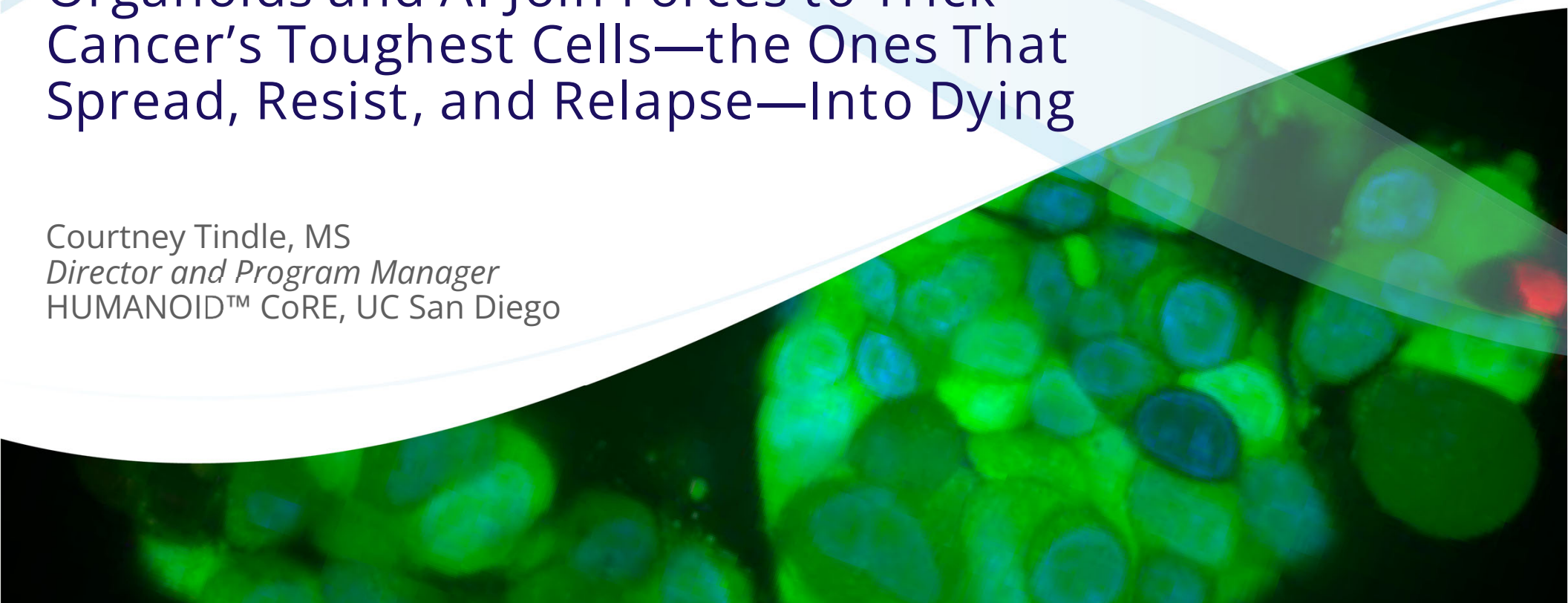


Organoids and AI Join Forces to Trick Cancer's Toughest Cells—the Ones That Spread, Resist, and Relapse—Into Dying

Courtney Tindle, MS
Director and Program Manager
HUMANOID™ CoRE, UC San Diego



About ATCC[®]



- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the “gold standard”
- Innovative R&D company featuring new product formats that support drug development and microphysiological systems
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 20 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

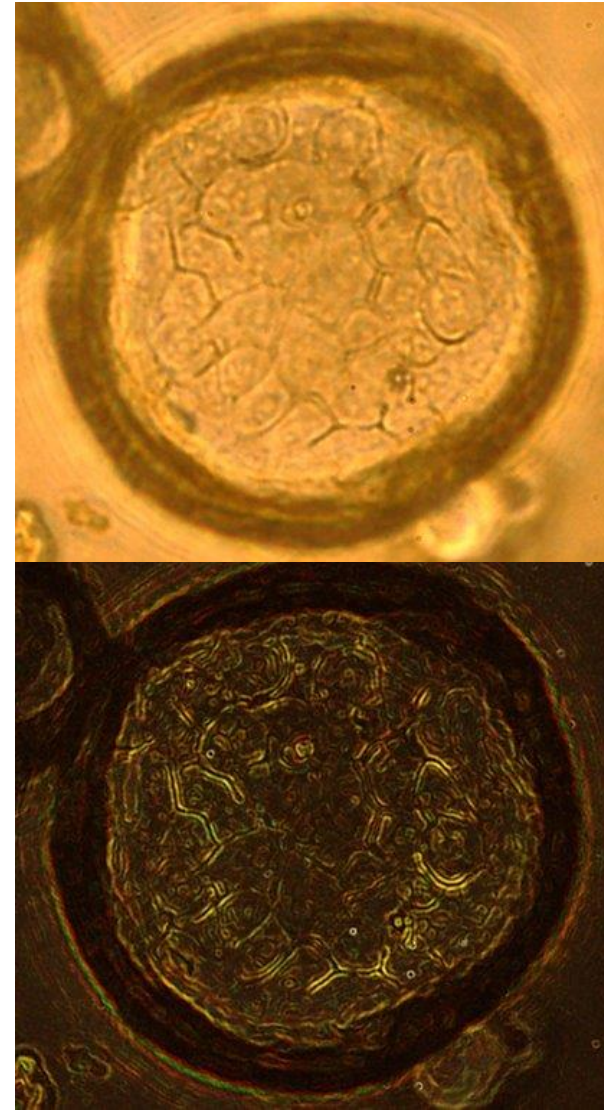


Introducing a 'Phase 0' in clinical trials with precise organoid-based disease models

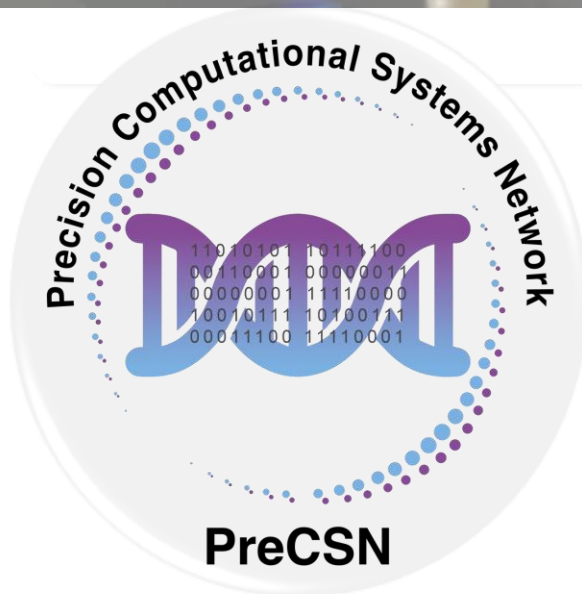
Presented by:
Courtney Tindle, M.S.
Director of HUMANIOD™

UC San Diego

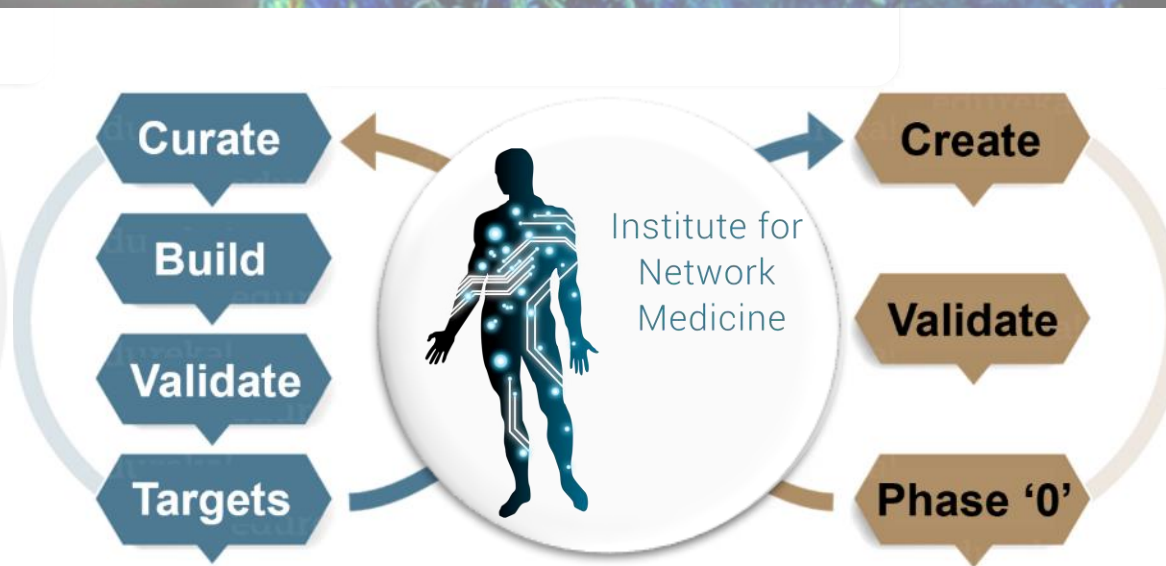
This work features cancer PDOs that were commercially obtained from The ATCC®.



PRECSN AND HUMANOID ENABLE PHASE 'ZERO'



USING BOOLEAN NETWORK
EXPLORER [BoNE]

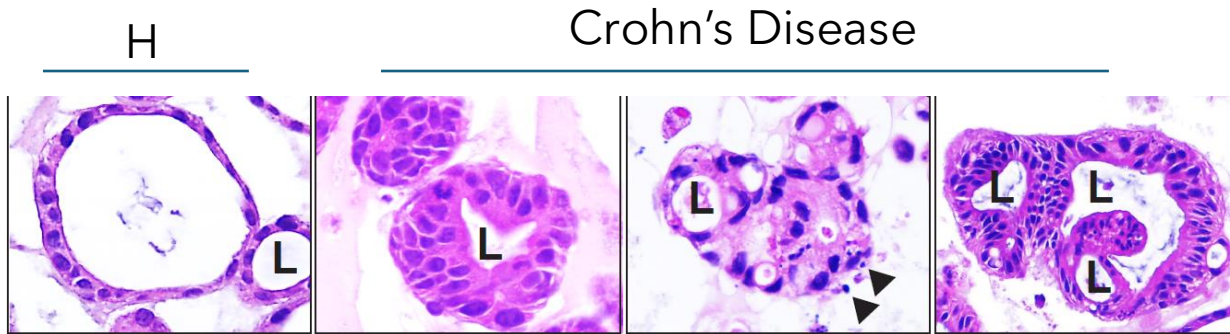


USING HUMAN ORGANOID-
BASED MODELS OF DISEASES

HOW DO WE CHOOSE WHICH MODELS

It's an equation: Passion + Public Need + Funding

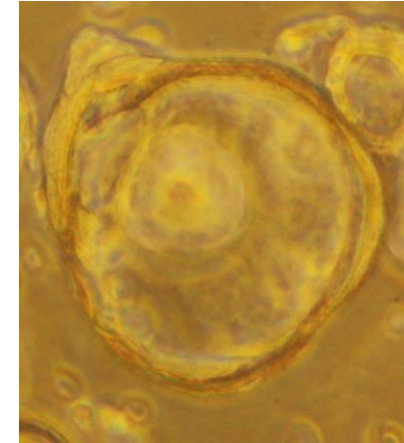
Gastrointestinal:



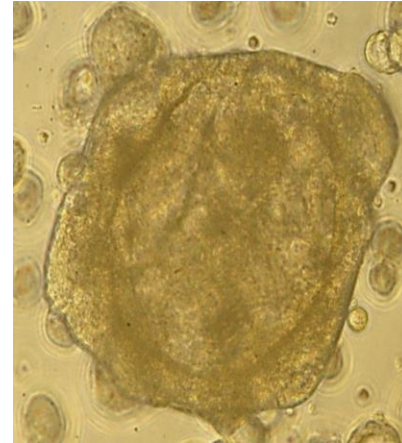
FAP Organoid



Ulcerative Colitis

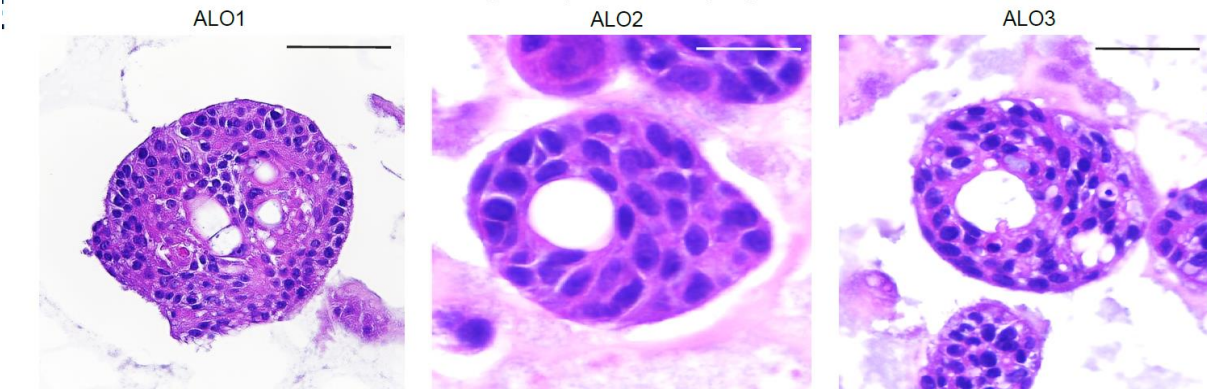


GEJ of BE

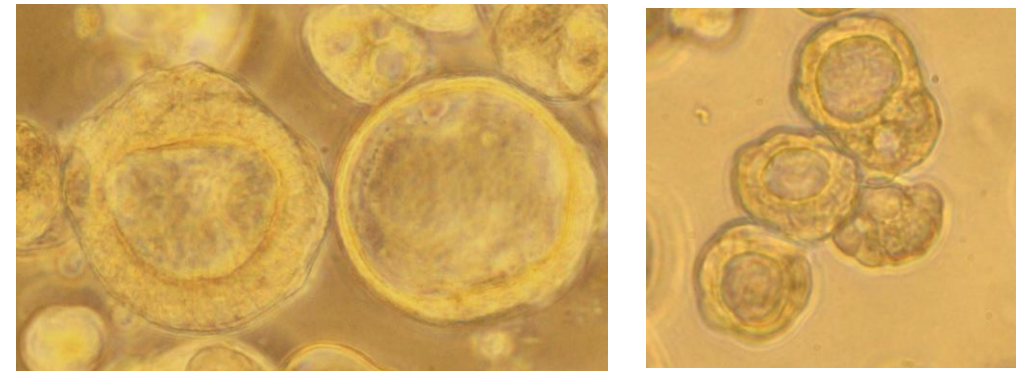


Respiratory:

Histologic analysis of 3D Morphogenesis



Multiple adenocarcinomas (ATCC's Resource)

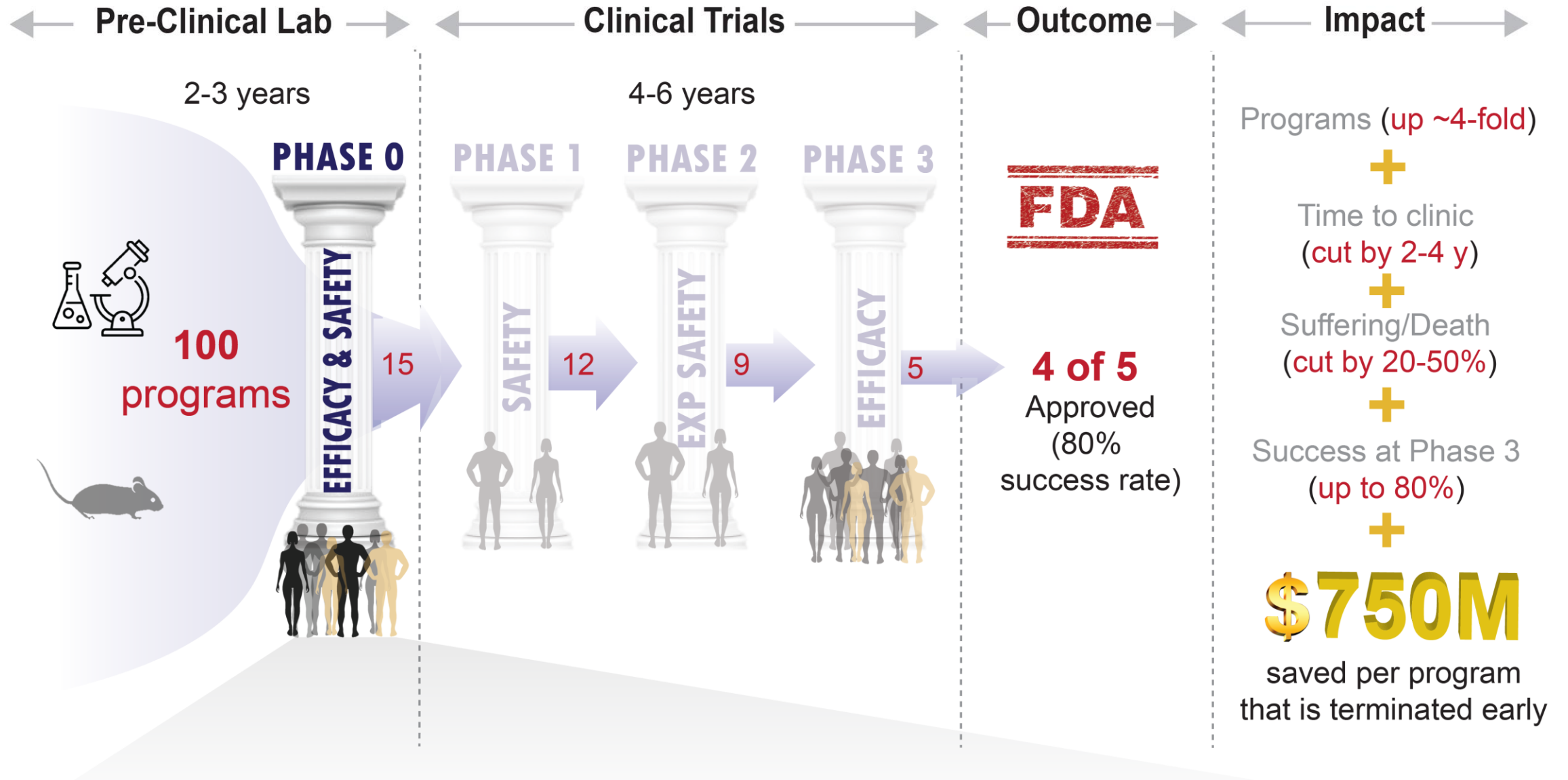


THE DRUG DISCOVERY PROCESS IS FLAWED

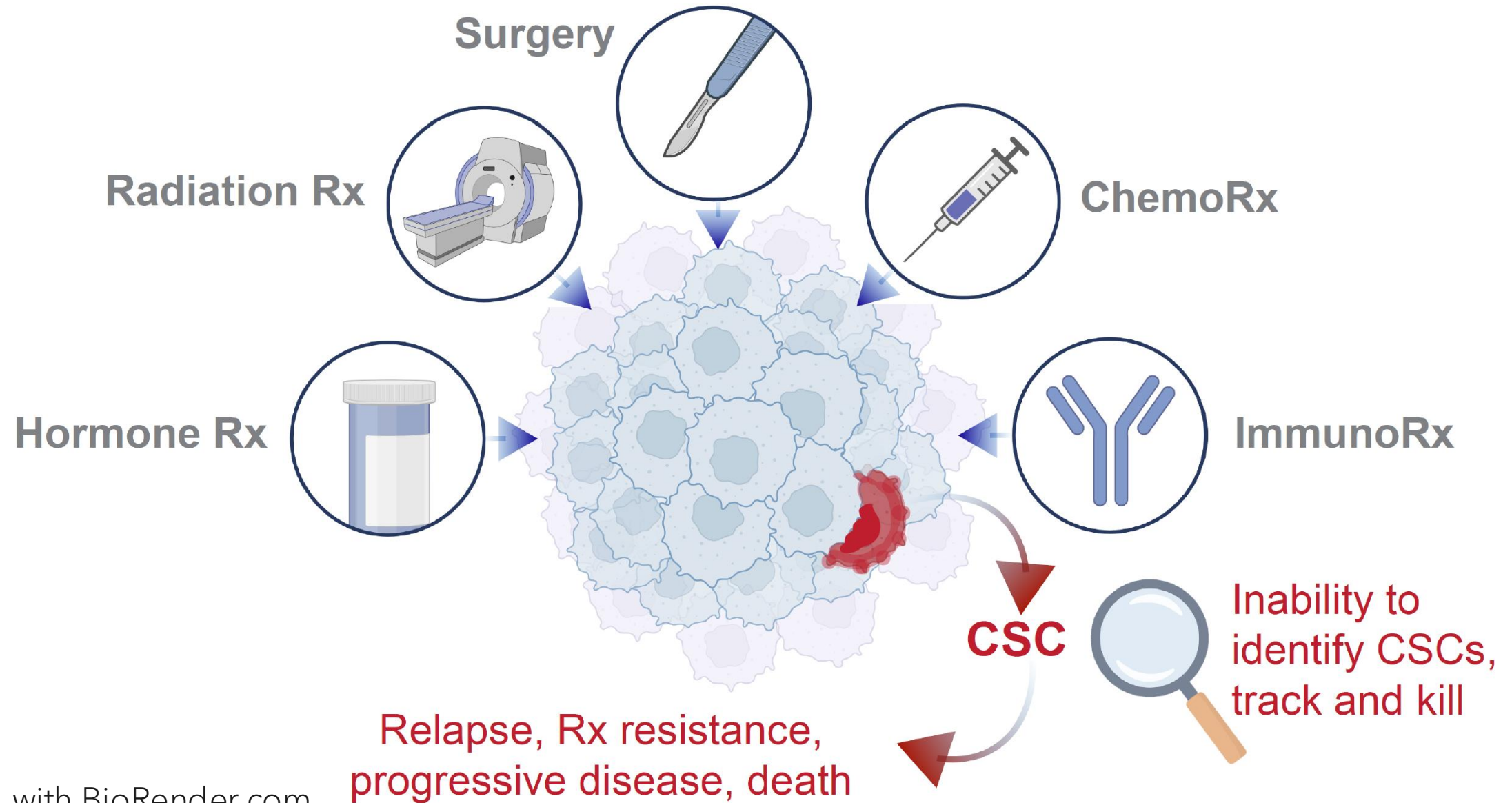
THE PROBLEM



TESTING EFFICACY EARLY ON CAN FIX IT

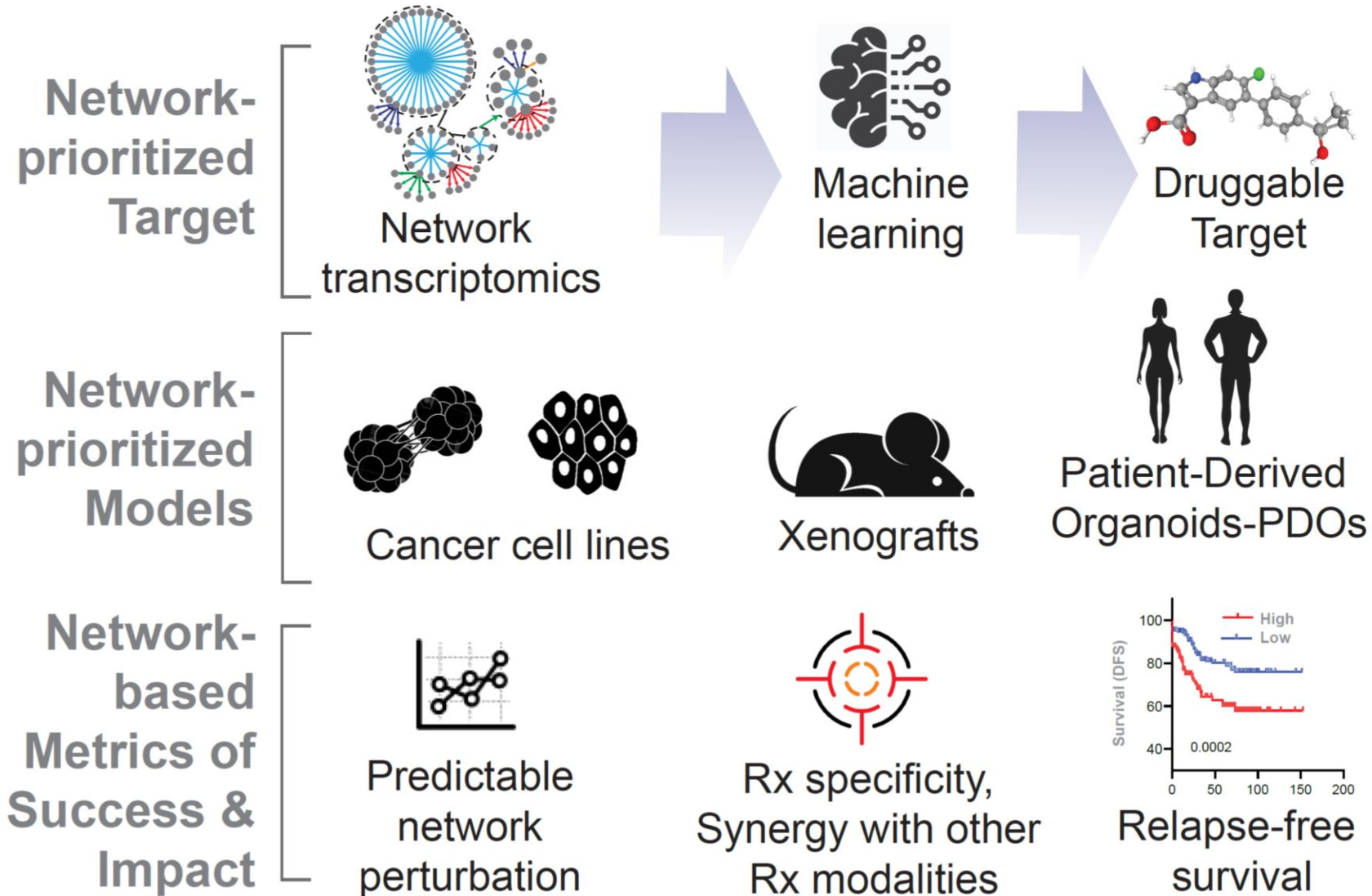


The Problem: Cancer Stem Cells escape Rx

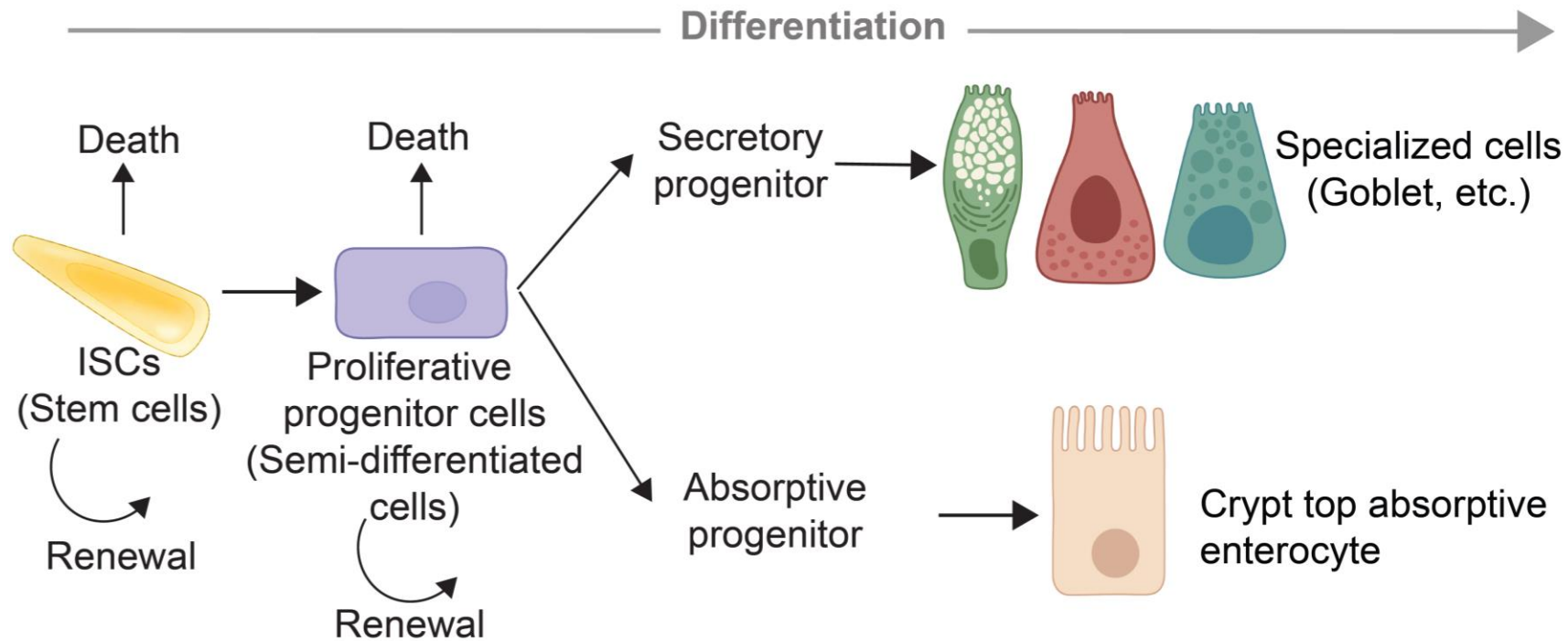


The Solution: Targeted CSC Differentiation

Network-guided identification of a first-in-class agent



WHAT ARE KEY 'EVENTS' THAT PUSH THE STEMNESS-DIFFERENTIATION AXIS?



MATHEMATICAL APPROACH: ABSENCE OF CDX2 EQUALS STEMNESS

The **NEW ENGLAND**
JOURNAL *of* **MEDICINE**

ESTABLISHED IN 1812

JANUARY 21, 2016

VOL. 374 NO. 3

CDX2 as a Prognostic Biomarker in Stage II and Stage III Colon Cancer

Piero Dalerba, M.D., Debashis Sahoo, Ph.D., Soonmyung Paik, M.D., Xiangqian Guo, Ph.D., Greg Yothers, Ph.D.,
Nan Song, Ph.D., Nate Wilcox-Fogel, M.S., Erna Forgó, M.D., Pradeep S. Rajendran, B.S., Stephen P. Miranda, B.A.,
Shigeo Hisamori, M.D., Ph.D., Jacqueline Hutchison, Tomer Kalisky, Ph.D., Dalong Qian, M.D.,
Norman Wolmark, M.D., George A. Fisher, M.D., Ph.D., Matt van de Rijn, M.D., Ph.D., and Michael F. Clarke, M.D.

Cell Stem Cell
In Translation

CDX2: Linking Cell and Patient Fates in Colon Cancer

Eric R. Fearon^{1,2,3} and Emina H. Huang^{4,5,*}

¹Division of Molecular Medicine and Genetics, Department of Internal Medicine

²Department of Pathology

³Department of Human Genetics

University of Michigan, Ann Arbor, MI 48109, USA

⁴Department of Stem Cell Biology and Regenerative Medicine

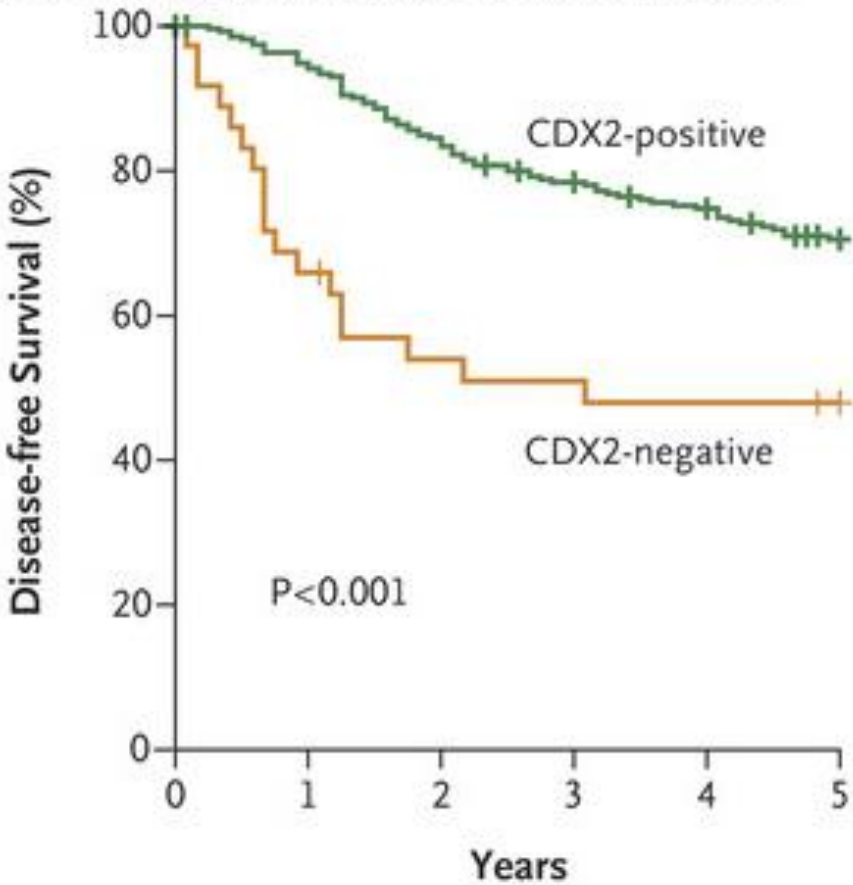
⁵Department of Colorectal Surgery

Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH 44195, USA

*Correspondence: huange2@ccf.org

<http://dx.doi.org/10.1016/j.stem.2016.01.011>

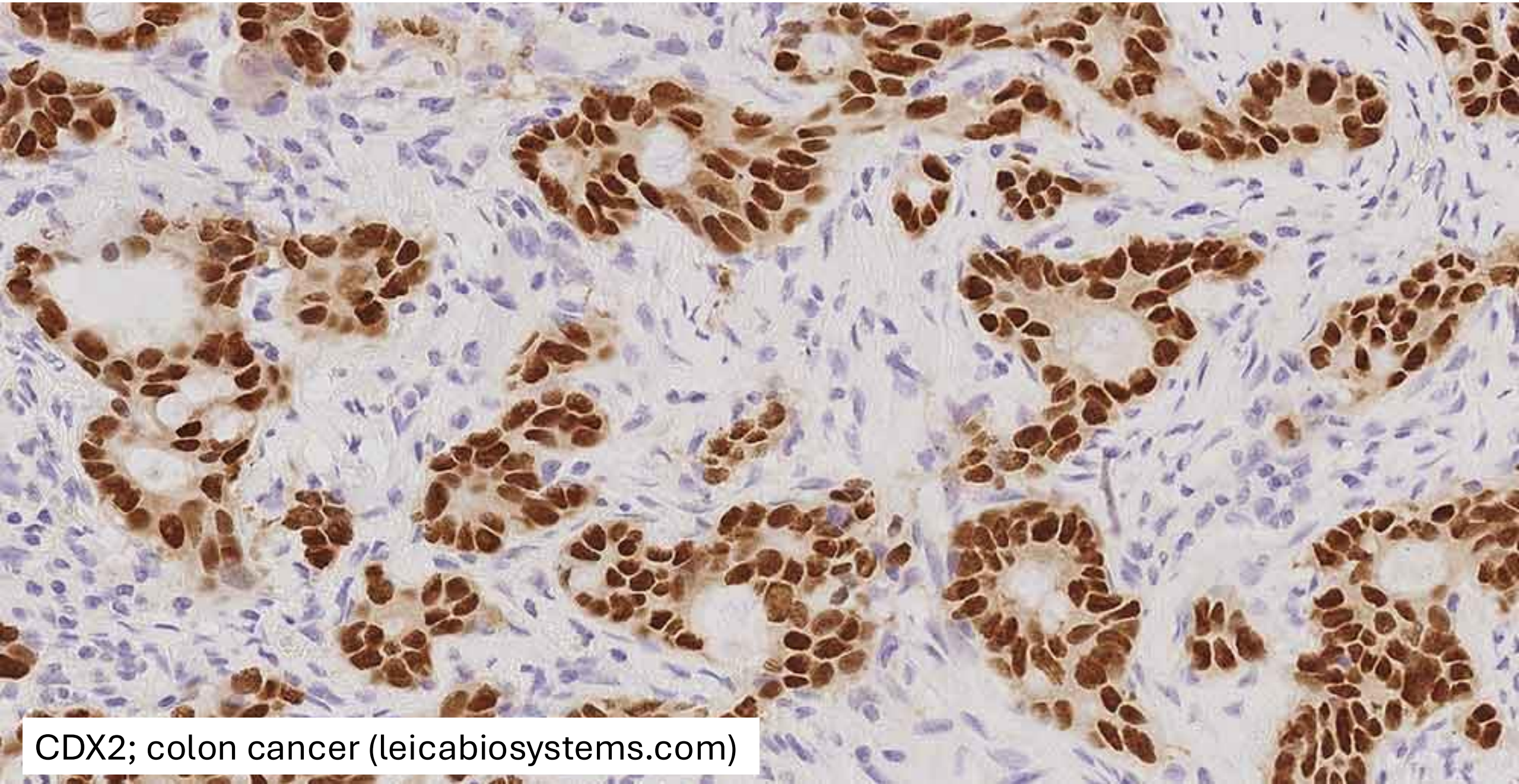
Disease-free Survival, According to CDX2 Expression



No. at Risk

CDX2-positive	276	258	225	199	182	150
CDX2-negative	38	23	18	17	16	15

CDX2 (CAUDAL HOMEBOX 2) IS A TF



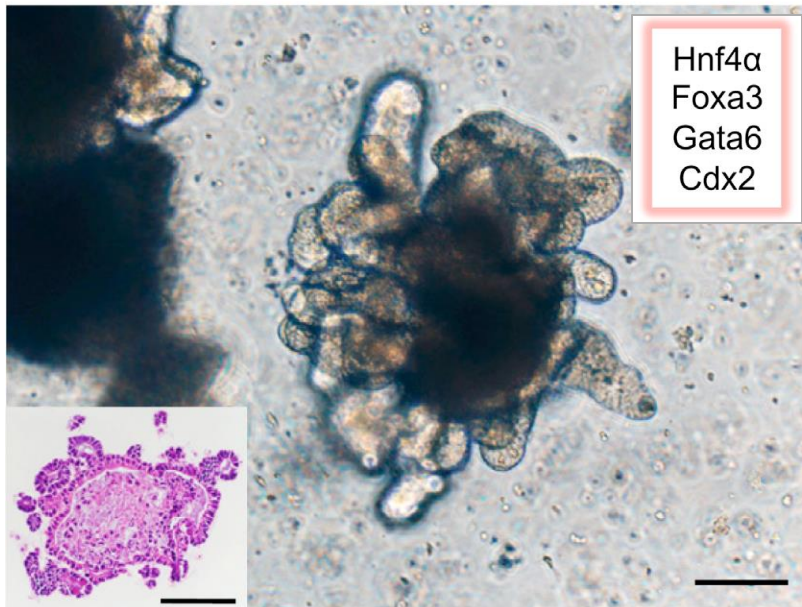
CDX2; colon cancer (leicabiosystems.com)

CDX2 IS ESSENTIAL FOR COMMITTED DIFFERENTIATION INTO INTESTINE

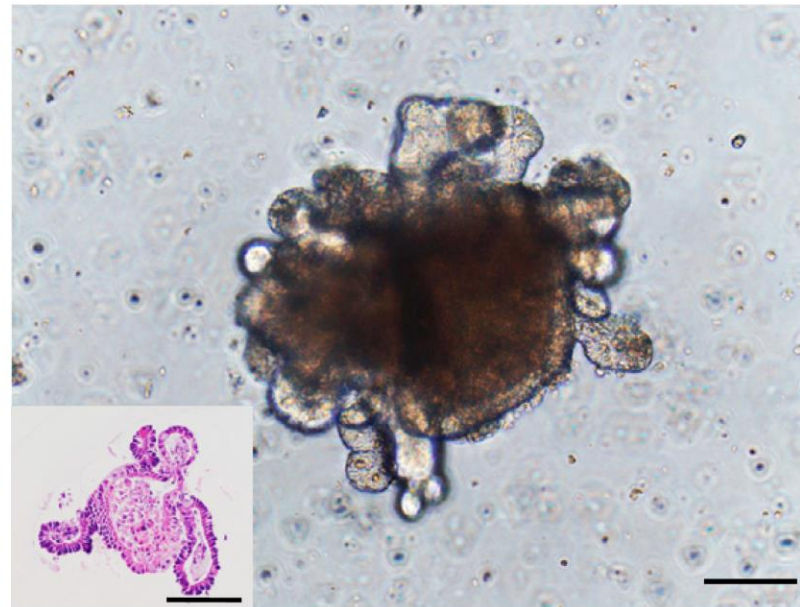
Cell Stem Cell

Article
Generation of Mouse and Human Organoid-Forming Intestinal Progenitor Cells by Direct Lineage Reprogramming

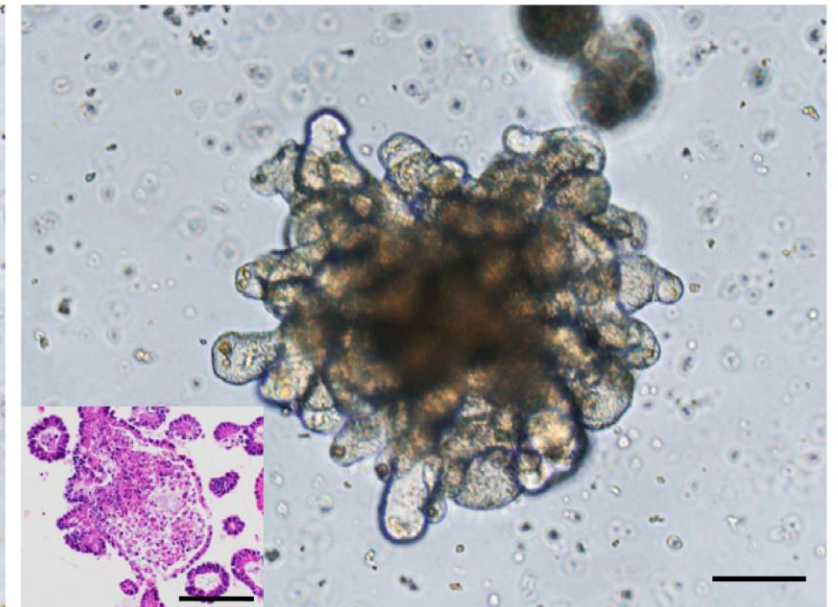
4F-MEF



Fetal intestine



Adult intestine



PREMISE AND STUDY RATIONALE

Step 1: A Rationalized Therapeutic Goal

Discovery and validation of CDX2 as a clinically actionable biomarker of colon epithelial differentiation, whose loss indicates stemness and carries poor prognosis.

N Engl J Med 2016; 374:211-222
DOI: 10.1056/NEJMoa1506597

CDX2-pos is associated with better DFS (n = 780; Stage II)

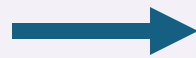
CDX2-neg is associated with benefit from chemotherapy (n = 1897; Stage II and III)

MULTIPLE PRIOR ATTEMPTS AT PHARMACOLOGICAL REINSTATEMENT OF CDX2 HAVE FAILED

NETWORK MODEL TO IDENTIFY TARGETS FOR DIFFERENTIATION RX



Step 2: Target Identification



Discovery of a clinically actionable therapeutic target to reinstate CDX2

Bioinformatics search for markers of CRC differentiation, using *CDX2* as a 'seed' gene, and based on the fulfilment of the "*CDX2*-neg implies *X*-neg" Boolean relationship identifies 42 putative target genes.

(**Model training:** n=1969 samples; normal =183; adenomas = 85; CRCs =1690).

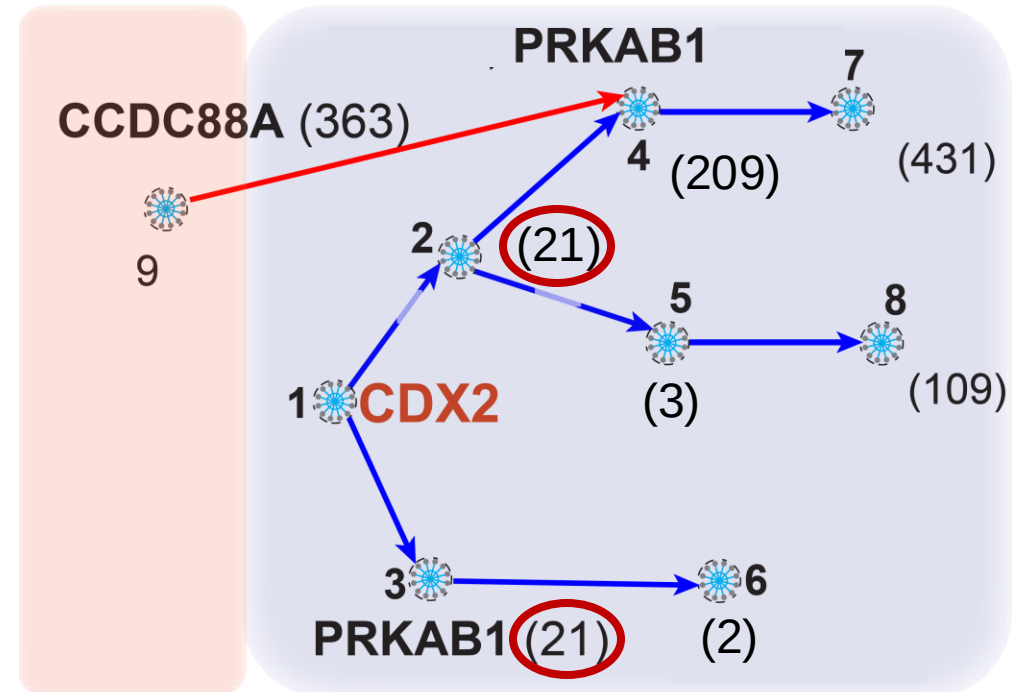
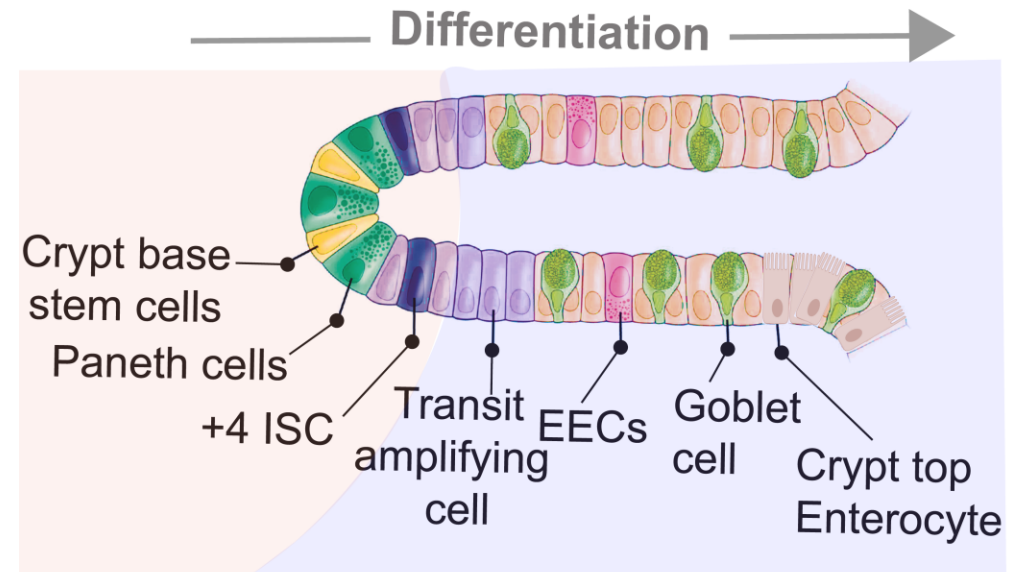
AN ACTIONABLE NETWORK MODEL FOR DIFFERENTIATION THERAPY

Boolean Implication Formula

CDX2 neg => **"X"** neg

Numbers in () indicate no. of genes in clusters

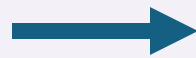
BOOLEAN LOGIC: If one of the 42 genes in clusters #2 and #3 are upregulated, **CDX2** must be upregulated;
CCDC88A must be downregulated



NETWORK MODEL TO IDENTIFY TARGETS FOR DIFFERENTIATION RX



Step 2: Target Identification



Discovery of a clinically actionable therapeutic target to reinstate CDX2

Bioinformatics search for markers of CRC differentiation, using *CDX2* as a 'seed' gene, and based on the fulfilment of the "*CDX2*-neg implies *X*-neg" Boolean relationship identifies 42 putative target genes.

(**Model training:** n=1969 samples; normal =183; adenomas = 85; CRCs =1690).

(**Model validation:** 1911 human; 107 mouse)

NETWORK-GUIDED TARGET VALIDATION



Step 3: Target Validation

Models

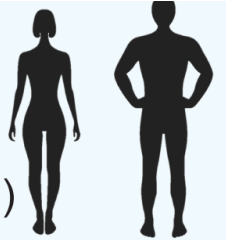


Hu CRC cell lines
(n = 4)



CRC
Xenograft

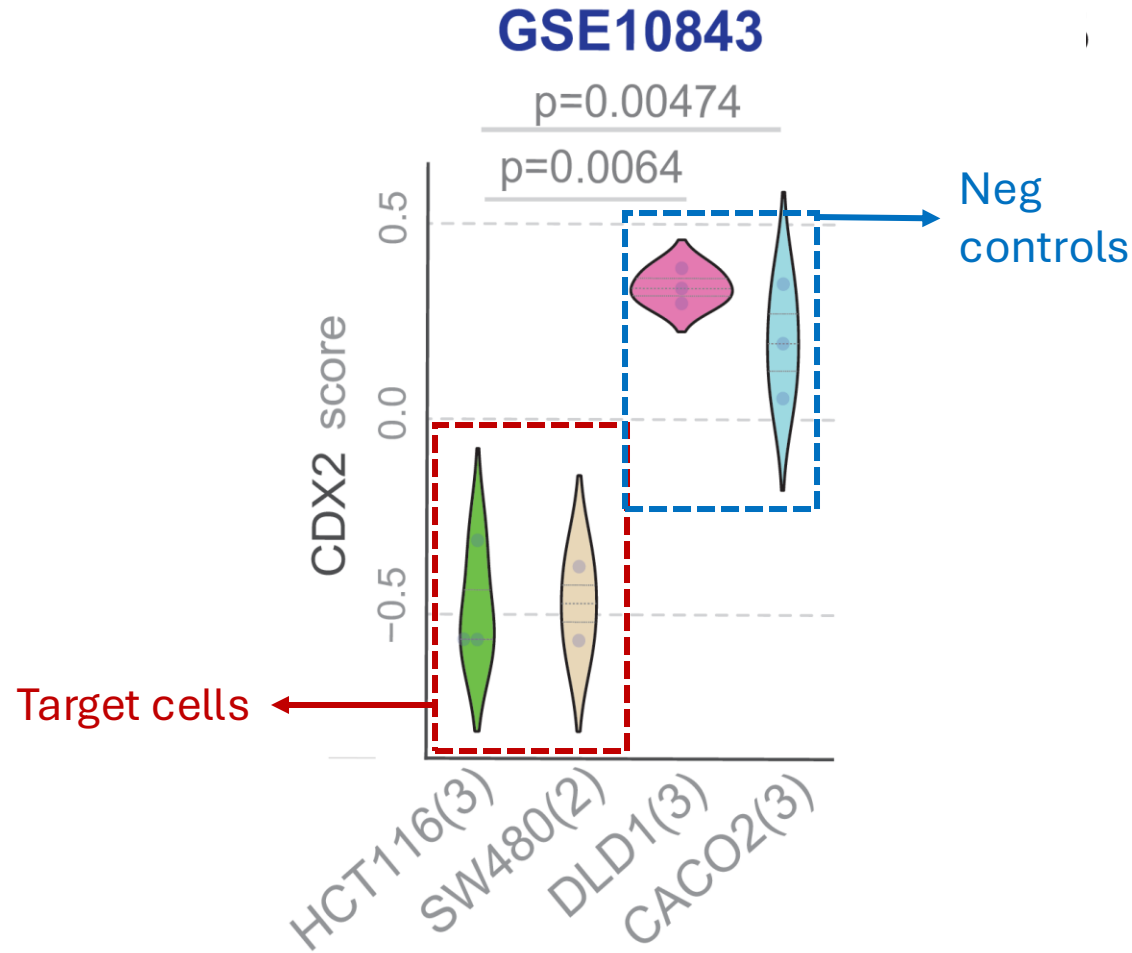
Patient-Derived
Organoids-PDOs
(healthy 3, CRC 23)



We Leveraged
The ATCC®
Catalog to
Acquire
Established
CRC Lines

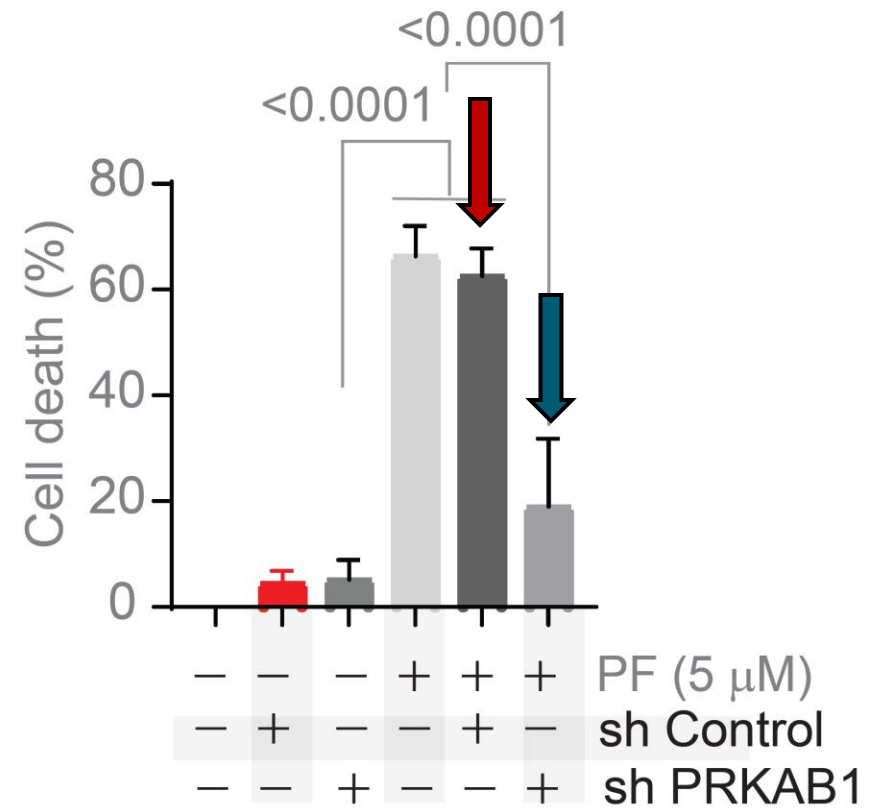
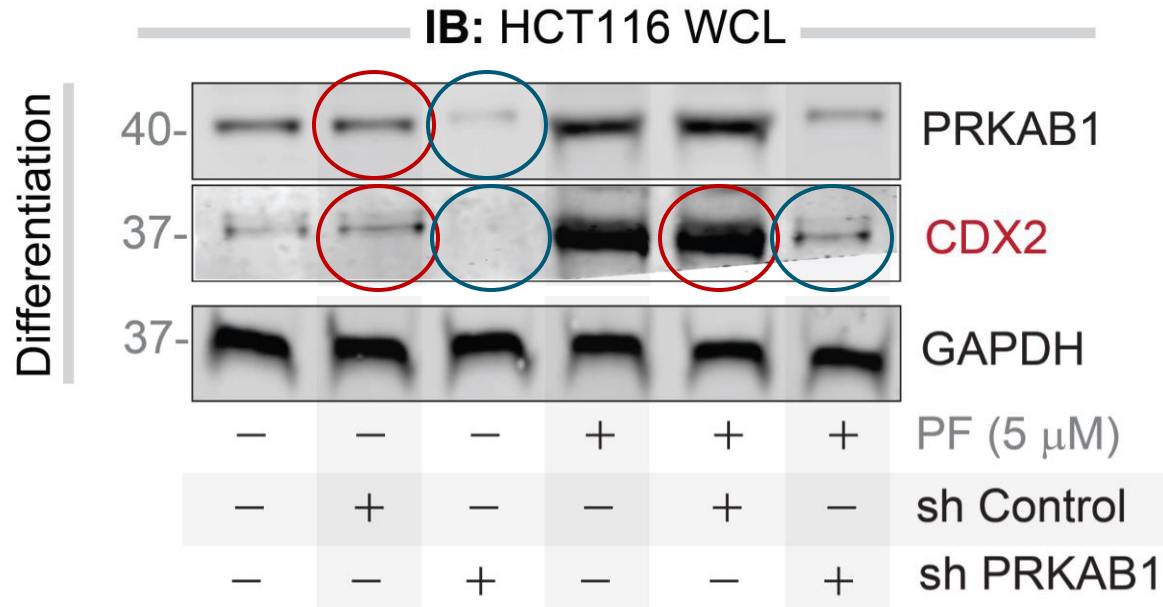
ATCC® Cat No.	Cell Line
CCL-247	HCT116
CCL-228	SW480 [SW-480]
CCL-221	DLD1
HTB-37	Caco-2

NETWORK-GUIDED MODEL SELECTION



- ✓ Impact of Rx was dose dependent
- ✓ No impact on DLD1 and Caco2 cells
- ✓ Induction of protein (CDX2 and other markers) by immunoblotting
- ✓ Late apoptosis as Mechanism of Death (FACS)

TARGET SPECIFIC ACTION



NETWORK-GUIDED MODEL SELECTION



Michael Bouvet



Siamak Amirfakhri



Saptarshi Sinha

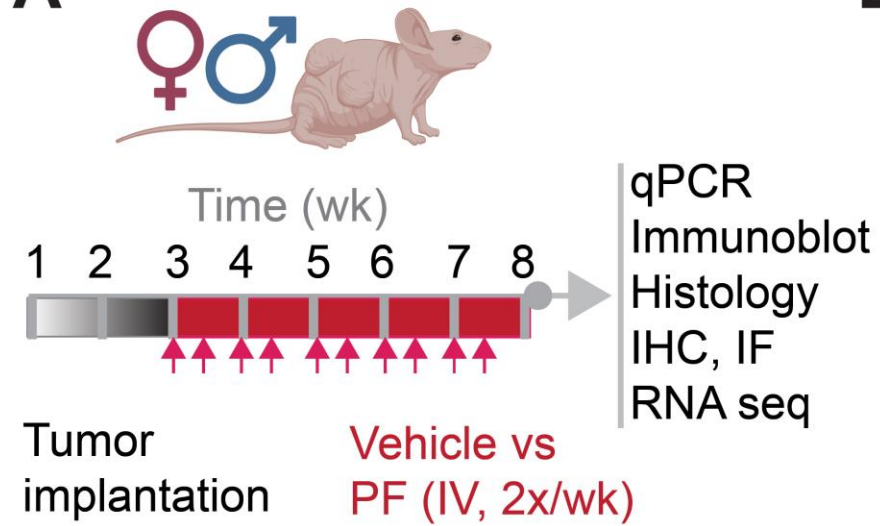


Joshua Alcantara

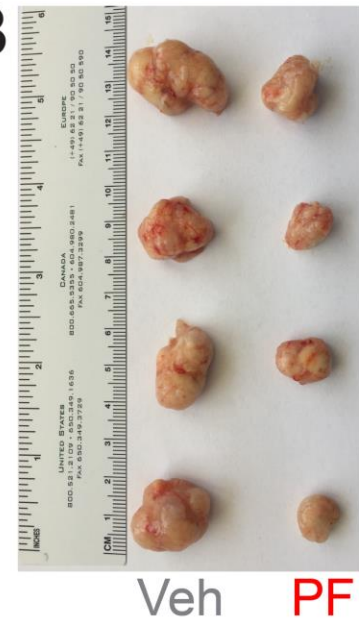


Vanessa Castillo-LFL

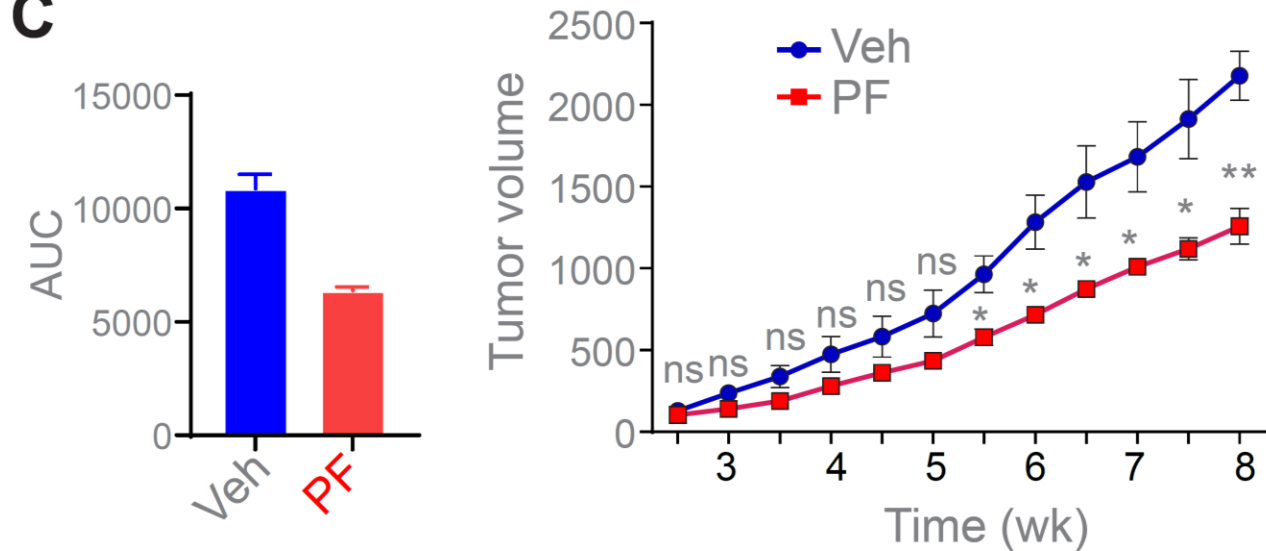
A



B



C

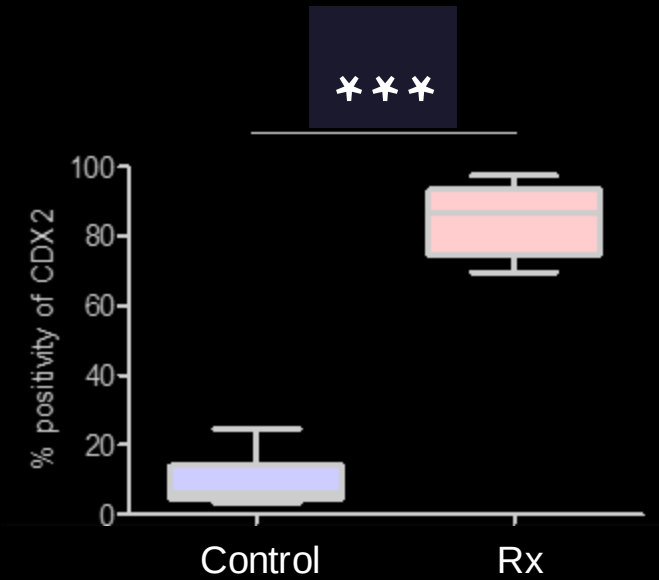
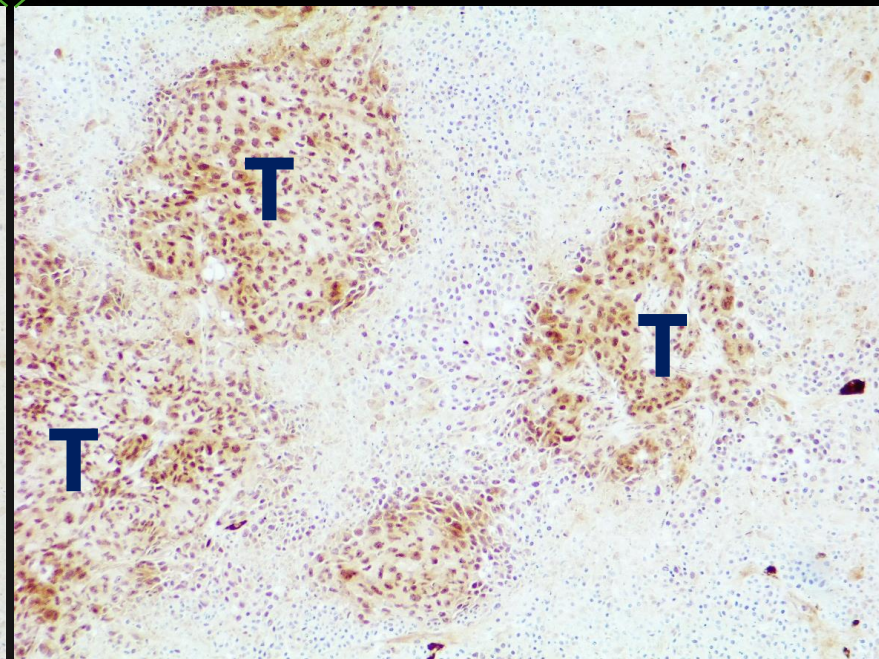
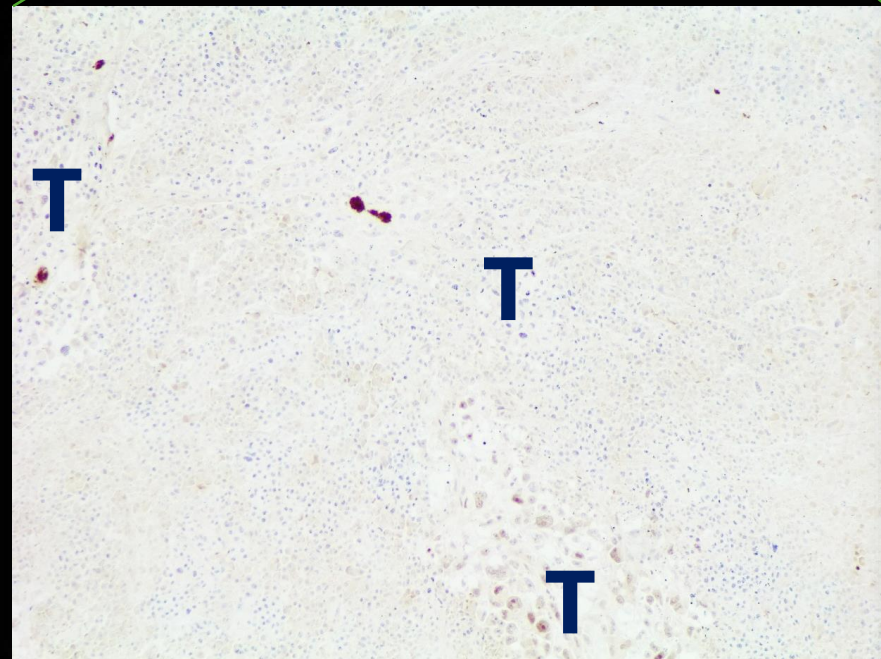
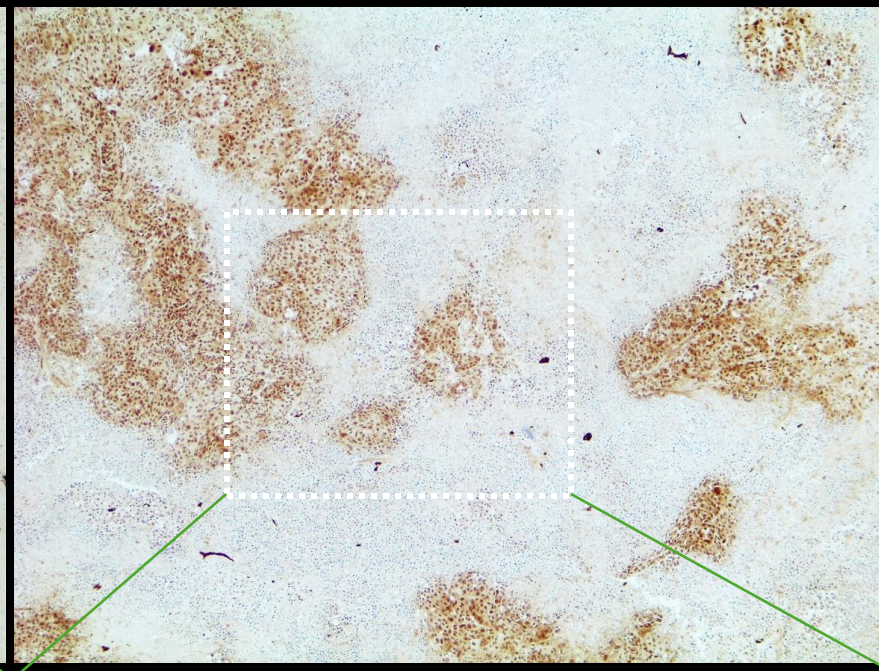
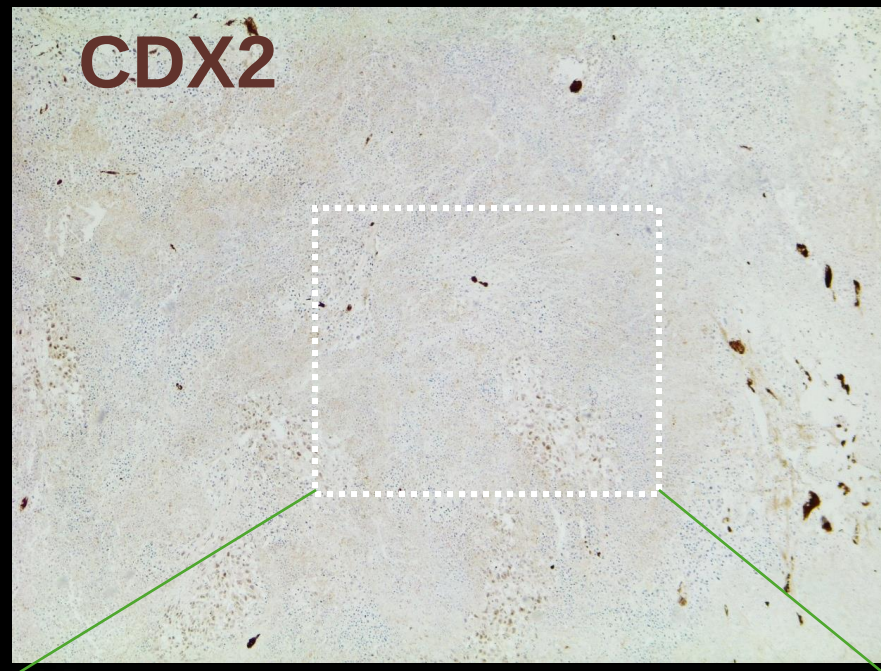


Vehicle

PF

**CDX2 is
successfully
induced in
HCT116 cell
xenotransplants**

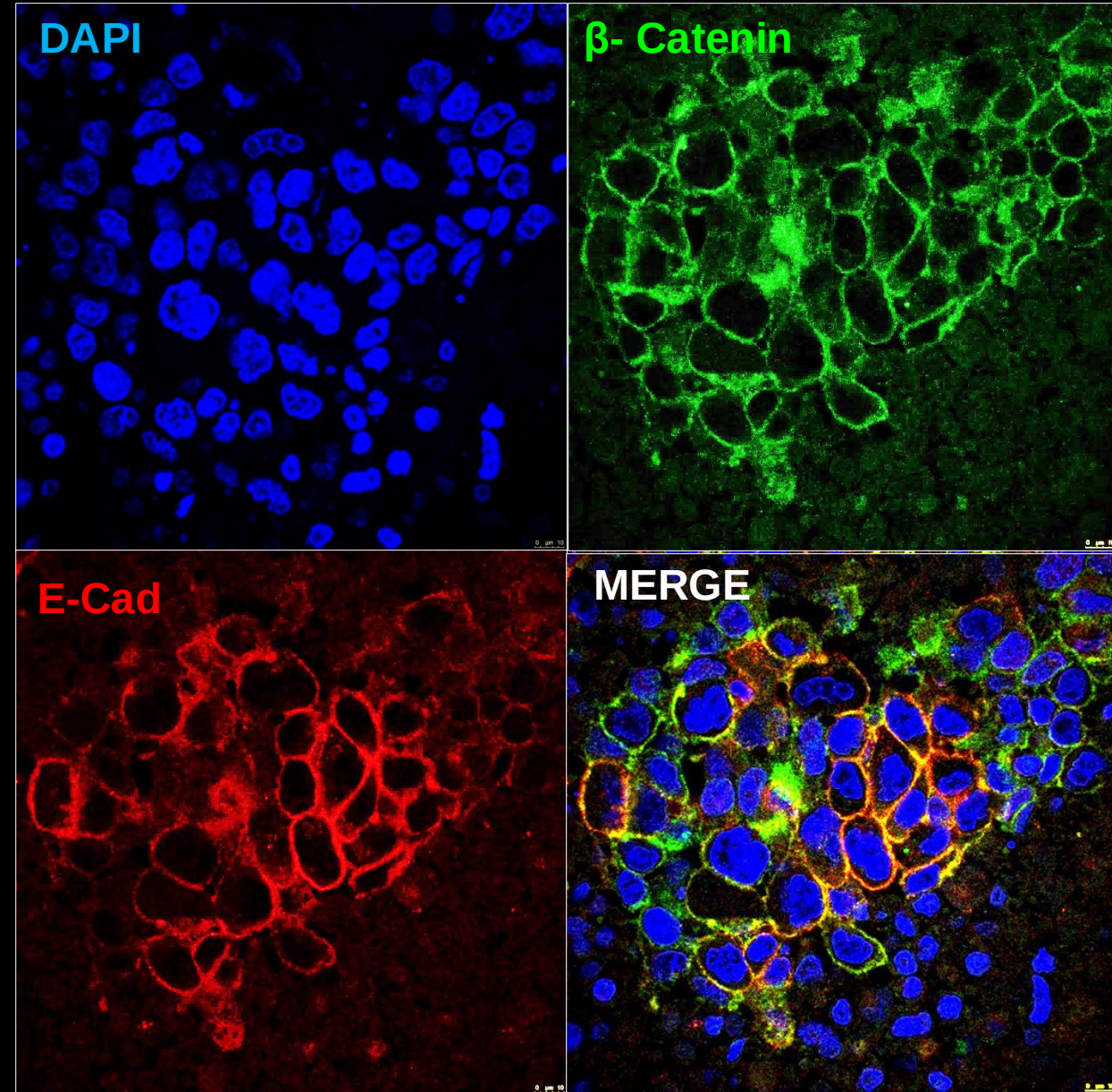
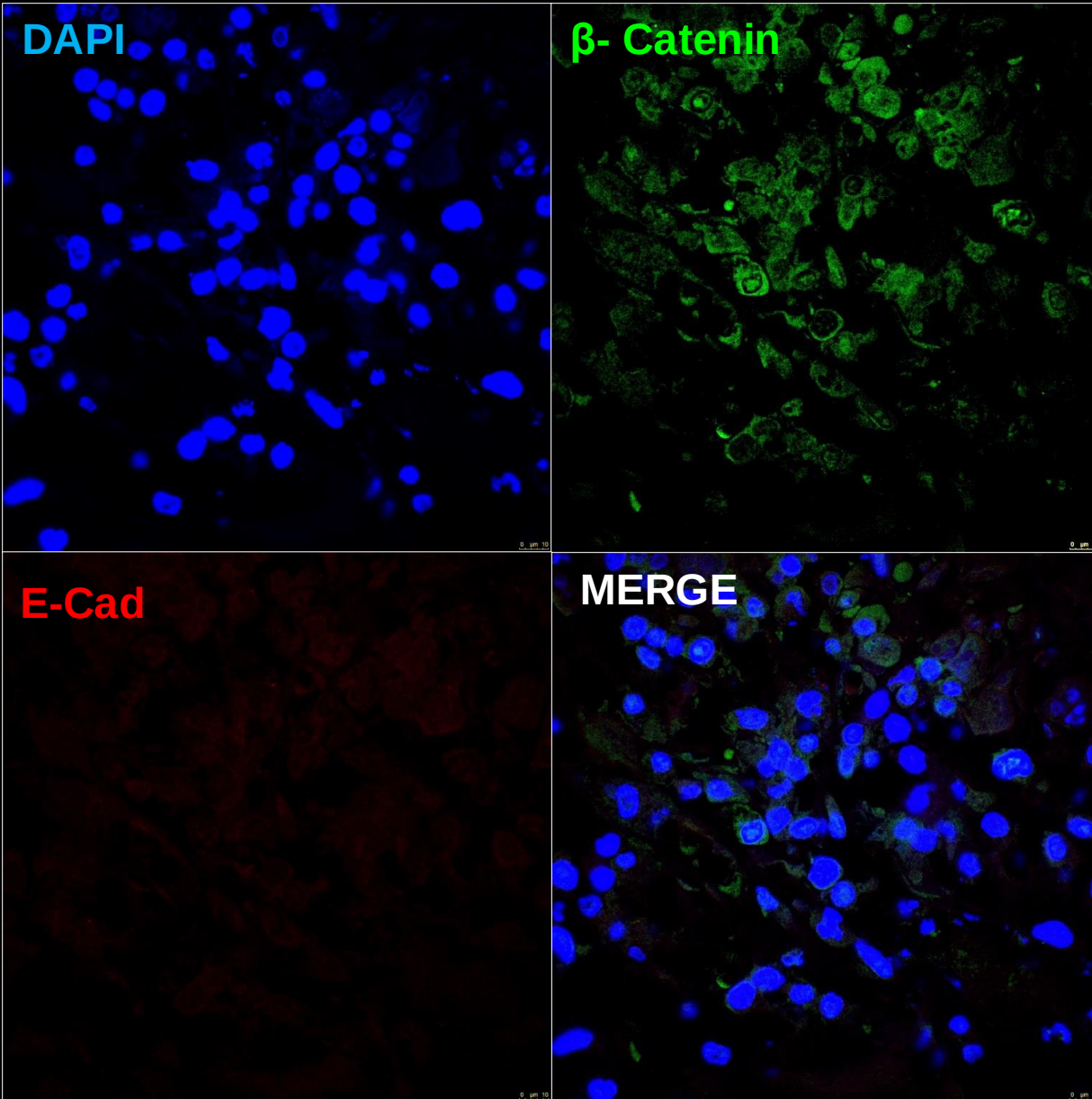
T = viable tumor



CDX2 induction promotes β Cat/E-Cadh localization at junctions

Veh-treated Xenografts

PF-Treated Xenografts



DOES IT WORK IN HUMAN PHASE 'ZERO' TRIAL?

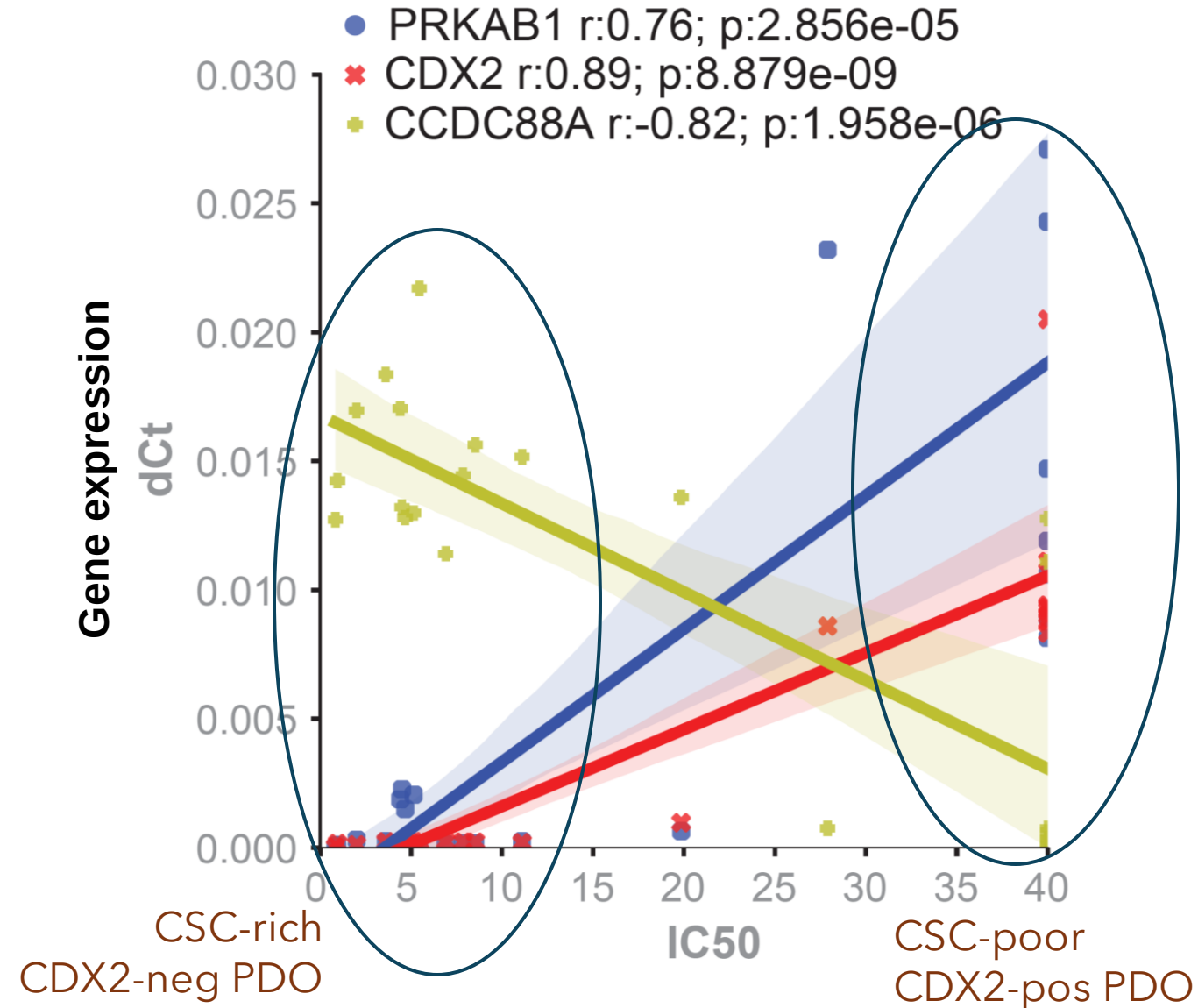
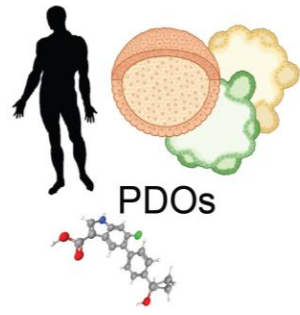


We Leveraged The ATCC® Catalog to Build a Cohort

ATCC® Cat No.	Cohort #	Age	Sex	Race	Primary Site
PDM-255™	1	73	F	White	Colon
PDM-356™	2	58	M	Unk	Colon
PDM-8™	2	75	M	Asian	Colon
PDM-191™	1	56	M	White	Rectum
PDM-264™	2	67	F	White	Rectosigmoid junction
PDM-5™	2	60	F	White	Colon
PDM-275™	1	73	F	White	Colon
PDM-4™	1	50	M	White	Colon
PDM-95™	2	61	M	Black	Colon
PDM-279™	1	51	M	Black	Colon
PDM-2™	1	68	M	White	Colon
PDM-50™	1	78	M	White	Colon

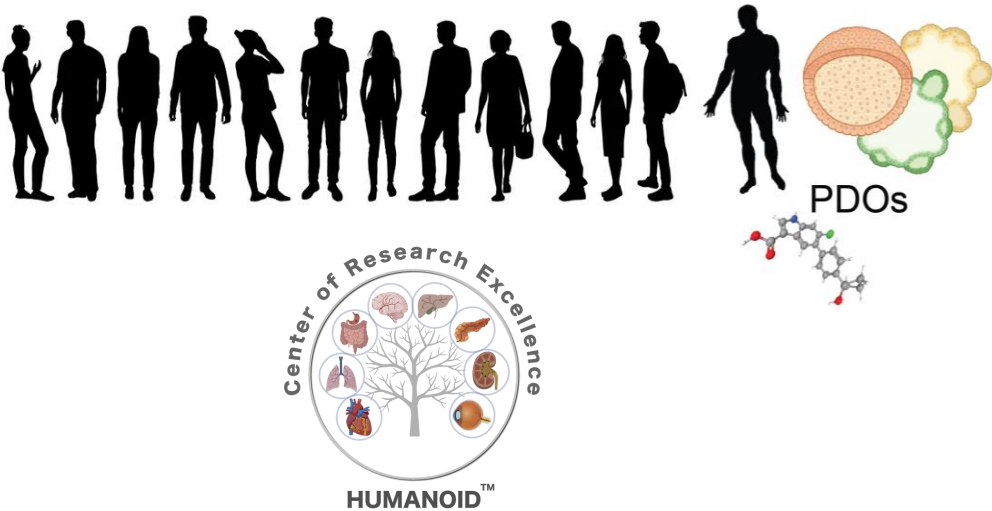
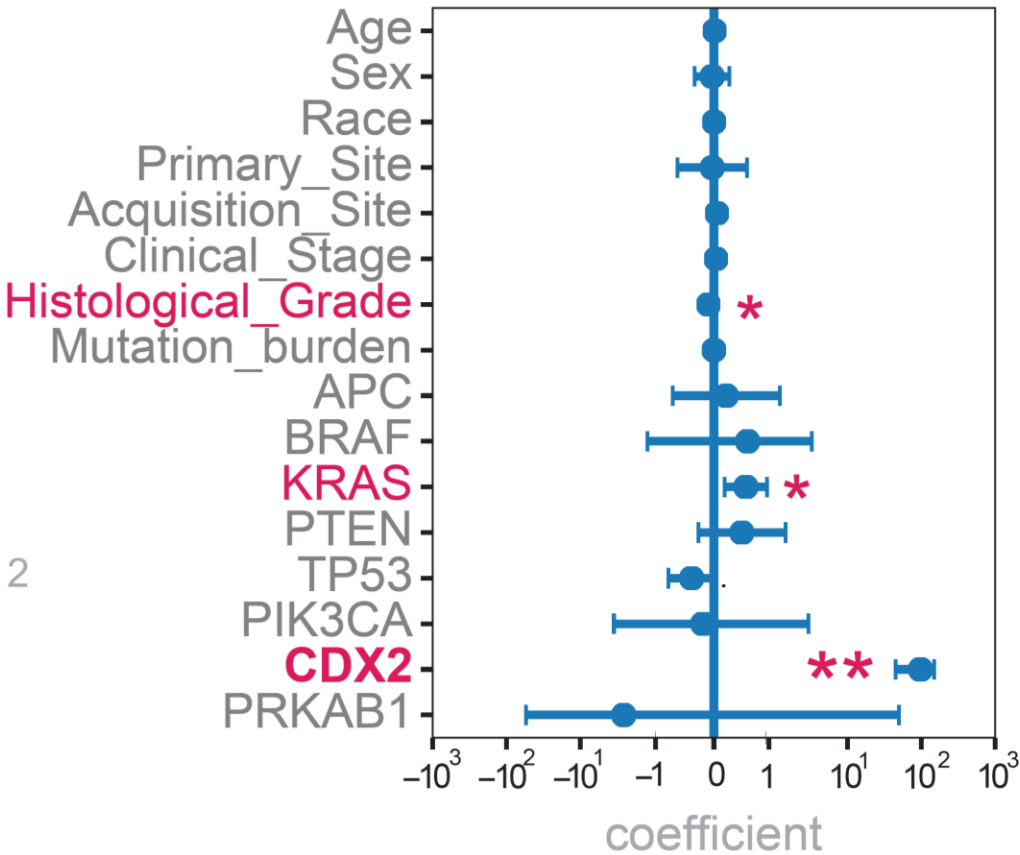
ATCC® Cat No.	Cohort #	Age	Sex	Race	Primary Site
PDM-9™	1	63	M	Asian	Colon
PDM-276™	2	54	M	Black	Colon
PDM-1™	1	75	M	White	Colon
PDM-185™	1	71	M	White	Colon
PDM-94™	2	67	M	White	Colon
PDM-7™	2	75	M	Black	Colon
PDM-257™	2	53	F	Unk	Rectosigmoid junction
PDM-363™	2	72	M	Unk	Colon
PDM-277™	2	76	F	White	Colon
PDM-359™	2	64	F	Unk	Colon
PDM-103™	2	51	F	Black	Colon

ONLY LOW CDX2 PDOs ARE SENSITIVE TO RX



CDX2, GRADE AND KRAS STATUS ARE CO-VARIATES

Multivariate analysis



Saptarshi Sinha

OBJECTIVE METRICS:

- ❑ Reinstatement CDX2;
- ❑ Reverse differentiation-axis;
- ❑ Reset the network



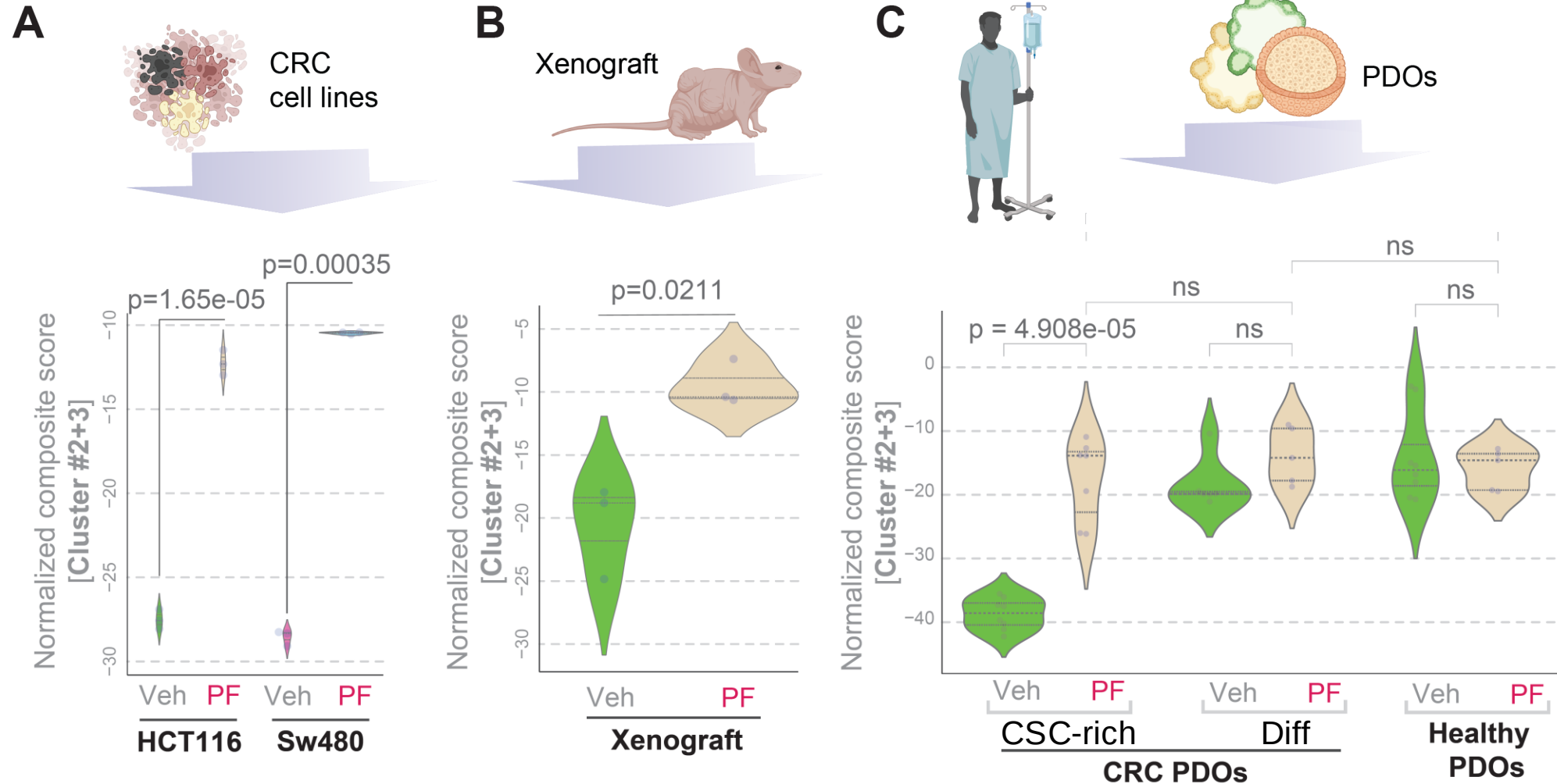
Sahar Taheri
(GSR, CSE)



Saptarshi Sinha
(PDF, CMM)

Impact on Network

[Induction of C #2+3]



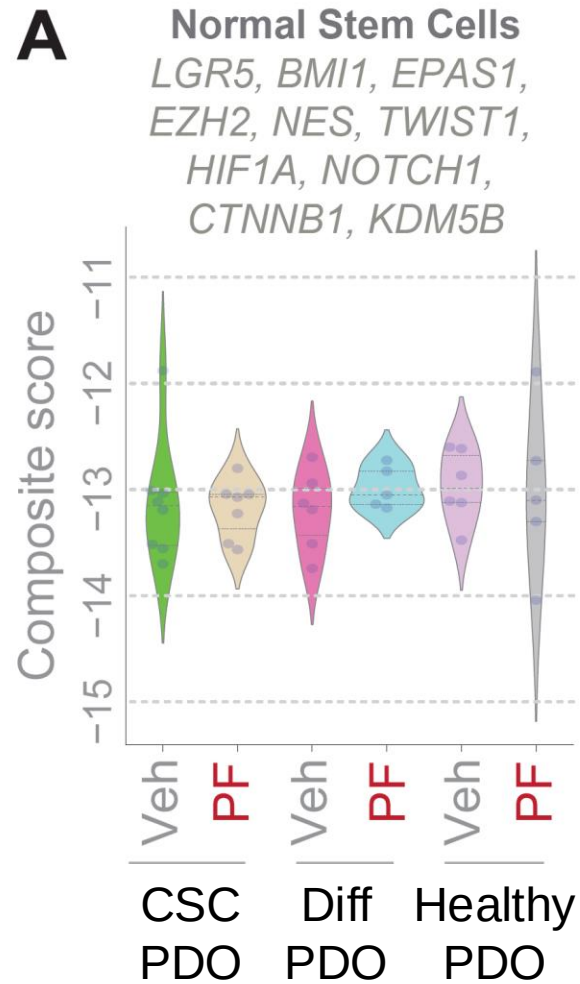
CAN WE MEASURE IMPACT?

1) SAFETY

2) EFFICACY [SAVE LIVES]

3) OTHER RX MODALITIES

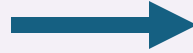
SELECTIVITY: RX KILLS CSCs; NOT NORMAL STEM OR DIFFERENTIATED CELLS



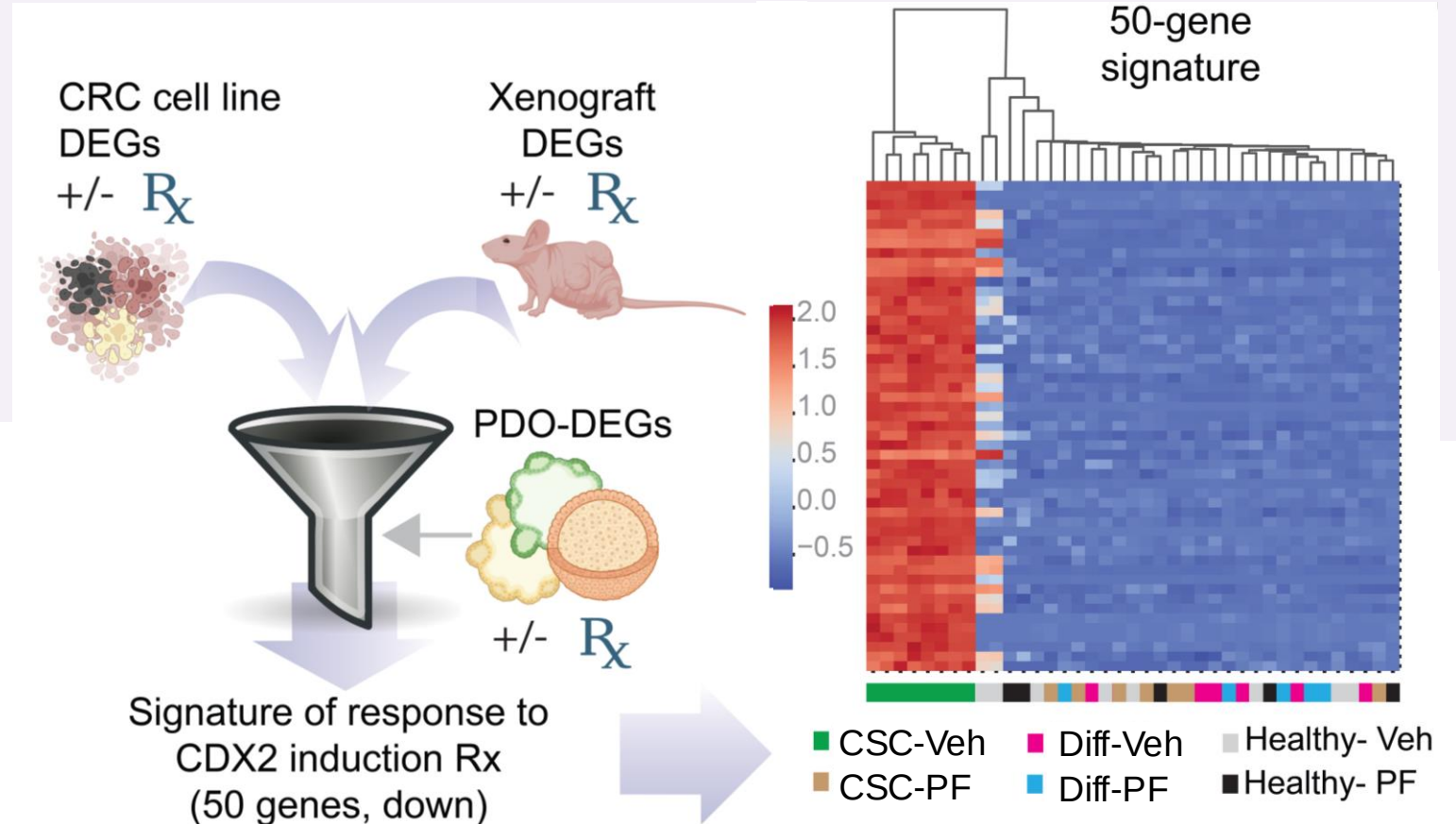
CAN WE MEASURE THE IMPACT OF RX ON LIVES OF PATIENTS?

Step 4: Impact of Differentiation Therapy

Estimation of the impact of therapeutic reinstatement of CDX2 on disease-free/relapse-free (DFS/RFS) and overall (OS) survival



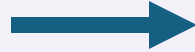
Integrated differential expression analysis between treated vs untreated samples using all 3 models



CAN WE MEASURE THE IMPACT OF RX ON LIVES OF PATIENTS?

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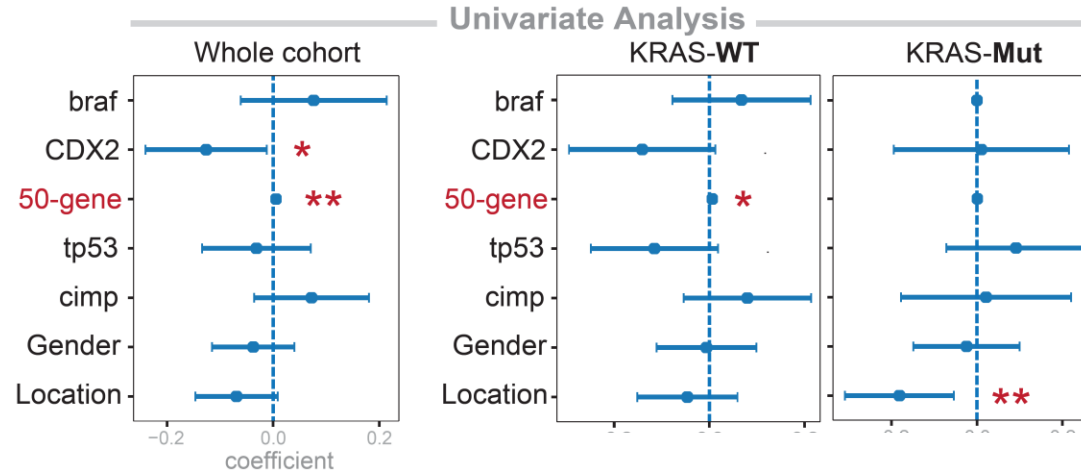
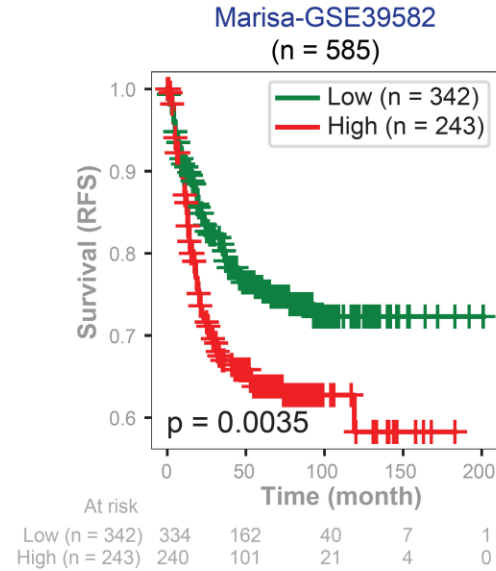
Derivation of a 50-gene signature of therapeutic response



Prognostic impact of the 50-gene signature assessed on 2472 unique subjects with CRCs in 10 independent cohorts

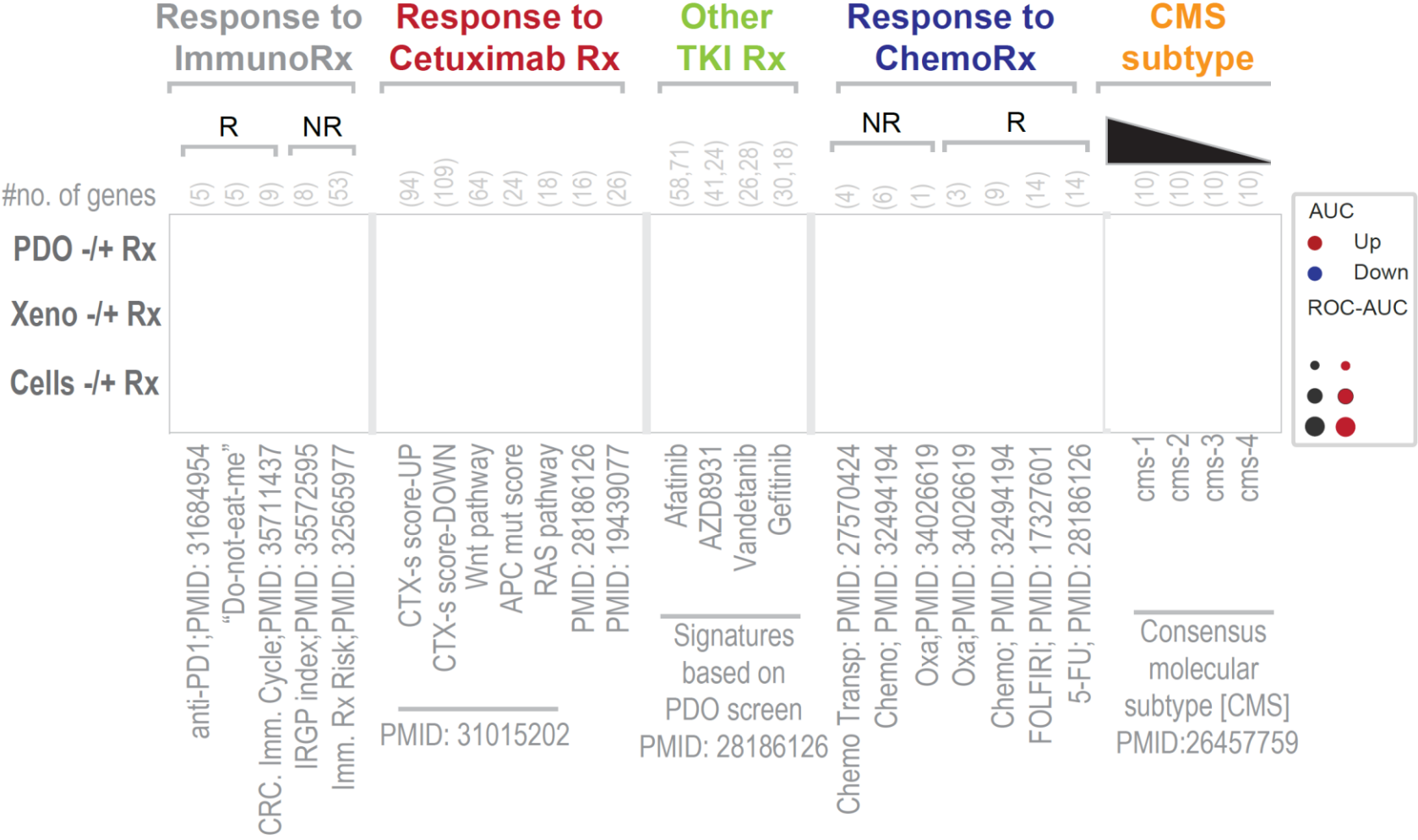
Univariate analysis based on the Cox-proportional hazards method on 585-patient cohort

SUPPRESSION OF 50-GENE SIGNATURE SHOULD IMPROVE SURVIVAL



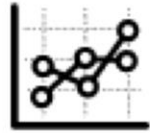
Saptarshi Sinha, Ph.D

EXPECT SYNERGY WITH EXISTING MODALITIES



Evaluating Our Metrics Of Success

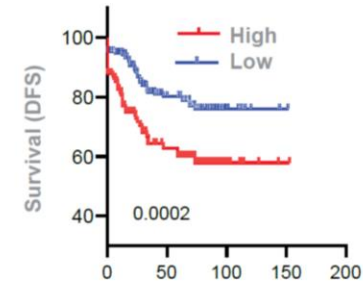
Network-
based
Metrics of
Success &
Impact



Predictable
network
perturbation



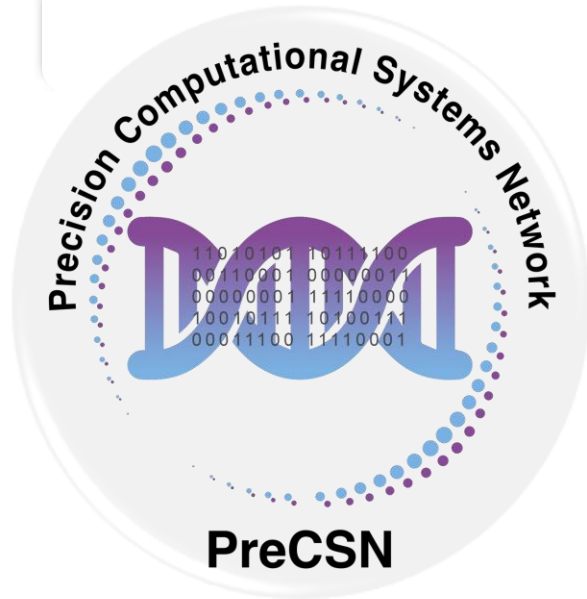
Rx specificity,
Synergy with other
Rx modalities



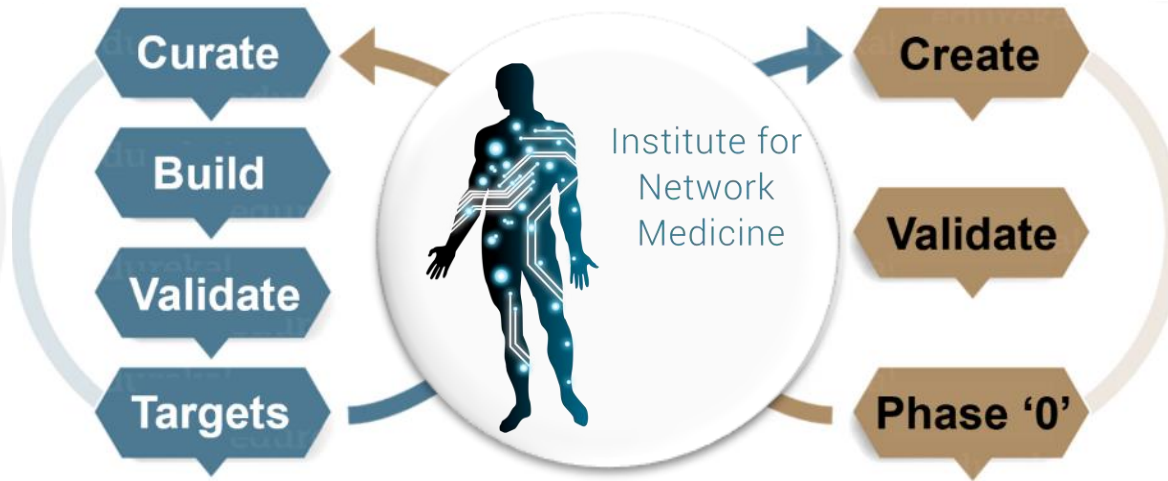
Relapse-free
survival

MEET US AT PHASE '0'

Thank You



USING BOOLEAN NETWORK
EXPLORER [BoNE]



USING HUMAN ORGANOID-
BASED MODELS OF DISEASES

Resources



Browse and search unreleased HCMI models at ATCC:
www.atcc.org/hcml-input

Email us which HCMI models are most relevant for your research

- Contact us at: hcml@atcc.org
- HCMI Searchable Catalog <https://hcml-searchable-catalog.nci.nih.gov/>
- New Paper! CANDiT: A Machine Learning Framework for Differentiation Therapy in Colorectal Cancer, Sinha et al. <https://doi.org/10.1016/j.xcrm.2025.102421>

