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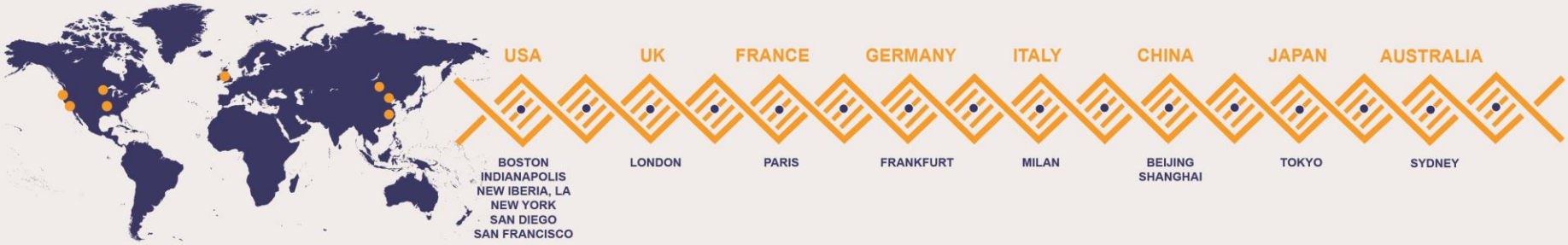
3 Strategies to Identify Predictive Biomarkers in the Preclinical Space

Mike Batey
Crown Bioscience



Speaker
Mike Batey

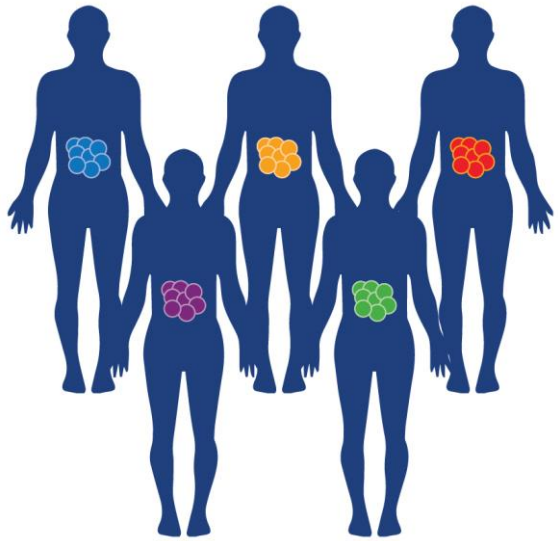
- Over 20 years experience in preclinical and translational science, business development, and marketing, across a variety of academic, industrial, and commercial roles
- Worked at Northern Institute for Cancer Research, Newcastle, making key contributions to the development of a number of novel anticancer drugs now licensed or in late stage clinical trials
- Previous roles have included working as an independent consultant to pharma and biotech, and Head of Oncology and Preclinical Imaging at Epistem

Webinar Overview

- Describe the importance of predictive biomarker development in early stage drug discovery
- Discuss how to use *in vitro* models to uncover genetic signatures of response and guide *in vivo* model selection for further validation
- Explore drug mechanism of action using patient-derived xenograft (PDX) models enabling clinical-preclinical data corroboration
- Review how identifying biomarkers early in drug development enables researchers to gain in depth insight into mechanism of action and pharmacodynamic response
- How to translate preclinical biomarkers into the clinic, making data informed decisions on trial design, and enabling identification and stratification of relevant patient populations

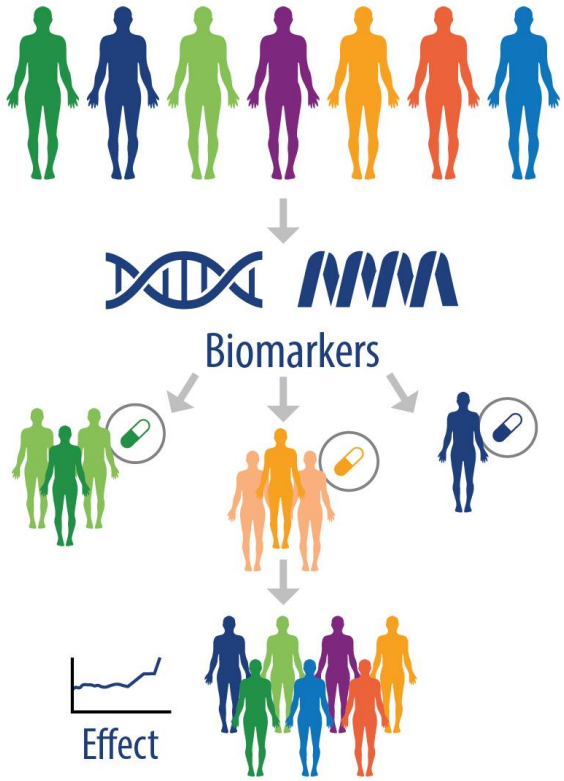
Heterogeneity in Cancer Treatment

- The majority of experimental cancer drugs fail during the later stages of clinical development, after considerable time and expense has been invested
- Typically, when diagnosed with cancer, patients have received similar treatment as others with the same type and stage of disease
- This approach does not accommodate the heterogeneity of cancer as a disease, or the genetic changes which can occur in individual patient tumors
- This diversity can help explain why the range of response in individual patients differs so widely from one case to another



Precision Medicine

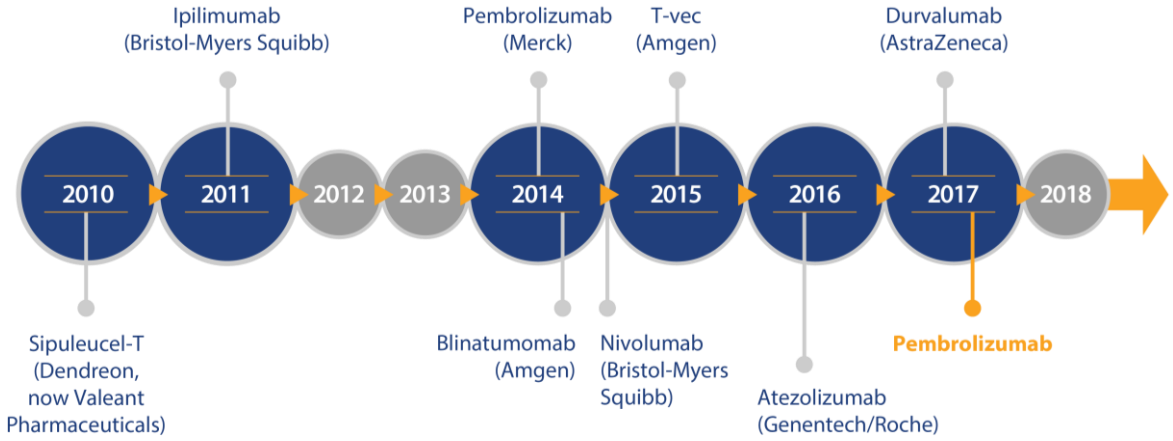
- This has led to the development of approaches targeting the right patient with the right drug at the right time – precision medicine
- Increased development of drugs targeting specific tumor characteristics
- Allows clinicians to select treatments based on a deeper understanding of patient disease:
  - Targeted therapy
  - Hormone therapy
  - Immunotherapy
- Over 6,000 compounds are currently in development, needing predictive biomarkers for patient stratification



Keytruda® Story

- A landmark for precision medicine and companion diagnostics (CDx)

Approved Immuno-Oncology Therapies



Pembrolizumab Approved for patients with unresectable or metastatic solid tumors

- Criteria: Tumors with MSI-H (high micro-satellite instability) or dMMR (mismatch repair deficiency)
- First ever cancer treatment approved by FDA on the basis of a common biomarker rather than the tumor cell of origin

Vitrakvi®
Another CDx Landmark

- First treatment to receive a tumor-agnostic indication at the time of initial FDA approval
- First TRK inhibitor approved for the treatment of:
  - Adult and pediatric patients
  - With solid tumors
  - With a TRK gene fusion without a known acquired resistance mutation
- 79% overall response rate (ORR) (n=121, 95% Confidence interval 72-85) including 16% complete response and 63% partial responses

> The approval of Vitrakvi is a testament to the relentless **prioritization of biology** in the drug development process. It is now even more critical to **screen patients** of all ages with advanced solid tumors for **actionable genomic insights** that could benefit their care or aid in their referral to clinical trials.
>
> **Josh Bilenker**
> MD, CEO Loxo Oncology **26 November 2018**

The Benefits of Early Biomarker Development

- Target validation and biomarker identification in early stage drug development is vital to identify promising candidate therapy at an early stage
  - Focus efforts on therapies with real potential
- The use of systems biology ensures appropriate target and model selection in the preclinical space
- Requires access to well characterized, large suite of models reflecting patient diversity
- Allows researchers to funnel their therapeutic through *in silico*, *in vitro*, *ex vivo,* and *in vivo* model systems in an intelligent way
  - Reduces costs and timelines and maximizes data value
- Translation of preclinical biomarkers into the clinic as CDx allows identification and stratification of relevant patient populations to give the best chance of clinical success

CrownBio: A Translational Model Systems Company

- Help clients develop biological insights through the most comprehensive and relevant collection of **model systems** (both laboratory and *in silico*) and **data**
- Trusted R&D partner to help advance therapeutic candidates in both preclinical and clinical phases

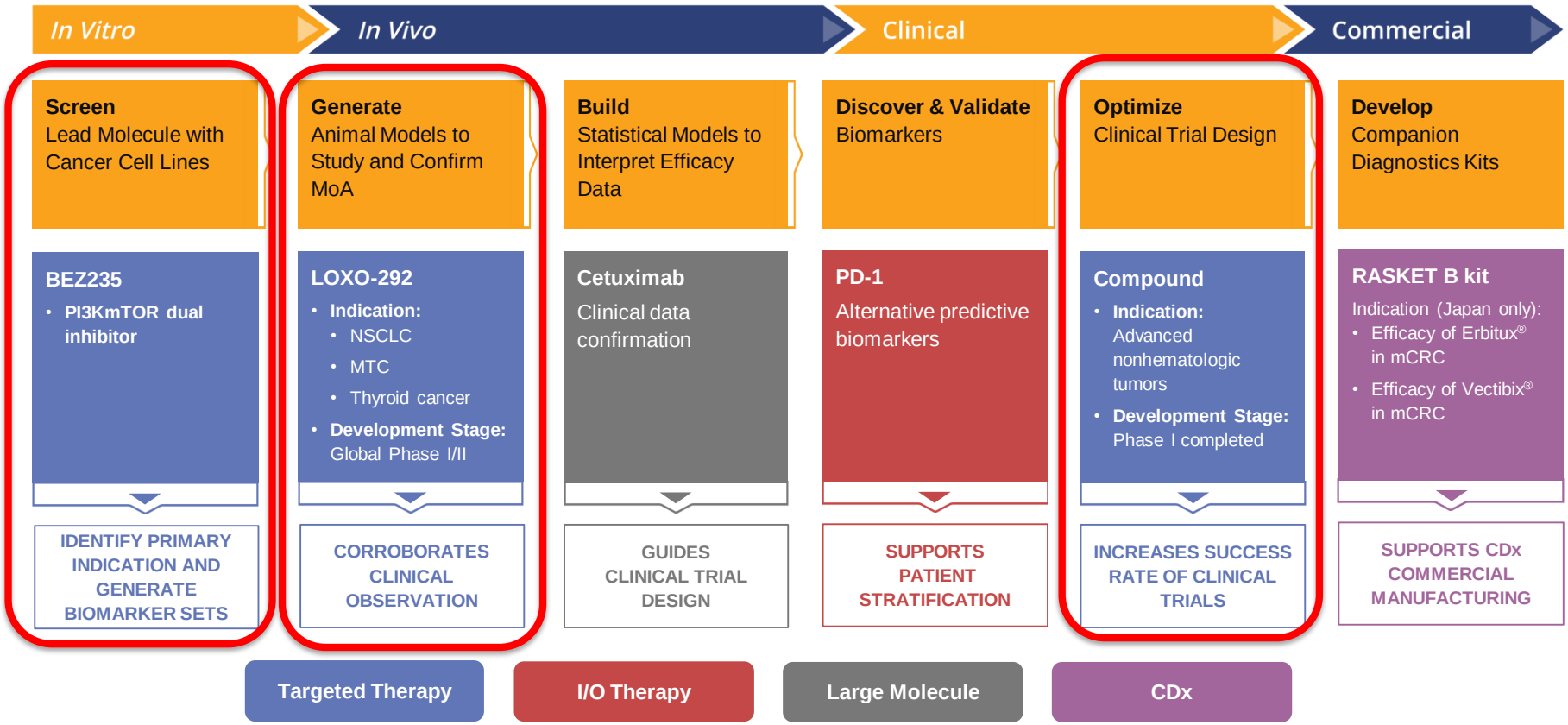
Genetic Aberrations	Immune-Deficiencies	Tumor Heterogeneity

ADDRESS

Laboratory Model Systems	***In Silico* Systems** (Methods + Data)
• ***In vivo*** model systems • ***In vitro*** assay systems • ***Ex vivo*** model systems • Organoid systems	• PDX and I/O efficacy databases • Genomic database • CVMD datasets • Biomarker discovery capabilities & track record • Data mining methods

Translational Platforms

Case Studies



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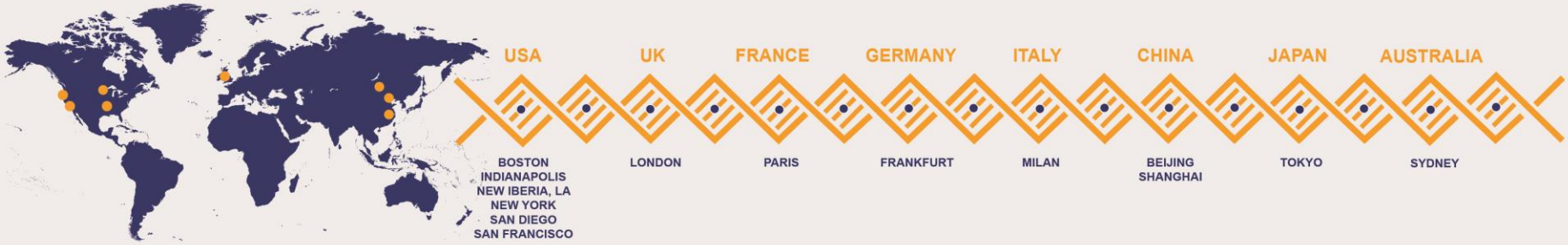
Case Study 1
Biomarker Identification from *In Vitro* Screening

Identifying the Primary Indication and Generating Biomarker Sets



Biomarker Identification from *In Vitro* Screening

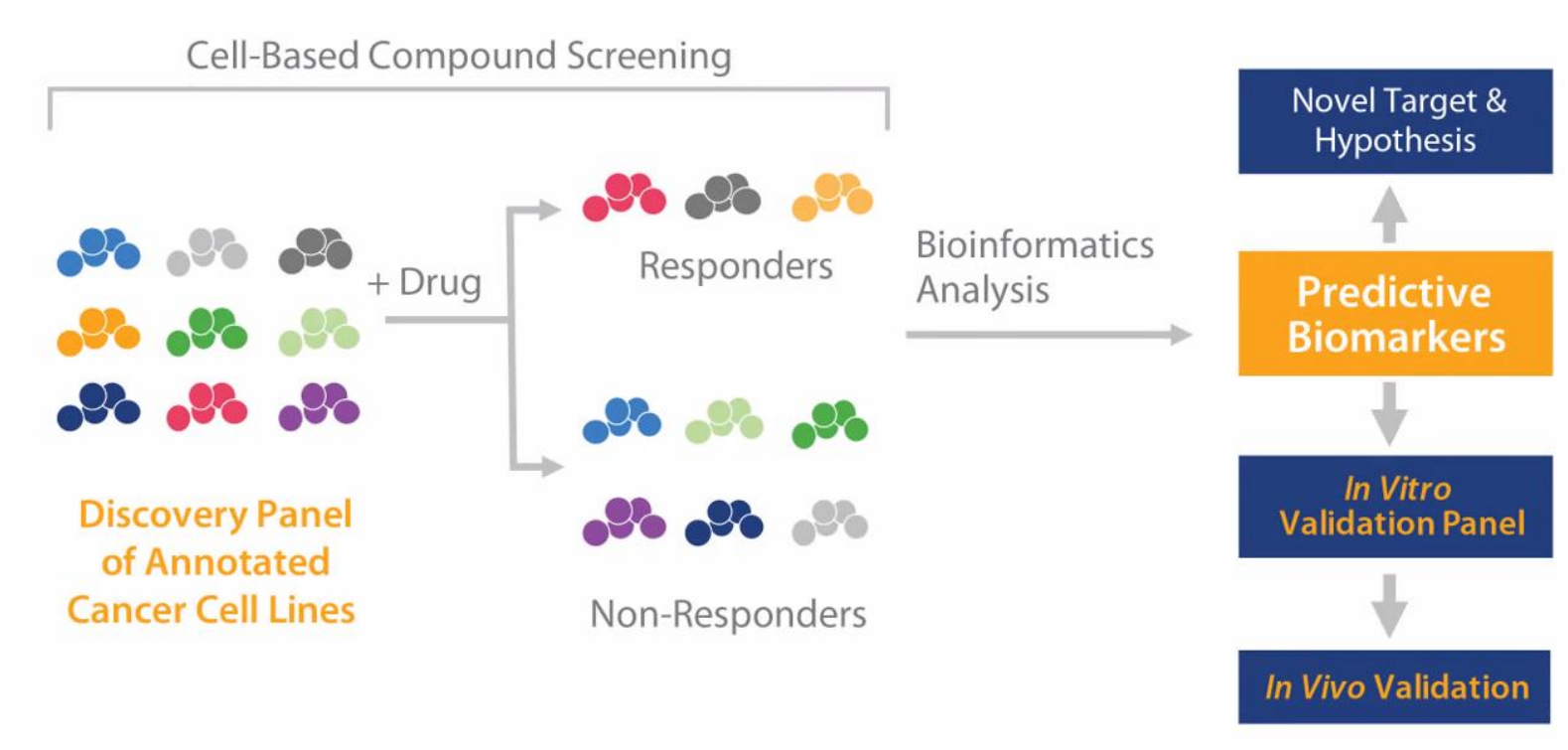
- BEZ235 is a PI3K/mTOR dual inhibitor
- Case Study 1 aimed to:
  - Predict response of cancer cell lines across a range of indications to BEZ235
  - Develop an understanding of the genetic signatures of responsive indications
  - Provide advice and guidance on future *in vivo* model selection

Case Study 1 Approach

- Examine the anti-proliferative activity of BEZ235 in 307 human cancer cell lines of different cancer types
- Correlate pharmacology data with genomic baseline information including:
  - Gene expression
  - Gene mutation
  - Copy number variation
  - Pathway/network activation

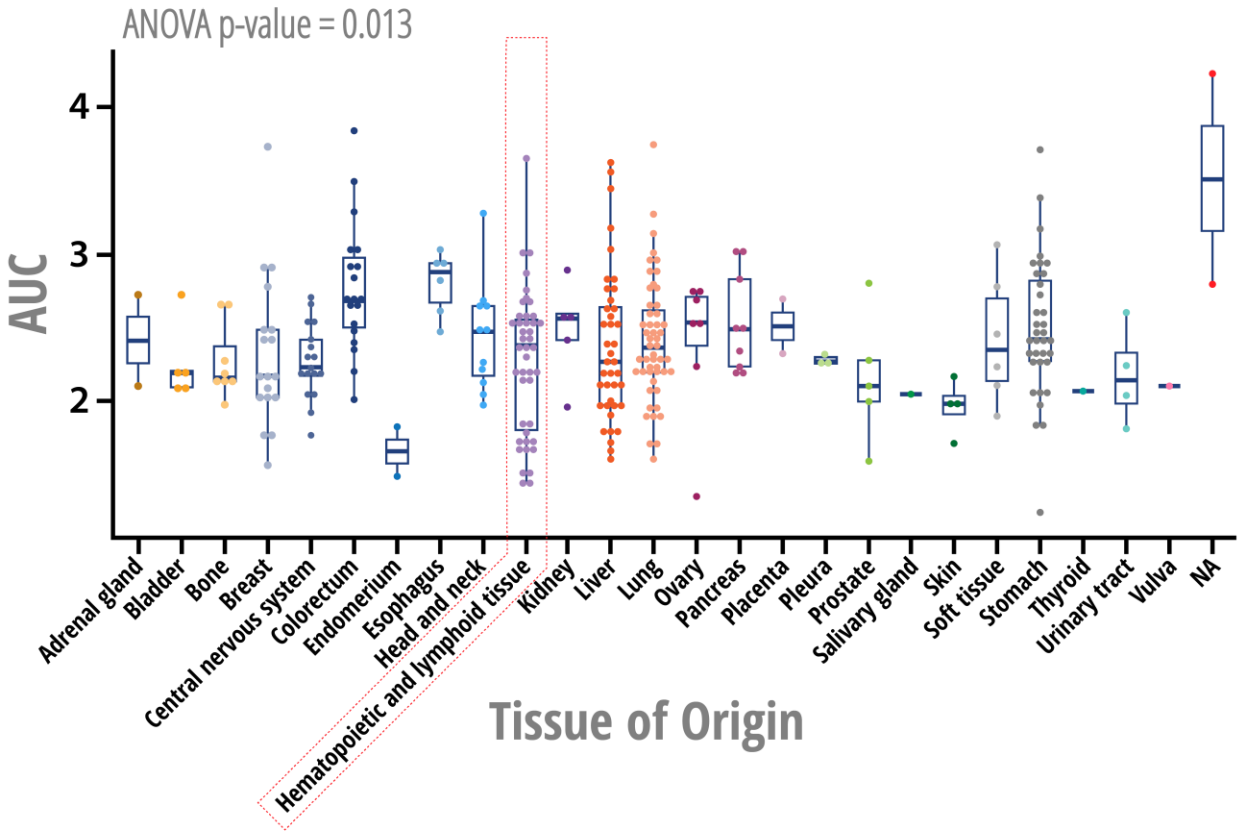
Biomarker Discovery - *In Vitro*

- *In vitro* biomarker discovery can be conducted using data from cell line and organoid screens



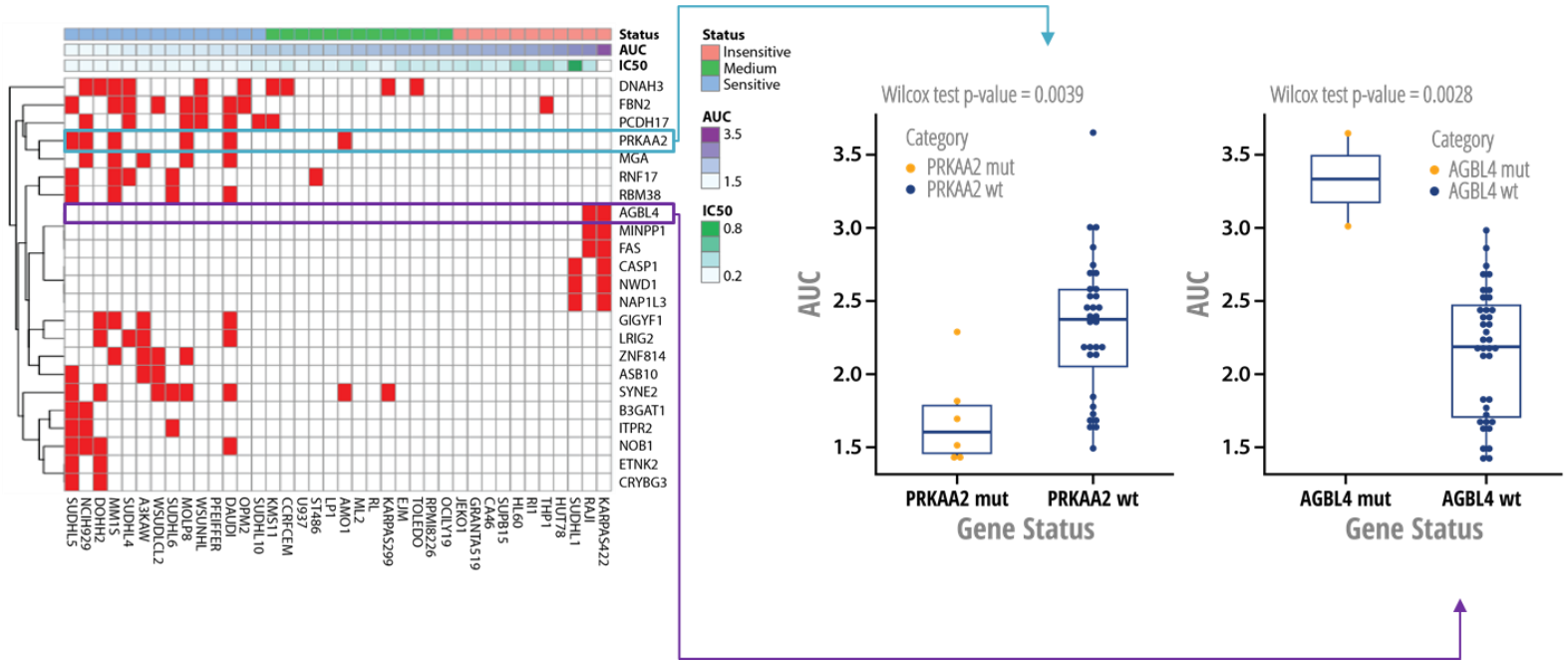
Finding Cancer Types Sensitive to Candidate Molecule

- Cell panel screening for BEZ235 in 307 cell lines indicated significant variance across cancer types



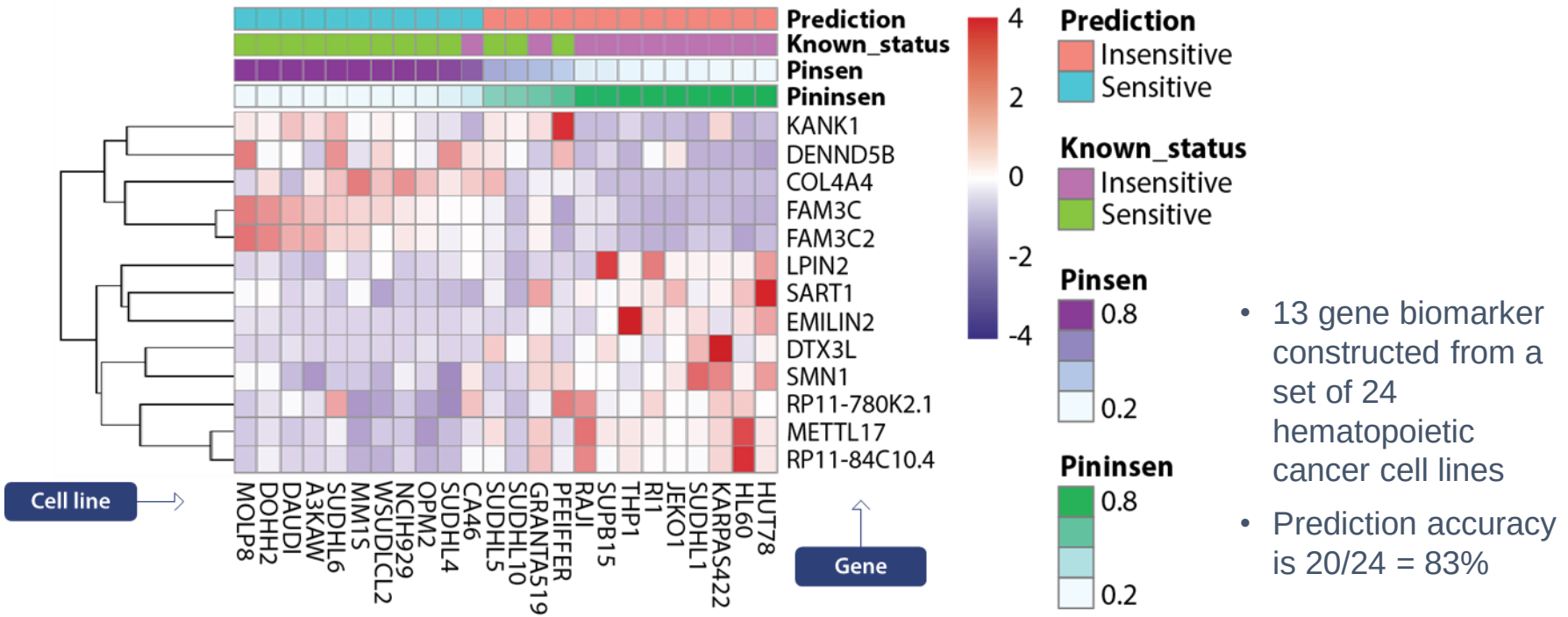
Mutational Status and BEZ235 Sensitivity

- Complete analysis of genomic mutational landscape performed and candidate genes nominated, out of over 20,000 genes sorted according to statistical significance
- Correlation and inverse correlations identified between response to test molecule and genetic mutations
- 23 genes identified whose mutational status strongly correlate with sensitivity to BEZ235



Transcriptome and Gene Expression Analysis

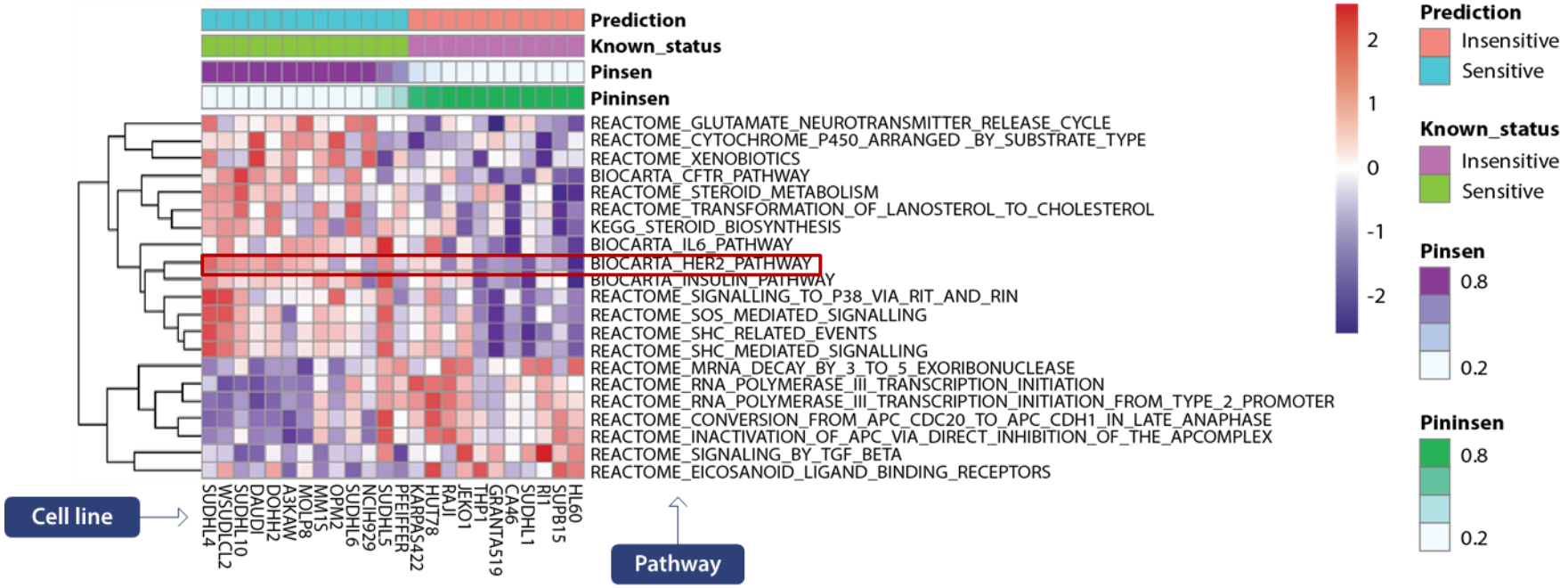
- 13-gene biomarker set expression analysis constructed
- Correctly predicts cell line response to BEZ235 in hematopoietic cancer cell lines



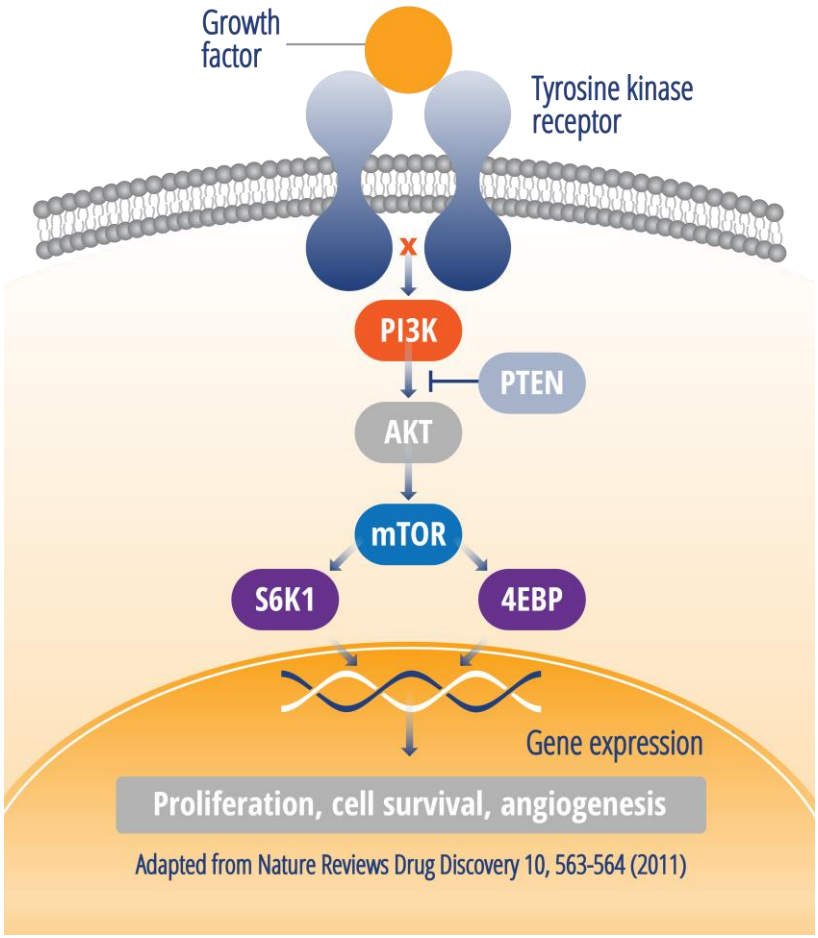
- 13 gene biomarker constructed from a set of 24 hematopoietic cancer cell lines
- Prediction accuracy is 20/24 = 83%

Pathway/Network Analysis

- 21 genes found which significantly differentiate in sensitive and insensitive cell lines



PI3K-Akt-mTOR Signaling Cascade



- One of the key pro-proliferation and pro-survival pathways involved in tumor growth and progression
- Activation of tyrosine kinase receptors recruits p85 subunit of PI3K to cell membrane
- mTOR one of the major downstream kinase of Akt
- mTOR activation leads to gene and protein expression profile modulation, which in most cases leads to tumor cell proliferation, survival, as well as activation of tumor angiogenesis
- BEZ235 is dual inhibitor of PI3K and mTOR

Case Study 1 Conclusions

- The Case Study results propose genetic signatures predicting sensitivity to BEZ235
- The mutation status of 23 cancer-associated genes strongly correlates with BEZ235 sensitivity
- 13 genes were identified whose expression levels significantly correlate with BEZ235 sensitivity
- 21 gene sets also identified which significantly differentiate in sensitive and insensitive cell lines
- The data allow determination of the most appropriate *in vivo* models to further investigate BEZ235

In Vitro Based Biomarker Discovery Summary

- CrownBio provides comprehensive screening of hundreds of commercial and proprietary cell lines covering over 30 cancer types
- In depth analysis of cell sensitivity to candidate molecules is identified, along with the corresponding NGS genomics data
- Optimized scoring matrix to nominate potential candidate indications/cancer types for further characterization
- Multiple tracks of unbiased, data driven statistical analysis in mutation, expression, and pathway to derive potential candidate biomarker sets for proper indications and potential patient selection criteria
- Preliminary screening that requires further iterative analysis – prelude to more in-depth functional and mechanistic studies *in vivo* and for clinical phase

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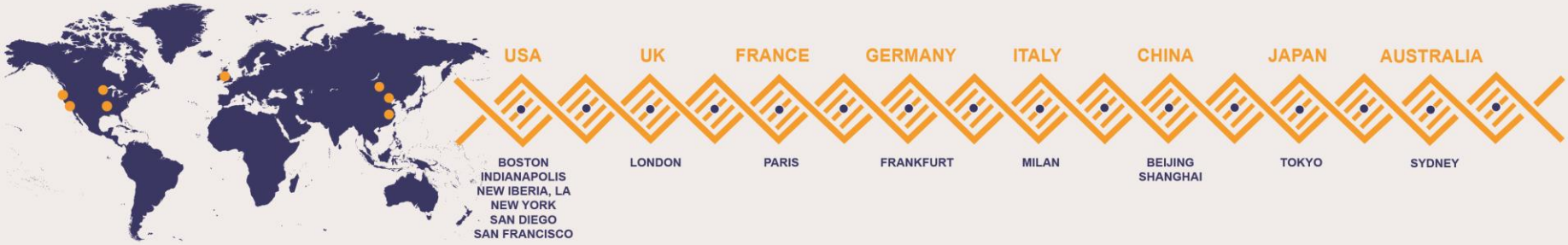
Case Study 2
Exploration of Drug Mechanism of Action using Unique PDX models

Corroborating Clinical Observations



Exploration of Drug Mechanism of Action using Unique PDX models

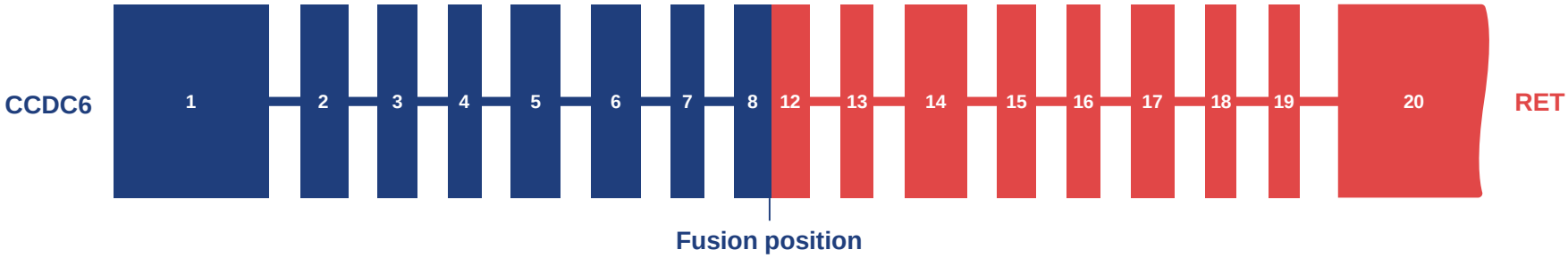
- Genomic alterations in the RET kinase, which include fusions and activating point mutations, lead to overactive RET signaling and uncontrolled cell growth
- RET fusions have been identified in a number of cancer types including NSCLC, and thyroid cancer
  - Activating RET point mutations account for approximately 60% of medullary thyroid cancer (MTC)
- Both RET fusion cancers and RET-mutant MTC are primarily dependent on a single activated kinase for their proliferation and survival
- This dependency, often referred to as “oncogene addiction,” renders such tumors highly susceptible to small molecule inhibitors targeting RET

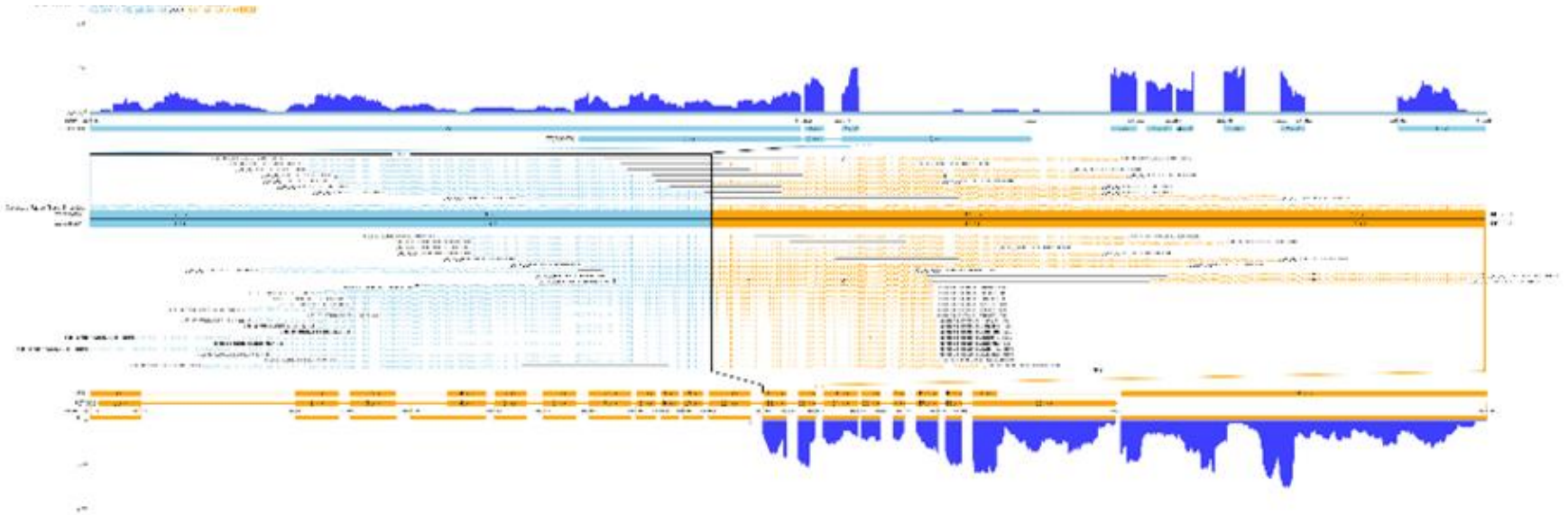
LOXO-292 MoA Confirmation Studies

- The LOXO Oncology drug selpercatinib (LOXO-292) was designed to inhibit native RET signaling, as well as anticipated acquired resistance mechanisms that could otherwise limit the activity of this therapeutic approach
- Case Study 2 aimed to confirm the mechanism of action of selpercatinib through:
  - Identification of an appropriate mouse model
  - Preclinical efficacy studies, in comparison with relevant standard of care cancer therapies

Recapitulating RET Fusion in a Unique PDX Model

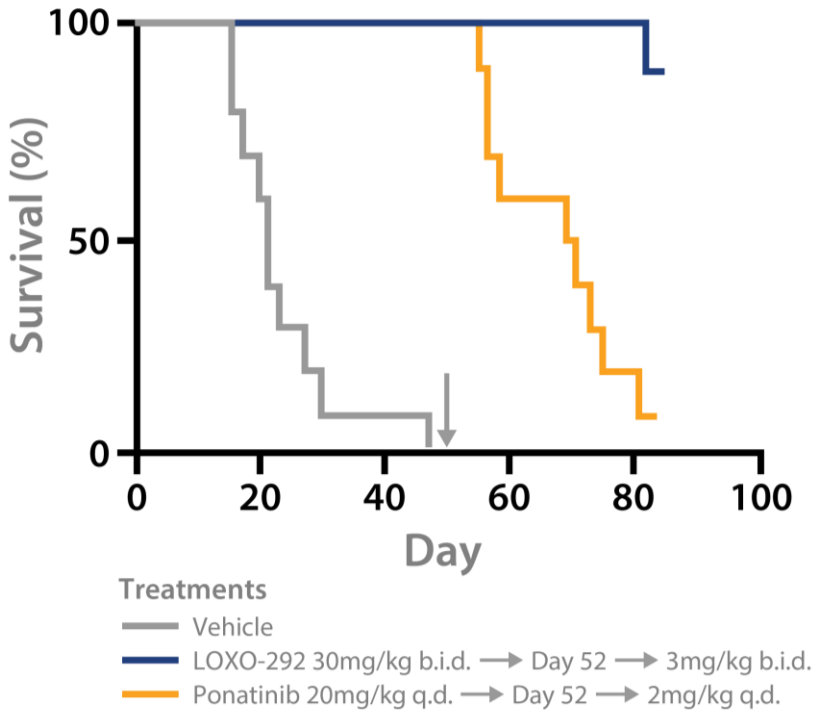
- PDX model CR2518





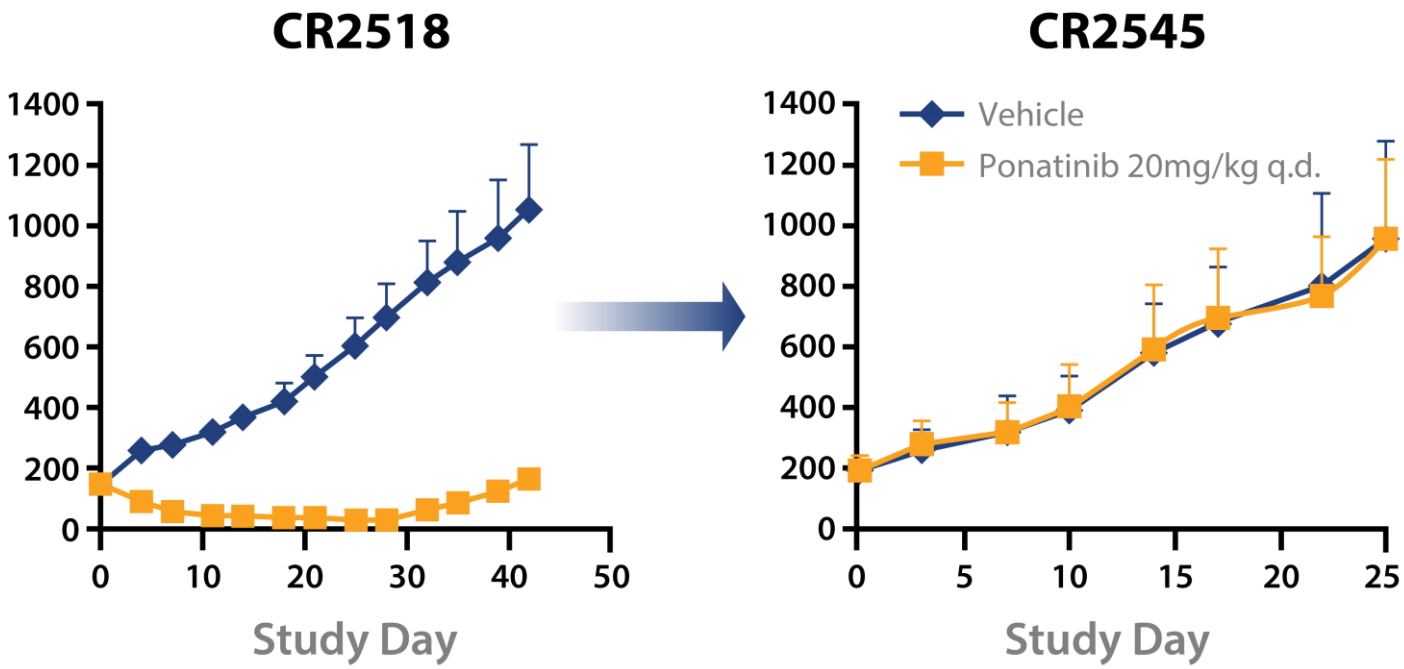
Orthotopic Brain Model Mimicking Metastasis

- PDX model CR2518 with CCDC6-RET fusion
- Orthotopic brain cancer model from CR2518 mimics metastasis
- LOXO-292 showed superior efficacy over standard of care (ponatinib)



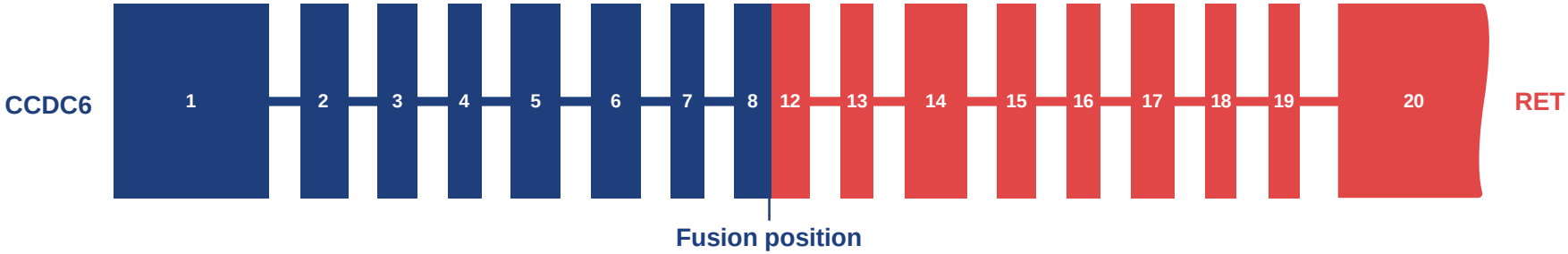
CR2518-Derived Treatment Resistant Model

- PDX model CR2545
- Induced drug resistance by extended ponatinib treatment

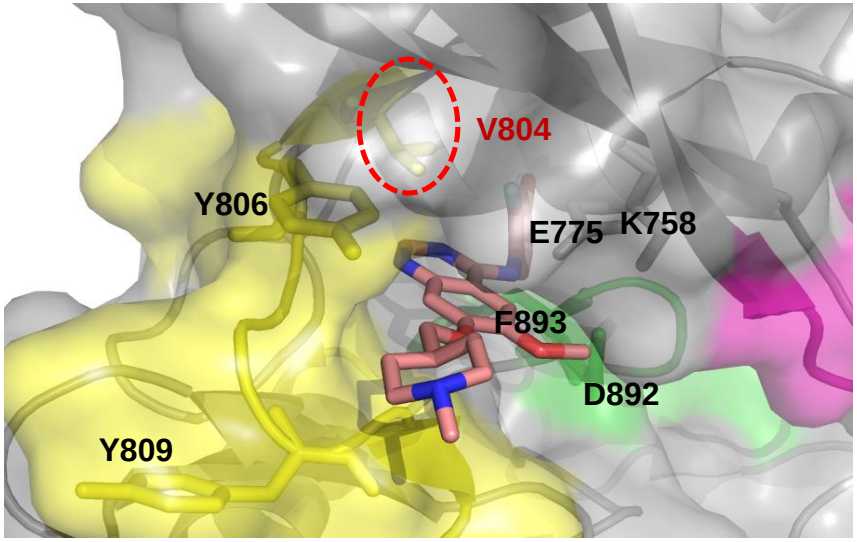


PDX CR2545 Genomic Profiling Identified Two Markers

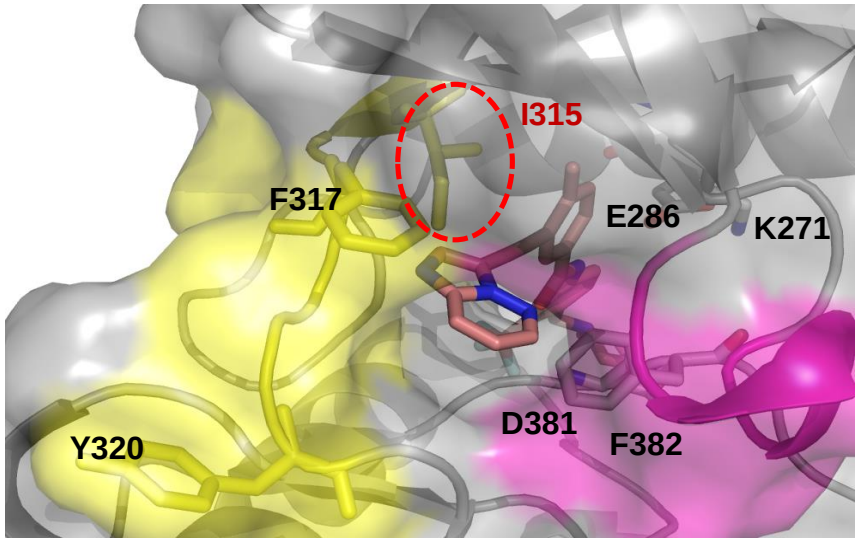
1. RET Fusion



2. RET V804M mutation



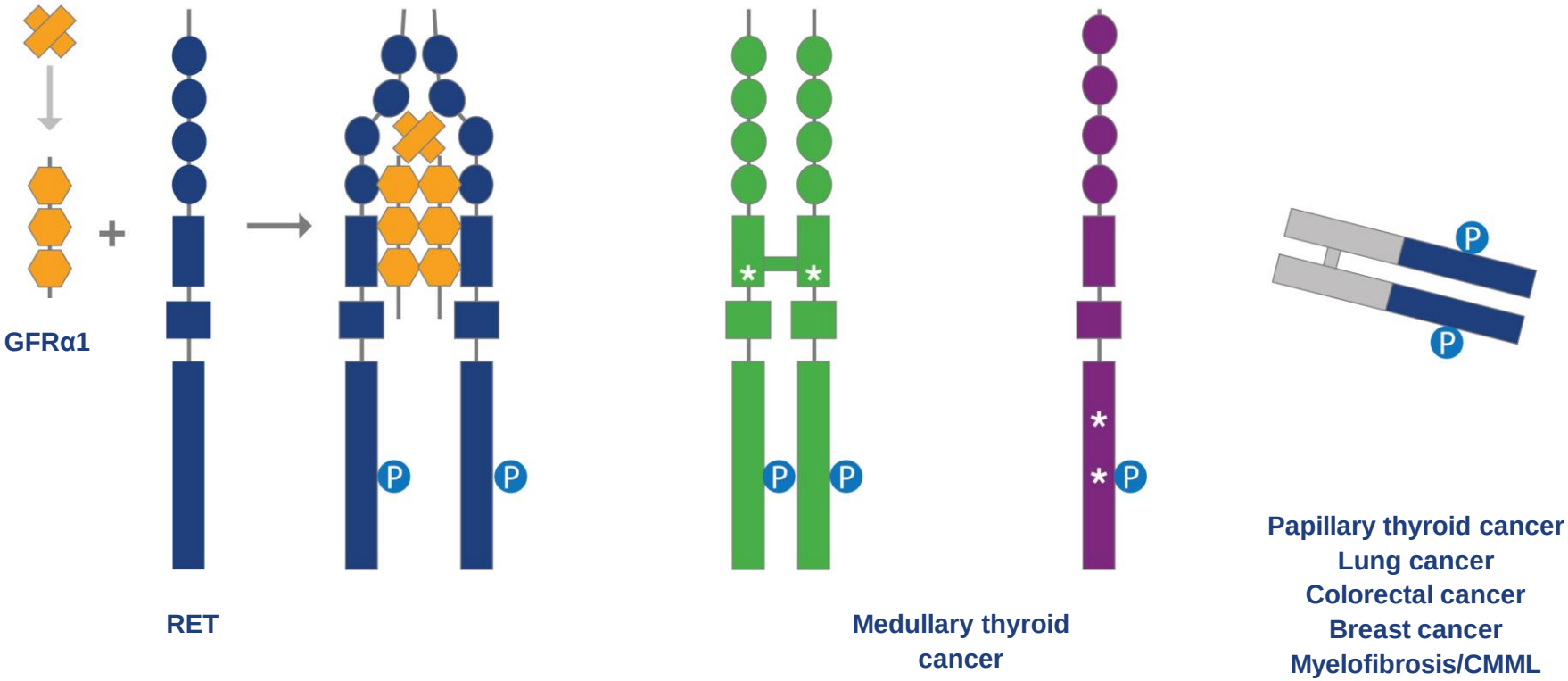
RET kinase: Vandetanib (PDB ID: 2IVU)



ABL: Ponatinib (PDB ID: 3IK3)

J Biol Chem. 2006 Nov 3;281(44):33577-87, Cancer Cell. 2009 Nov 6;16(5):401-12

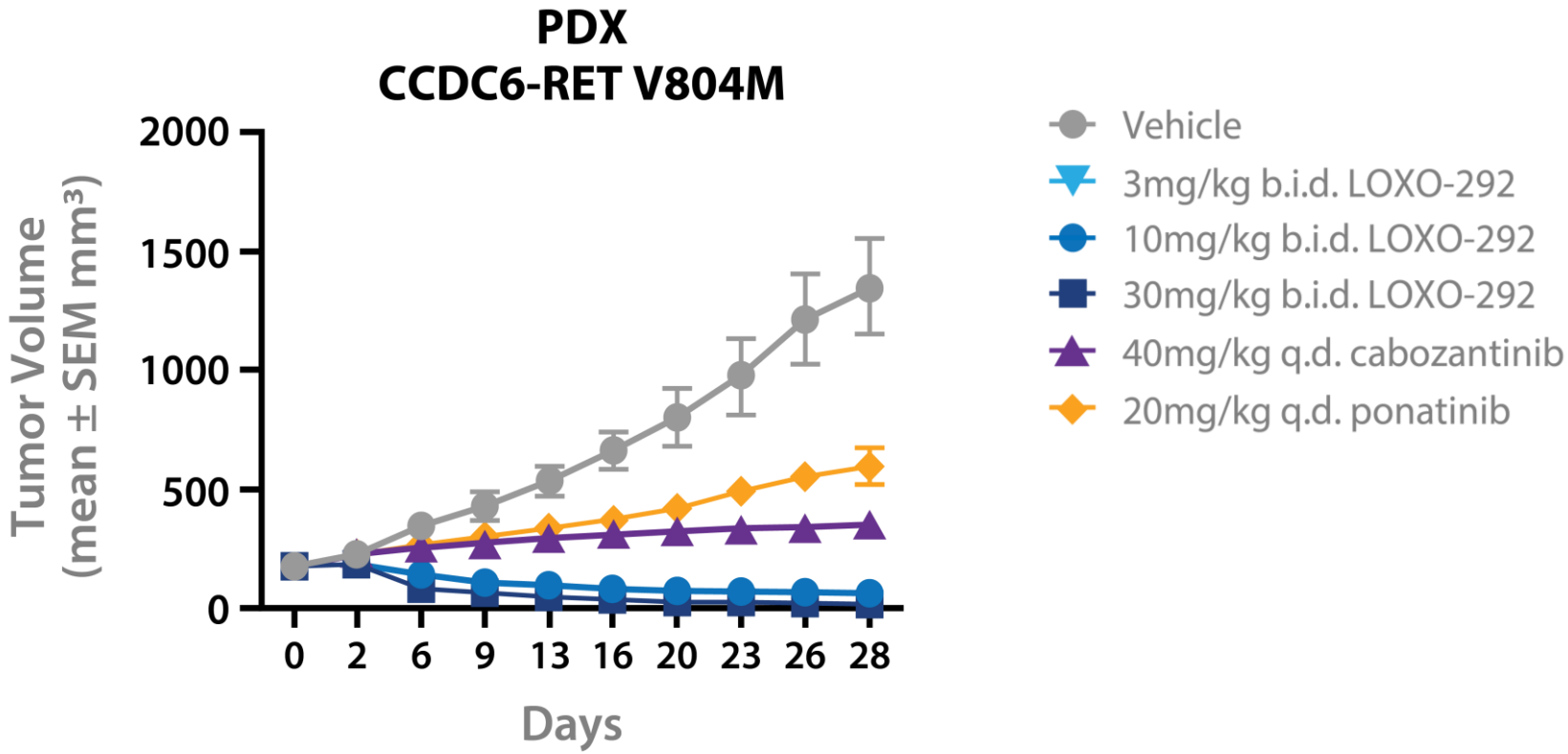
RET Alterations in Human Cancers



- **V804M** confers a gain of function of the RET protein, resulting in increased kinase activity, cell transformation, and is considered a gate keeper due to lack of response to some inhibitors, including cabozantinib and vandetanib

LOXO-292 Efficacy Studies with CR2545

- Dose dependent tumor growth inhibition in PDX model CR2545 harboring RET fusion and activating mutation



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Clinical Data Corroboration
LOXO-292 in RET-Altered Cancers

	RET Fusion-Position Cancers			RET-Mutated MTC	No Known Activating RET Alteration
	All	NSCLC	Other		
Enrolled	49	38	11	29	4
Eligible for Response Evaluation	47	38	9	29	3
ORR	**70%** (61%–89%)	**68%** (51%–83%)	**78%** (40%–97%)	**59%** (39%–77%)	**0%** (0%–71%)
Confirmed ORR	64%	66%	71%	56%	0%
CR	—	—	—	2	—
uCR	—	—	—	–	—
PR	30	25	5	13	—
uPR	3	1	2	2	—
SD	10	8	2	8	2
PD	2	2	—	2	1
Not Evaluable	2	2	—	2	—

Data cut-off:
April 2, 2018;
Follow-up as of July 19, 2018.
(TC +NSCLC)

NSCLC = non-small-cell lung cancer; **MTC** = medullary thyroid cancer; **CR** = complete response; **PR** = partial response; **SD** = stable disease; **PD** = progressive disease; **ORR** = objective response rate

Case Study 2 Conclusions

- Case Study 2 successfully demonstrated the MoA of LOXO-292
- A novel PDX model was developed (CR2545) by inducing existing PDX CR2518 through extended ponatinib treatment
- PDX model CR2545 confirmed to carry both a RET fusion and mutation
- Both models bearing driver mutations were used to compare the TGI of the drug candidate with SoC
- LOXO-292 showed superior efficacy over SoC
- Clinical-preclinical data corroboration was performed
- LOXO-292 is currently being studied in the global LIBRETTO-001 Phase 1/2 trial

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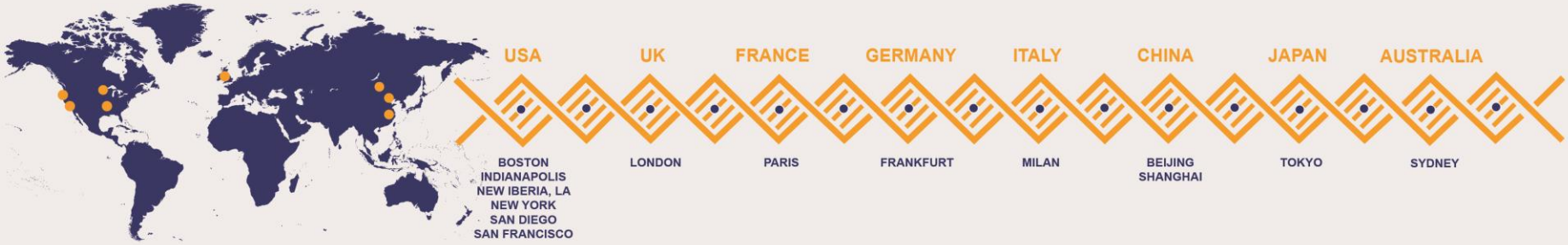
Case Study 3
Optimizing Trial Design via Hypothesis-Free Biomarker Discovery

Increasing Clinical Success Rate

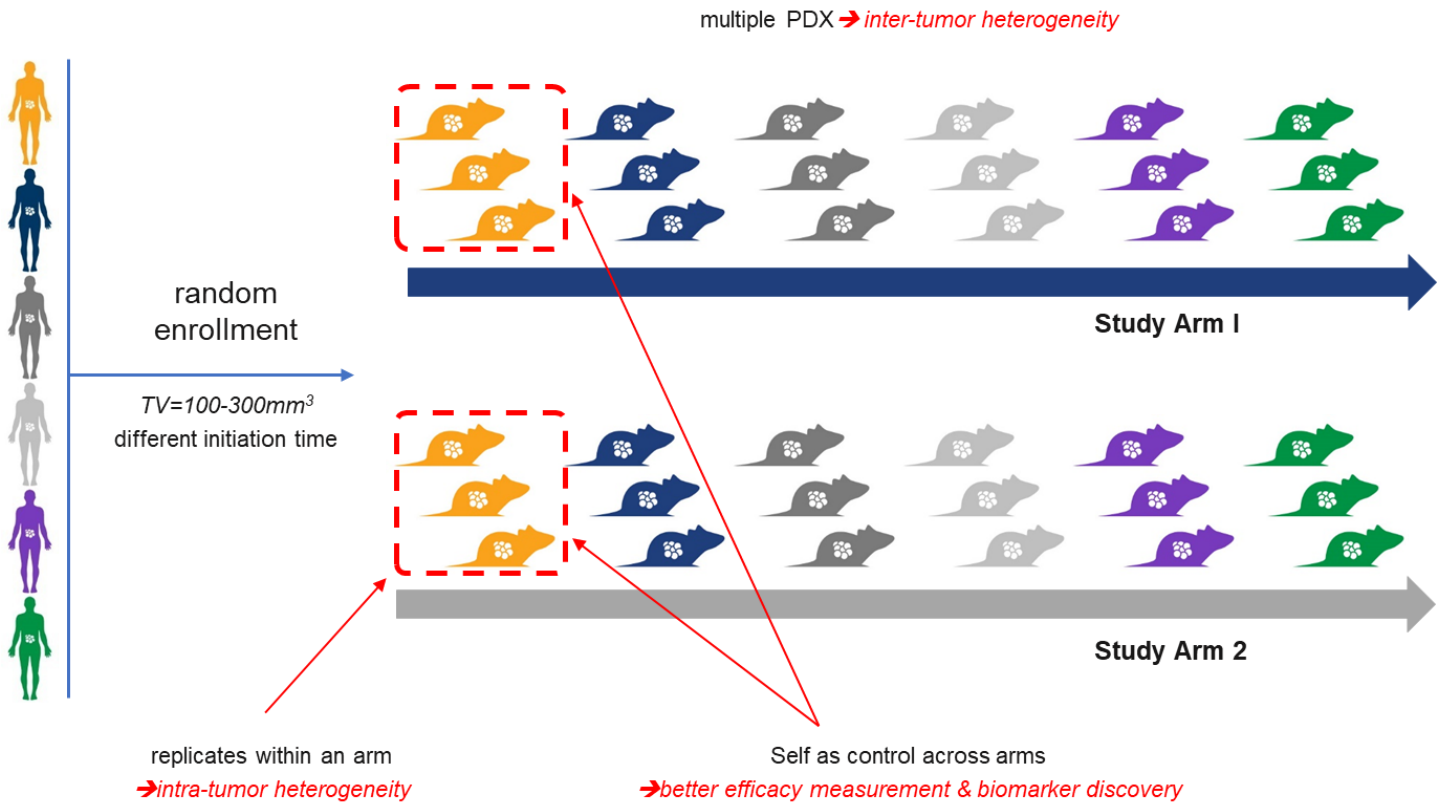


Retrospective Biomarker Analysis

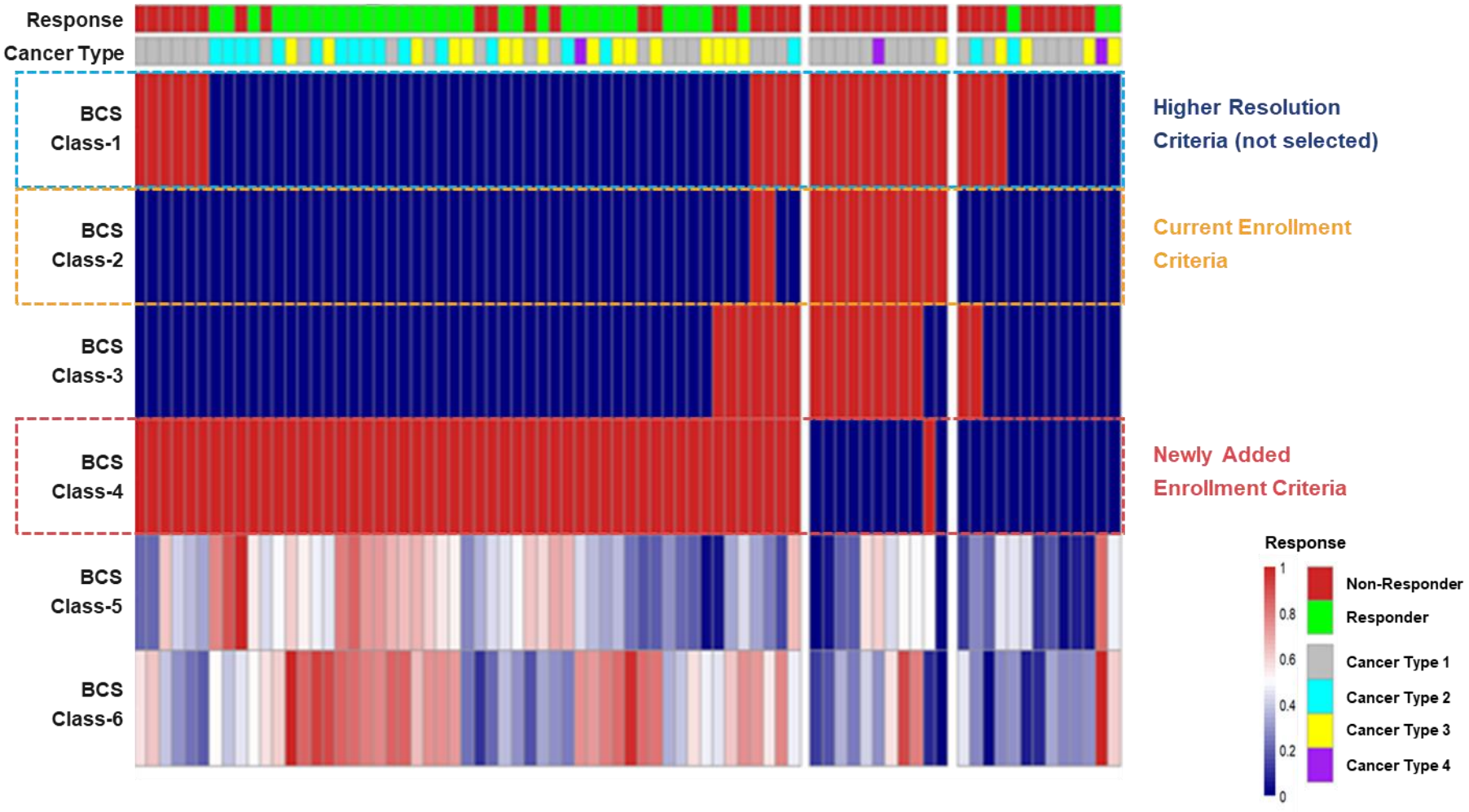
- Phase 1 trial of antitumor activity of Compound Y being conducted in patients with investigated indication
- Case Study 3 performed retrospective biomarker analysis to see if criteria could be improved upon
- Approached using hypothesis free biomarker discovery, using the following tools:
  - Mouse clinical trial design and implementation
  - Sample collection and pathology analysis
  - NGS and bioinformatics analysis
  - Genomic profiling
  - Hypothesis free biomarker discovery

In Vivo Biomarker Discovery using MCT

- This is a 3:3 mouse clinical trial (MCT) design (3 in vehicle arm, 3 in drug arm)
- We have developed a statistical framework that maximizes the utilization of MCTs for efficacy evaluation and biomarker discovery



Identified Improved Enrollment Criteria & Indication Expansion



Case Study 3 Conclusions

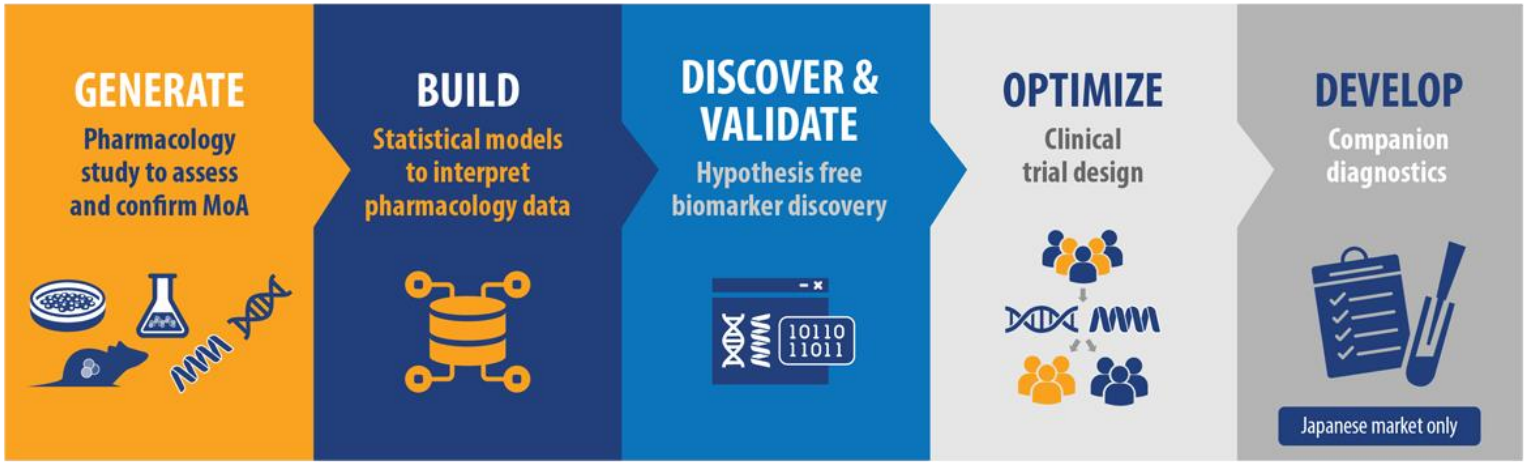
- By using MCT and *in silico* analysis, trial enrollment criteria were updated
- The drug indication was expanded, opening the trial to a larger group of relevant patients
- The original biomarker selection criteria were confirmed
- The analyses improved the accuracy, with the newly discovered criteria better defining the patient subset

Advantages & Benefits of MCT/ *In Silico* Biomarker Pipeline

- Enrollment criteria adjustment, ensuring appropriate patients are brought into studies
- Can allow for indication expansion, bringing in more patients and expediting clinical trials
- In depth analysis can lead to increased success rates and trial accuracy
- Potential to rescue and repurpose previously “unsuccessful” candidate drugs

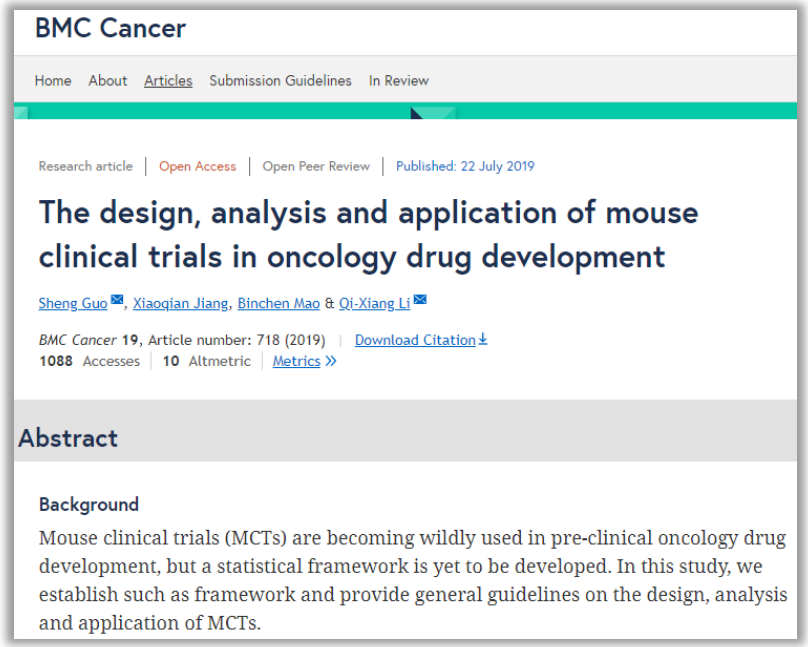
Biomarker Discovery and CDx

- Combines expertise of CrownBio's biomarker discovery and MBL's companion diagnostics (CDx) development and regulatory submission
- Identifies clinically actionable biomarkers **early in drug development**, in the most appropriate preclinical models
- Enables downstream evaluation of CDx in clinical trials, ultimately **de-risking drug development**



Biomarker Discovery Track Record

- CrownBio has internally established state-of-the-art methodologies for biomarker discovery from:
  - *In vitro* cell line screening
  - *In vivo* mouse clinical trials (MCT)
- Our proven track record includes:
  - Over 30 client projects
  - Proven ability of MCT to identify actionable biomarkers
  - 2 biomarkers discovered at CrownBio currently in clinical trials
  - MCT methodology paper
  - Automated *in vitro* analysis platform



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The design, analysis and application of mouse clinical trials in oncology drug development

Sheng Guo, Xiaoqian Jiang, Binchen Mao & Qi-Xiang Li

BMC Cancer **19**, Article number: 718 (2019) | Download Citation

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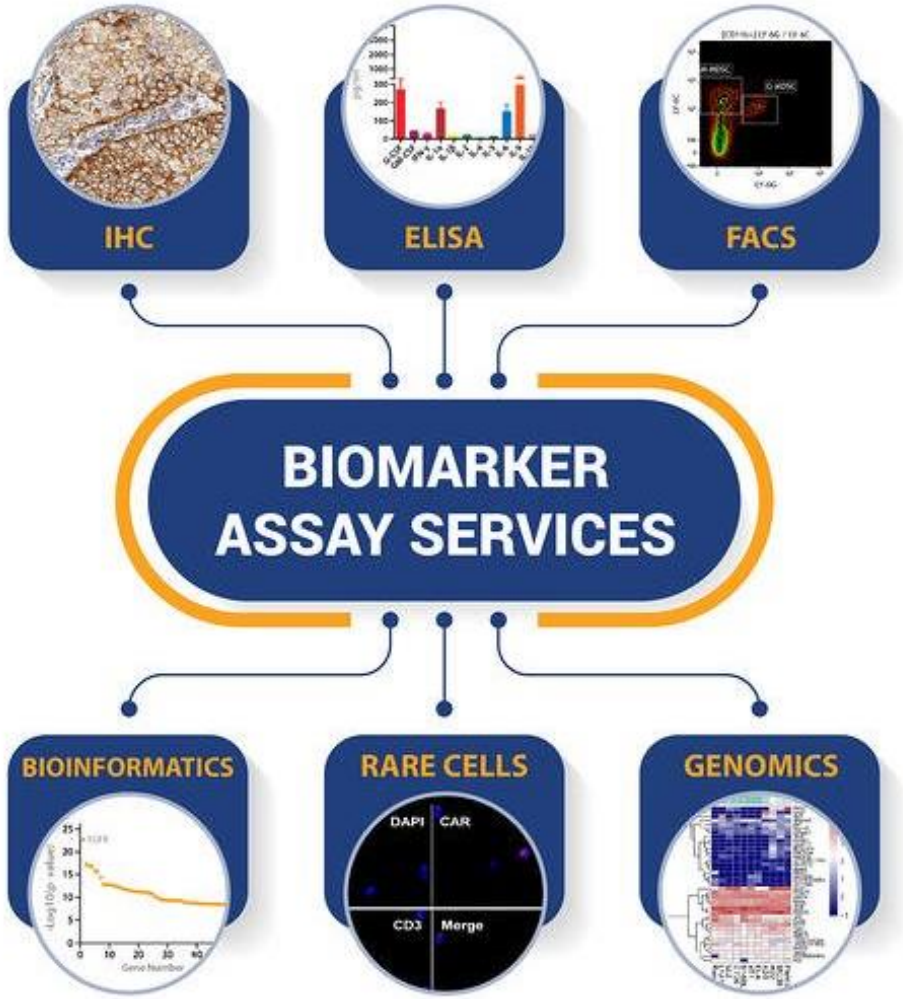
Abstract

Background

Mouse clinical trials (MCTs) are becoming wildly used in pre-clinical oncology drug development, but a statistical framework is yet to be developed. In this study, we establish such as framework and provide general guidelines on the design, analysis and application of MCTs.

Biomarker Core



- Multi-parametric flow cytometry for immunophenotyping
- Immunohistochemistry, high throughput imaging/analysis, and digital pathology
- Singleplex and multiplex ELISA-based cytokine and chemokine profiling
- Genomic analysis via highly sensitive PCR-based, real-time PCR, and NGS technologies
- Bioinformatics to guide biomarker discovery and validation, statistical analyses, and preclinical study/clinical trial design
- Rare cell analyses for detection and monitoring of CTC, CAR-T cells, etc.
- Complete blood count

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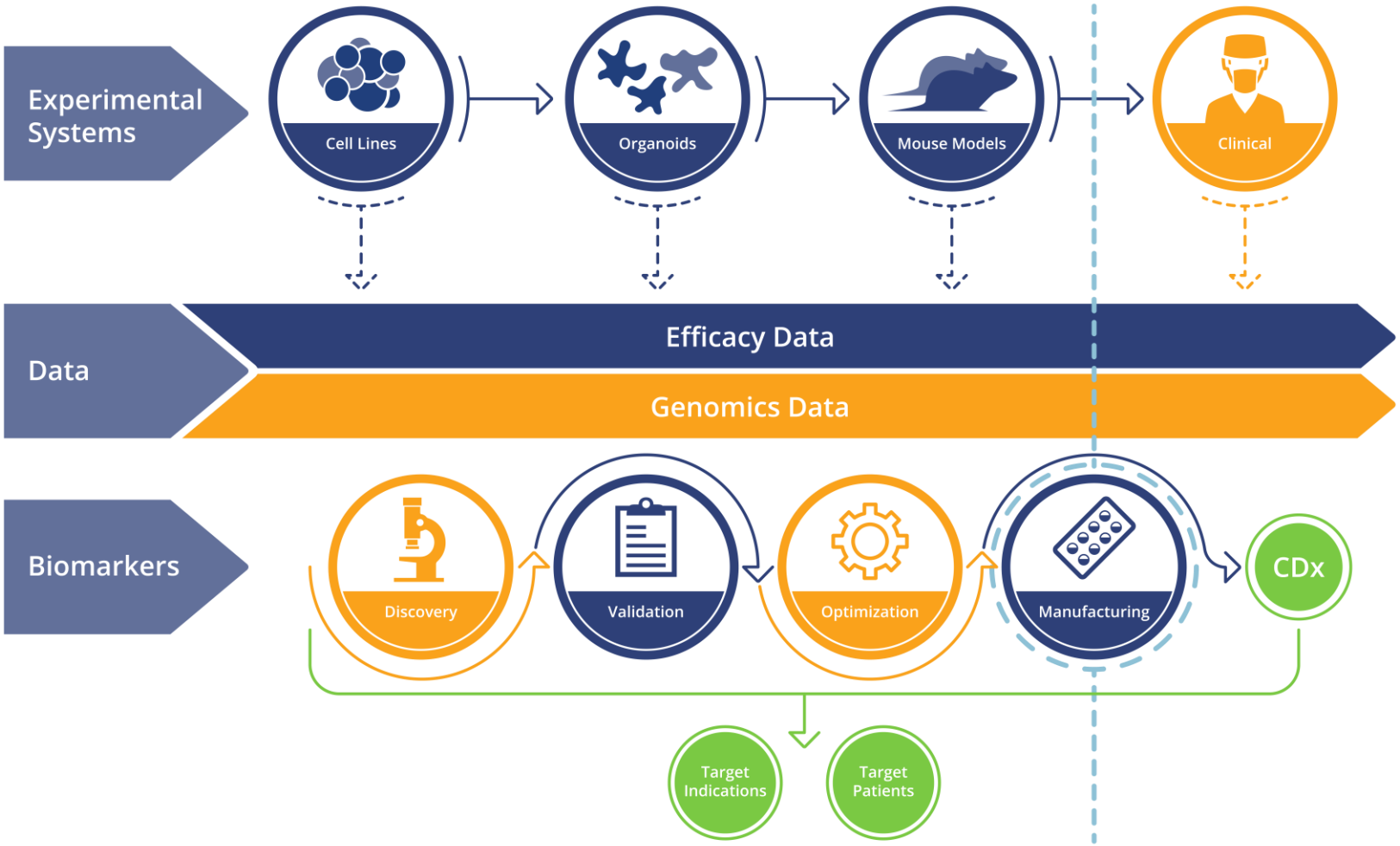
An Integrated Biomarker Discovery and Commercial Manufacturing Platform



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