disease status	Clin Stage	Path stage	NAC	NAC Response	Sens(S) or Res(R)	ChemoXRT	ChemoXRT response	Status
M	Urothelial w/50% SCCa	Post chemo	T3N3	pT3bN3	Y	NR	R	N	na	DOD
M	Small cell NE	Pre-NAC	T2	cT0	Y	CR	S	Y	CR	Alive (CIS) NED 19 mos
M	Urothelial	Pre-Chemo	T3bN+	na	Y	NR	R	N	na	Alive with disease
M	Urothelial w/15% SCCa	Pre-chemoXRT	T3b	na	N	na	na	Y	NR	Alive with dx 11 mos
F	Urothelial	No treatment	T3b	na	N	na	na	N	na	DOD
F	Urothelial w/sarcoma	No treatment	T3bN3M1	na	N	na	na	N	na	DOD
M	Urothelial	Pre-RC	T3bN2	pT3aN0	N	na	na	N	na	Alive; NED 4 mos
M	Urothelial	Post-Pembro; no RC	T2N+	na	N	na	na	N	na	DOD
M	Urothelial	Pre-chemo for M1	T2N3M1	na	Y	NR	R	N	na	Progressed; AWD
M	Urothelial w/90% SCCa	No treatment	T2bM1	na	N	na	na	N	na	DOD
F	Urothelial	Pre-chemoXRT	T2	na	N	na	na	Y	pending	Dx status pending
M	Urothelial	Pre-BCG	T1	na	N	na	na	N	na	Alive NED 8 mos
M	Urothelial	Post-chemo; pre-RC	T3	pT4aN2	Y	NR	R	N	na	Alive; NED 5 mos
M	Urothelial w/focal SCCa	Treated one cycle NAC	T3bN2	na	Y	NR	R	N	na	DOD
										Alive; Dx status

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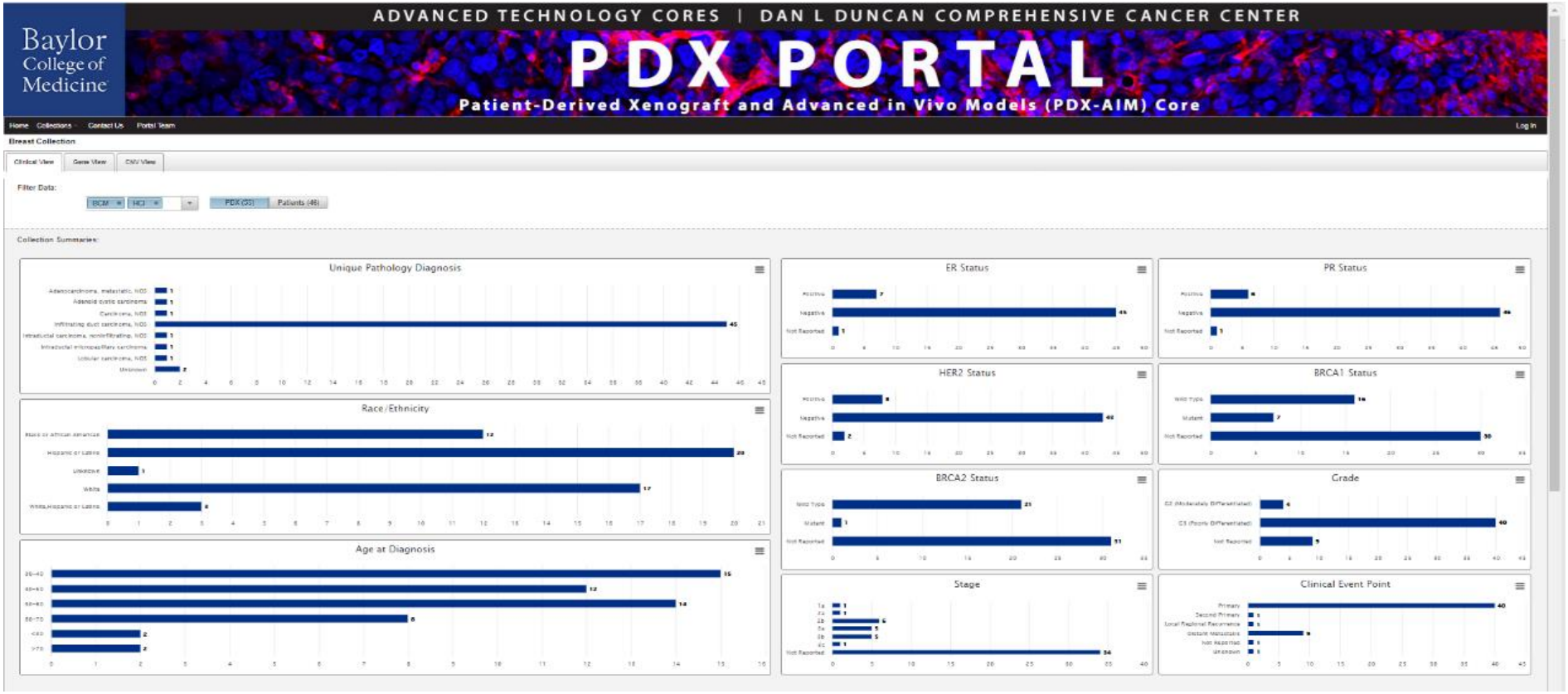
BCM PDX Portal: Collection Summary Page

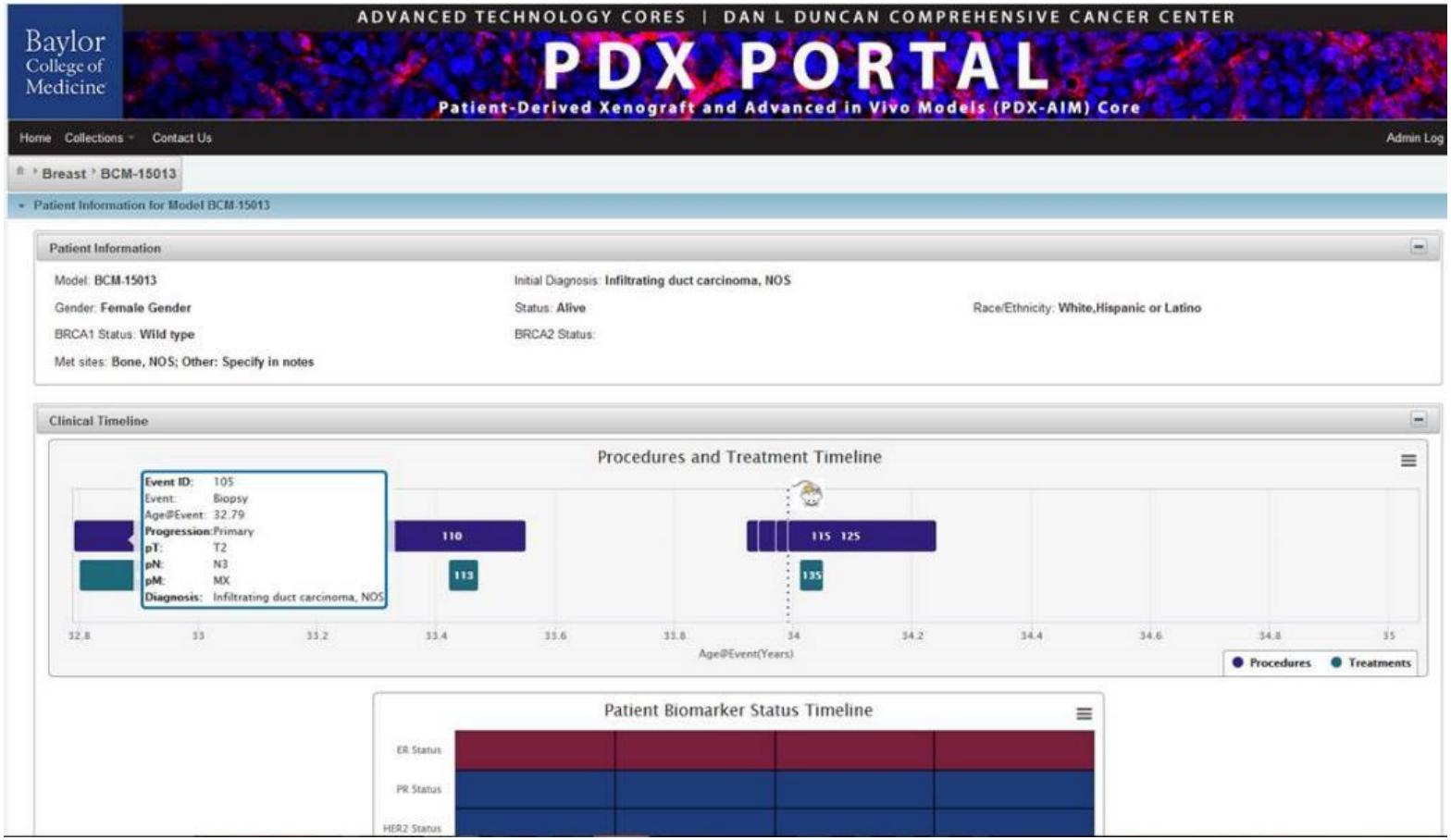


Heidi Dowst
Apollo McOwiti
Kerri Zheng
Ram Srinivasan
John Landua

Lacey Dobrolecki
Alaina Lewis
Christina Sallas
Ana Hernandez-Herra
Alphi Kuriakose

Chen Huang
Bing Zhang

BCM PDX Portal: Patient Clinical Information View



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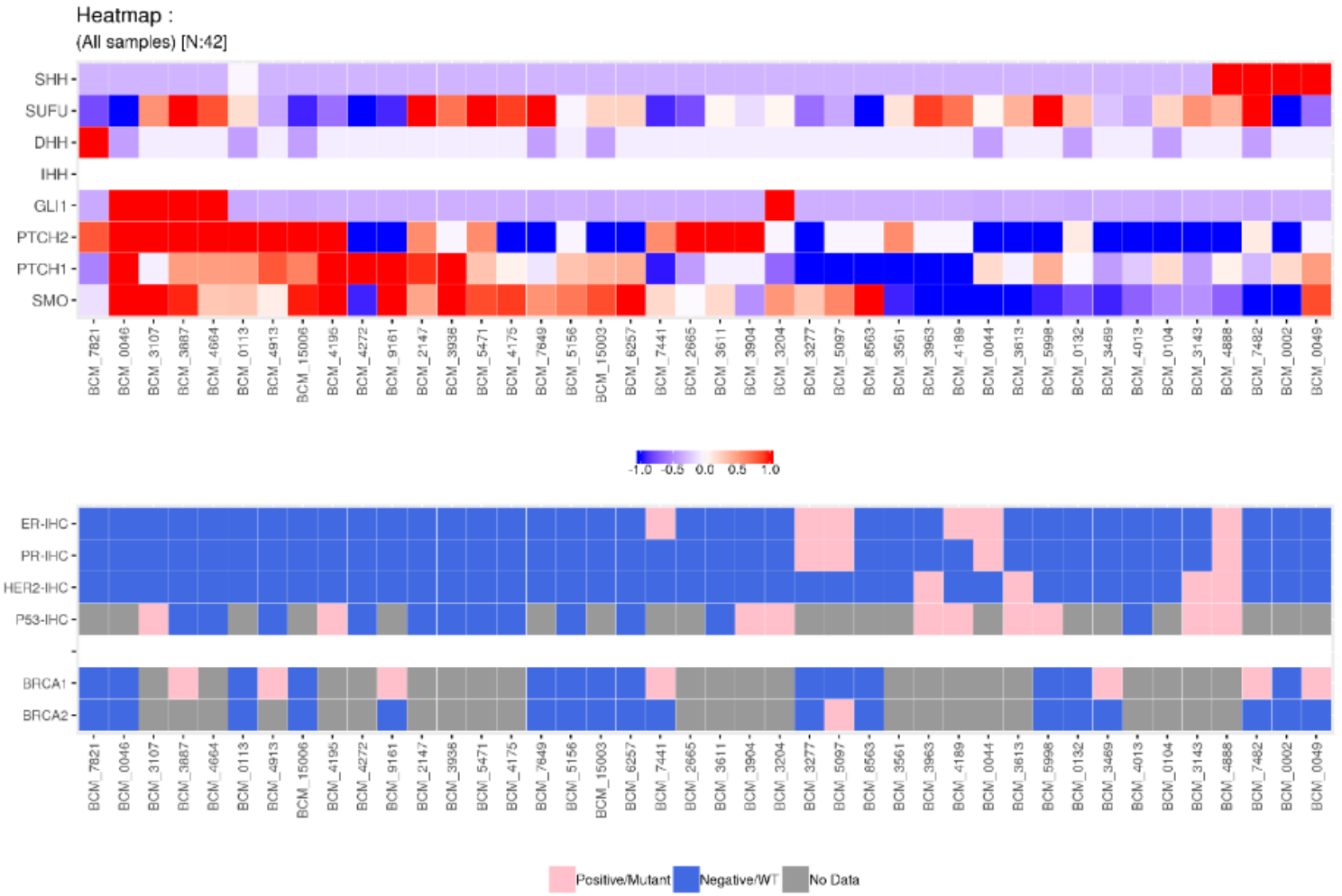
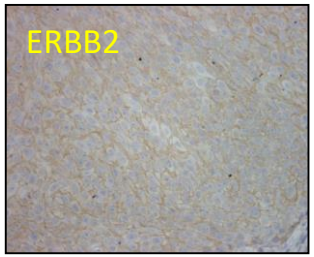
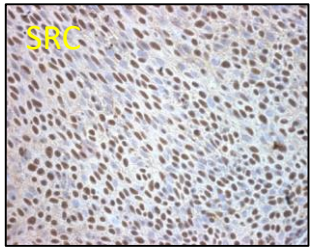
BCM PDX Portal: Gene Expression View

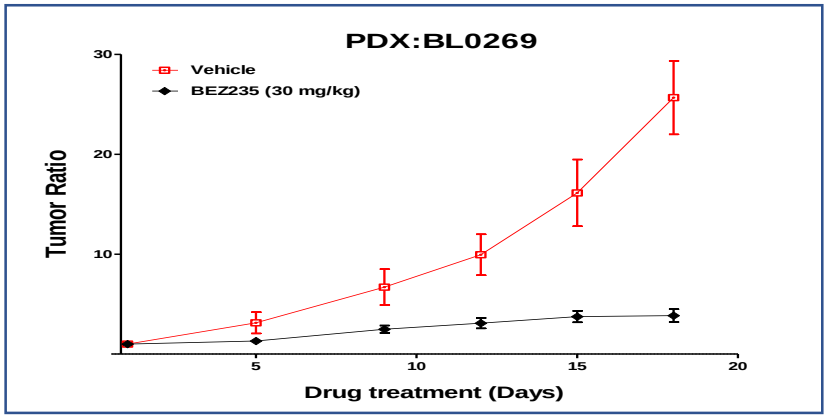


Screening for effective targeted therapies

PDXs		BL0269
ERBB2		+++
Protein		
	mRNA	51.2582
ERBB3		weak
Protein		
	mRNA	46.4868
FGFR3	Protein	Dirty ++ (30% cells)
	mRNA	158.192
SRC		++++
Protein		
	mRNA	22.964
EphB4		
Mouse		N/A
Human		POSITIVE
PIK3CA		H1047R

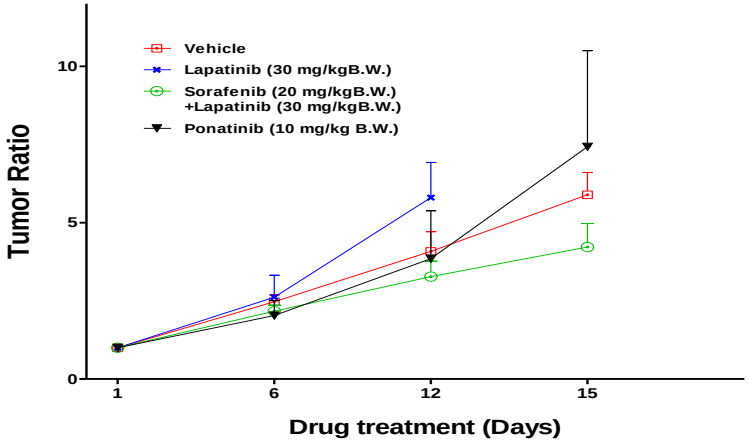






PDX BL0269 has overexpression of ERBB2 and SRC, and PIK3CA mutation. Only PIK3CA inhibitor BEZ was effective.
(In the table, the numbers are RNA seq results; the "+" is the IHC staining results)

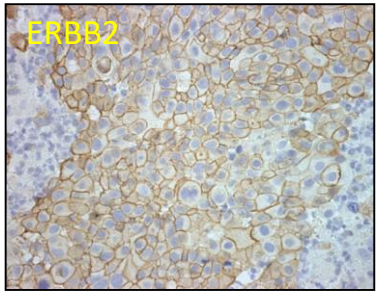
BEZ235: PIK3CA inhibitor
Lapatinib: EGFR and ERBB2 inhibitor
Sorafenib: Raf inhibitor
Ponatinib: Src inhibitor

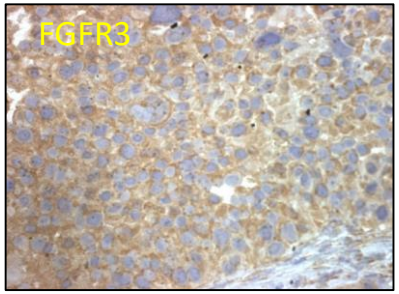


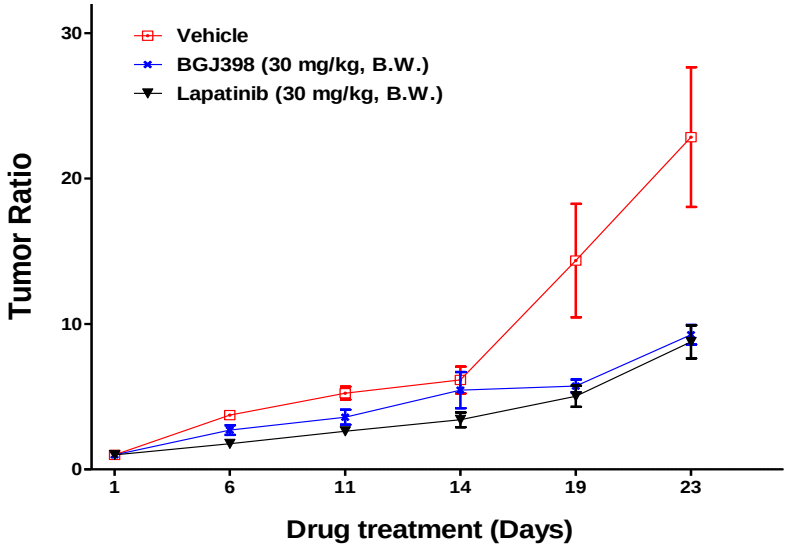
Courtesy Chong-xian Pan, UC Davis

Repurposing FDA-approved drugs

PDXs		BL0440
ERBB2	Protein	+++
	mRNA	158.051
ERBB3	Protein	++
	mRNA	46.0066
FGFR3	Protein	+++
	mRNA	80.387
SRC	Protein	5%+
	mRNA	70.0928
EphB4	Mouse	NEGATIVE
	Human	NEGATIVE
PIK3CA		





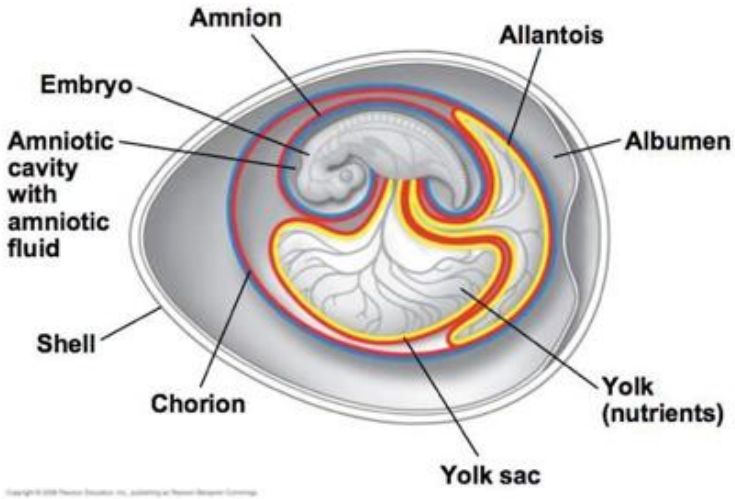


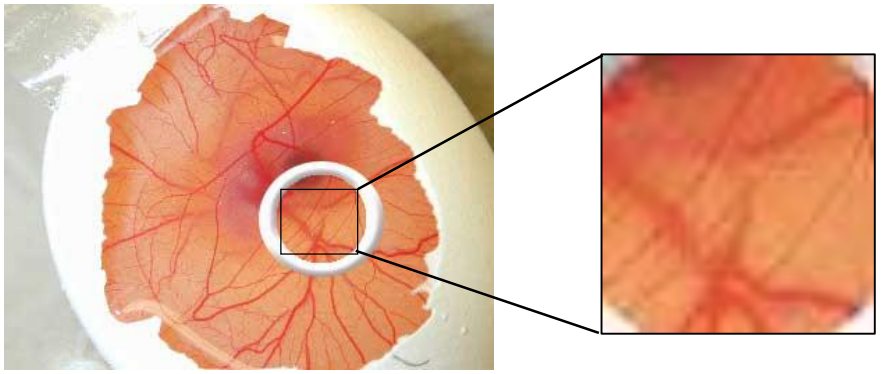
PDX BL0440 has overexpression of ERBB2, ERBB3 and FGFR3. Both lapatinib and FGFR3 inhibitor BGJ398 were effective.

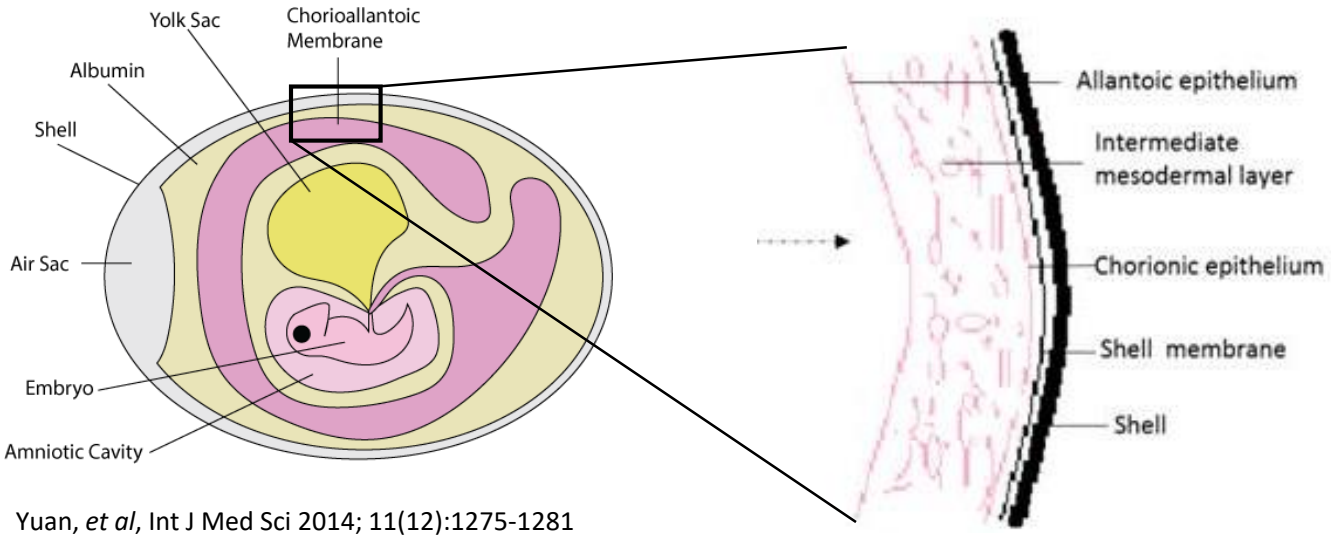
BGJ398: FGFR inhibitor
Lapatinib: EGFR and ERBB2 dual inhibitor.

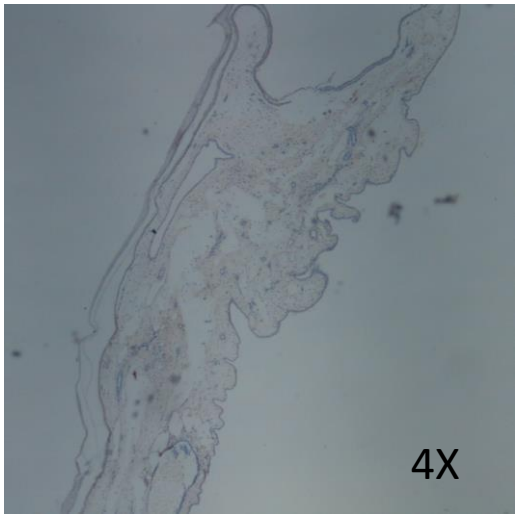
Courtesy Chong-xian Pan, UC Davis

The Chorioallantoic Membrane (CAM)



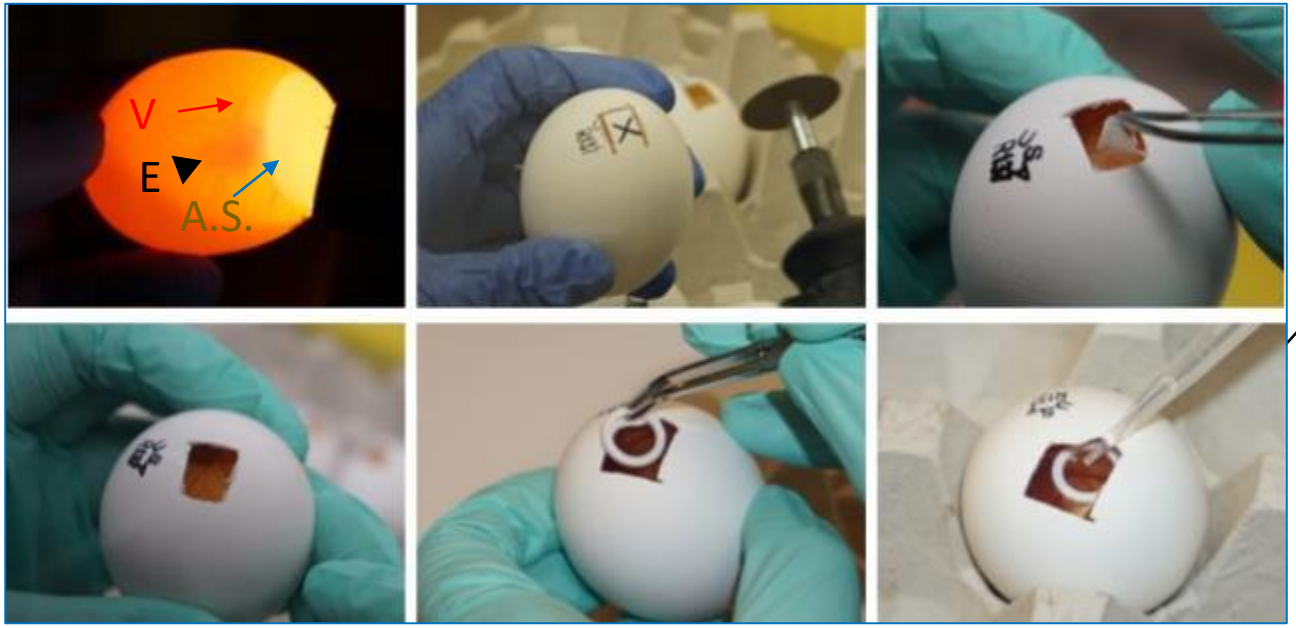


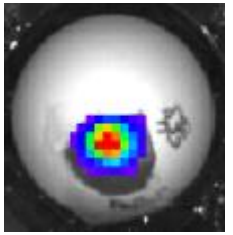


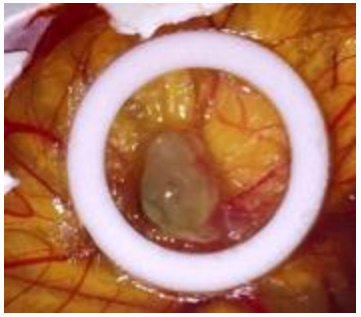


Yuan, *et al*, Int J Med Sci 2014; 11(12):1275-1281

Preparing the Chorioallantoic Membrane (CAM) for Growth of Cell Lines and PDX

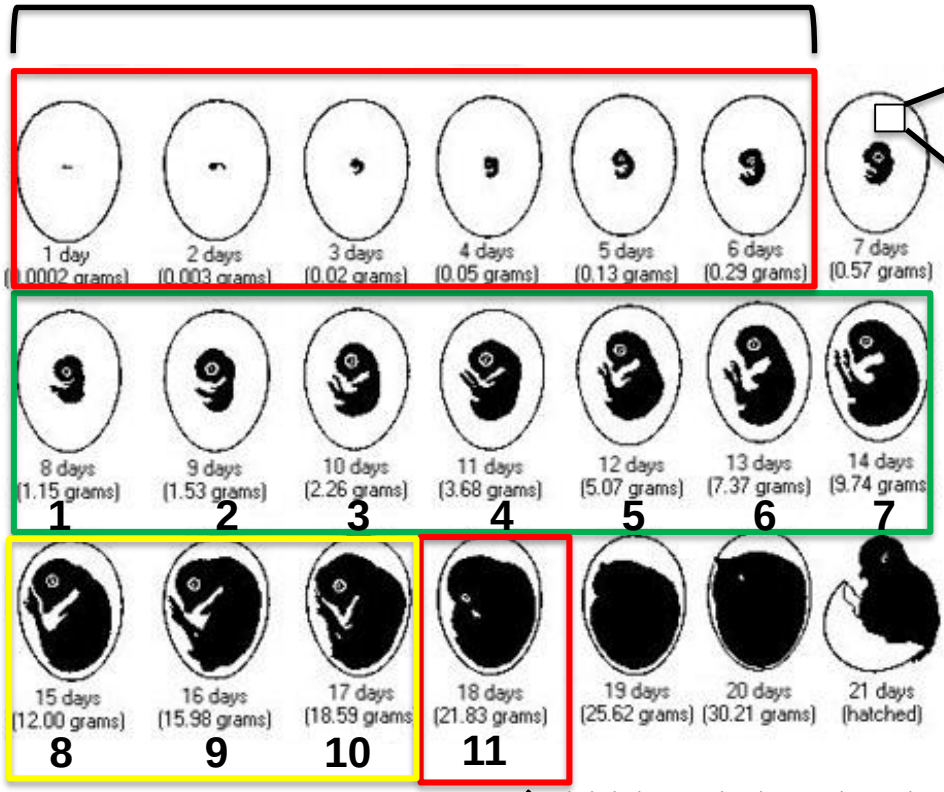






Li, M., Pathak, R. R., Lopez-Rivera, E., Friedman, S. L., Aguirre-Ghiso, J. A., Sikora, A. G. **The In Ovo Chick Chorioallantoic Membrane (CAM) Assay as an Efficient Xenograft Model of Hepatocellular Carcinoma**. *Journal of Visualized Experimentation* (104), e52411, doi:10.3791/52411 (2015).

CAM - The "Window of Opportunity"

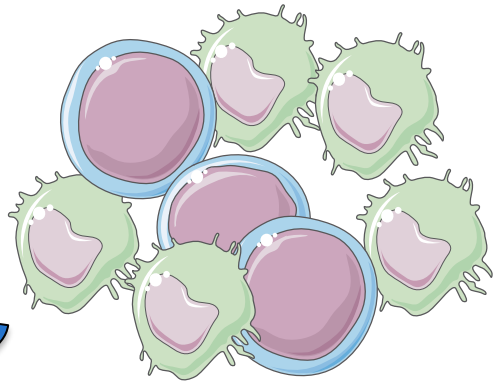


Mississippi State University Extension Service

Engraftment Day

Post-Graft Day:

"Immune Infiltrate"



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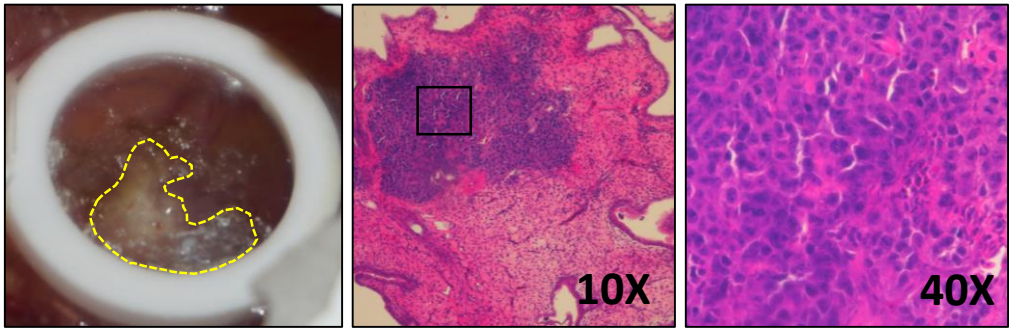
CAM

- Angiogenesis
- Tumor xenograft models
  - Glioblastoma, pancreatic cancer, melanoma, and osteosarcoma, HCC
- Both in ovo and ex ovo techniques
- Relatively high incidence of embryonic death after manipulation of the egg
  - Chick embryo mortality rates ranging from 25 – 50%
- BCM bladder CAM inventory
  - 101 tumors (51 viably frozen for future engraftment)
  - 31/50 (62%) attempts engrafted

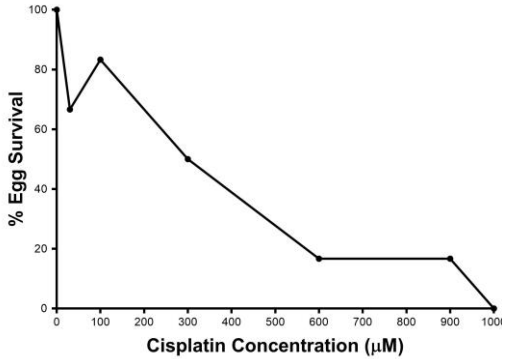
Prathak, et al J. Vis. Exp. (104), e52411

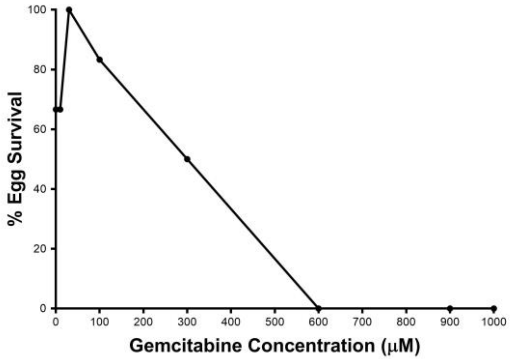
PDX-8035

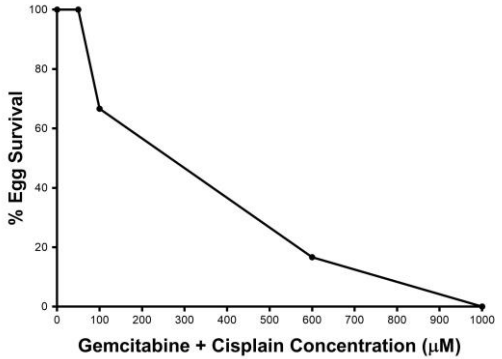


Patient-derived xenograft on chorioallantoic membrane. Bladder tumor grown on CAM after seven days of culture. Hematoxlin and eosin stained section of CAM-engrafted bladder cancer histology at 10X and 40X.

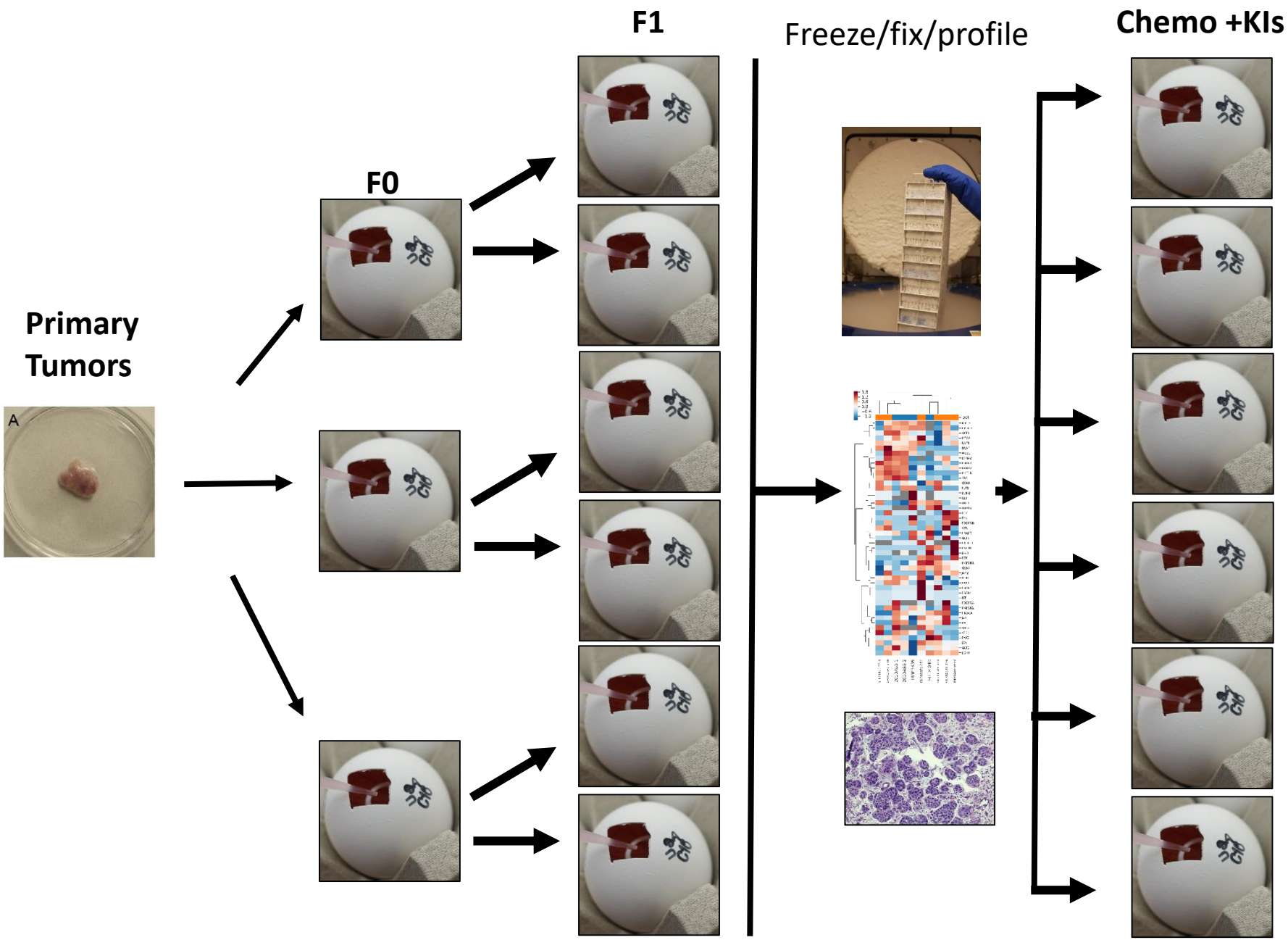
"Kill" curves



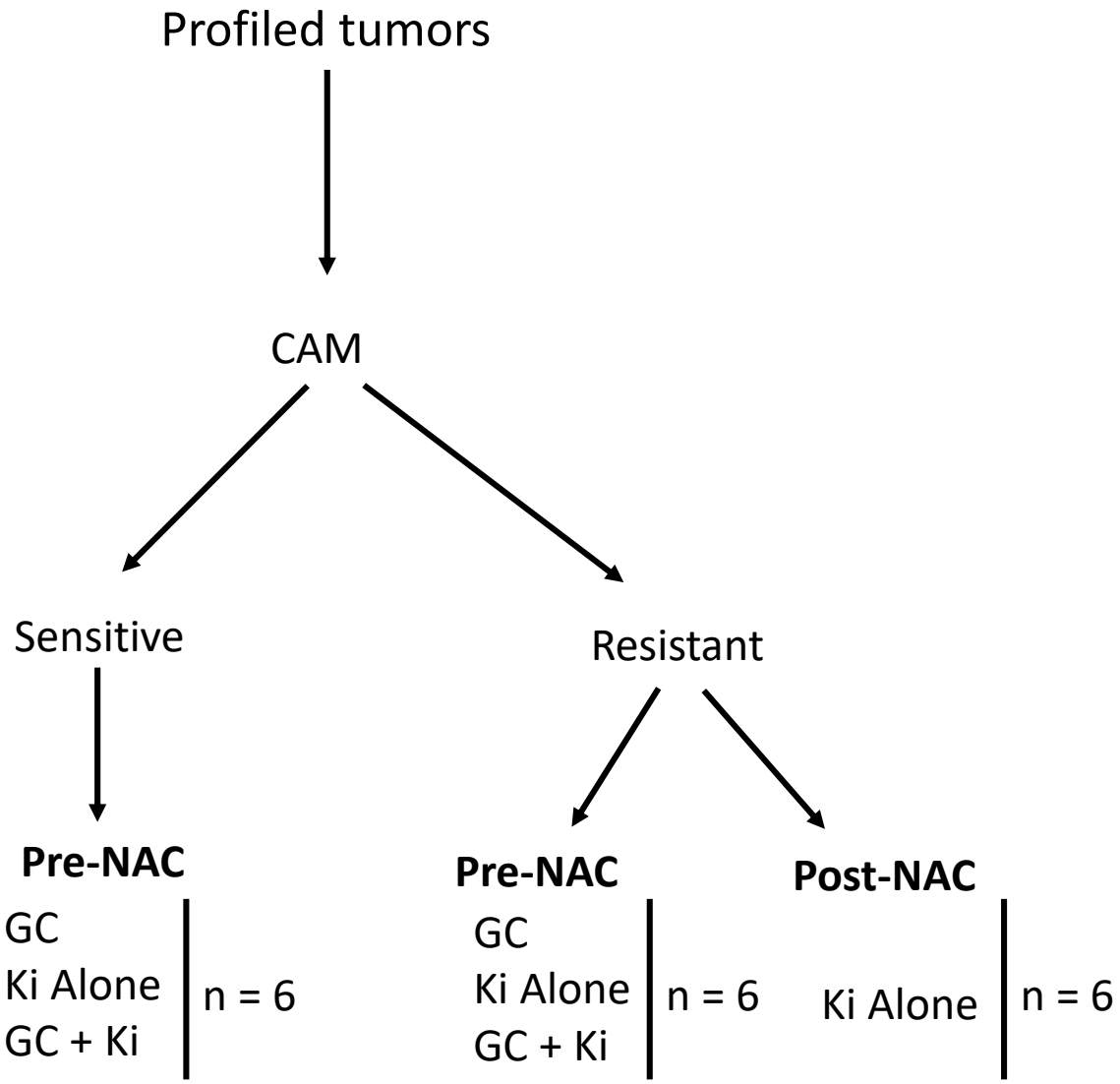




Bladder Cancer PDX Treatment Plan



CAM PDX Workflow



AZD4547 (FGFR)
18 nM - 30 uM
Abemaciclib
(CDK4/6)
0.12 - 2.7 uM
Afatinib (EGFR)
5.72 nM - 50 uM

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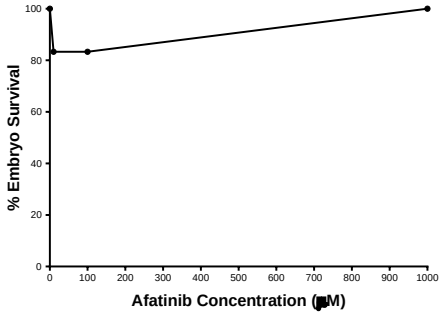
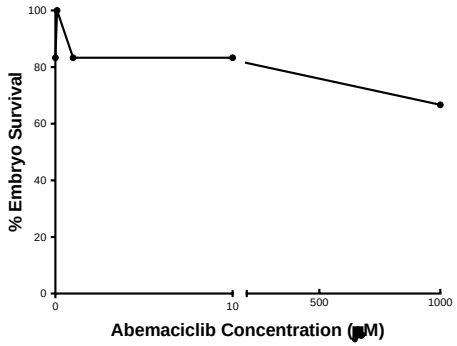
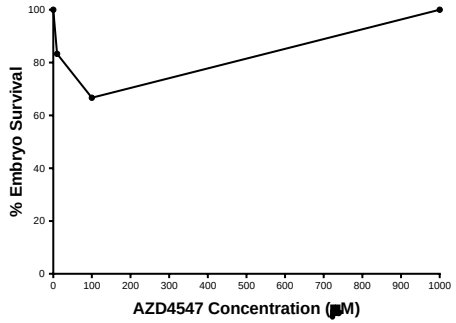
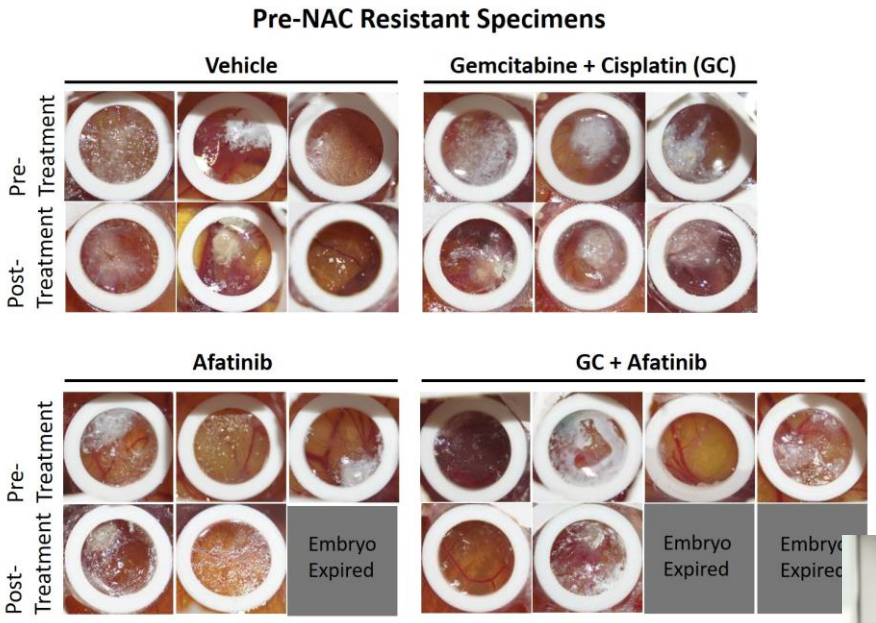
ADVANCED TECHNOLOGY CORES

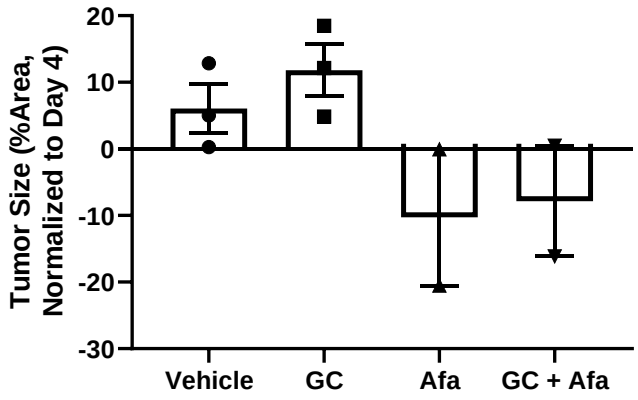
TKI on CAM Proof of Concept

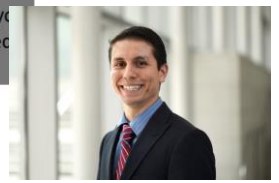


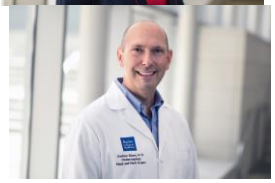




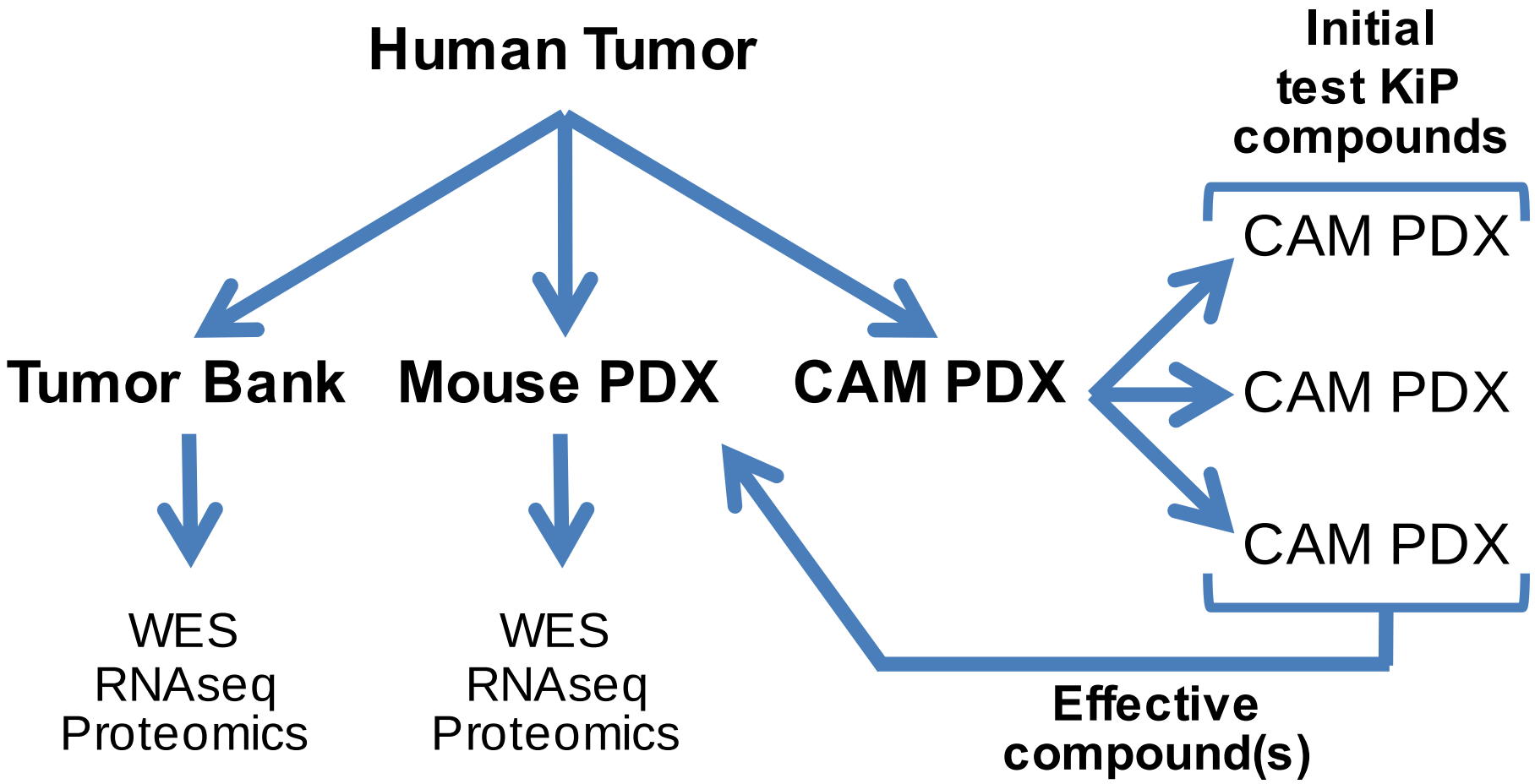




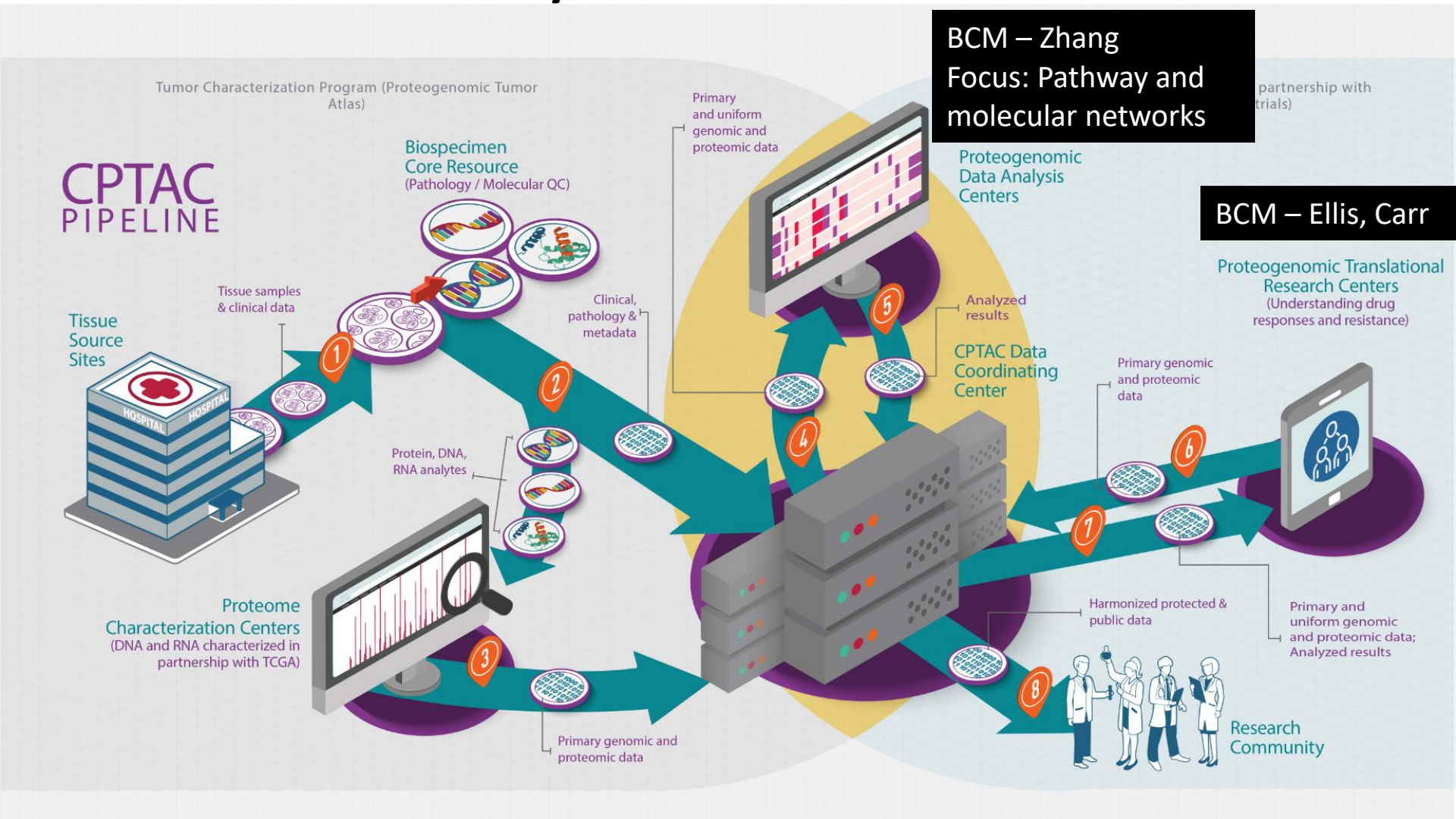




PDX Workflow



CPTAC –Clinical Proteomic tumor Analysis Consortium



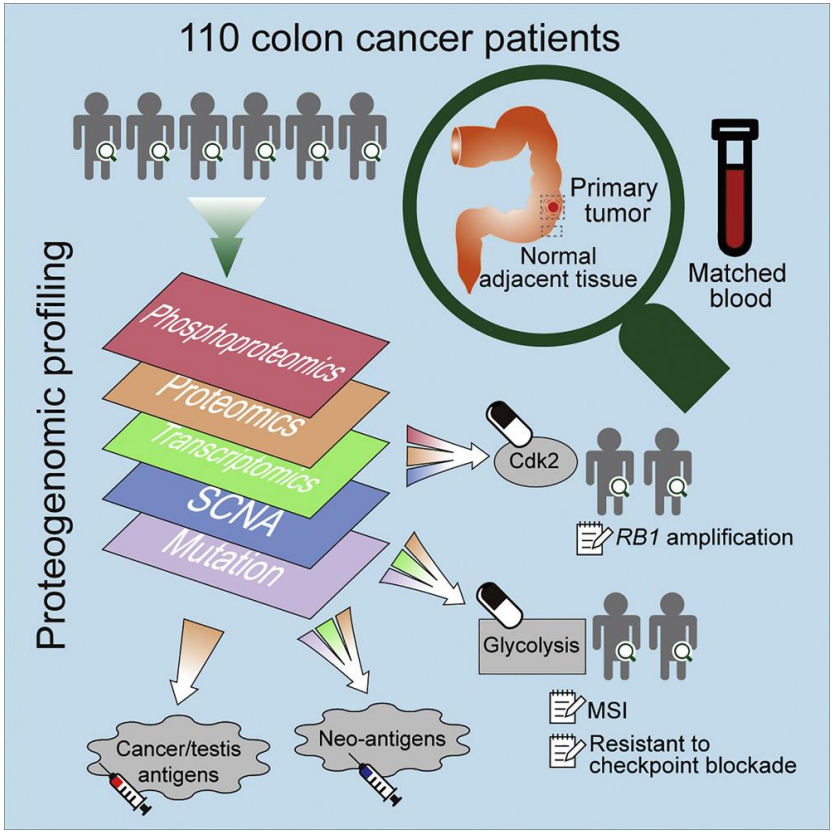
https://proteomics.cancer.gov/programs/cptac

CPTAC - Colorectal Cancer

Resource

Cell

Proteogenomic Analysis of Human Colon Cancer Reveals New Therapeutic Opportunities

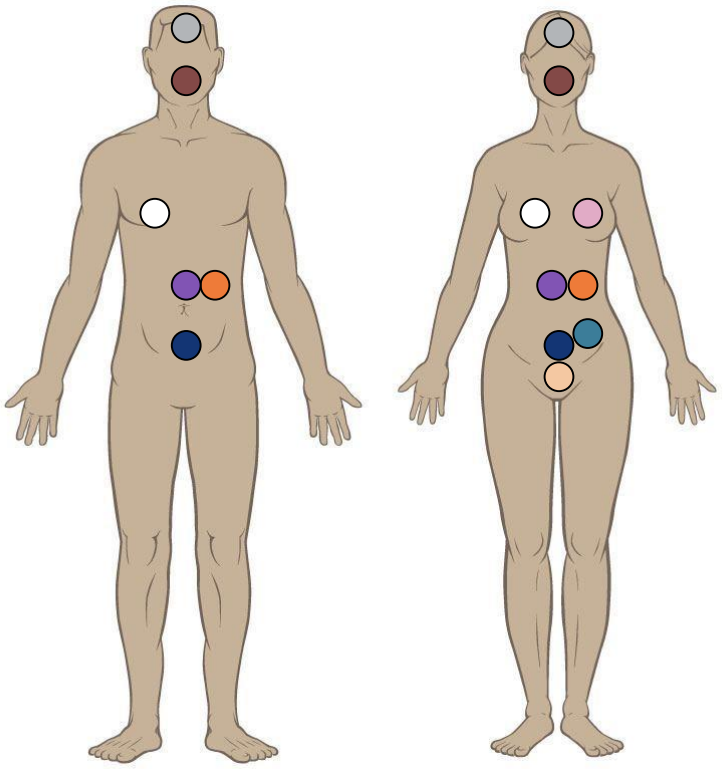


Highlights

- Systematic identification of colon cancer-associated proteins and phosphosites
- Proteomics-supported neoantigens and cancer/testis antigens in 78% of the tumors
- Rb phosphorylation is an oncogenic driver and a putative target in colon cancer
- Glycolysis inhibition may render MSI tumors more sensitive to checkpoint blockade

Vasaikar S, …Zhang, Cell, 177:1035-49, 2019

Clinical Proteome Tumor Analysis Consortium (CPTAC)

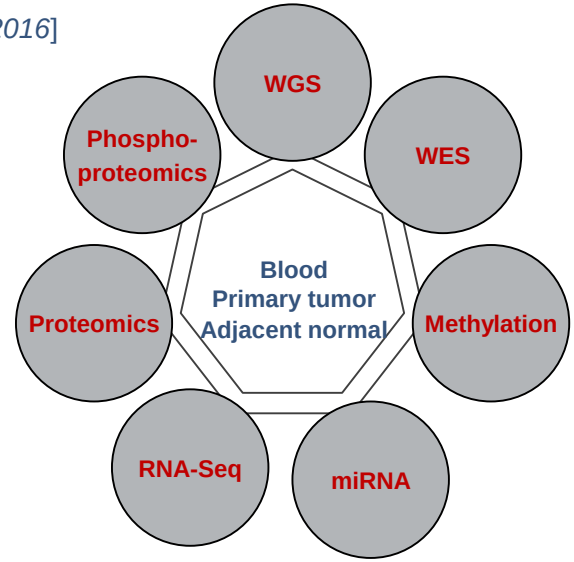


- Colorectal [*Zhang et al., Nature, 2014; Vasaikar et al., Cell, 2019*]
- Breast [*Mertins et al., Nature, 2016*]
- Ovary [*Zhang et al., Cell, 2016*]

- Kidney
- Uterus
- Lung

- Head and neck
- Pancreas
- Brain

……



CPTAC V04.02 – Bladder

Inclusion criteria

- New dx, untreated, undergoing primary cytoreductive surgery
- New tumor event in an existing or concurrent patient
- Recurrence, metastasis or second primary

Exclusion criteria

- No prior cancer within last 12 months except BCE
- No prior systemic therapy for another cancer within last 10 yrs

Blood and urine collection required

11.3 Appendix B: Summary of Key Study Criteria

CPTAC code	Cancer	Percent Tumor Nuclei	Percent Total Cellularity	Percent Necrosis	Maximum Tumor Ischemic Time (min)	Normal Tissue
BLCA	Bladder Urothelial Carcinoma	≥80%	≥50%	≤20%	30 (45 for robotic)	Normal adjacent tissue as feasible

CPTAC Research protocol version 04.00 6/7/2019

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Conclusions

- Proteomic profiling identifies clusters with similar protein kinase expression patterns
- KiP assay offers high throughput mass spec to identify druggable kinases and candidate kinase inhibitors
- Mouse PDX models established with proof of concept testing kinase inhibitors stratified by chemosensitivity.
- CAM PDX offers high throughput testing of chemotherapy and kinase inhibitors
- CPTAC for bladder cancer 2020-21