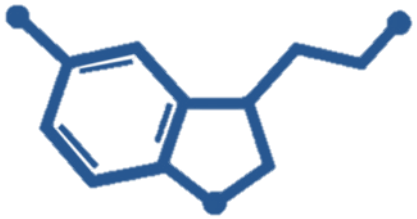
Metabolite Biomarkers of Response (BoRs) To Optimize Metastatic Breast Cancer Treatment



Cancer and Evolution Symposium

Elizabeth O'Day, MPhil, PhD

Oct. 16th, 2020

Disclosures

I am the CEO and Founder of Olaris, Inc. Olaris is a private company developing “Biomarkers of Response” (BoR) to optimize treatment decisions. I receive financial support and equity from the company.

22%

Despite access to therapies, **less than 22% of metastatic breast cancer (mBC) patients survive 5+ years**

WHY?

RESISTANCE Leads to Poor Outcomes

Today

ER+ metastatic BC patients are prescribed CDK4/6 inhibitors

20% of patients are **intrinsically resistant**

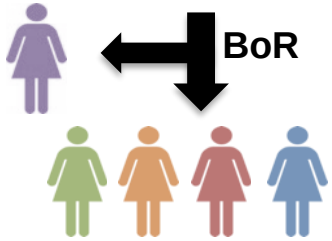
All patients **acquire resistance**

- **Poor outcomes**
- **Increased adverse effects**
- **Increased healthcare costs**

The Future

Each patient is screened prior to treatment

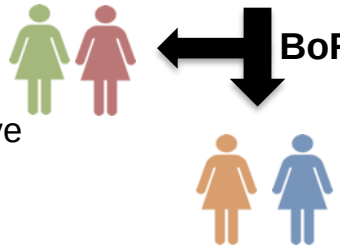
“resistant” patients receive alternative treatment



SCREEN BEFORE TREATMENT

“responders” receive CDK4/6 inhibitor

“resistant” patients receive alternative treatment



MONITOR ON TREATMENT

“responders” receive CDK4/6 inhibitor

- **Improved outcomes**
- **Reduced adverse effects**
- **Reduced healthcare costs**

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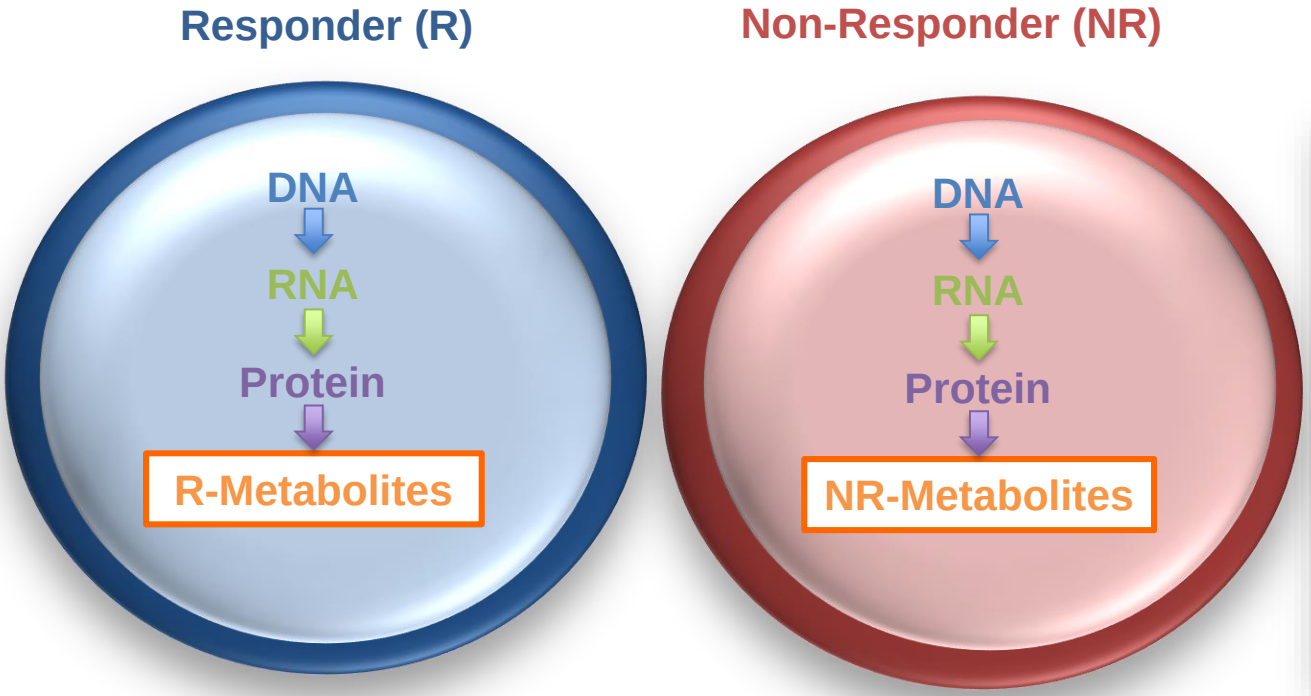
Why Metabolites?



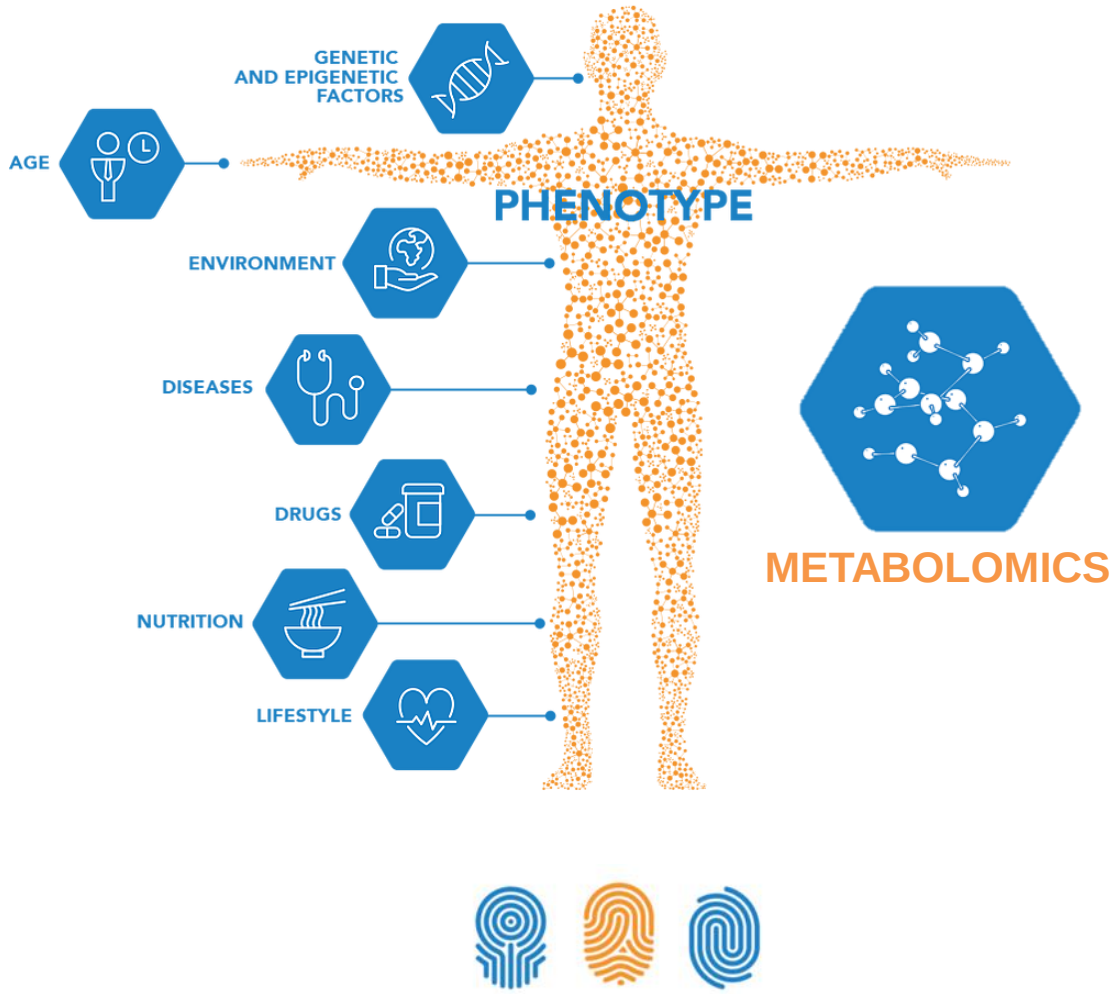
Biomarkers:

DNA/RNA:
what **could** happen

Protein:
what **makes it** happen

Metabolites:
what ***is*** happening

Factors Beyond Genetics Influence Drug Response



Metabolomics provides a fingerprint of disease

Not Your Grandfather's Metabolomics

Pioneering methods using NMR and MS increase metabolome coverage in a highly **reproducible** manner. Proprietary **BoR** algorithm combines top features from multiple ML algorithms to create more **accurate** classifications

metabolites

MDPI

Article

Evaluation of Non-Uniform Sampling 2D ^{1}H–^{13}C HSQC Spectra for Semi-Quantitative Metabolomics

Bo Zhang [1], Robert Powers [2,3] and Elizabeth M. O'Day [1,*]

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2 Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304, USA; rpowers3@unl.edu
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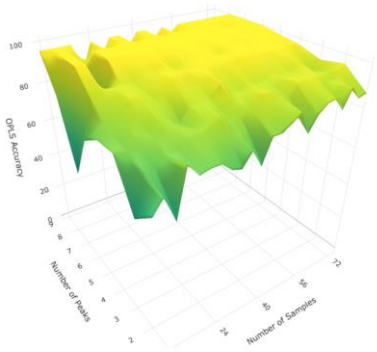
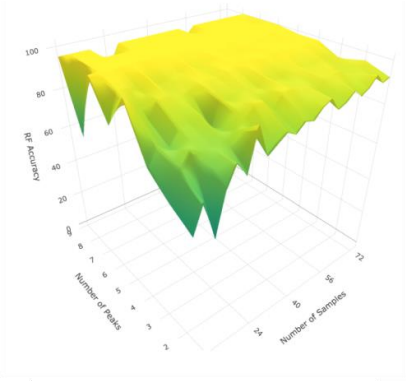
check for updates

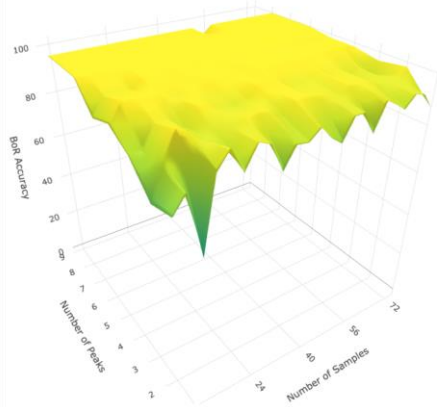
Abstract: Metabolomics is the comprehensive study of metabolism, the biochemical processes that sustain life. By comparing metabolites between healthy and disease states, new insights into disease mechanisms can be uncovered. NMR is a powerful analytical method to detect and quantify metabolites. Standard one-dimensional (1D) ^{1}H-NMR metabolite profiling is informative but challenged by significant chemical shift overlap. Multi-dimensional NMR can increase resolution, but the required long acquisition times lead to limited throughput. Non-uniform sampling (NUS) is a well-accepted mode of acquiring multi-dimensional NMR data, enabling either reduced acquisition times or increased sensitivity in equivalent time. Despite these advantages, the technique is not widely applied to metabolomics. In this study, we evaluated the utility of NUS ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) for semi-quantitative metabolomics. We demonstrated that NUS improved sensitivity compared to uniform sampling (US). We verified that the NUS measurement maintains linearity, making it possible to detect metabolite changes across samples and studies. Furthermore, we calculated the lower limit of detection and quantification (LOD/LOQ) of common metabolites. Finally, we demonstrate that the measurements are repeatable on the same system and across different systems. In conclusion, our results detail the analytical capability of NUS and, in doing so, empower the future use of NUS ^{1}H–^{13}C HSQC in metabolomic studies.

OPLS-DA



Random Forest



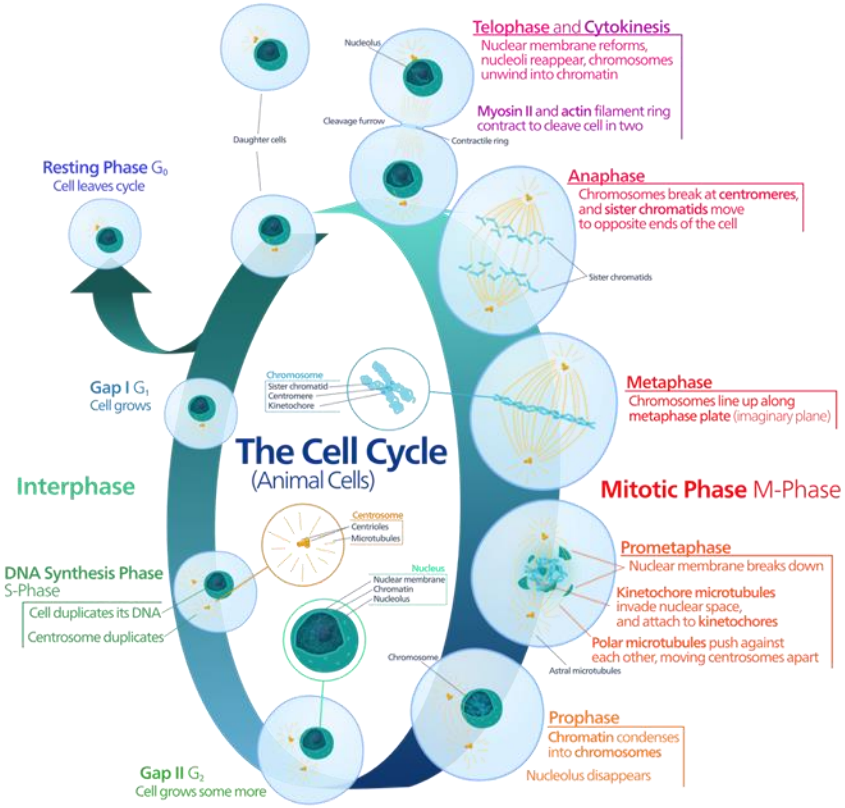


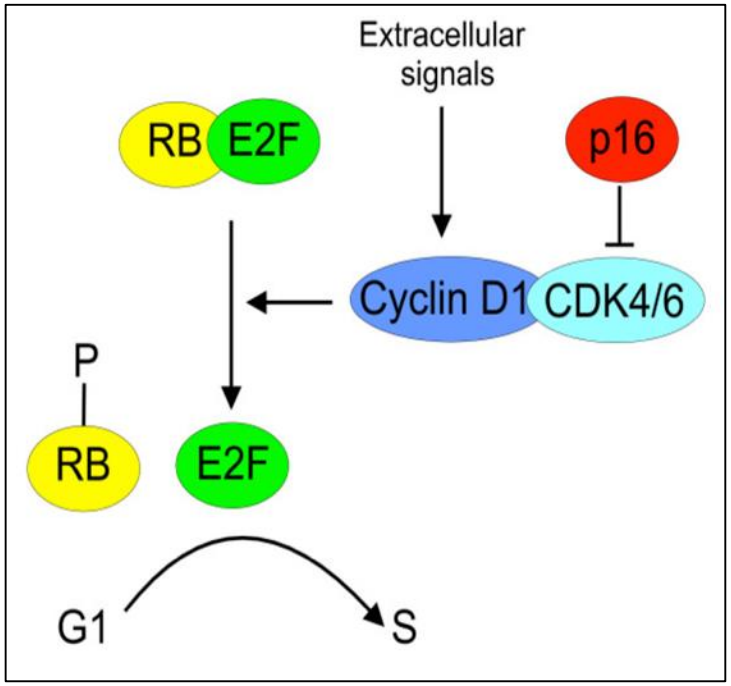
Olaris BoR

More stable/accurate predictions across sample sizes and features

Case Study: CDK4/6 BoR

CDK4/6 Inhibitors Block Cell Cycle Progression





Inhibiting the cell cycle is a long-standing strategy for cancer therapy

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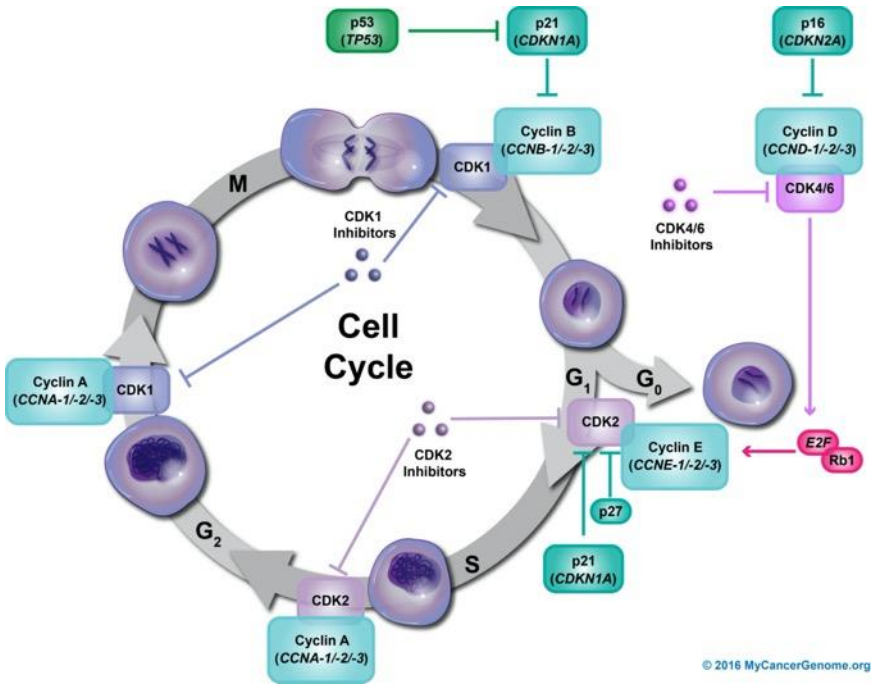
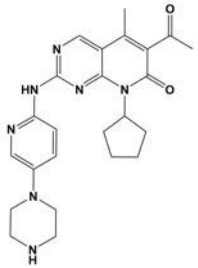
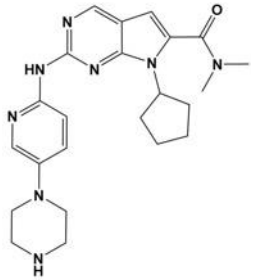
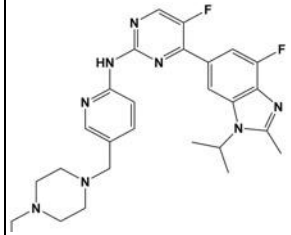
New “selective” CDK4/6 Inhibitors Block Cell Cycle Progression



Structure	Drug	CDK IC50
	Ibrance (palbociclib) Pfizer	CDK1: >10μM CDK2: >10μM CDK4: 9-11nM CDK6: 15nM
	Kisqali (ribociclib) Novartis	CDK1: >100μM CDK2: >50μM CDK4: 10nM CDK6: 39nM
	Verzenio (abemaciclib) Eli Lilly	CDK1: >1μM CDK2: >500nM CDK4: 2nM CDK6: 5nM

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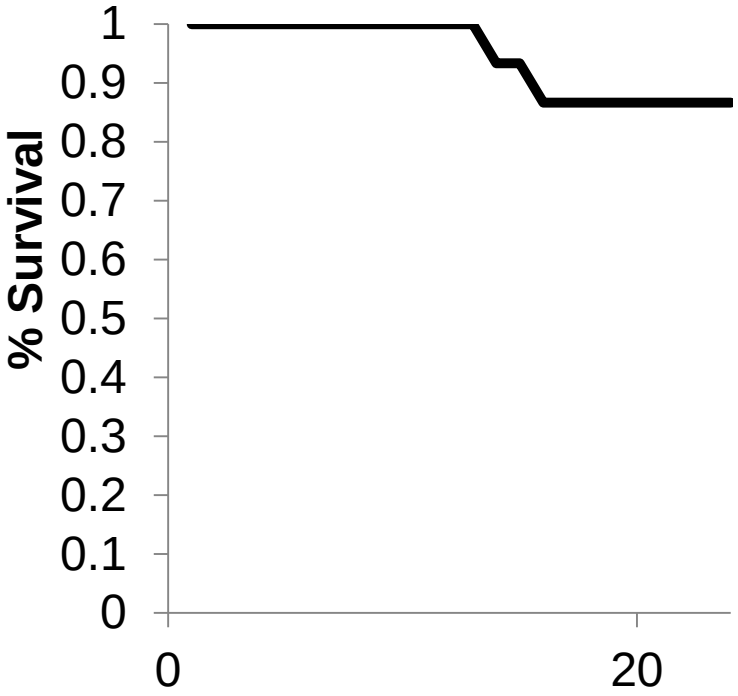
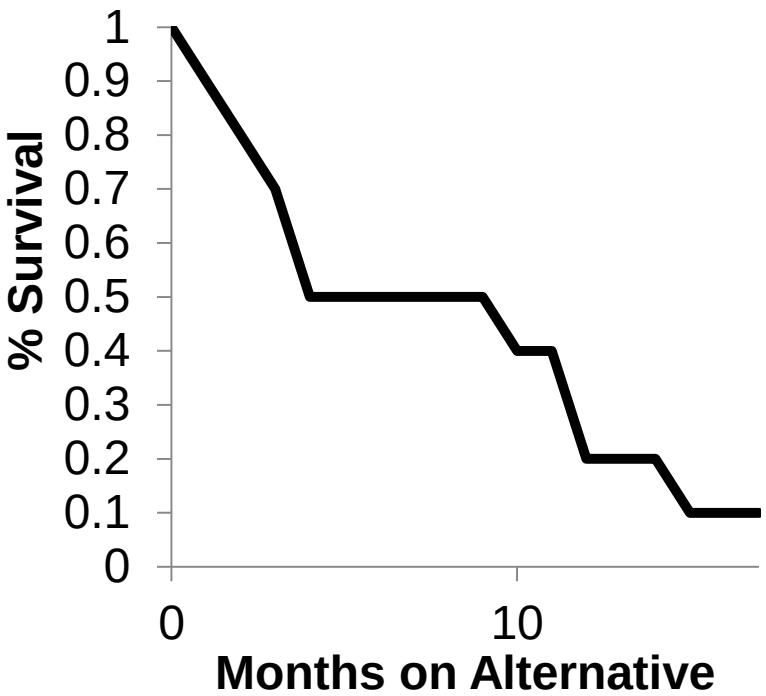
Critical Need to Identify CDK4/6 NR

CDK4/6 Responders



CDK4/6 Non-Responders



O'Day, E.M., et al (2019) ASCO Poster

- **87% of Responders** [patients who saw tumors decrease within first 6 months of treatment] are **alive** + 2 years
- **90% of Non-Responders** [patients who saw tumors increase within first 6 months of treatment] are **deceased** within 12-15 months

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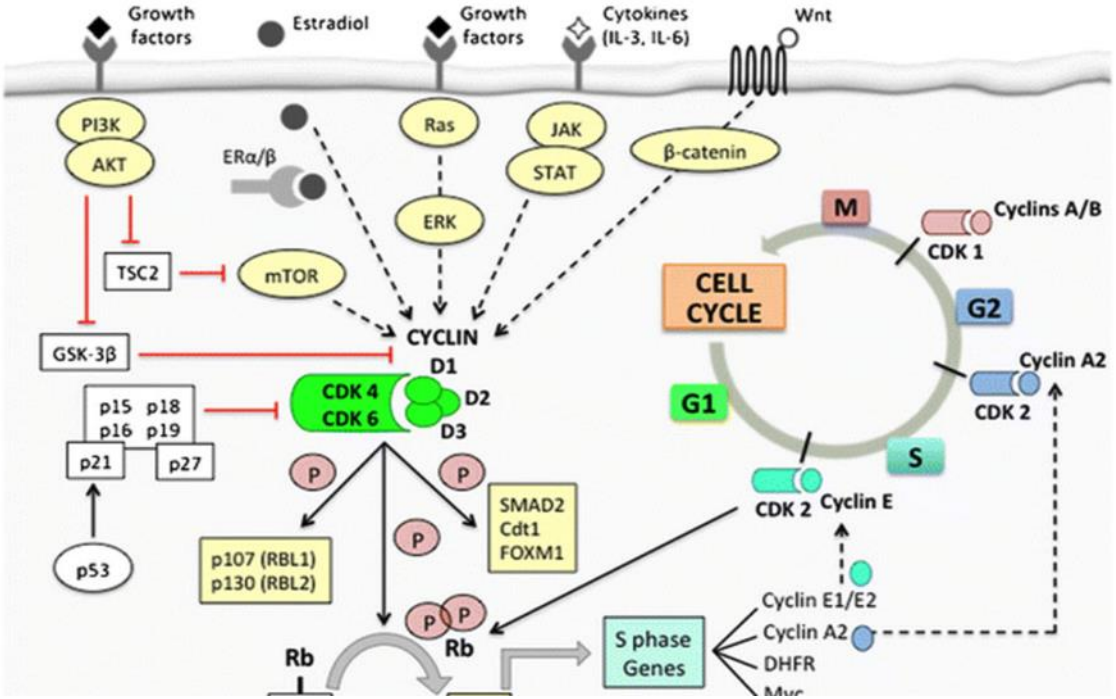
Current CDK4/6i Biomarkers Fail to Predict R vs NR



Garrido-Castro, A., et al (2017) Curr Breast Cancer Rep. 9 (1) 26-33.

Proposed Biomarker	Observations
ER+ positivity	In phase 1 study, abemaciclib monotherapy (N=47), only 11 of 36 ER+ patients experienced clinical benefit. In PALOMA-2 and PALOMA-3 trials, benefit from palbociclib did not differ by ER IHC expression
Luminal gene expression, Rb status, Cyclin E/ CDK2 amplification	Most breast cancer cell lines with luminal gene expression are sensitive to palbociclib (some are ER-). Cell lines with low Rb levels are less sensitive to CDK4/6 inhibitors. CDK2 can substitute for CDK4/6. Cyclin E and CDK2 can phosphorylate Rb to escape block. Each marker needs to be tested in clinic and can be difficult to measure and set "cut-offs".
Cyclin D1 amplification and/or loss of p16	In PALOMA-1 (N=165) and separate phase II (N=37) neither cyclin D1 nor p16 were predictive of benefit or PFS with palbociclib

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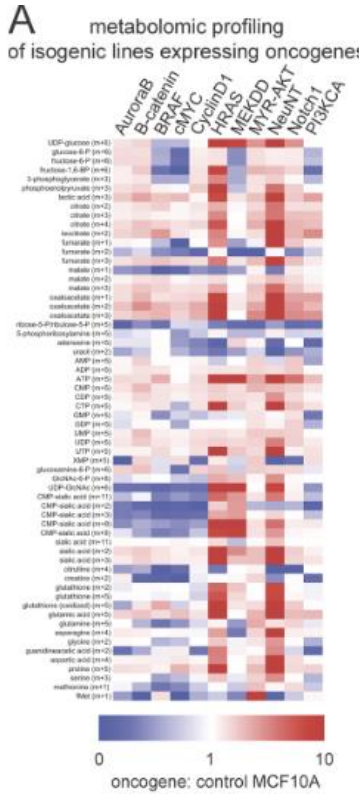
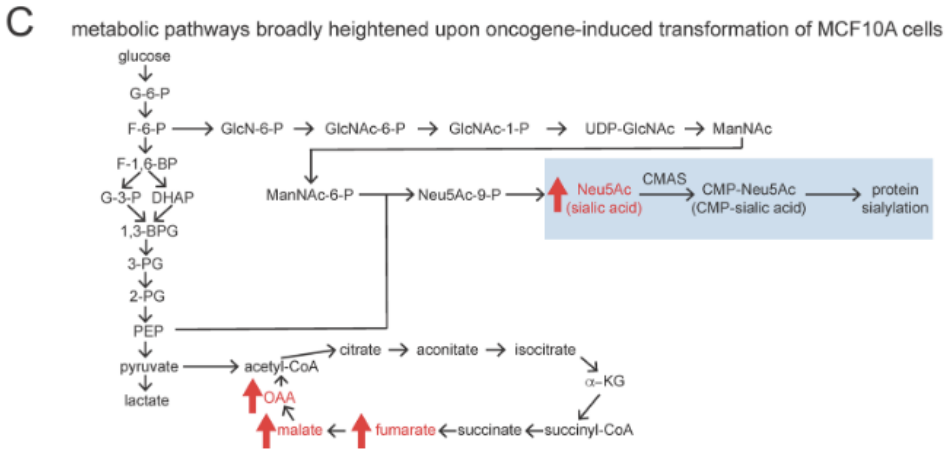
Metabolites Are The End Product of Genomic Mutations

ACS chemical biology
ACS Editors' Choice
Letters
pubs.acs.org/acschemicalbiology

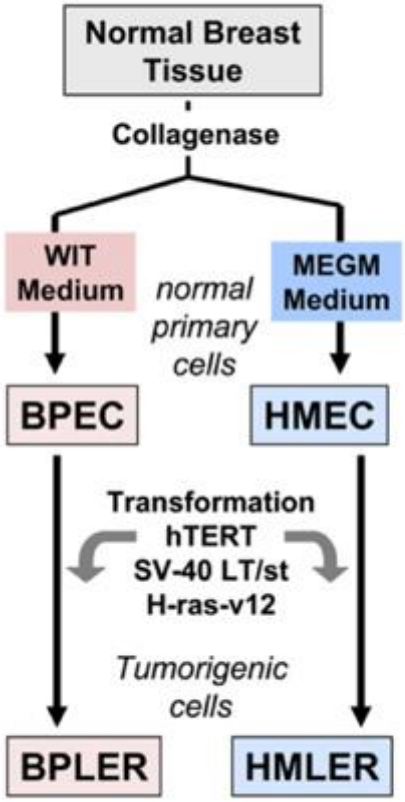
Protein Sialylation Regulates a Gene Expression Signature that Promotes Breast Cancer Cell Pathogenicity

Rebecca A. Kohnz,[†] Lindsay S. Roberts,[†] David DeTomaso,[‡] Lara Bideyan,[†] Peter Yan,[†] Sourav Bandyopadhyay,[§,∥] Andrei Goga,[§,∥] Nir Yosef,[‡] and Daniel K. Nomura*[,†]





Metabolites Are Influenced by the environment

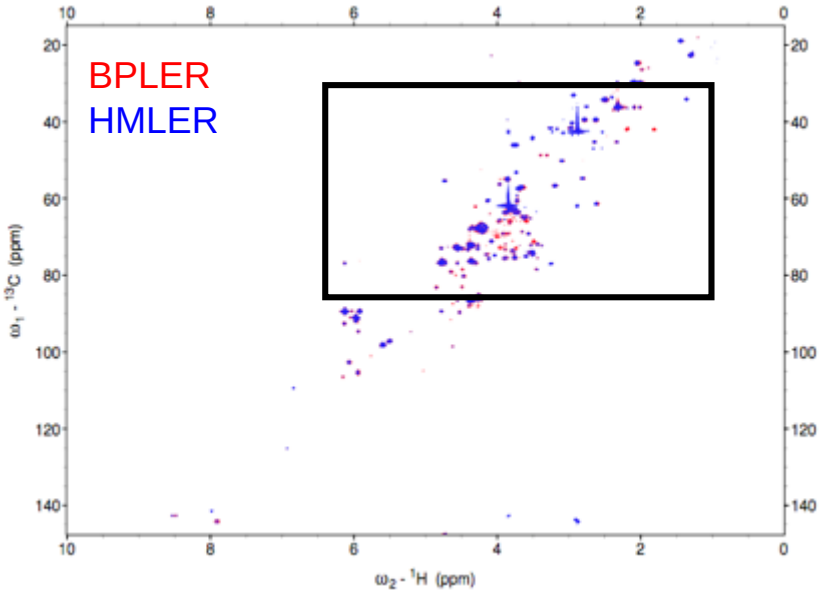


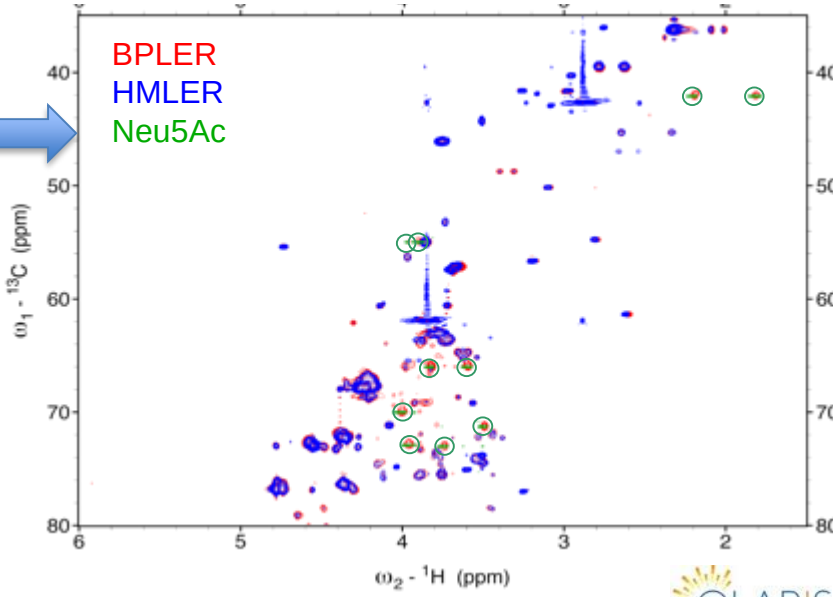
number of mice with tumors

Cells injected	BPLER	HMLER
10^6	9/9	5/9
10^5	9/9	0/12
10^4	9/9	0/12
10^3	9/9	0/12
10^2	10/12	-

Ince, T., et al (2007)

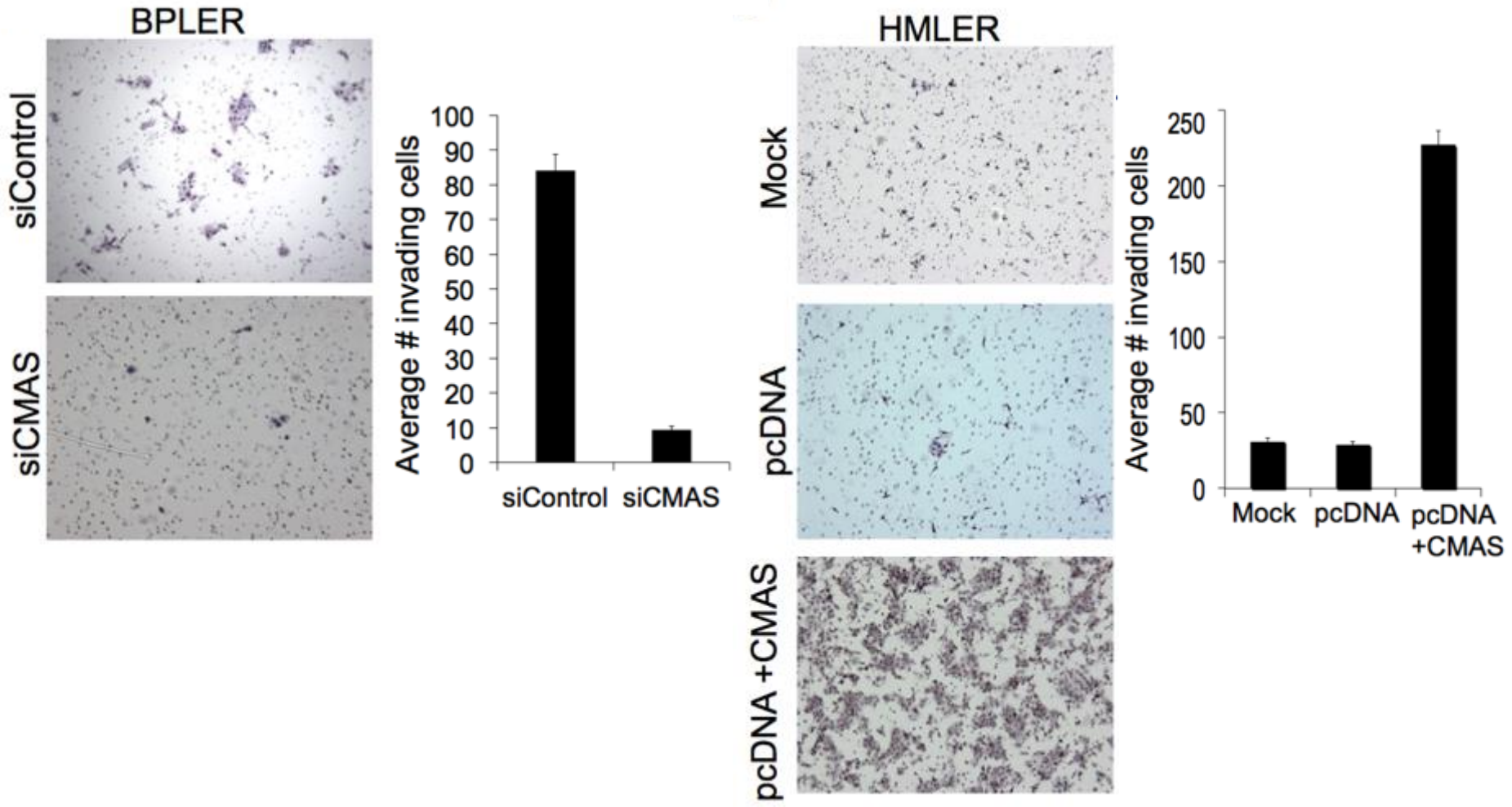
Neu5Ac increased in BPLER





O'Day, E.M. et al. (2018) OncoTargets & Therapy

Metabolic Insights Can Uncover Disease Mechanisms



O'Day, E.M. et al. (2018) OncoTargets & Therapy

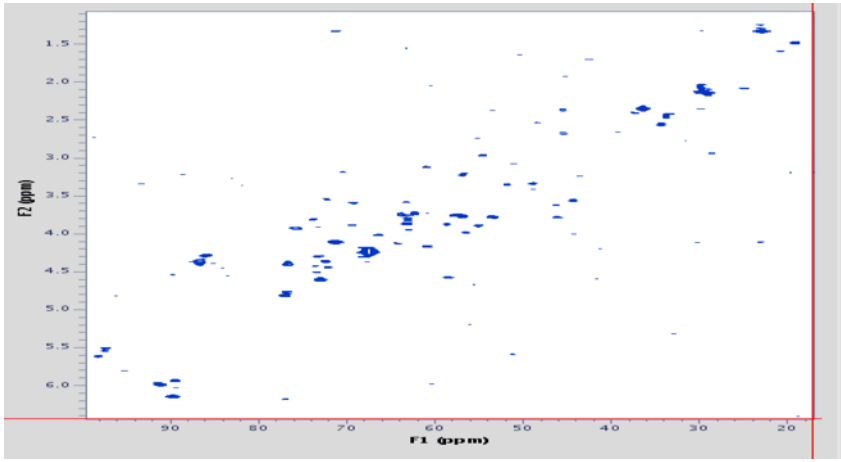
Learning From Current Patients To Help Future Patients

- Receive baseline (BL) and 2 months post treatment (2M) plasma sample
- Isolate metabolites
- Detect and quantify Jane's & Jill's metabolites using NMR & MS
- Correlate differential metabolites with clinical outcomes

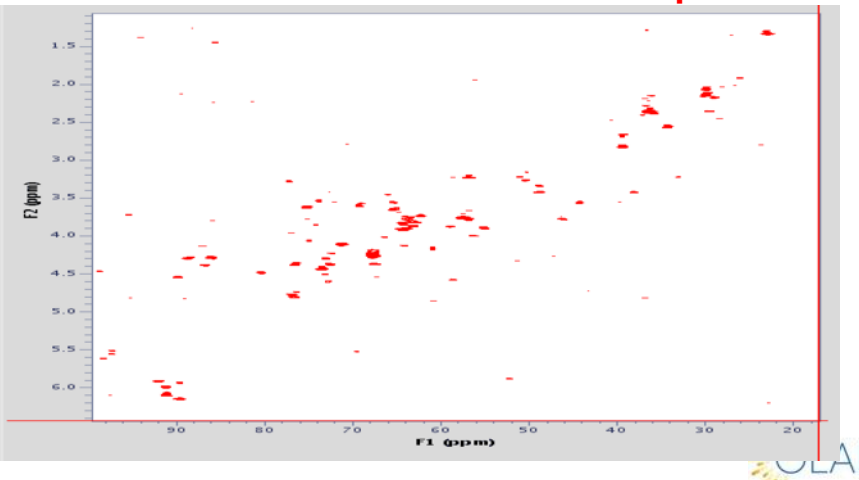
- Jane on AI + CDK 4/6 inhibitor
  Jane's tumor shrinks (R)
  N=15

- Jill on AI + CDK 4/6 inhibitor
  Jill's tumor **did not** shrink (NR)
  N=9

Metabolic Profile of a Responder

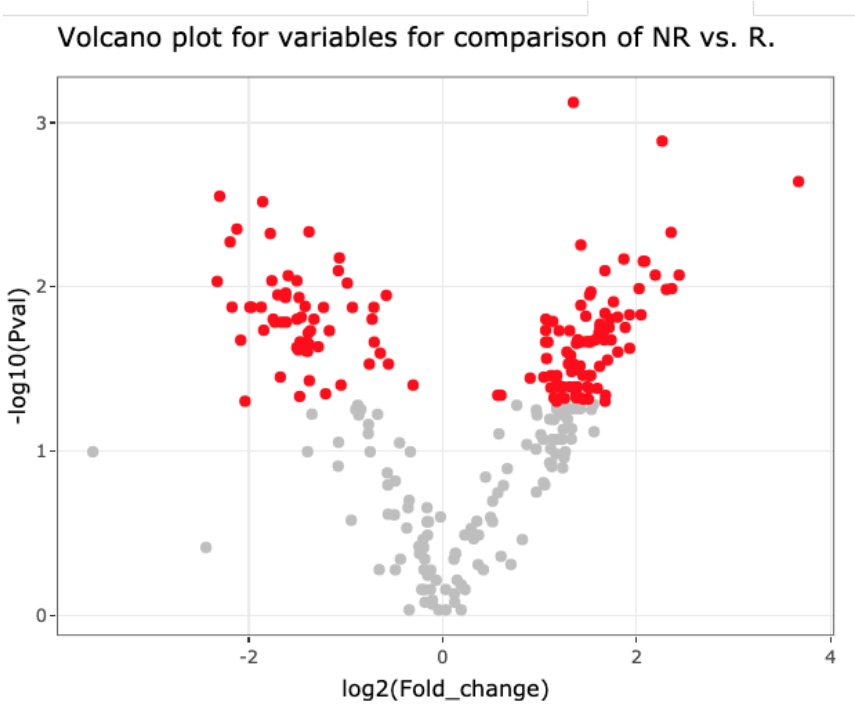


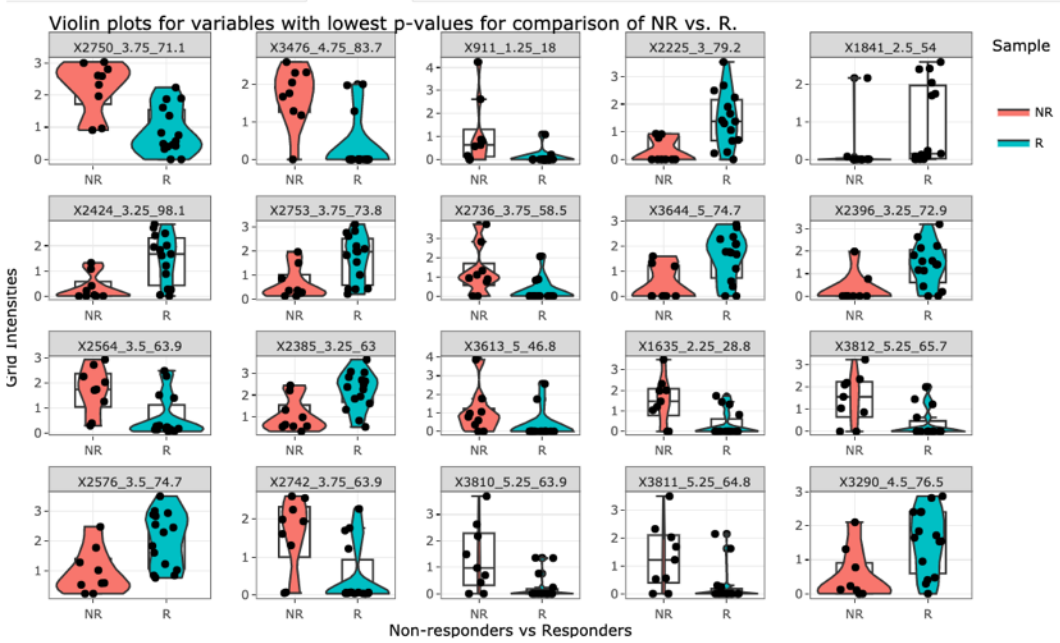
Metabolic Profile of a Non- Responder



Analyzing Metabolite Biomarkers

Identify differential grids for R vs. NR

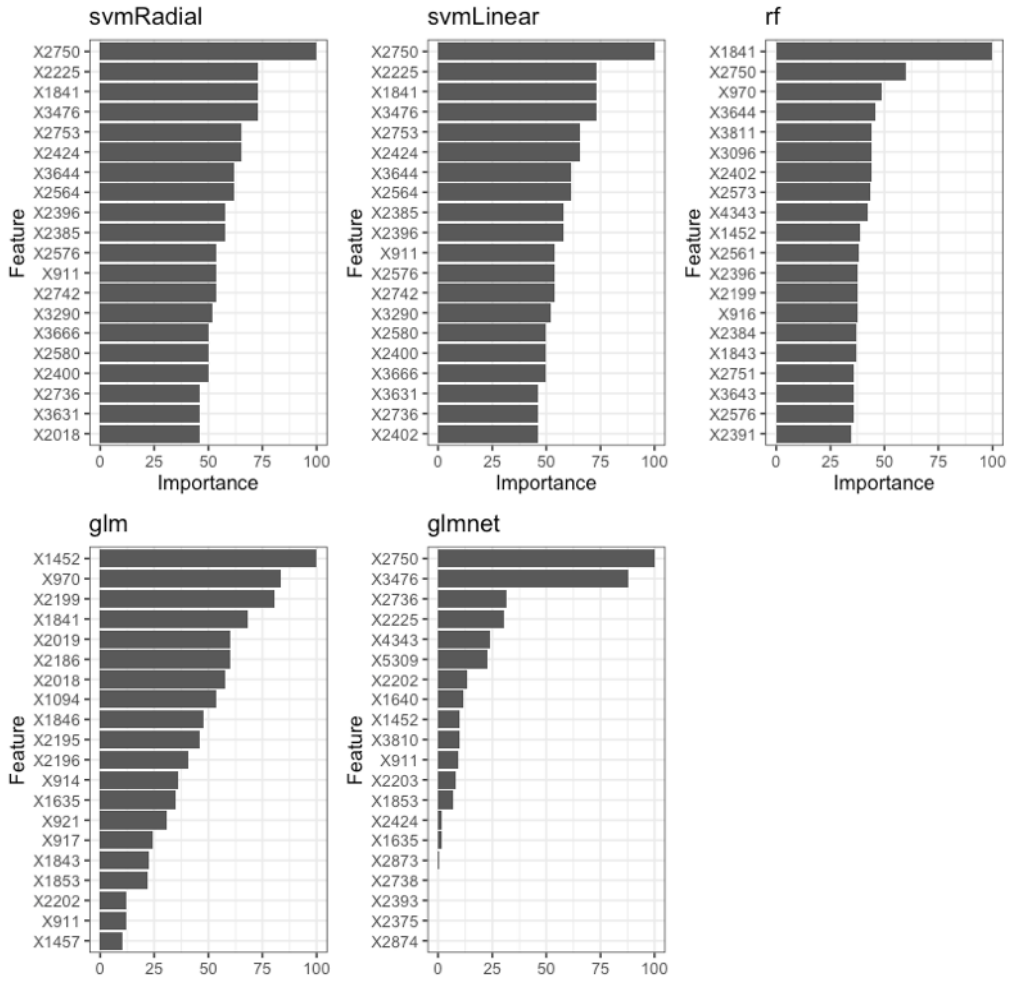




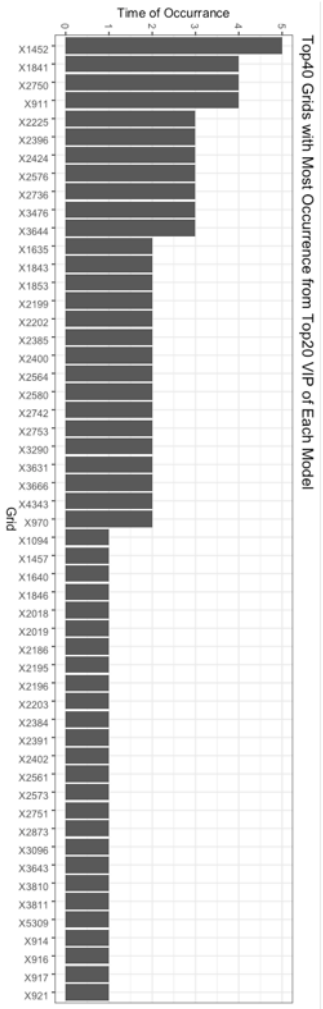
146 R vs NR (p <0.05)
14 expected by chance

“Learning” from Machine Learning→ Clear Box ML

Perform predictive modeling



Identify most significant features



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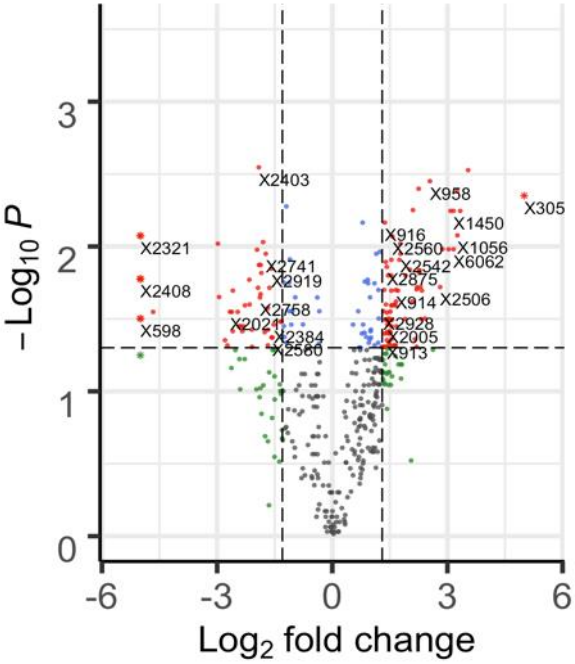
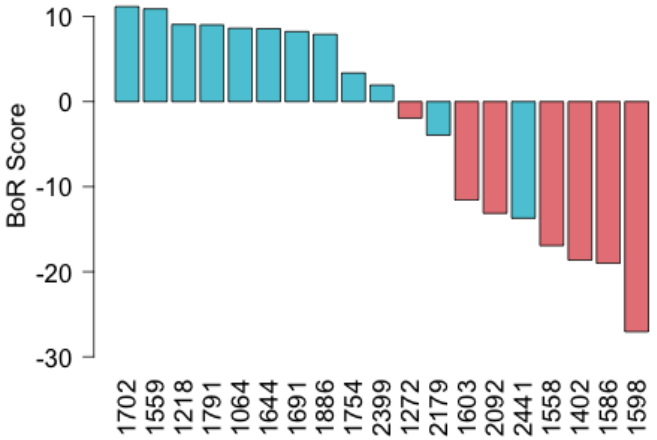
CDK4/6 BoR Differentiates R vs. NR

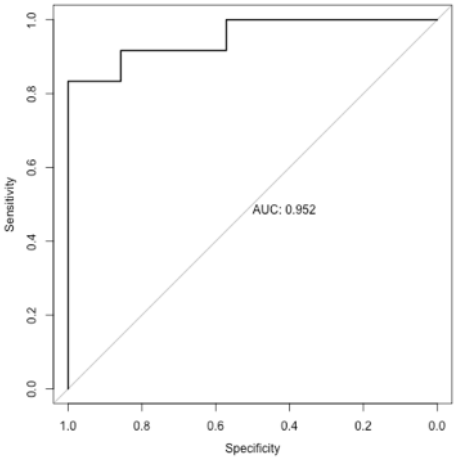
R vs NR Metabolites



Patients BoR Score



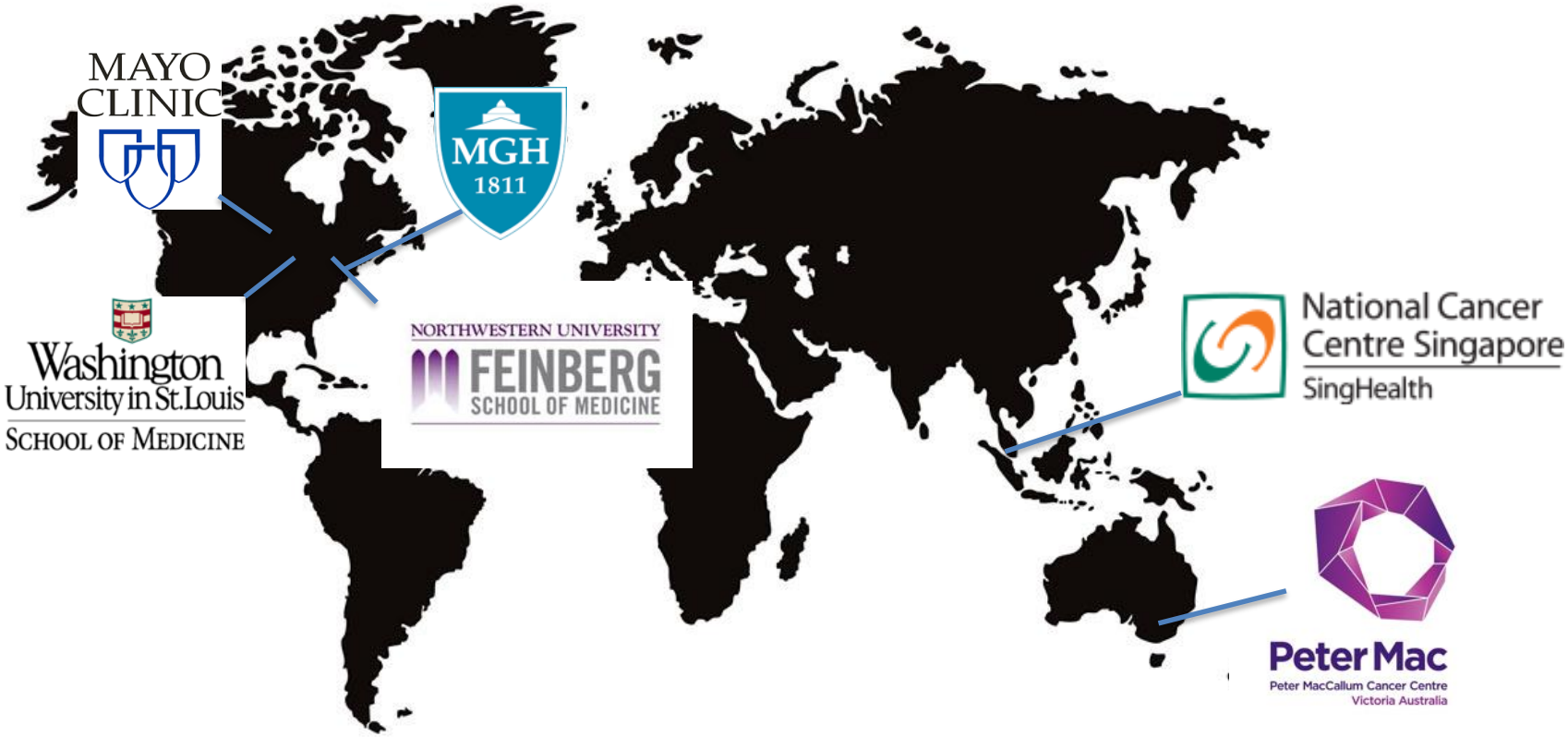
ROC Analysis



O'Day, E.M., et al (2019) ASCO Poster

CDK4/6 BoR differentiates R vs. NR with 95.2% predictive accuracy

We need to collaborate & conduct multi-site studies to uncover meaningful biomarkers



We are working with world-leading breast oncologists across the globe to validate & test CDK4/6 BoR

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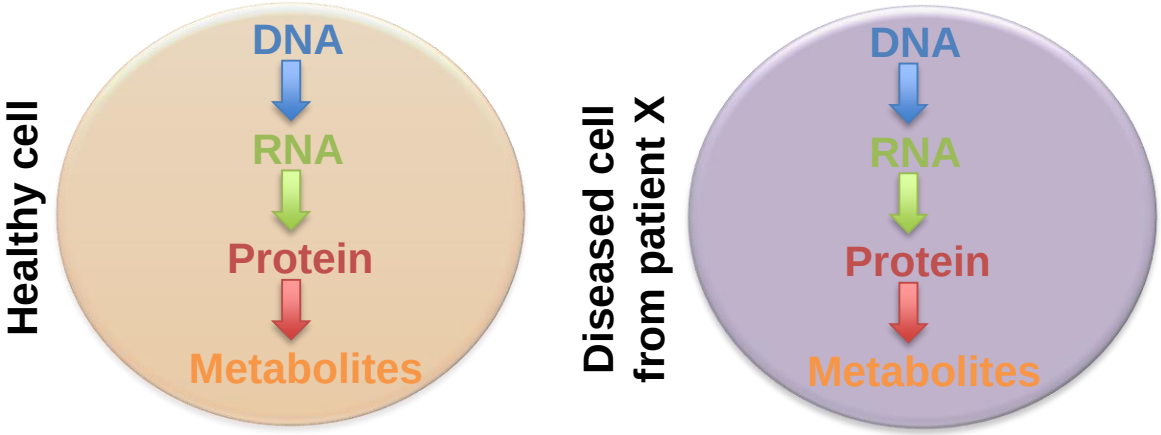
Why study metabolism?

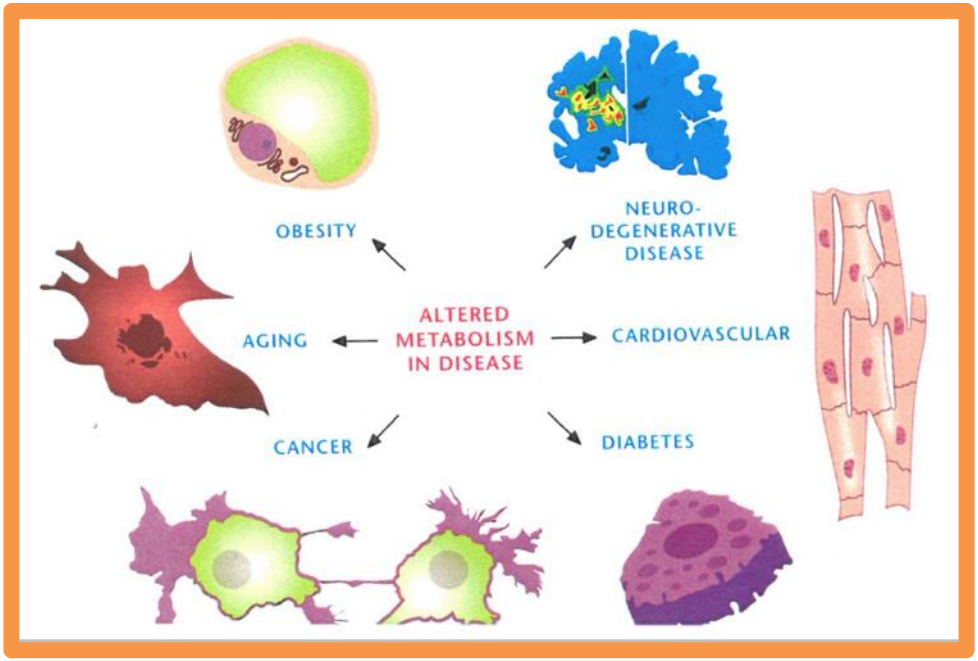
Metabolism dictates phenotype



DNA/RNA: what could happen

Protein: what makes it happen

Metabolites: what is actually happening



Altered metabolism is linked to many common diseases

- **Metabolites provide diagnostics**
- **Metabolic enzymes provide therapeutic targets**

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Future Of Medicines

BoR on every drug product

Acknowledgements

Bo Zhang, PhD
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Ciara O'Sullivan, MB BCh/BAO (Mayo)

Yap Yoon Sim, MD (National Cancer Center Singapore)

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HUMAN METABOLIC PATHWAYS

Thank You

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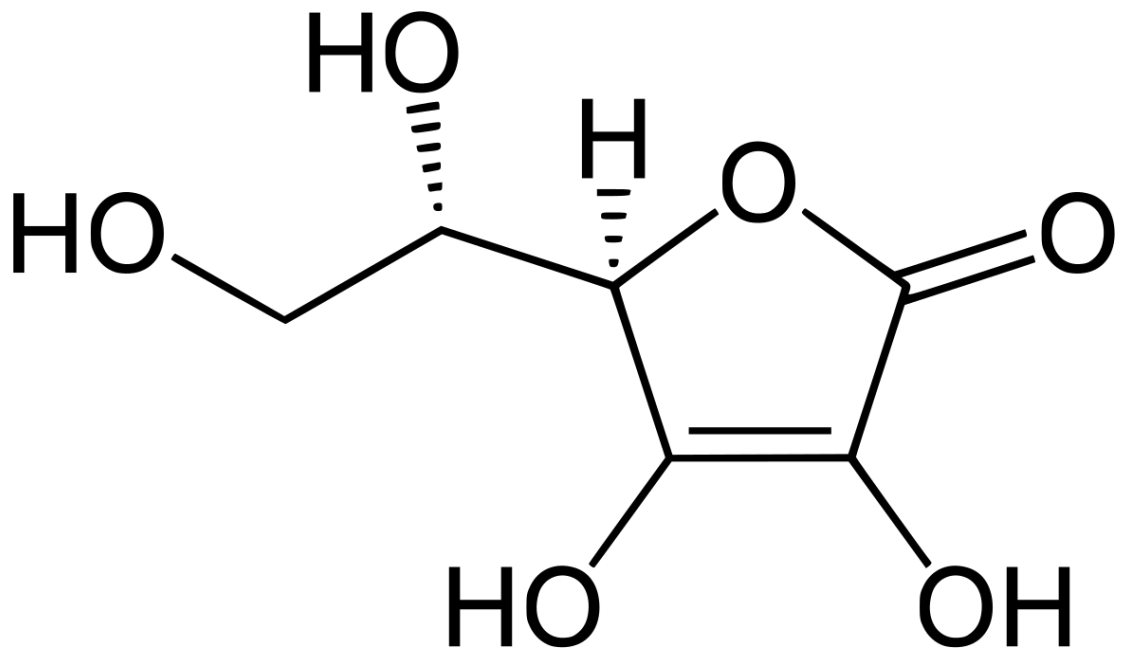
Metabolites Are Powerful Biomarkers



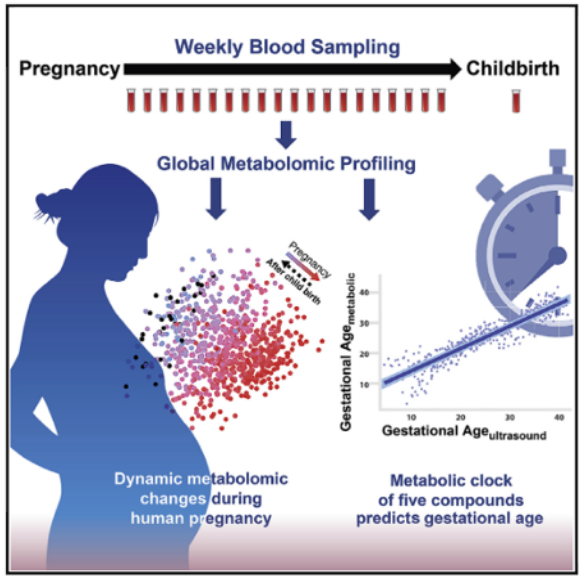
Let's avoid another vitamin C missed opportunity

Metabolites Are Powerful Biomarkers

Cell

Metabolic Dynamics and Prediction of Gesta Age and Time to Delivery in Pregnant Wome

Graphical Abstract



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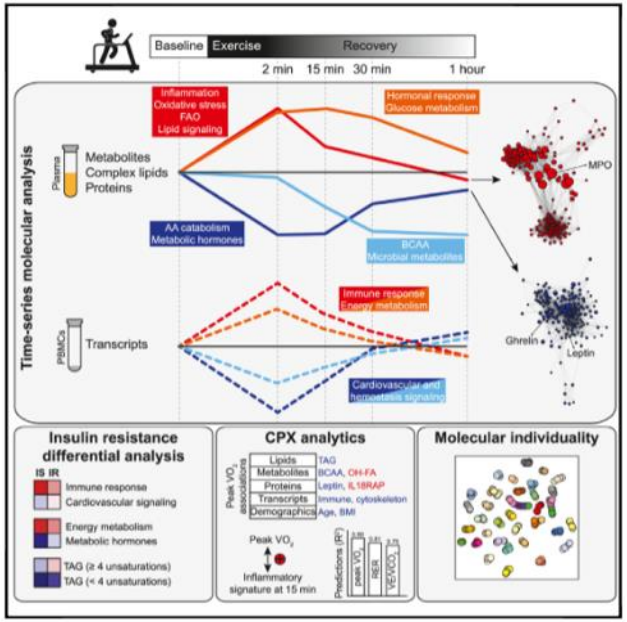
In Brief

Identification of blood m
pregnant women that ca
predict gestational age a
insights into pregnancy
undetected by ultrasoun

Cell

Molecular Choreography of Acute Exercis

Graphical Abstract



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In Brief

Longitudinal multi-o
characterize the mol
associated with acut

OLARIS26

BoR Report: Reliable Data For Clinicians

Example Patient Report

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USA/CANADA: +1.866.OLARIS1
International: www.olaristherapeutics.com/contact
www.olaristherapeutics.com
CLIA Number ##########

ER+/HER2- Metastatic Breast Cancer BoR Report

Patient/ID: Patient, 1603
Gender: Female
Date of Birth: 1/1/1956
Diagnosis: metastatic ER+/Her2- BC

Report #:
Ordering Physician: Dr. First Last Name
Pathologist: Dr. First Last Name
Specimen Type: Plasma

Olaris BoR Score uses a metabolite-profiling platform to determine the expression of a panel of metabolites and calculates a score ranging from 0-100.

The findings are applicable to women who have ER+/HER2- metastatic breast cancer.

Clinical Experience: The likelihood of response shown below are from a retrospective study that included 21 ER+/HER2- metastatic breast cancer patients who were treated with CDK4/6 inhibitors, Ibrance (palbociclib) or Kisqali (ribociclib).

Your baseline (BL) BoR Score compared to other patient
Responders (R) and Non-Responders (NR)

If Olaris BoR Score is:
- Greater than 0 → R
- Less than 0 → NR

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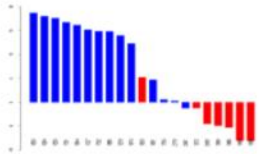
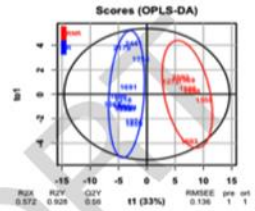
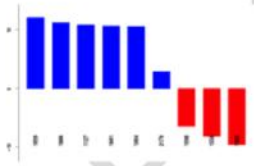
CLIA Number ##########

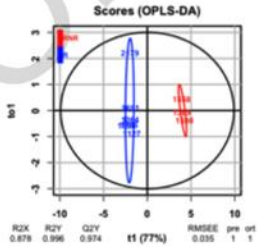
BL CDK4/6 class BoR Score: +7



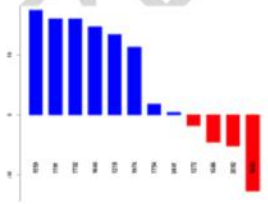


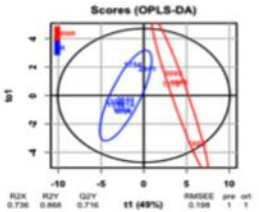
BL Ibrance BoR Score: +4





BL Kisqali BoR Score: -1





BL PI3K BoR Score:

BL mTOR BoR Score:

Biomarkers Accelerate Drug Development

Therapeutic group		Biomarkers										
		Phase 1 to Phase 2			Phase 2 to Phase 3			Phase 3 to approval			Overall	
		Total phase transitions	$POS_{1,2}$, %	(SE, %)	Total phase transitions	$POS_{2,3}$, %	SE, %	Total phase transitions	$POS_{3,APP}$, %	(SE, %)	POS, %	(SE, %)
Oncology	No biomarker	9349	28.0	(0.5)	4773	17.4	(0.5)	1159	33.6	(1.4)	1.6	(0.2)
	With biomarker	1136	43.5	(1.5)	742	38.8	(1.8)	77	63.6	(5.5)	10.7	(1.9)
	All	10 485	29.7	(0.4)	5515	20.3	(0.5)	1236	35.5	(1.4)	2.1	(0.2)
Metabolic/ endocrinology	No biomarker	1532	44.5	(1.3)	1438	33.9	(1.2)	1086	52.0	(1.5)	7.9	(0.8)
	With biomarker	7	57.1	(18.7)	2	50.0	(35.4)	15	20.0	(10.3)	5.7	(13.9)
	All	1539	44.6	(1.3)	1440	34.0	(1.2)	1101	51.6	(1.5)	7.8	(0.8)
Cardiovascular	No biomarker	1241	39.6	(1.4)	1027	37.9	(1.5)	962	62.2	(1.6)	9.5	(1.0)
	With biomarker	7	85.7	(13.2)	5	100.0	(0.0)	2	100.0	(0.0)	85.7	(13.2)
	All	1248	39.9	(1.4)	1032	38.2	(1.5)	964	62.2	(1.6)	9.5	(1.0)
CNS	No biomarker	2181	40.4	(1.1)	2050	30.2	(1.0)	1141	51.1	(1.5)	6.2	(0.6)
	With biomarker	42	54.8	(7.7)	42	28.6	(7.0)	15	53.3	(12.9)	8.3	(6.4)
	All	2223	40.7	(1.0)	2092	30.2	(1.0)	1156	51.1	(1.5)	6.3	(0.6)
Autoimmune/ inflammation	No biomarker	2506	38.9	(1.0)	2106	25.4	(0.9)	964	63.7	(1.5)	6.3	(0.6)
	With biomarker	9	55.6	(16.6)	14	35.7	(12.8)	5	60.0	(21.9)	11.9	(16.8)
	All	2515	39.0	(1.0)	2120	25.5	(0.9)	969	63.7	(1.5)	6.3	(0.6)
Genitourinary	No biomarker	359	34.3	(2.5)	287	28.9	(2.7)	212	66.5	(3.2)	6.6	(1.5)
	With biomarker	5	80.0	(17.9)	0	N.A.	N.A.	0	N.A.	N.A.	N.A.	N.A.
	All	364	34.9	(2.5)	287	28.9	(2.7)	212	66.5	(3.2)	6.7	(1.5)
Infectious disease	No biomarker	1961	39.7	(1.1)	1453	34.7	(1.2)	1069	75.1	(1.3)	10.4	(0.9)
	With biomarker	6	66.7	(19.2)	27	44.4	(9.6)	9	100.0	(0.0)	29.6	(16.8)
	All	1967	39.8	(1.1)	1480	34.9	(1.2)	1078	75.3	(1.3)	10.5	(0.9)
Ophthalmology	No biomarker	180	52.2	(3.7)	274	34.7	(2.9)	207	74.9	(3.0)	13.6	(2.8)
	With biomarker	1	0.0	(0.0)	3	33.3	(27.2)	0	N.A.	N.A.	N.A.	N.A.
	All	181	51.9	(3.7)	277	34.7	(2.9)	207	74.9	(3.0)	13.5	(2.8)
Vaccines (infectious disease)	No biomarker	733	40.8	(1.8)	761	32.9	(1.7)	609	85.4	(1.4)	11.4	(1.3)
	With biomarker	0	N.A.	N.A.	5	0.0	(0.0)	0	N.A.	N.A.	N.A.	N.A.
	All	733	40.8	(1.8)	766	32.6	(1.7)	609	85.4	(1.4)	11.4	(1.3)
Overall	No biomarker	20 042	34.7	(0.3)	14 169	26.8	(0.4)	7409	59.0	(0.6)	5.5	(0.2)
	With biomarker	1213	44.5	(1.4)	840	38.6	(1.7)	123	60.2	(4.4)	10.3	(1.6)
	All	21 255	35.2	(0.3)	15 009	27.4	(0.4)	7532	59.0	(0.6)	5.7	(0.2)

Wong, C.H., et al (2019) *Biostatistics*

Biomarkers increase the Probability of Success (PoS) At Every Stage of Clinical Development

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