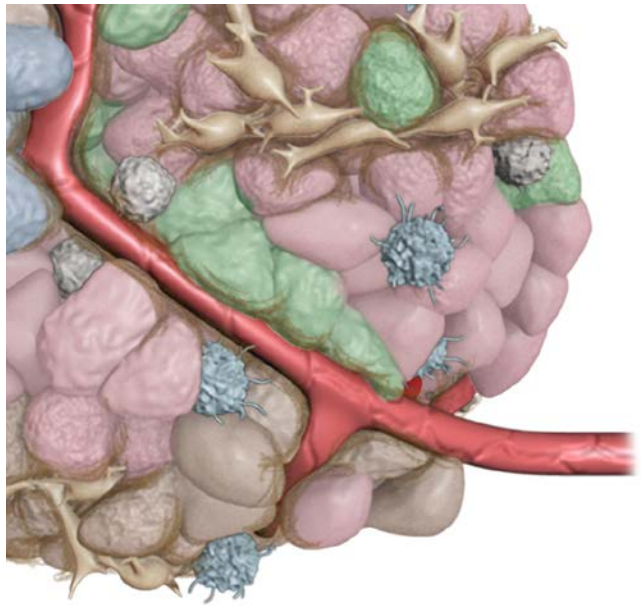
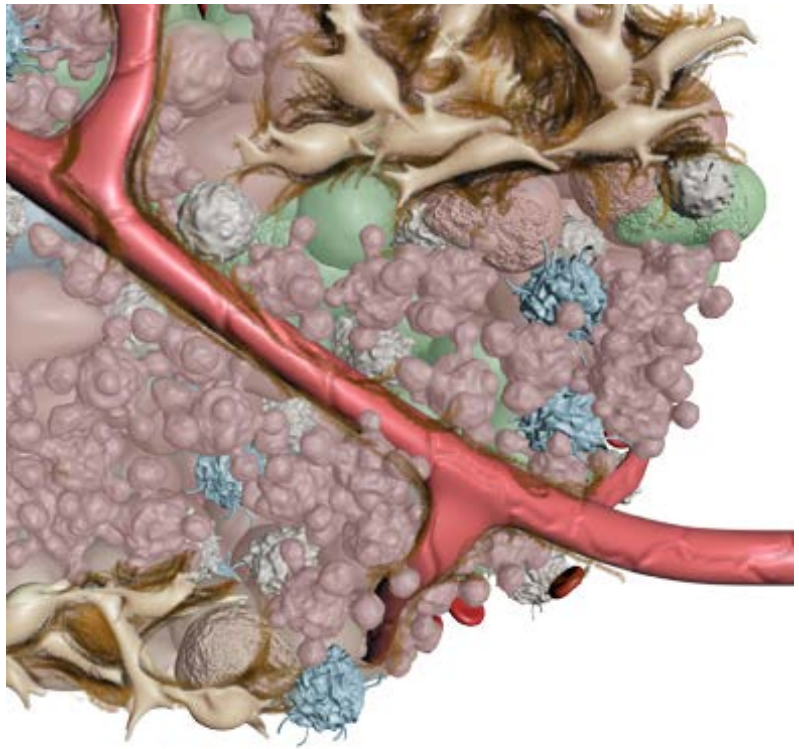


Extending the utility of PARP inhibitors

Gordon Mills
Knight Cancer Institute



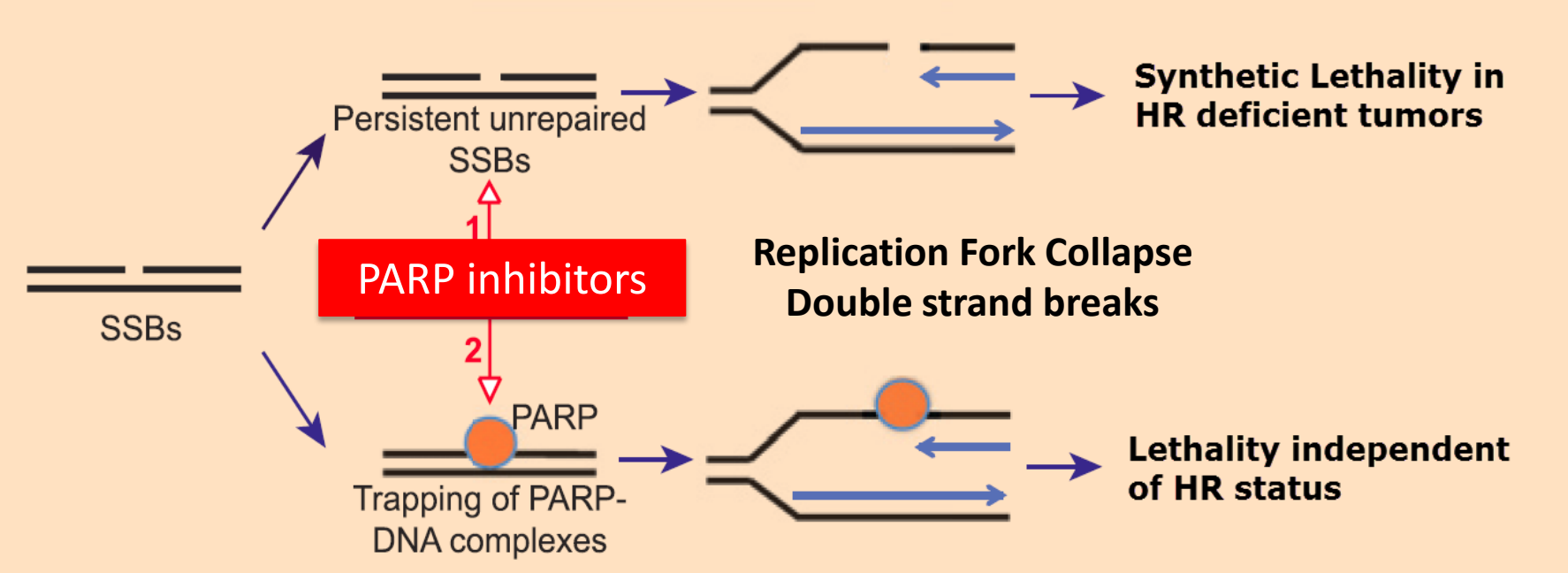
Rachel Davidowitz

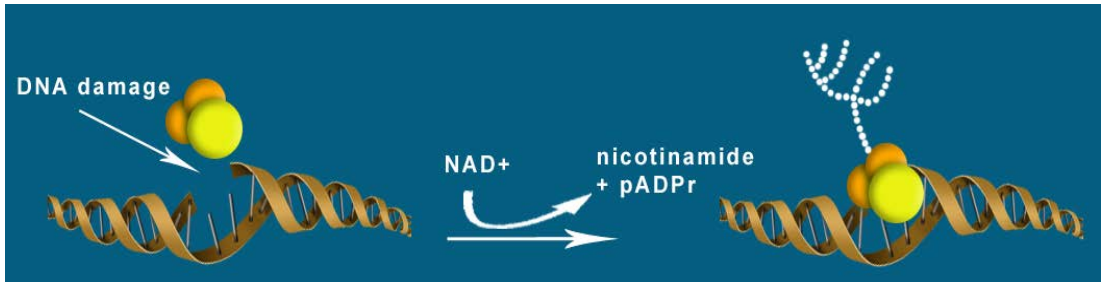
POTENTIAL CONFLICT OF INTEREST DISCLOSURES

- **Financial Relationships**
  - **SAB/Consultant:** AstraZeneca, Catena Pharmaceuticals, Critical Outcome Technologies, ImmunoMET, Ionis, Medimmune, Nuevolution, Pfizer, Precision Medicine, Signalchem Lifesciences, Symphogen, Takeda/Millennium Pharmaceuticals, Tarveda,
  - **Stock/ Options/Financial:** Catena Pharmaceuticals, ImmunoMet, SignalChem, Spindle Top Ventures, Tarveda
  - **Licensed Technology** HRD assay to Myriad Genetics
  - **Sponsored Research:** Abbvie, Adelson Medical Research Foundation, AstraZeneca, Breast Cancer Research Foundation, Critical Outcomes Technology, Illumina, Ionis, Immunomet, Karus Therapeutics, Komen Research Foundation, Pfizer, Nanostring, Takeda/Millennium Pharmaceuticals, Tesaro

I will discuss off label use and/or investigational use of drugs

Dual mechanisms of action of PARPi



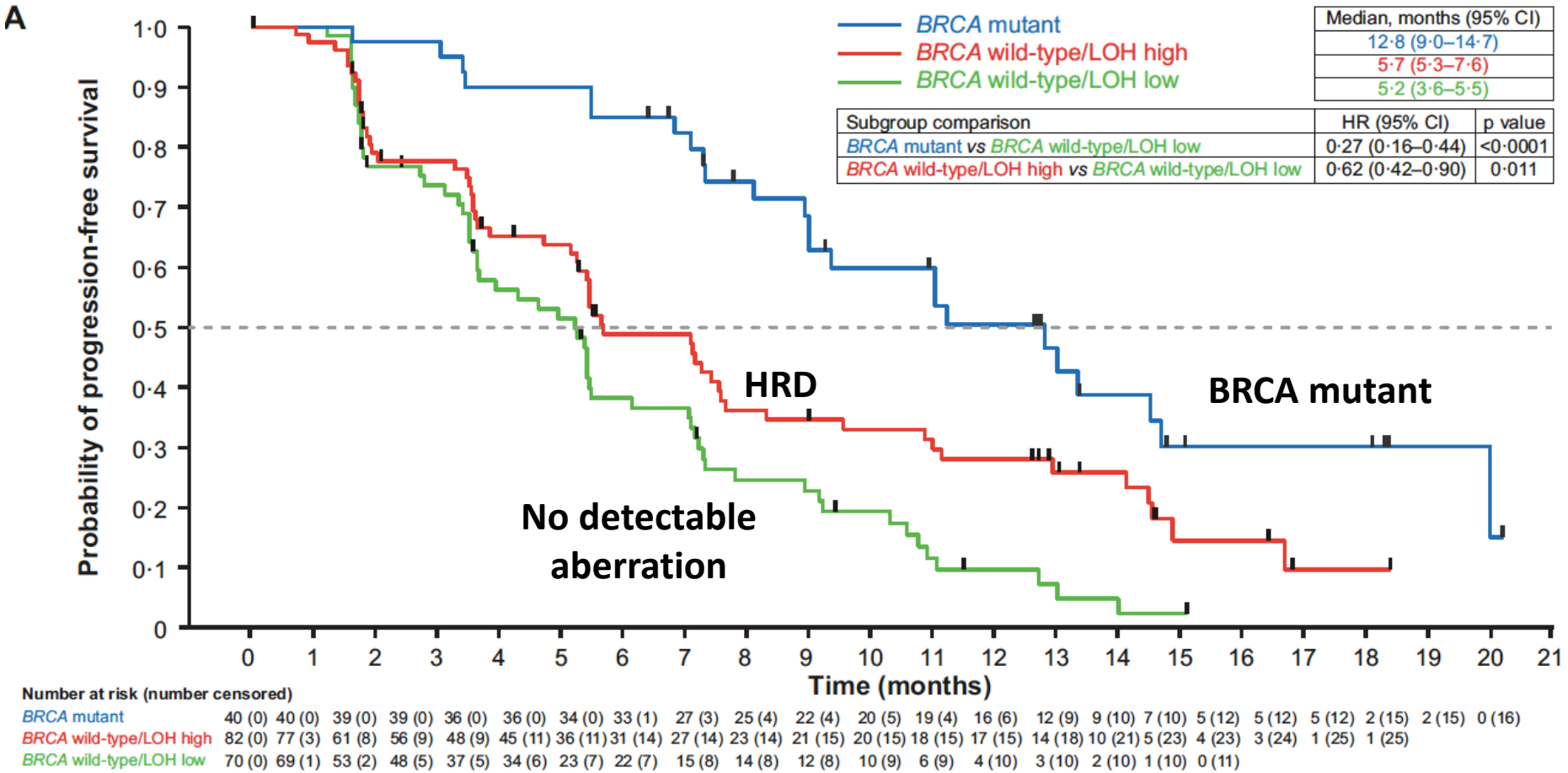


ADP ribosylation required for PARP to leave DNA

Trapped PARP creates "toxic" double strand breaks

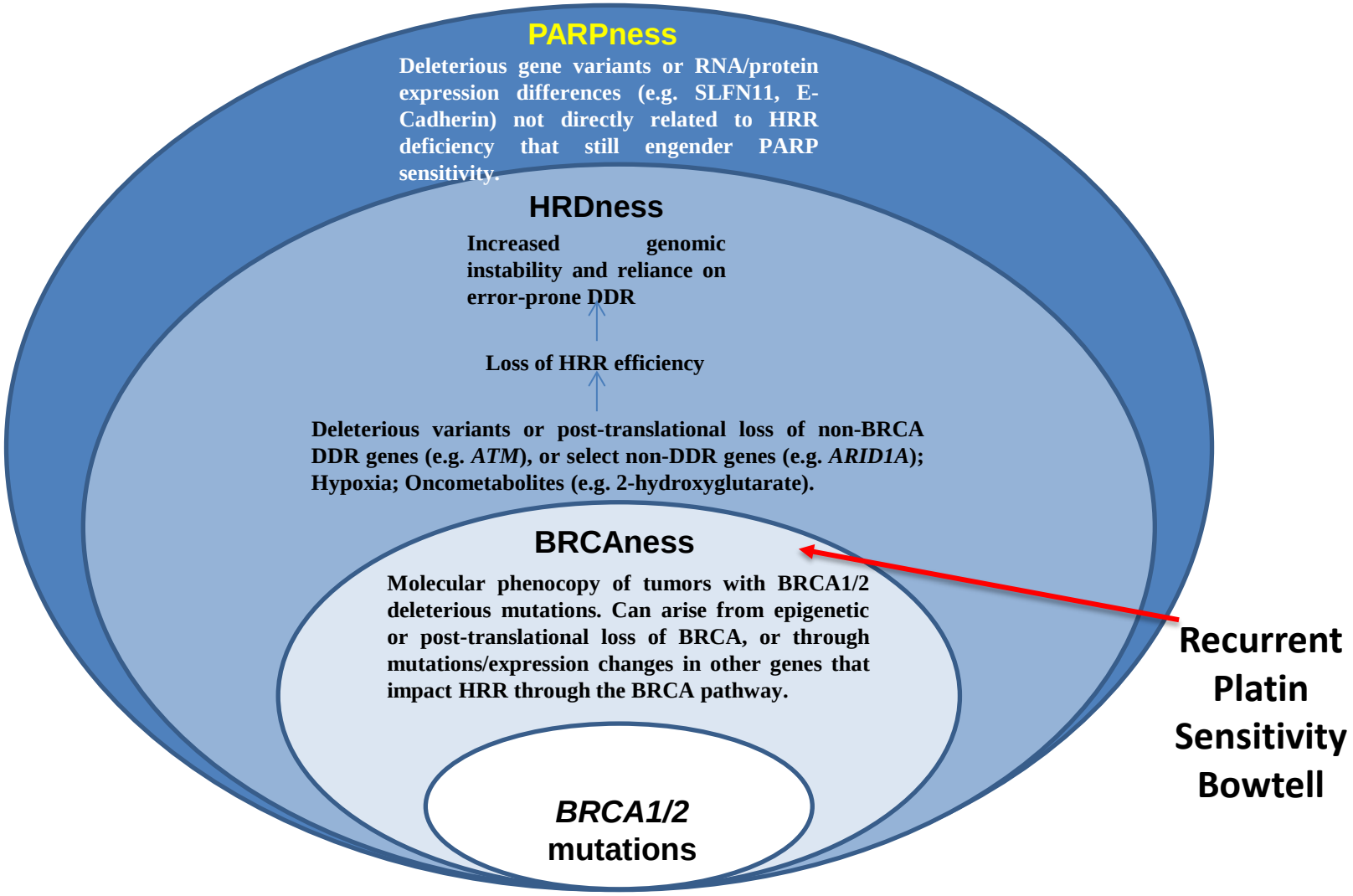
Can PARP activity be extended beyond HRD

PARP inhibitor responses are transient
Ariel 2 Rucaparib Ian McNeish Lancet:
LOH high is HRD assay performed by Foundation Med



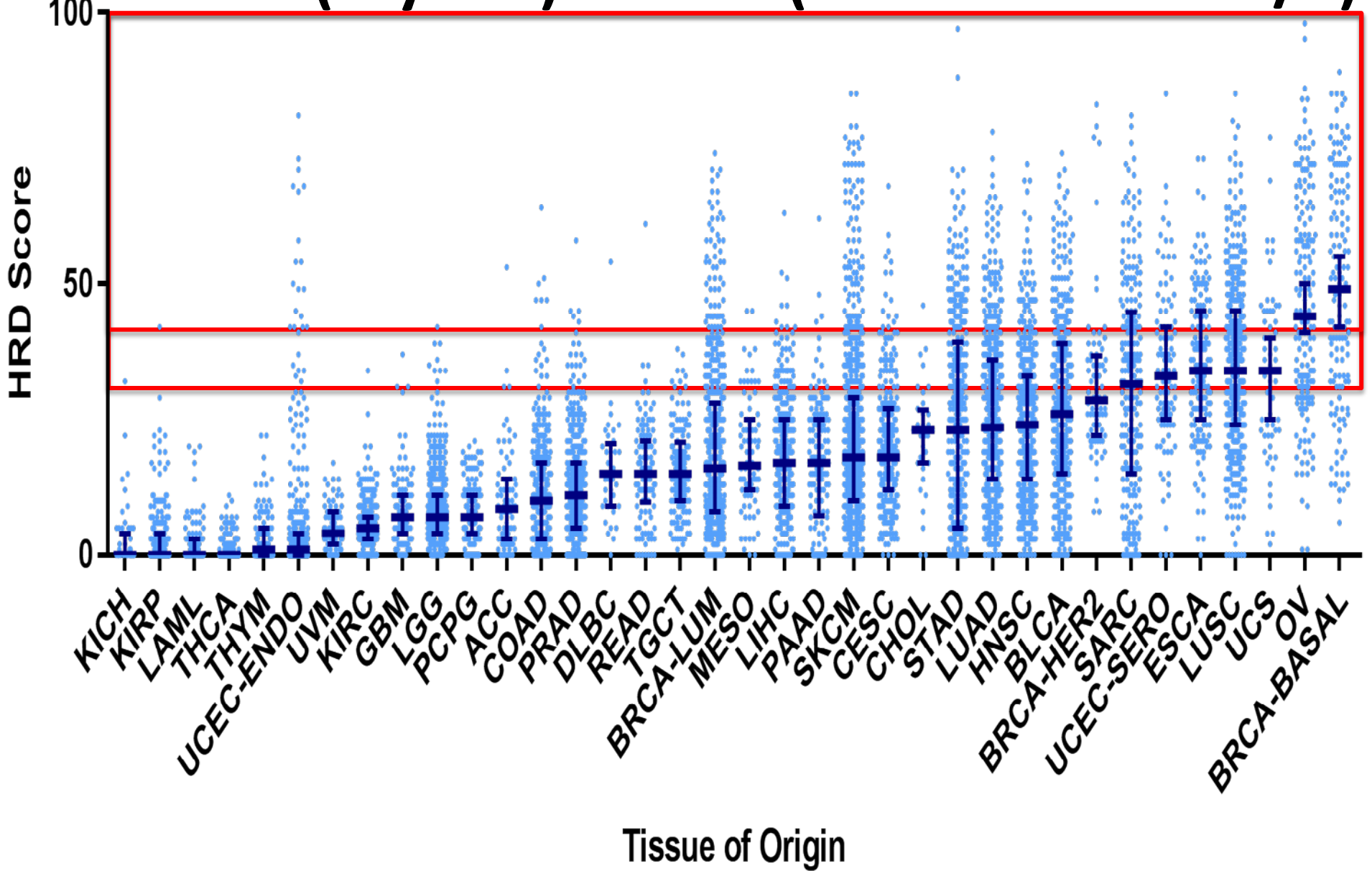
Conclusion: Germline BRCA1/2 is strongest predictor of benefit
HRD positivity identifies an additional population with significant benefit
A population of patients without HRD show modest benefit

Categorizing Predictive Biomarkers of Response for PARP inhibitors



Subpopulations of tumors are HRD

Cut off 42 (Myriad) and 31 (based on BRCA1/2)

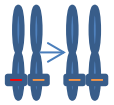


Classes of PARP inhibitor resistance

PARP Inhibitor Resistance

Restoration of HR Activity

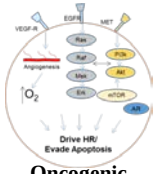
DIRECT



Reversion-to-WT mutations in HR genes and hypomorphic mutants (e.g. BRCA1/2, RAD51)

Promoter demethylation of HR genes

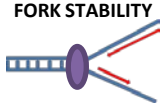
INDIRECT



Oncogenic signaling driving HR gene activity.
53BP1 loss restoring HR activity.

Mitigation of Replication Stress

FORK STABILITY

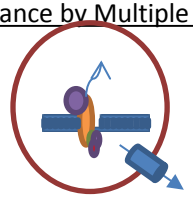


\+

CELL CYCLE CONTROL

Decreased proliferation with increased fork protections via mechanisms such as EZH2 loss , MLL3/4 loss, and/or increased dependence on ATR/CHK1.

Resistance by Multiple Mechanisms



Mechanisms inherent to PARP, such as mutations in catalytic or drug binding domain.
Mechanisms that hinder PARylation and release PARP from DNA, such as PARG-mediated.
P glycoprotein /MDR/ABC drug efflux transporters.
Biomarkers of resistance of unknown mechanism include loss of SLFN11 and loss of EMT signature.

Reconstitution of Rad51 foci
Healing of BRCA1/2 ,PALB2, Rad51C, Rad51D Demethylation of BRCA1/2 promoter
Upregulated hypomorphic mutant BRCA1/2 alleles
Loss of shield complex: 53BP1, RIF1, Rev7 (MAD2L2), FAM35A and C20orf196 complex

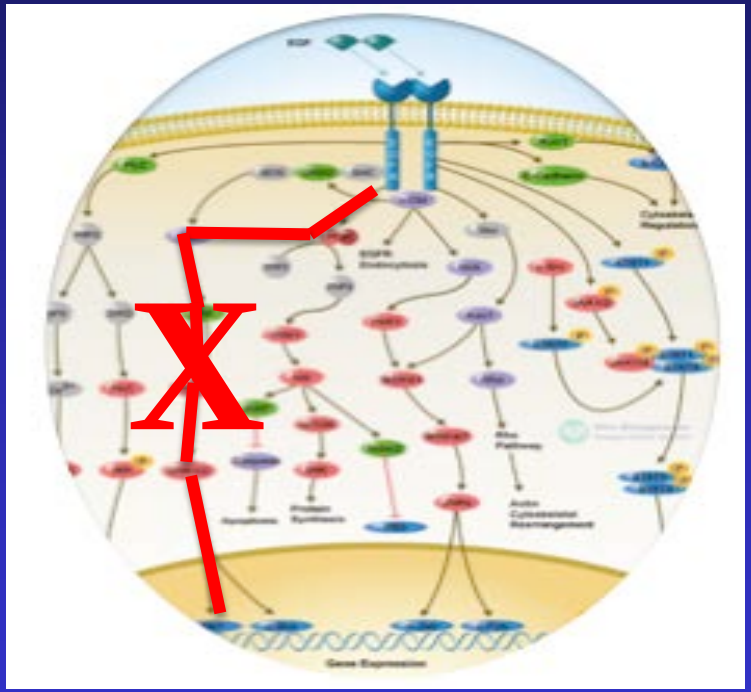
Loss of MLL3/4 (PTIP and MUS81 effectors)
Loss of EZH2 (MRE11 nuclease effector),
Protects BRCA2 and not BRCA1
Decreased proliferation
BRCA2 and Rad51 but not BRCA1 play a role in replication fork protection

PARP loss
PARP mutations:
PARG reverses ADP ribosylation of PARP and releases PARP from DNA
P glycoprotein/MDR/ABC transporters overexpression and fusions
SLFN11 loss
EMT

Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations Yossi Yarden and Arthur Lander

Interdict a critical pathway mediator



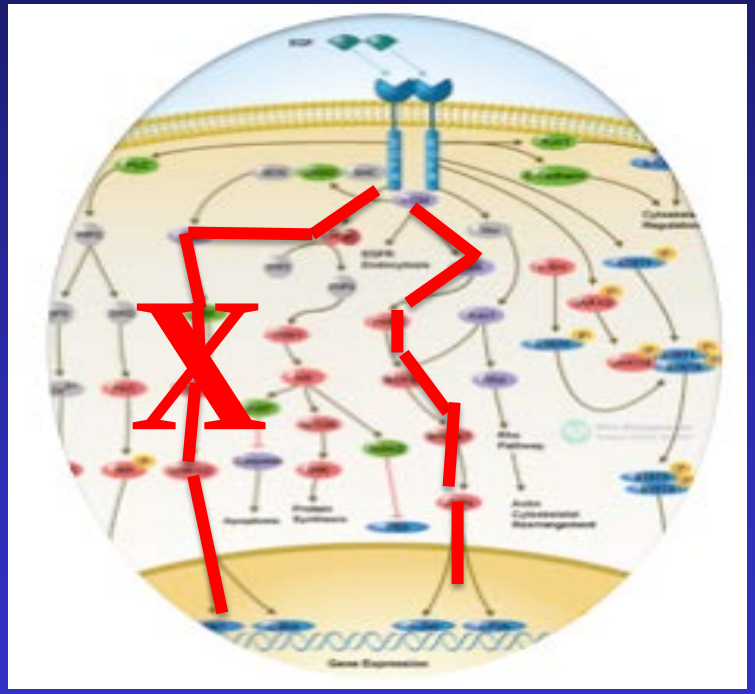
Mathematical modeling indicates that by chance during phylogeny many/most molecules in cell/organism will be blocked by mutation or environmental stress

Thus response to single targeted therapy is expected to be short and transient as observed!

Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations Yossi Yarden and Arthur Lander

Cells adapt by using an alternative pathway

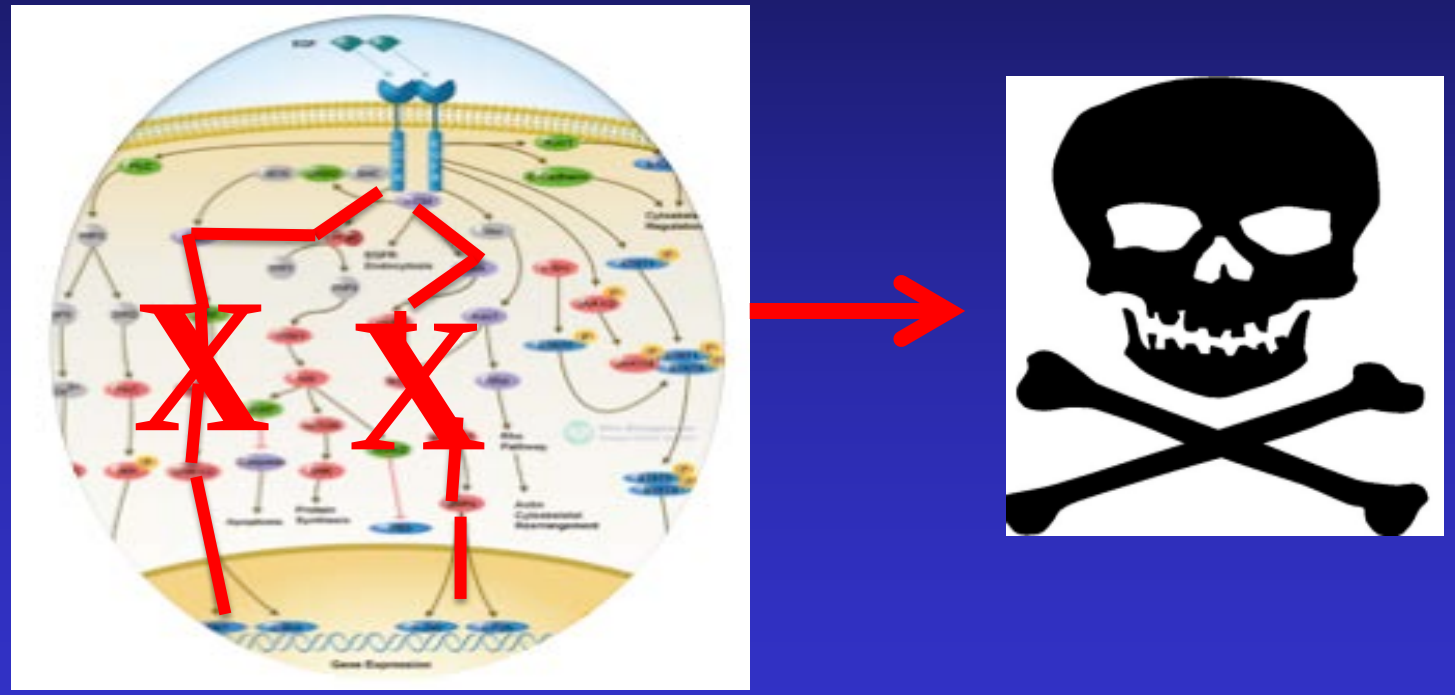


Chance that both the original target and the adaptive response will be “hit” randomly (mutation or environmental stress) is vanishingly low

Adaptation can occur at the protein level which is best assessed by post translational modification

Rational combinatorial therapy will be required to fulfill the promise of targeted therapy

Systems are robust to individual perturbations but are susceptible to multiple perturbations Yossi Yarden and Arthur Lander



Rational drug combinations will be required to convert transient responses into durable responses

A PLATFORM TO FACILITATE TARGETING ADAPTIVE RESISTANCE TO INCREASE UTILITY OF TARGETED THERAPEUTICS

Cells in 2D, 3D, in vivo, or patient tumors

↘

Add drug

Early time points: target engagement

Medium time points: adaptive responses

Late time points: genomic resistance

↘

Harvest cells for Omic analysis

DNA, RNA, protein, metabolomics

G. Gao

HUMAN PROTEOMICS ATLAS: RPPA

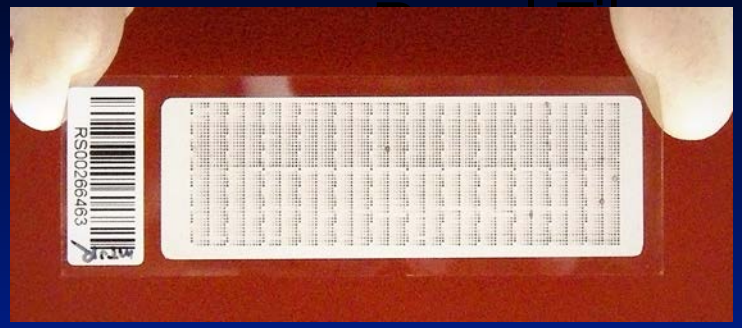
Quantitative high throughput multiplexed inexpensive ELISA

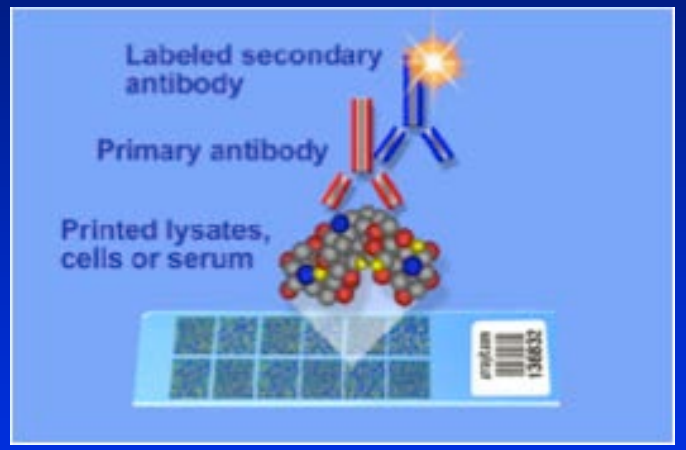
416 validated antibodies

Dot blot: less sensitive to degradation

Requires high quality validated antibodies and robotics

No Spatial orientation: combined tumor and stromal signature





Tcpaportal.org

Search Cancer Proteome Atlas

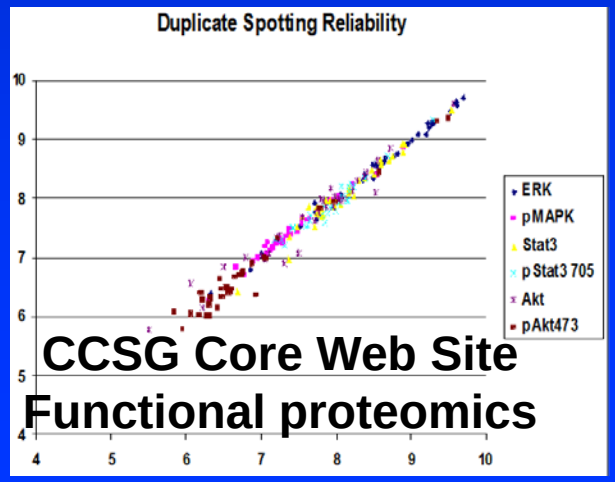
TCGA and internal patient samples (>10,000) with extensive DNA, RNA, miRNA, and clinical data

Cell lines with RNASeq and drug data

1200 cell lines

Broad Cancer Cell Line Encyclopedia

144,000 samples in total



Rank-Sum Analysis of AZD2281 and BMN673

5 representative cell lines were treated with 2 doses for 72 and 96 hours in 2D and 3D cultures. Lysates were collected and analyzed by RPPA for 191 antibodies. High levels are represented in Red. >50,000 data points

Data is ratio of treated to untreated

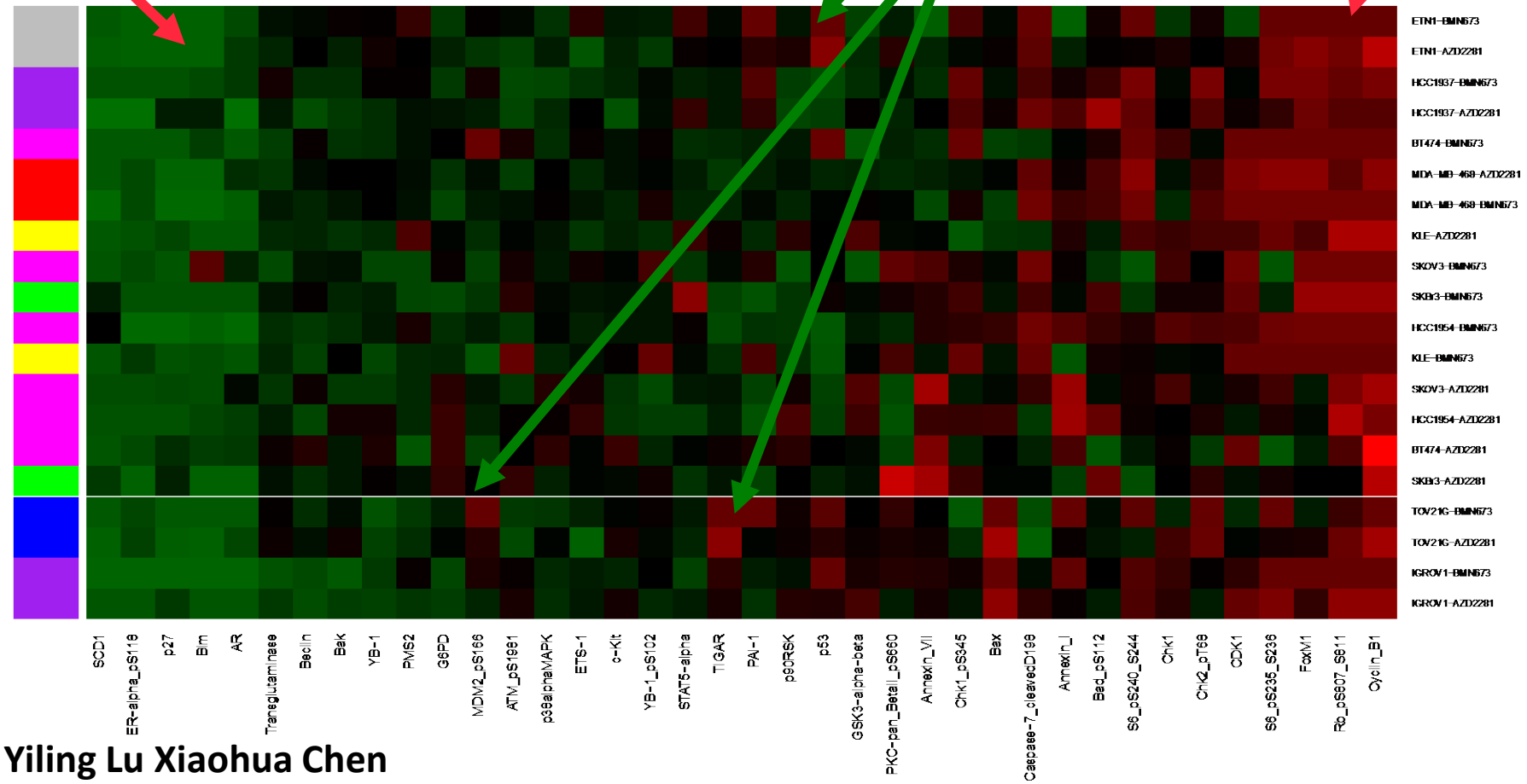
Samples are ordered based on adding all antibody scores

Only significant changes presented

Public

Private

Public



Yiling Lu Xiaohua Chen

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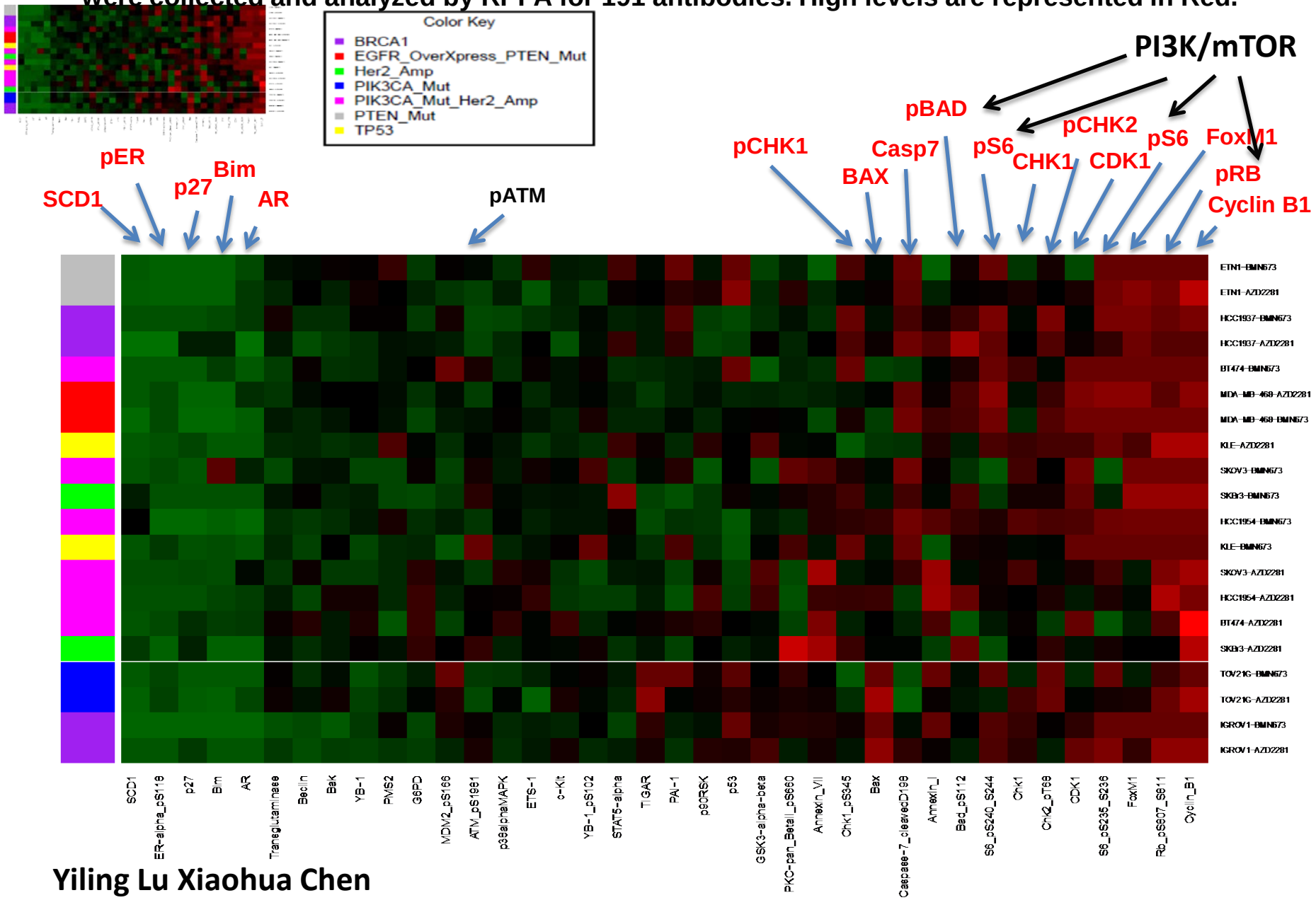


Yiling Lu Xiaohua Chen

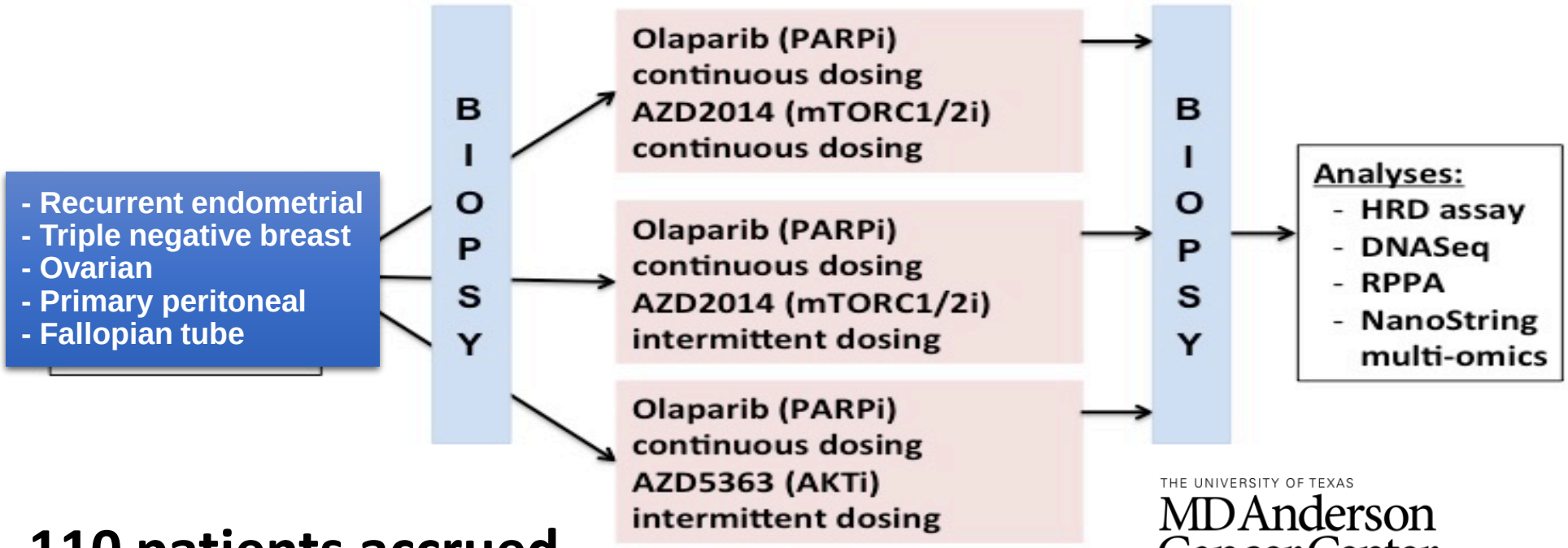
SU2C: Olaparib and BKM120: Olaparib and BYL719

30-35% RR for OC: Not dependent on BRCA1/2 status

(Lotus AND PAKT AKTi and taxol)

OCTOPUS – PARP/PI3K pathway combinations

Shannon Westin



110 patients accrued

RR ~ 30% for OC, 50% for EC for AZD536?

Prolonged responses over 2 years

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

AstraZeneca

Rank-Sum Analysis of AZD2281 and BMN673

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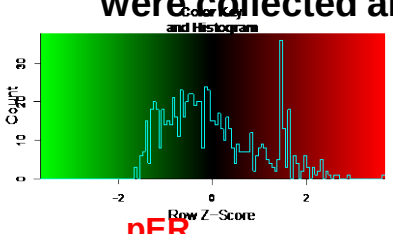
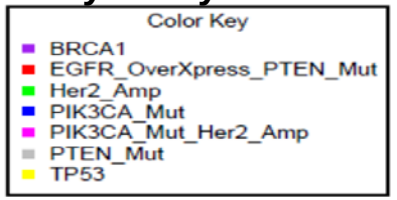




Joan Brugge

pATM

Mek

pER

Bim

p27

AR

SCD1

pCHK1

Casp7

pBAD

BAX

pS6

CHK1

pCHK2

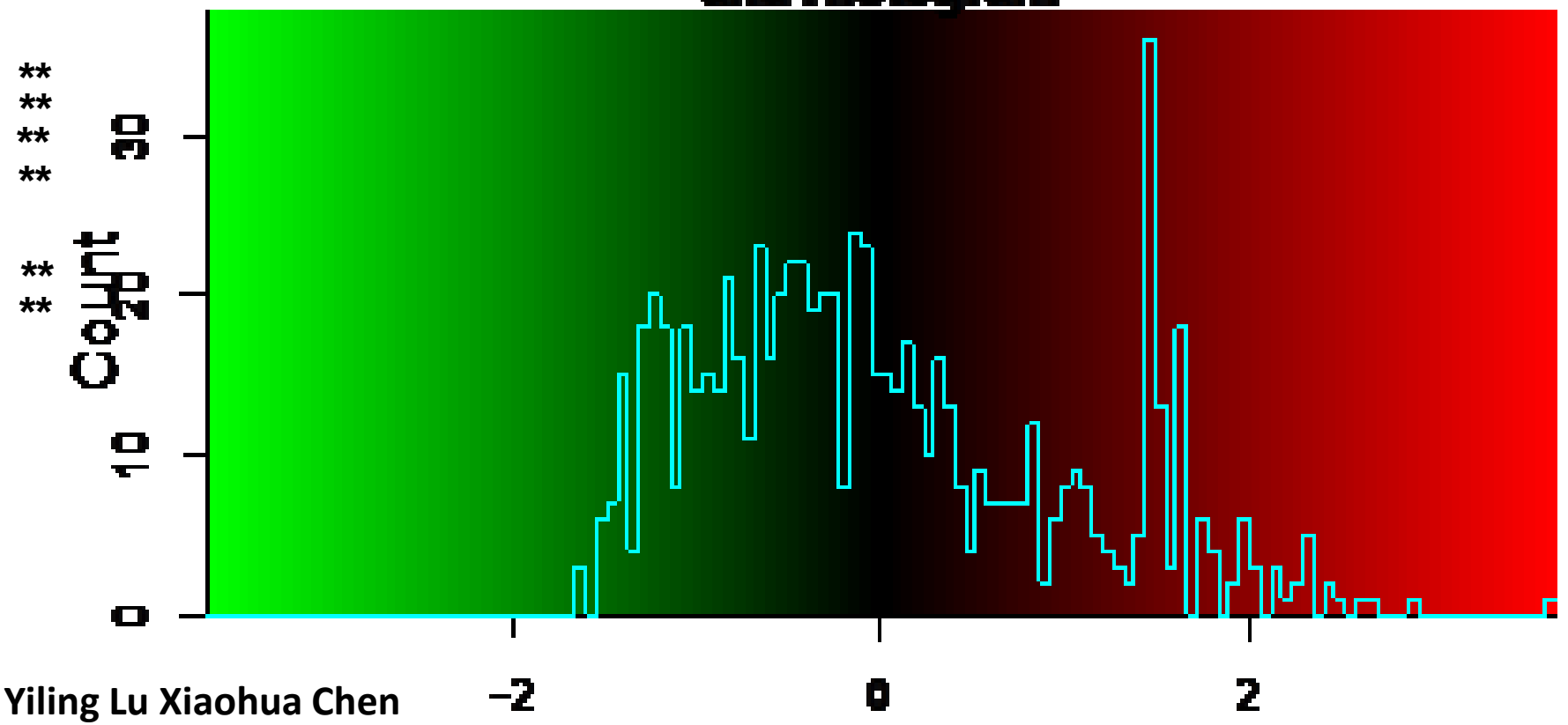
CDK1

pS6

FoxM1

pRB

Cyclin B1



Yiling Lu Xiaohua Chen

PARP plus MEK inhibitors are synergistic in vivo

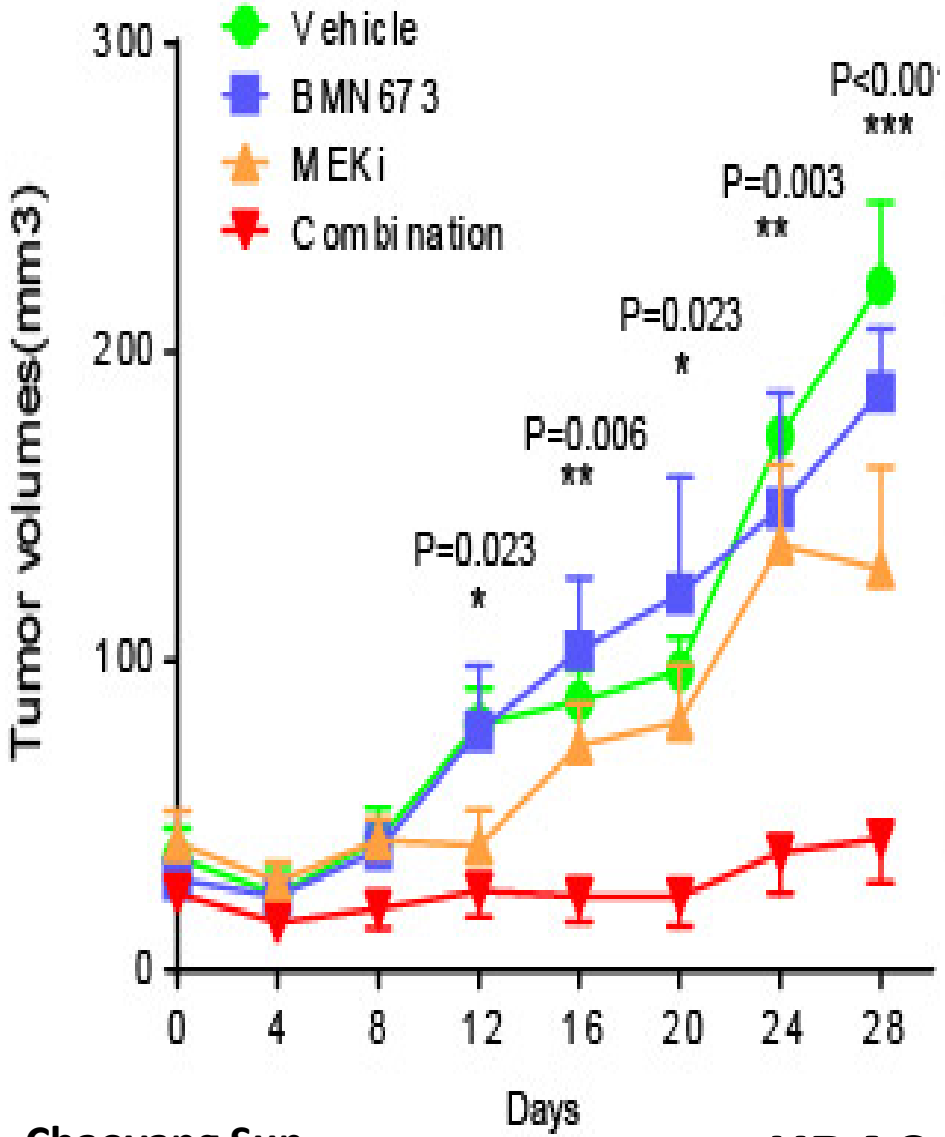
RAS pathway activation induces replication stress

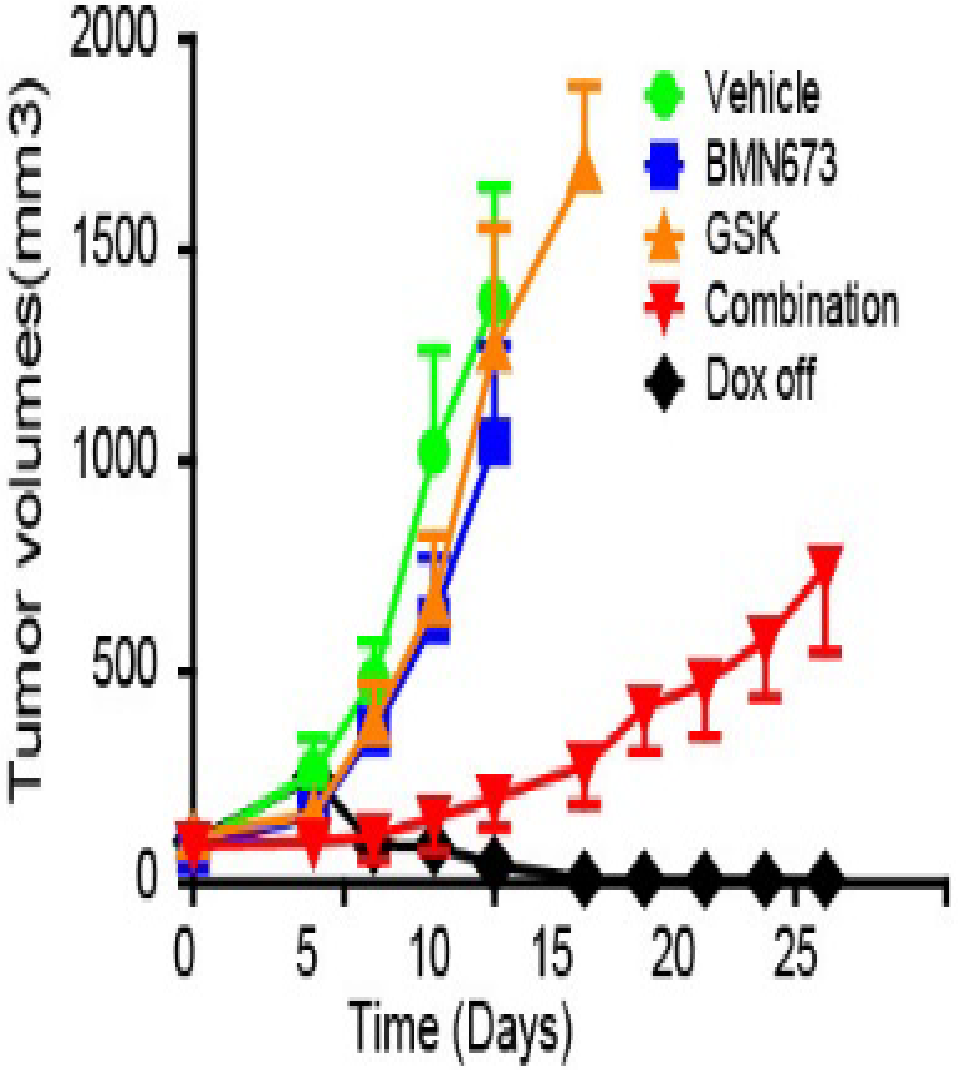
RAS pathway activation increases HR

RAS pathway activation is indicative of PARP resistance

PARP resistant cells acquire RAS mutations and increased signaling

Inhibiting MEK or ERK increases PARP activity in RAS mutant or PARP resistant cell lines





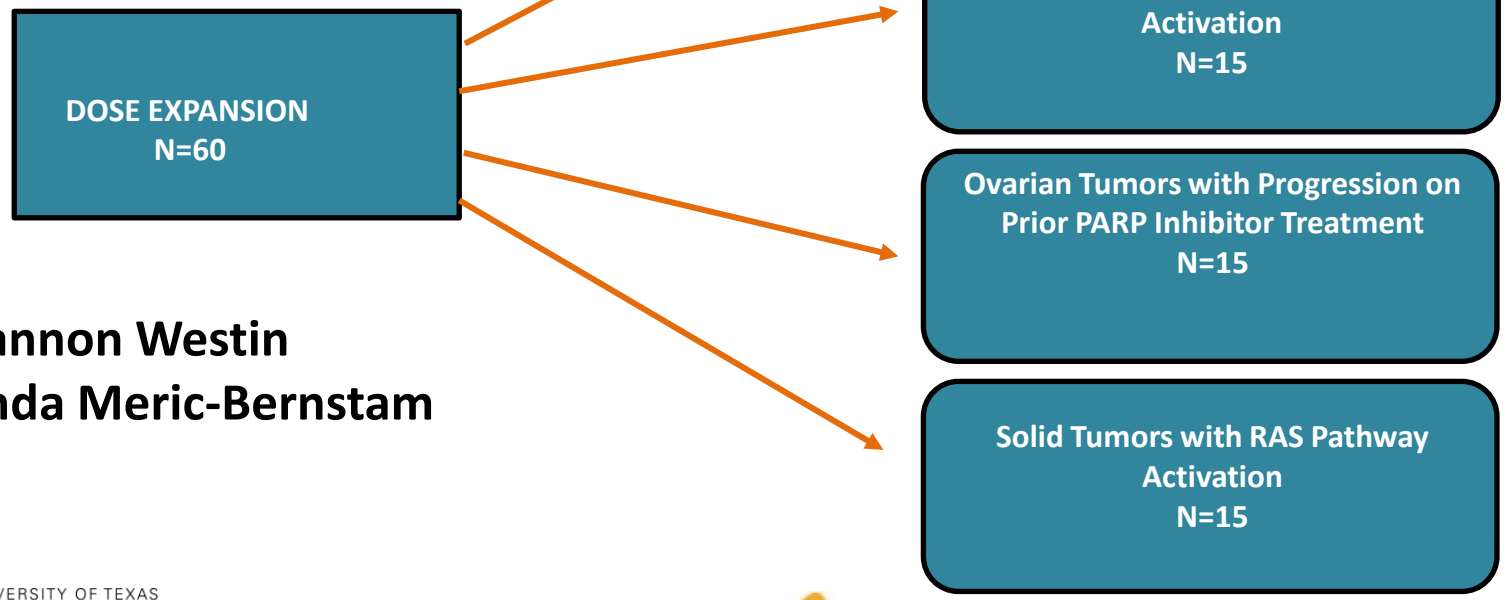
Chaoyang Sun
Dong Zhang
Yong Fang

KRAS OVCAR8

KRAS HPDE Pancreas

SOLAR study: selumetinib and olaparib in RAS activated tumors

Original observation 4/8/2015
CRC Approved, IRB 3/1/17
FDA no Objection
SIV May 30 2017
First in human Nov 2017



Shannon Westin
Funda Meric-Bernstam

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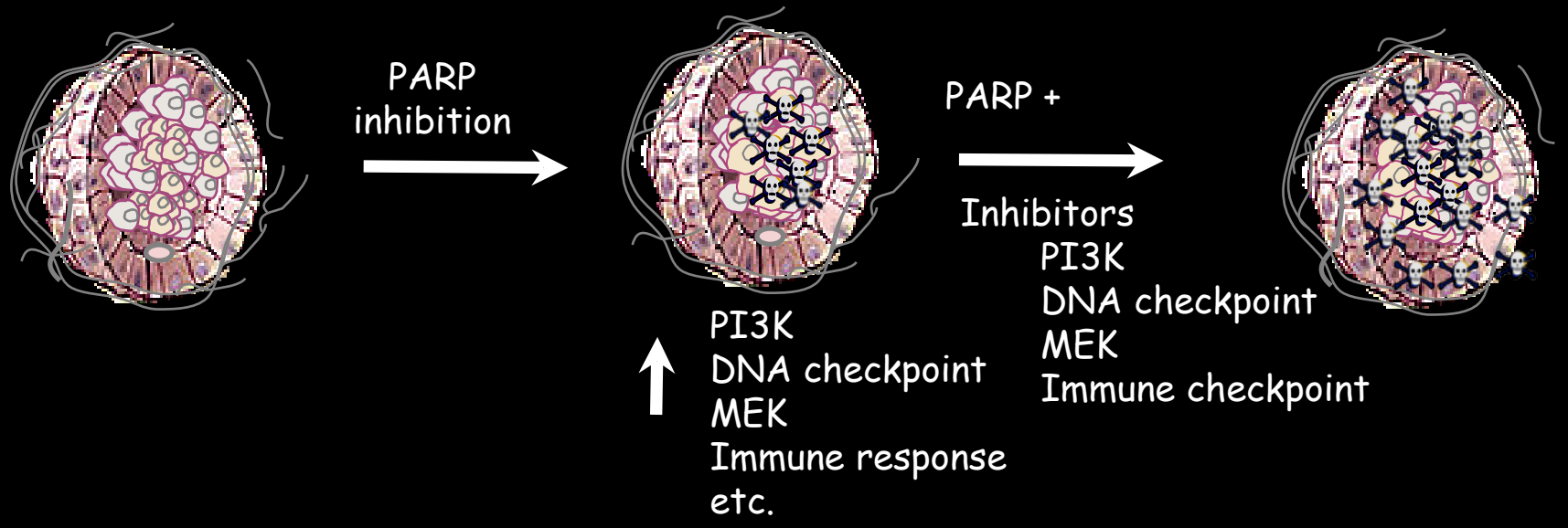
Rational Strategy for Combination Therapies



Blocking critical signaling nodes "rewires" signaling pathways

Rewired networks contribute to cellular resistance to targeted therapeutics

Induced signaling events represent "vulnerabilities" that can be exploited leading to synthetic lethality

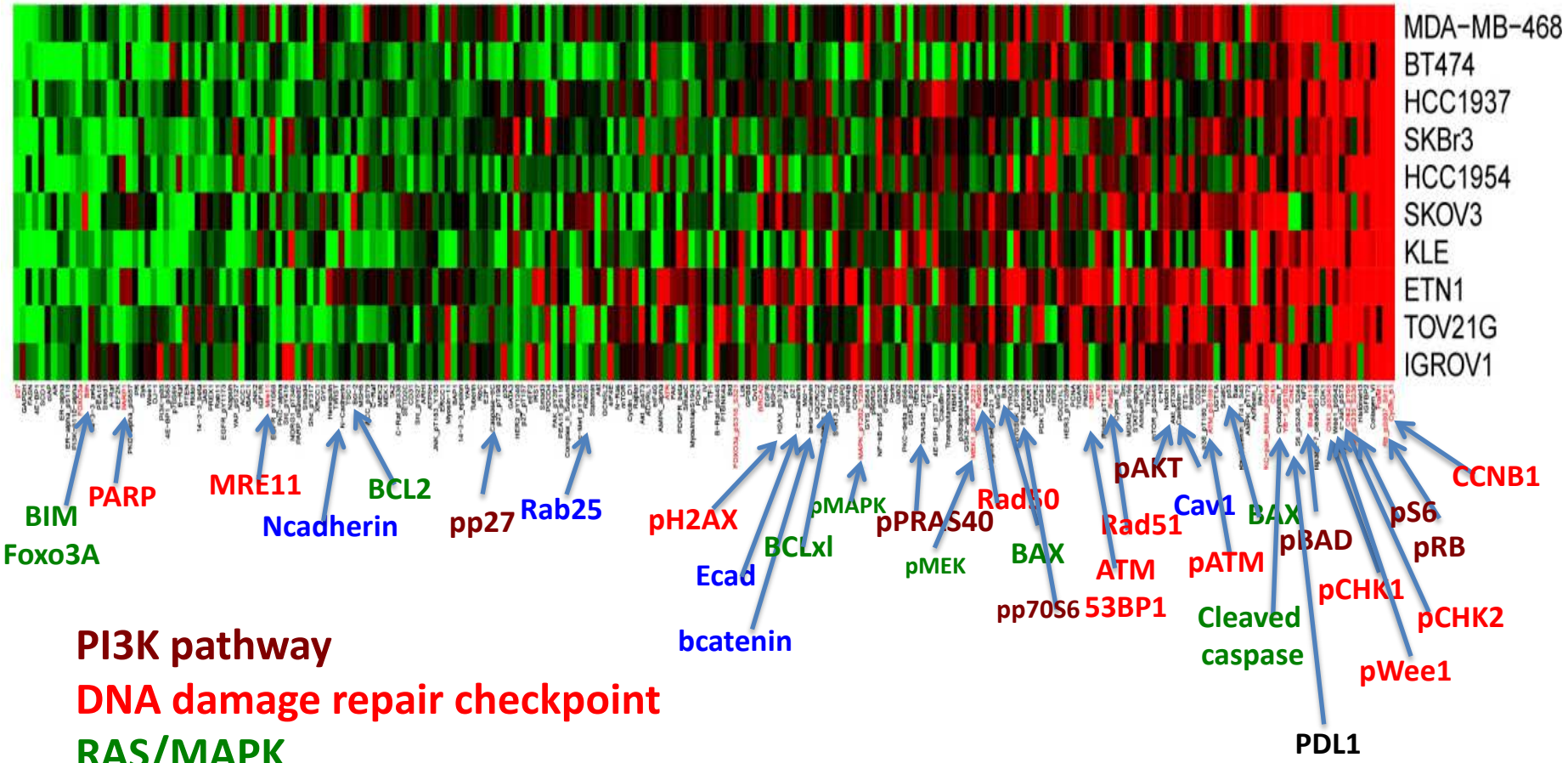
Adaptive responses can be restricted to specific tumor subpopulations

Combinatorial Adaptive Resistance Therapy
CART

Combinations with PARPi

- PI3K/AKT/mTOR inhibitors
- MEK ERK inhibitors
- DNA damage checkpoint inhibitors
- Immune checkpoint inhibitors
- BET inhibitors
- Anti-apoptotic inhibitors
- Angiogenesis inhibitors
- HSP90 inhibitors
- HDAC inhibitors
- Azacytidine
- HER2 inhibitors
- Chemotherapy/radiation to induce double strand breaks

Adaptive responses to PARP inhibitors could be used to select rational combinations



PI3K pathway

DNA damage repair checkpoint

RAS/MAPK

Apoptotic pathway

STING/Immune

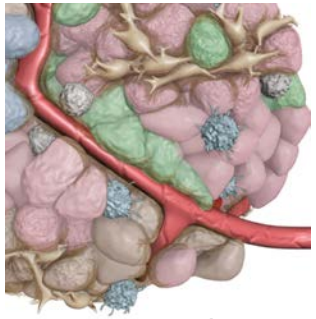
EMT

Predictor of response

Marilyne Labrie
Yiling Lu

REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem

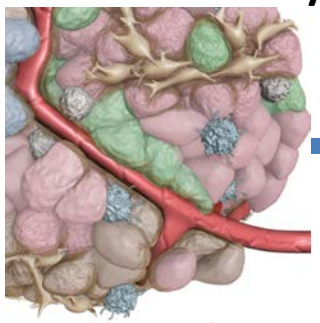


Limited systems information

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem



Limited systems information

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Adaptation to Therapeutic stress

PARPi

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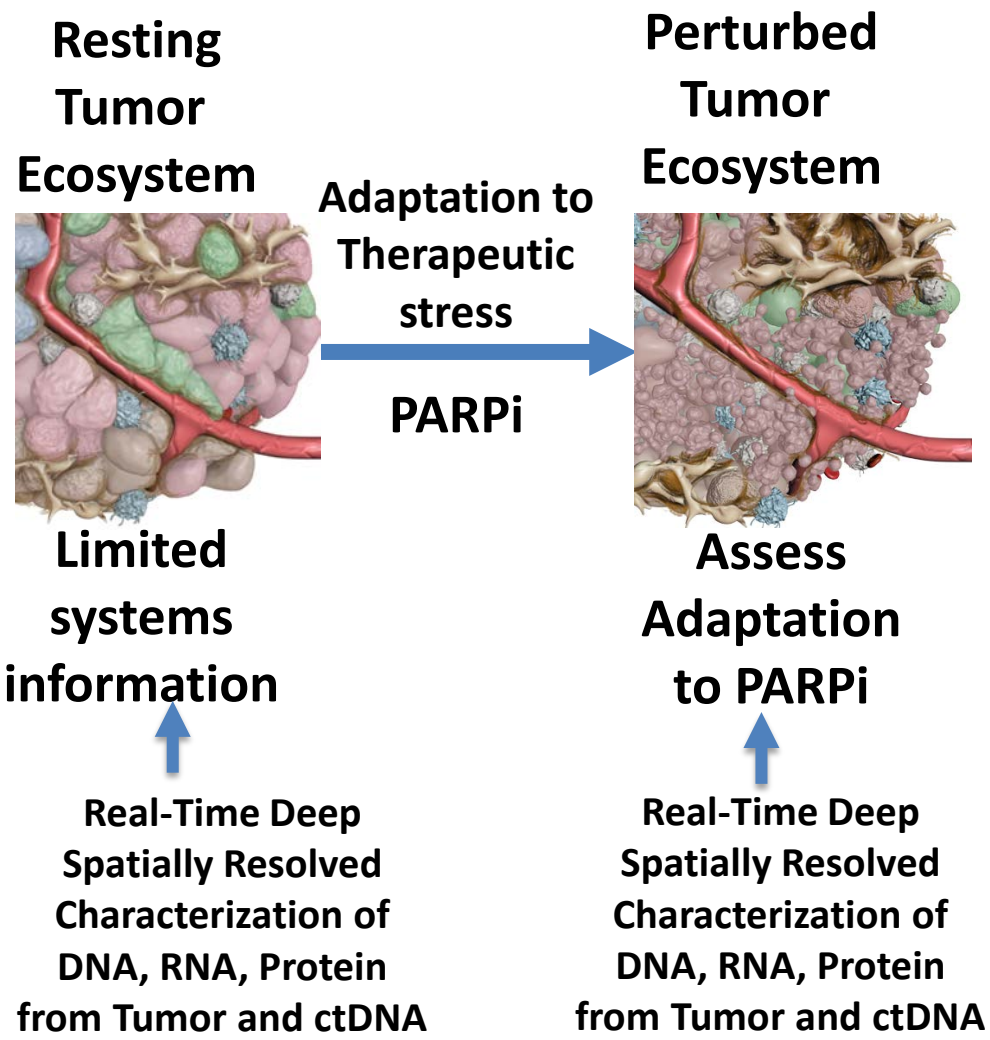

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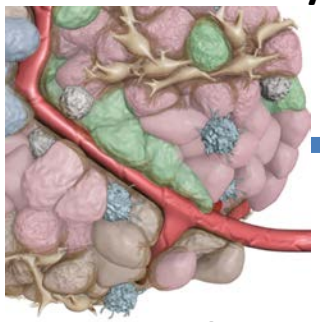

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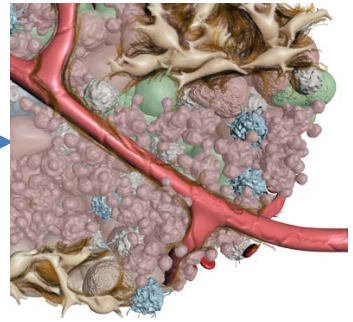
PARPi

Perturbed Tumor Ecosystem



Assess Adaptation to PARPi

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

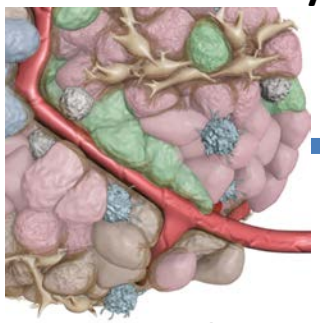
Immune cell infiltration

Target the adaptive response PD1/PDL1

Combination selected based on tumor evolution

REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem



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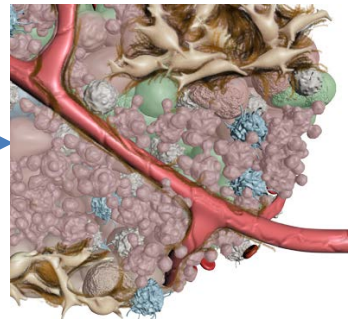
Perturbed Tumor Ecosystem



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Immune cell infiltration

PI3K pathway

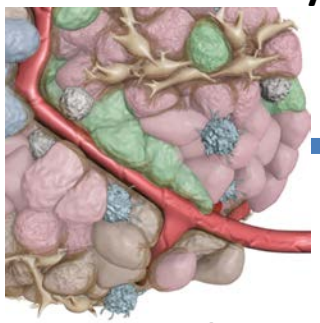
Target the adaptive response PD1/PDL1

mTOR/AKT

Combination selected based on tumor evolution

REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE

Resting Tumor Ecosystem



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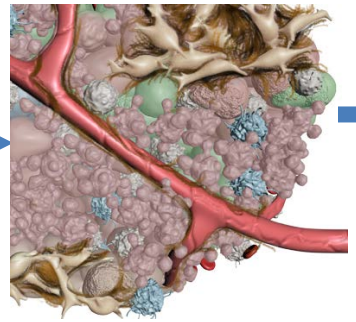
Adaptation to Therapeutic stress

PARPi

Perturbed Tumor Ecosystem



Assess Adaptation to PARPi

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PI3K pathway

RAS/MEK pathway

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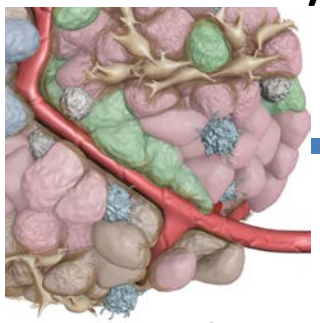

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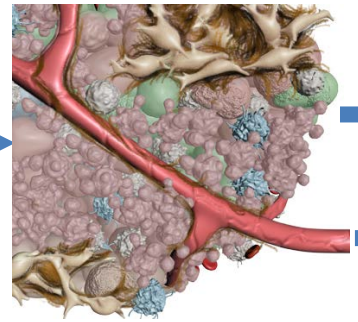


Assess Adaptation to PARPi

Real-Time Deep Spatially Resolved Characterization of DNA, RNA, Protein from Tumor and ctDNA

Immune cell infiltration → Target the adaptive response PD1/PDL1

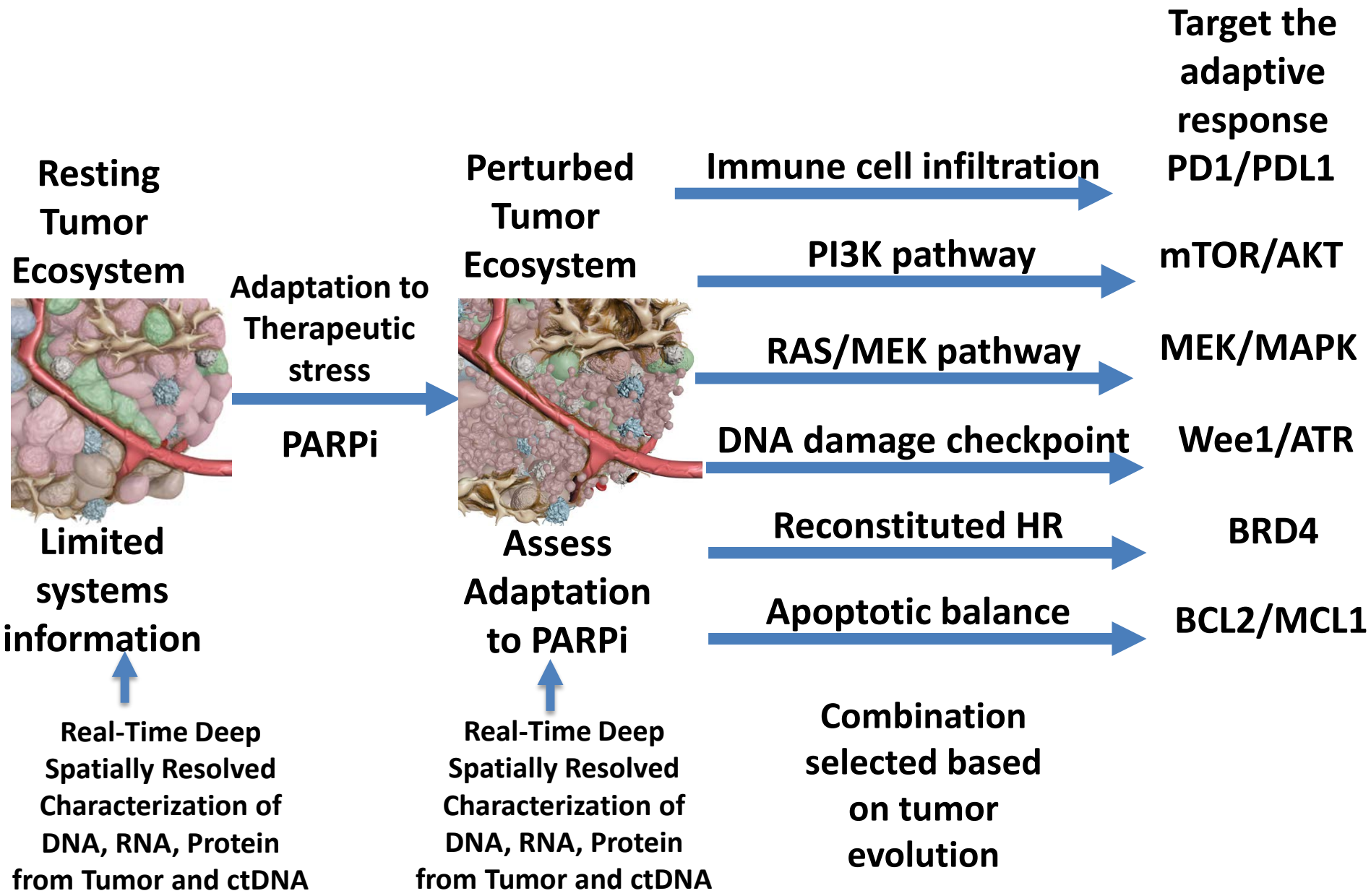
PI3K pathway → mTOR/AKT

RAS/MEK pathway → MEK/MAPK

DNA damage checkpoint → Wee1/ATR

Combination selected based on tumor evolution

REAL TIME SELECTION OF DRUG COMBINATIONS BASED ON ADAPTIVE RESPONSE



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