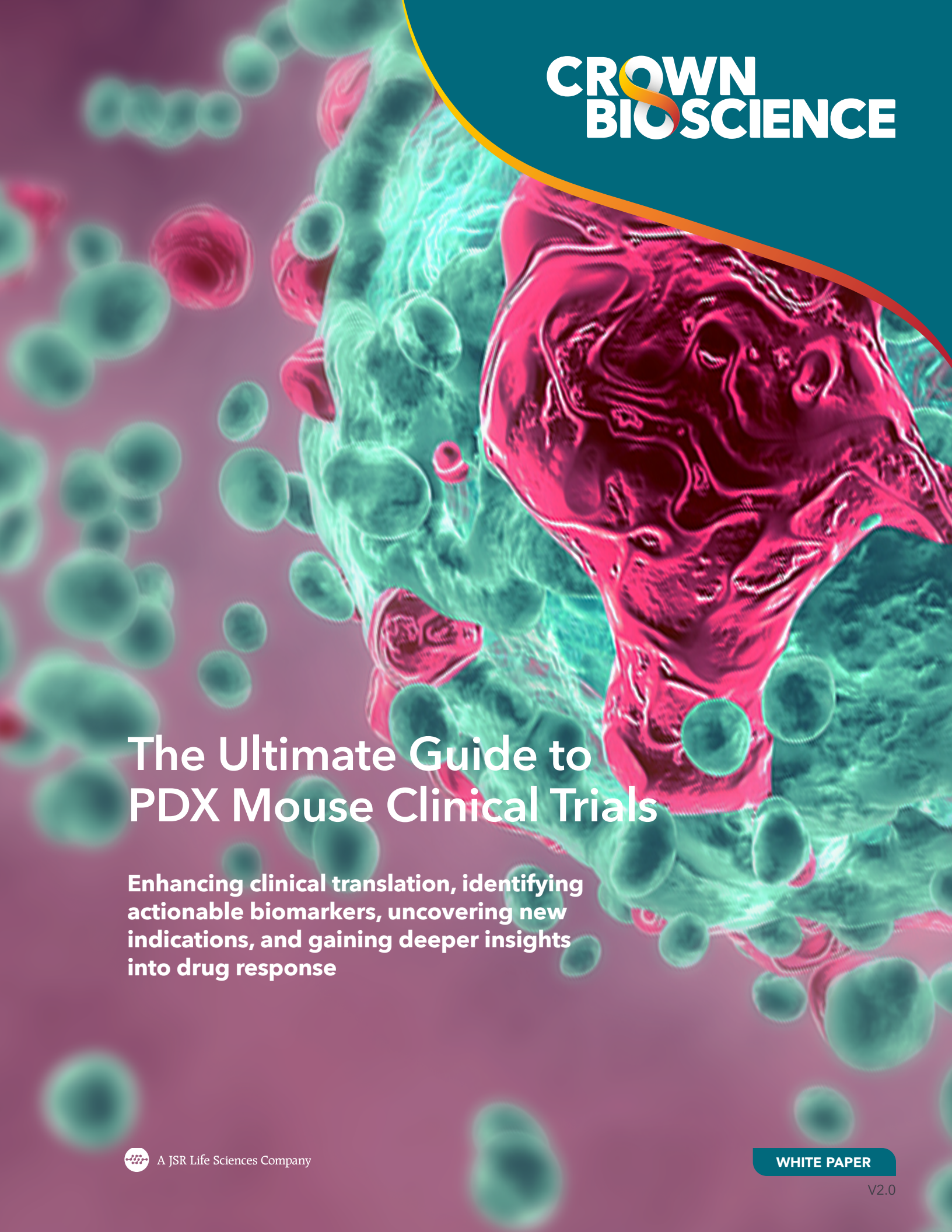


CROWN BIOSCIENCE

The Ultimate Guide to PDX Mouse Clinical Trials

Enhancing clinical translation, identifying actionable biomarkers, uncovering new indications, and gaining deeper insights into drug response

A JSR Life Sciences Company

WHITE PAPER

V2.0

Improving the translatability of your preclinical efficacy studies to optimize clinical success

New oncology agents face higher attrition rate than drugs in other therapeutic areas[1], highlighting the urgent need for improvements in drug discovery and development.

A key factor contributing to these failures is the lack of clinical efficacy, even when drug candidates demonstrate strong activity in preclinical studies. **Figure 1** illustrates that the highest failure rate for oncology agents occurs in Phase II, where efficacy in patients is evaluated for the first time. This highlights the critical need for more predictive preclinical models, as current models often fall short in forecasting clinical outcomes. The gap in translatability between preclinical and clinical results may be attributed to the type of preclinical model used and how effectively it is employed.

Traditional cell line tumor models have been routinely used in cancer drug discovery, and cancer research in general, for many years. They are derived from immortalized cell lines, which are grown *in vitro* in 2D structure and implanted into mice **(Figure 2)**.

Cell line models provide an excellent tool for early stage *in vivo* drug discovery, such as pharmacological testing. Activity information can be carried forward from *in vitro* studies, and subsequently assessed *in vivo* in the context of host determined factors, such as ADME and pharmacokinetics (PK).

However, adaption to 2D tissue culture changes the cell lines, potentially compromising clinical relevance[2]. The selection pressure imposed by culturing heterogeneous tumor cells on plastic, results in the outgrowth of homogeneous clones of cells that are no longer representative of the original tumor.

Patient-Derived Xenografts: A More Translational Tumor Model

Patient-derived xenograft (PDX) models are created from patient tumors which are implanted directly in immunocompromised mice **(Figure 2)**. Unlike cell line models, these models are never manipulated to grow *in vitro*, therefore, do not undergo the selection pressures of growing on plastic, which enhances their clinical relevance. PDX models are fully characterized for pathology, growth characteristics, and response to novel and standard of care (SoC) treatments, and are also genetically/genomically annotated for gene expression, gene copy number, mutations, and fusions via NGS technologies **(Table 1)**. Numerous studies have confirmed that PDX models preserve both the genomic integrity and heterogeneity of original patient disease, and that PDX model data closely resemble patient clinical response [3,4].

Retrospective analyses have confirmed the predictive power of PDX models for tumor response to treatment[3,4,5], substantiating the high relevance of PDXs as surrogate experimental models for human disease, and their improved predictability compared with cell line models[6].

Overall this means that PDXs provide a more translational *in vivo* model, with cohorts of PDX models reflecting the diversity and heterogeneity seen within oncology patients, and also offering a better representation of the human population.Therefore, PDX models are a better tool for late stage drug development – providing highly predictive preclinical data on potential drug responders before moving to clinical trials.

PDX models are routinely used across preclinical drug discovery from target discovery to translational mouse clinical trials (MCTs) **(Table 1)**. Large biobanks of PDX models, characterized with genomic, molecular and phenotypic data including tumor growth, standard of care response, histopathology and patient information are key to facilitating patient population and clinical studies.

Figure 1: The Probability of Drug Candidate Success During Clinical Trials[1]

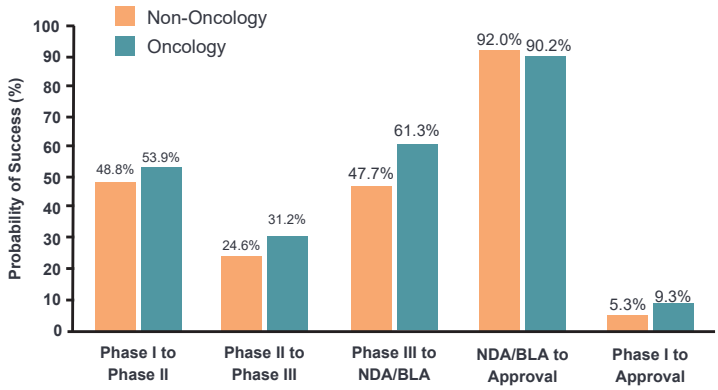
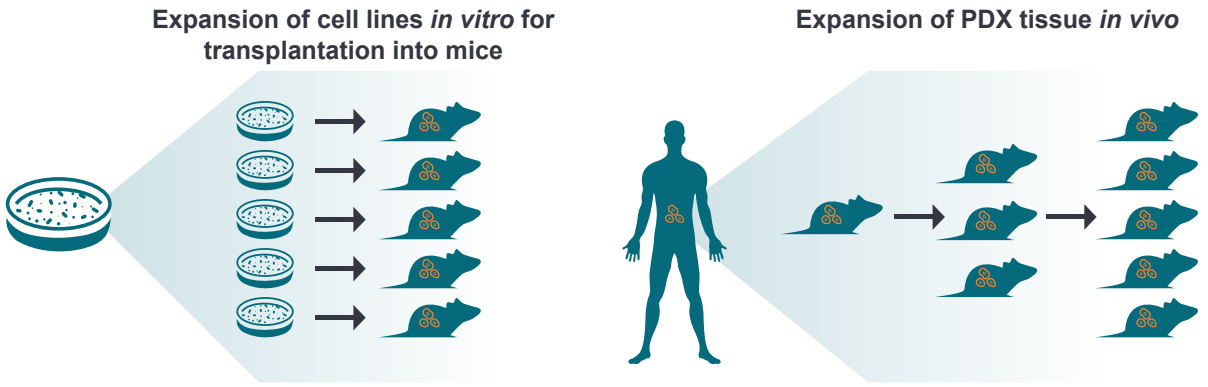


Figure 2: Traditional Tumor Cell Line Models vs PDX Models



Comparison	Cell line models	PDX
Origin	Cell lines maintained in 2D culture	Patient-derived tissue maintained as *in vivo* xenograft
Legacy	Established history in literature, generally available in commercial cell banks	Limited history established in literature and limited access to some PDX biobanks
Clinical Relevance	Potentially compromised by long-term culture *in vitro*	Higher fidelity due to *in vivo* environment by lack of *in vitro* culture

Table 1: Key Features and Applications of PDXs for Drug Discovery

Key Features of PDXs

- Utilize tissue derived directly from patients to create mouse avatars
- Enhanced relevance due to absence of 2D monolayer cell culture
- Represents the **variability in heterogeneity** seen in patient populations
- Preserves both the genomic integrity and heterogeneity of original patient disease with high correlation to clinical outcomes
- Offers more accurate **representation of tumor architecture** than *in vitro* and other *in vivo* options
- PDX biobanks are **fully characterized** for pathology, growth characteristics, genomic annotations and response to novel and SoC treatments
- Living biobanks allow **expansion and multiple use** of each model
- PDX tissue can be used subcutaneous/orthoptic, in TMAs, *ex vivo* or to create organoids

PDX Applications for Drug Discovery

- Drug Efficacy
- Resistance and metastasis modelling
- Target identification & validation
- MoA, PK/PD
- Mouse clinical trials
- Biomarker discovery
- Humanized models for immunotherapeutics
- *Ex vivo* or organoid generation for in vitro screening
- Patient stratification
- Personalized medicine
- Population studies
- Co-clinical trials

Mouse Clinical Trials using PDX Models

By leveraging the clinical relevance of PDXs, we can re-evaluate the paradigm of using preclinical models for drug discovery. Historically, novel agents have been assessed for activity using a small number of xenograft models, with a large number of subjects in each arm. Instead we can now move to mouse clinical trials (MCTs) which assess a large number of models with a small number of subjects in each arm.

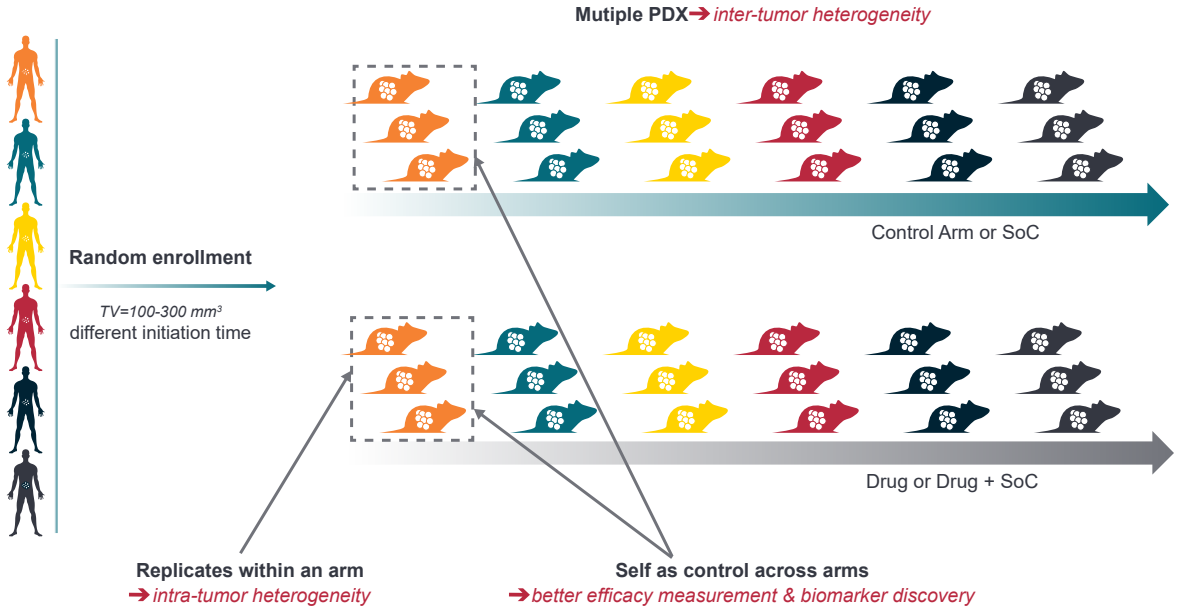
MCT are human surrogate clinical trials, utilizing cohorts of PDX models within a randomized, controlled, and statistically powered setting. Each PDX model reflects the pathology of the original patient (behaving as a patient avatar), and the cohort of patient avatars represent a diversity of the human patient population. Utilizing this method to test a new therapy or combination regimen provides predictive data on subgroups of responders and non-responders which can be used to guide clinical strategies and patient stratification.

In a human trial, a cohort of patients with different backgrounds and heterogeneous disease are treated with the same novel agent/treatment regimen and response outcomes measured, e.g. Progression-Free Survival (PFS) or Overall Survival (OS). Using a large number of different PDX models, with a small number of subjects each, within a MCT allows each model to represent a different patient, more faithfully reproducing the human trial system **(Figure 3)**.

MCTs using small numbers of subjects were validated in a seminal study from Gao *et al* at Novartis[7]. More than 60 treatment regimens, including single agents and combinations, were tested in over 250 models in a 1x1x1 design (e.g. one mouse per model per treatment group).

MCTs can be applied to test a range of different hypotheses, including targeting a single mutation/feature/target across multiple cancer types or targeting a single cancer with different mutations/features. Here we describe a step by step guide to design the most appropriate design for drug discovery and development.

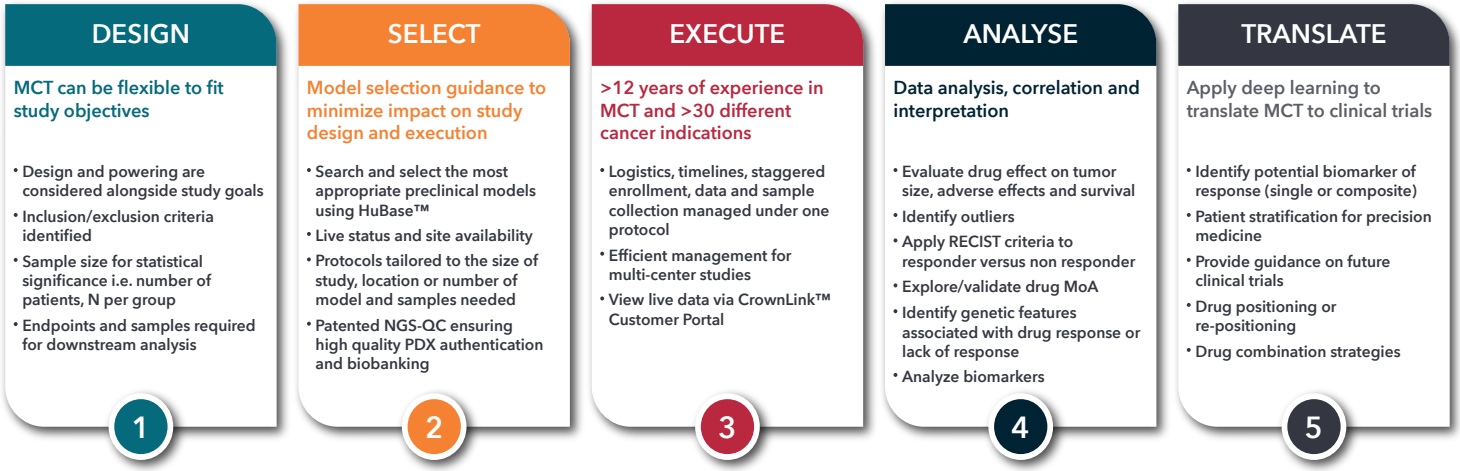
Figure 3: A Sample PDX Mouse Clinical Trial Study Design



Guide for MCTs

MCTs are widely used for preclinical oncology drug development to improve clinical trial translatability. As such, the design, execution, analysis and application of MCTs needs expertise and experience to ensure successful outcomes[8]. The following steps may assist you in planning a MCT as well as analyzing and interpreting the results **(Figure 4)**.

Figure 4: Step by Step Guide for MCTs



Designing a MCT and Selecting Models

There are multiple types of MCTs, each with their own design, utility, and drawbacks, thus it is important to select the right method for any given drug development program. Optimal study design and analysis for drug development was published by Guo *et al.* providing in-depth guidance on a statistical framework for MCT[8]. Our MCT and bioinformatics teams provide expert advise on how to optimise your MCT as part of Step 1 in our MCT guide.

n + n MCTs

The basis of any MCT is to enroll PDXs from different patients, so that each model represents a different individual, and the group represents the heterogeneity of the clinical population. Each model then receives the treatment being tested and the required outcomes are measured.

A typical **3+3** MCT is shown in **Figure 3**. Here, each model has a control or comparator arm with 3 mice per group from the same PDX. The comparator arm is often a SoC therapy, with the treatment arm being SoC with a novel agent added, to mimic Phase II trials. Due to having a comparator arm, this trial type lends itself to an easier determination of "response". A **1+1** design also allows a large study to be conducted, across a wide range of tumor types, while limiting the number of models used. Depending on the number of arms needed, the design can also be extended to **1+1+1**.

A MCT with n>1 per group can be designed to allow additional endpoint sampling, PK/PD, assess inter as well as intra-tumor heterogeneity or simply to increase statistical accuracy when the number of models are limiting. For example, to achieve 80% power, a minimum of 28 PDX models are needed for a 1+1 design, whereas 11 PDX models would be needed for a 3+3 design, i.e. 3 models per group[8,9]. The higher the number of PDX the more the representation of inter-tumor heterogeneity and potential for biomarker discovery[9]. In addition, the more potent a therapeutic response the fewer the number of PDX models are needed. For example, to achieve 80% statistical power of 0.05 significance level in a 3+3 design, 40 models would be needed to achieve 10% efficacy compared to 5 PDXs to achieve 30% efficacy. Our team can advise on optimizing the design and selecting the right models, including designing a trial for drug combinations.[10]

Indication-Driven MCT

An indication-driven MCT evaluates whether an agent works in only one specific type of cancer, which may be driven by a range of different mutations **(Figure 5)**. This type of MCT provides a framework for biomarker discovery within one disease indication, with responders and non-responders within the single cancer type identified. Another benefit of an indication-driven MCT is that it allows the assessment of the dependence of your agent on a specific target in the case of target-negative (or target-low) PDXs.

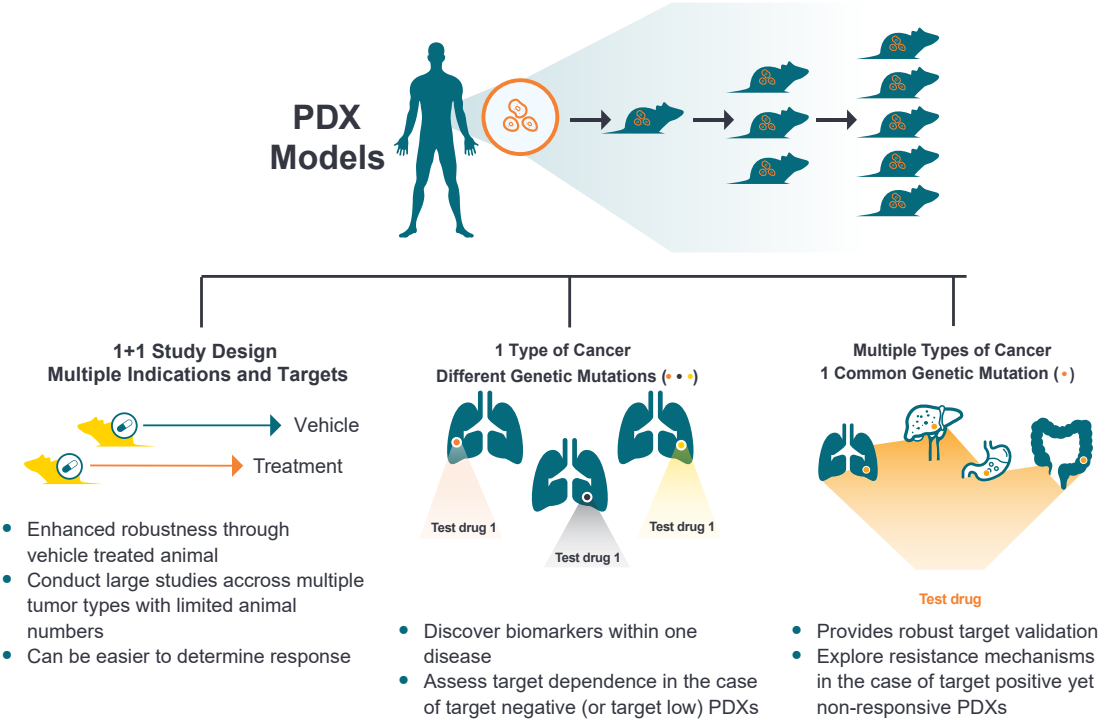
The drawbacks of an indication-driven MCT are that, inherent in the study design, it does not allow cross-indication exploration. This approach might also require a higher number of models, or of animals, if target incidence is low.

Target-Driven MCT

A target-driven MCT is independent of cancer type. This approach provides robust target validation - evaluating whether a target/common genetic mutation is present across a range of cancer types, whether the target is engaged, and if there is a downstream effect **(Figure 5)**. Another benefit of a target-driven MCT is that it provides a framework for exploration of resistance mechanisms in the case of target-positive PDX models where expected activity is not seen.

One drawback of this MCT method is that is not likely to support robust indication selection, given that the target of interest may be across multiple different cancer types.

Figure 5: Design a MCT Based on Study Objectives



Leverage the Largest PDX Collection

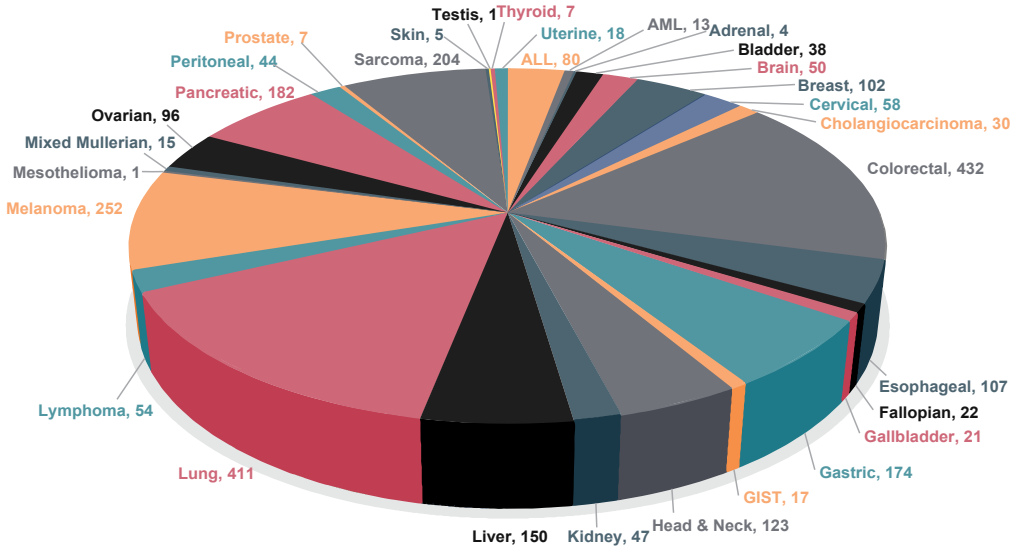
Selecting the right models for a MCT **(Step 2)** plays a crucial role in the design of your MCT. Crown Bioscience provides a complementary online database, Hu**Base**™, to explore the world's largest commercial collection of clinically relevant PDX models, allowing access to over 2,400 global PDX models across >30 cancer types **(Figure 6)**. The rich clinical information and characterization data allow you to tailor your selections based on indication, drug responses, patient histories, and multiomics data for precision in your studies.

Our PDX models exhibit robust and consistent growth characteristics, are fully recoverable from cryogenic preservation, and have sufficient material banked down at multiple passages (master bank) to ensure our ability to perform multiple constant studies. We utilize our patented NGS-QC method for model authentication, contamination monitoring and mouse cell content evaluation to ensure all our PDXs are maintained at the highest quality standards. Due to our large operational capacity and ongoing recruitment to studies, we have a large number of models maintained *in vivo* available for expansion for new studies, thus shortening timelines.

Full Annotation List:

- RNA-seq
- Whole genome sequencing (WGS)
- U219 gene chip array analysis (mRNA)
- SNP6.0 array analysis
- miRNA profiling
- Whole exome sequencing (WES)
- Transcriptome sequencing
- Short Tandem Repeat (STR) genotyping
- Phenotyping, including HLA test
- Primary blood test results
- Primary marrow morphology
- Patient & model treatment and post treatment
- Gene fusion and mutation
- Growth curves
- SoC response curves

Figure 6: Overview of PDX Cancer Types and Number of Models



Data at your Fingertips

Study tracking and data monitoring for complex studies like MCT are effectively implemented through CrownLink™ portal, which provides access to your preclinical *in vivo* and biomarker data in one central secure location during during **step 3** of our MCT Guide. Key features include:

- **Integrated Study Portal:** Share important study data results and communicate with project teams securely at any time
- **Simple Charting:** See how simple and fast it can be to create study data notes
- **Convenient Access:** Save time and work more efficiently with online access to study data
- **Document Management:** Quickly enter data and develop a repository for all your study data and document needs
- **ISO 27001 Certified:** Meet the highest standards for maintaining data confidentiality and secure sharing of information.

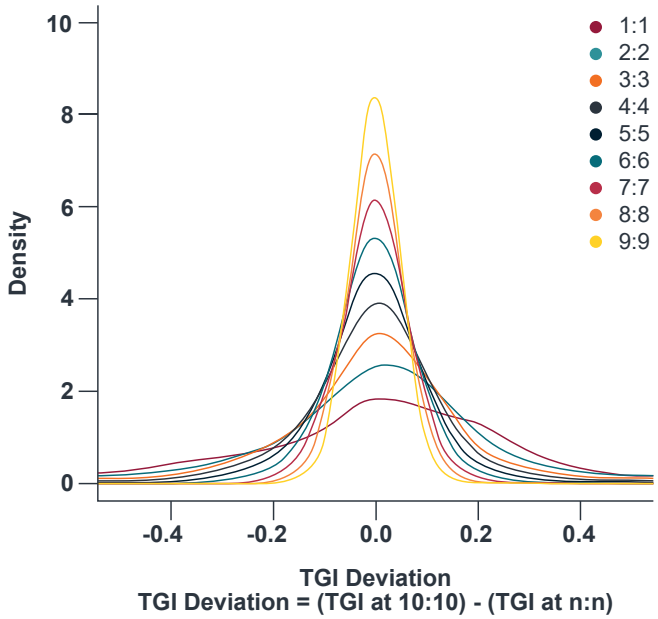
Endpoint Analyses

Clinical trials endpoint-based approaches to analyze drug efficacy include overall survival (OS), progression free survival (PFS), and objective response rate (ORR), which considers percent complete and partial response. PDX studies also use endpoint-based approaches; however, they employ unique endpoints, such as tumor growth inhibition (TGI), ΔT/ΔC (TV changes relative to initial volume for drug group and vehicle group), growth rate difference/ratio or % TV change at the end of a trial or on a particular day.

Endpoint-based approaches (e.g. ORR and TGI) focus on achieving accurate efficacy measures for individual PDX models, and it has been established that accuracy in measuring TGI increases when more mice are used. Here we show an analysis of multiple MCT study designs which demonstrate how accurate the TGI value can be calculated for a n+n design. Starting with a 9+9 design, the true TGI value was calculated by using all 18 mice. One mouse was then randomly sampled from each group, and the TGI was calculated for this 1+1 design along with the difference from the true TGI value (i.e. the TGI deviation). This was repeated for all possible combinations and with other study designs (e.g. 8+8, 7+7 etc.) to derive a distribution of TGI deviations **Figure 7**[8]. The overall conclusion is that when more mice are used, the better the TGI accuracy.

Although endpoint analyses are convenient and valuable in MCTs, they do not take advantage of the full dataset that is generated. Reducing multiple TV-day data points into a single number such as ORR or TGI clearly sacrifices information. Furthermore, variability in growth and drug response between PDXs (inter-tumor heterogeneity) and between mice (intra-tumor heterogeneity) is not considered. Since MCTs are clustered longitudinal studies and provide additional information that human trials cannot, it is therefore necessary to develop alternative approaches to analyzing MCT data.

Figure 7: TGI Accuracy Increases with More Mice



To unlock the power of MCT, a linear mixed models (LMMs) can be used to model and explicitly describe heterogeneity in growth and drug response between PDXs and between mice of the same PDX for these clustered longitudinal studies. (Check our White Paper for details: How to Optimize Mouse Clinical Trials Through Statistical Endpoints and Study Design.) Examples of applying LMM are described in the following case studies.

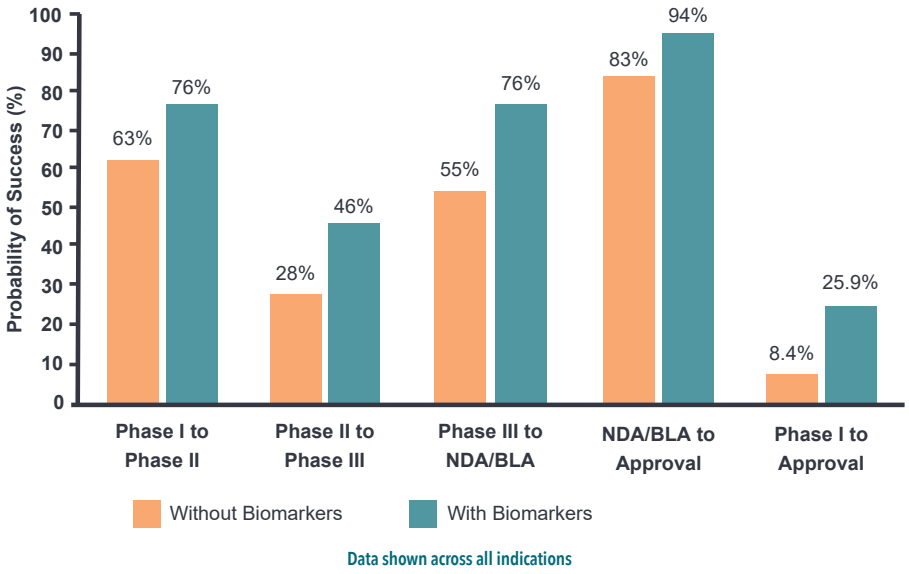
MCTs Facilitate Biomarker Discovery

As noted by Gao *et al*., biomarker discovery is an important part of modern oncology drug development, especially with the move towards personalized medicine. MCTs provide added value above efficacy readouts by facilitating sample collection for biomarker discovery. MCTs provide patient-derived material which, following treatment, can be classified into responders or non-responder populations and interrogated for genomic or proteomic differences between the groups, providing insights into drug mechanism of action and potential resistance phenotypes. Our bioanalysis lab provides ready to use immunoassays and analysis for standard biomarkers from a range of tissue types, as well as flexible and comprehensive multi-omics methodologies and cutting-edge technologies to meet your specific research and drug development need.

The real power of a MCT approach is the ability to capture and leverage patient-to-patient variability preclinically, which is seen universally in the clinic. Identifying a biomarker during drug development can greatly increase your chances of success in the clinic. **Figure 8** shows the probability of a investigational agents success during clinical trial (across all indications), with or without biomarker selection[1]. Those trials with biomarkers show a greater success than those trials without, particularly in the difficult Phase II to Phase III progression, where inclusion of a biomarker almost doubles the chances of a successful trial.

MCTs provide a highly useful tool for retrospective biomarker analysis, due to PDX models reflecting the heterogeneity observed in the patient population. Data analysis can provide powerful prospective inclusion/exclusion criteria for clinical trials to allow more careful trial planning and control, to potentially improve success rates. Moreover, the use of MCTs may allow for a cost effective way to explore translational hypotheses.

Figure 8: Clinical Trial Success With or Without Biomarkers



Data shown across all indications

Case Study 1: Biomarker Discovery in MCT with LMMs to Reinterpret Cetuximab Response in Gastric Cancer

There are very few effective and reliable targeted therapies currently available to treat gastric cancer (GC). Trastuzumab is effective, but only in the subset of GCs which overexpress HER2, which is around 20% of patients.

Cetuximab was originally tested in a Phase III trial, but did not reach the trial endpoints, failing to show significant OS or PFS benefit for SoC plus cetuximab[9]. Therefore, an indication-driven MCT looked to rediscover cetuximab utility in GC, by exploring biomarker correlations in responder versus non-responder populations across 20 PDX models.

The MCT clearly identified GC PDX models which did and did not respond to cetuximab **(Figure 9)**[9]. Following treatment with cetuximab, 4/20 (20%) models showed an almost complete response to therapy (ΔT/ΔC <0), whilst 16/20 (80%) of PDX models showed partial or complete resistance (ΔT/ΔC >30%) **(Figure 10)**. Immunohistochemistry (IHC) staining for EGFR status and EGFR copy number analysis by FISH show that responders had EGFR amplification and overexpression as well as high copy number, with the non-responders showing opposite- low copy number levels and little/no EGFR expression **(Figure 9)**.

Mutation analysis of common oncogenes associated with the EGFR pathway (KRAS, BRAF, c-MET, EGFR, AKT, and PI3KCA) did not reveal any aberrations that could easily explain the non-response of the majority of the GC PDX models to cetuximab. Results from the entire MCT cohort showed that all 4 responder PDX models had:

- High EGFR copy number
- High EGFR relative mRNA expression
- High EGFR IHC score

However a weak correlation was observed between TGI and EGFR expression **Figure 11**. When a LMM is applied that models a gene's effect on tumor growth EGFR was confirmed as the top ranked gene **Figure 12**.

These MCT results correspond with published Phase II trial of cetuximab combined with cisplatin and capecitabine, where 7 out of 44 gastric cancer patients had high EGFR expression and exhibited a reduction in TV[11]. In a further Phase III randomized open label trial for advanced gastric cancers comparing capecitabine and cisplatin (XP) with XP plus cetuximab, it was concluded that the addition of cetuximab provided no additional benefits[12]. However, when the results are reinterpreted for patients with high EGFR protein expression scores (>200), this revealed that cetuximab did provide clinical benefit for those gastric cancer patients with high EGFR expression.

Figure 9: Cetuximab Responders vs Non-Responders in Gastric Cancer MCT

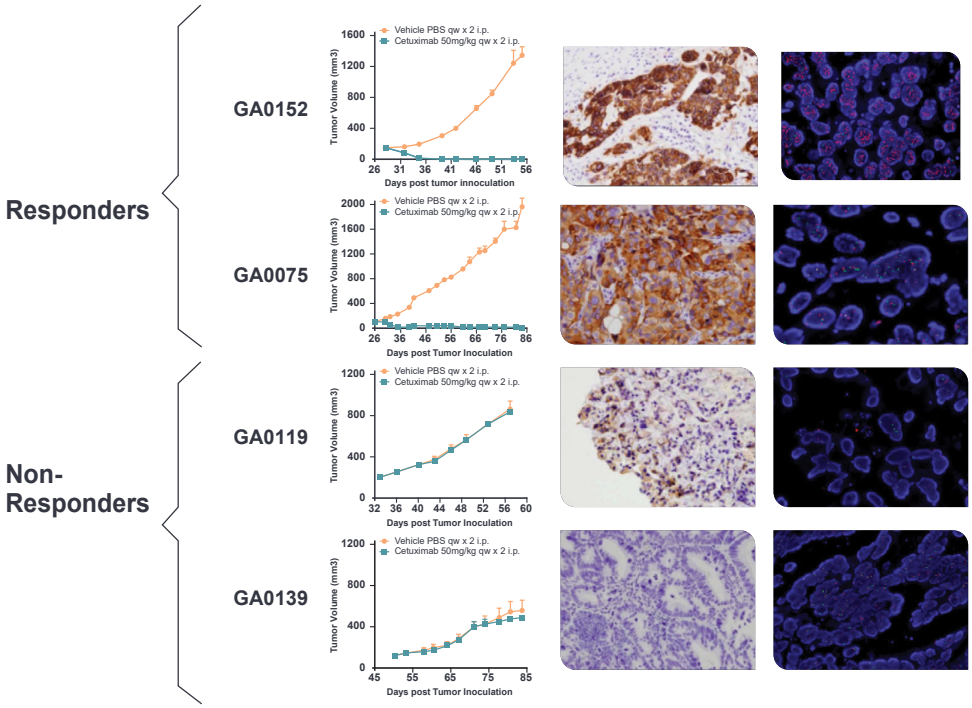


Figure 10: A Subset of Cancers with EGFR Amplification and Overexpression Respond to Cetuximab

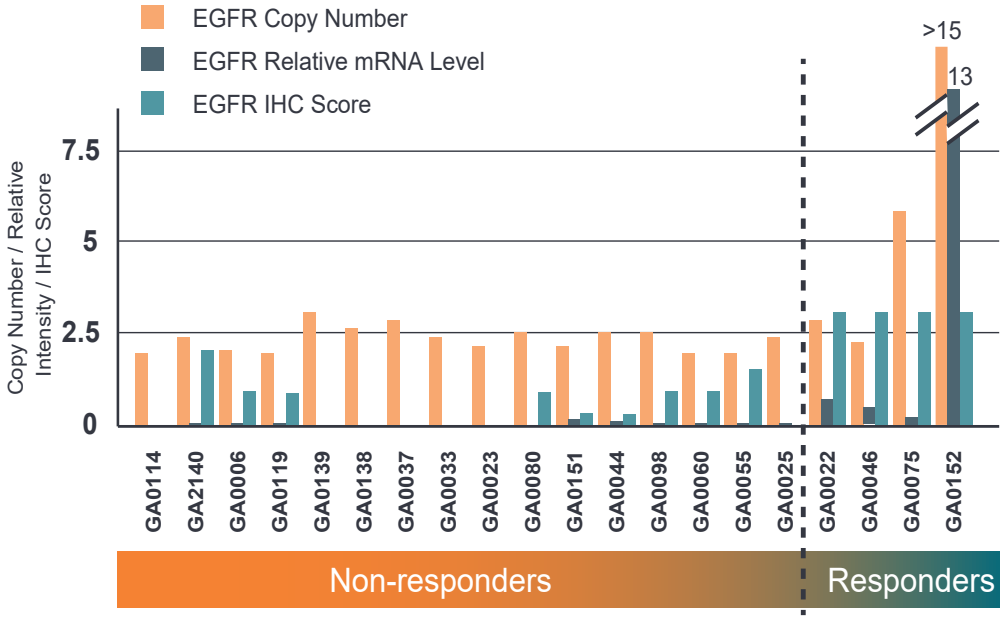
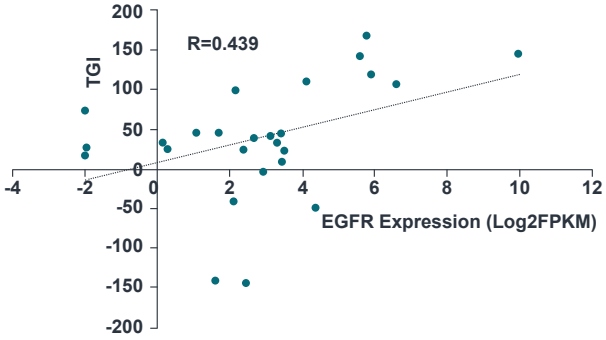
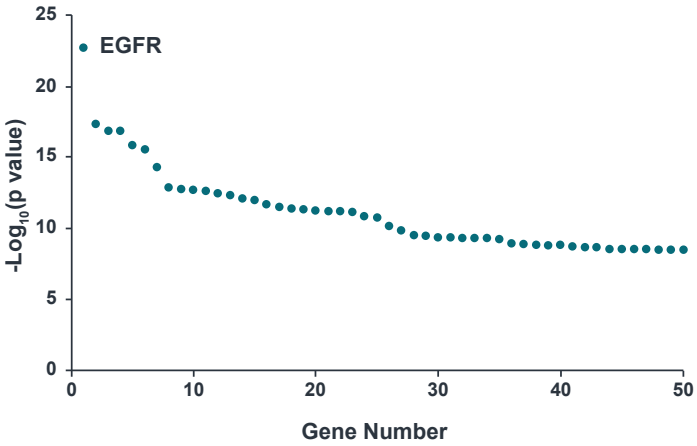


Figure 11: Weak Correlation Between TGI and EGFR Expression



Metric	Cor	Ranking (in ~20k Genes)
TGI	0.439	157

Figure 12: The LMM for Biomarker Discovery Ranks EGFR as the Top Gene



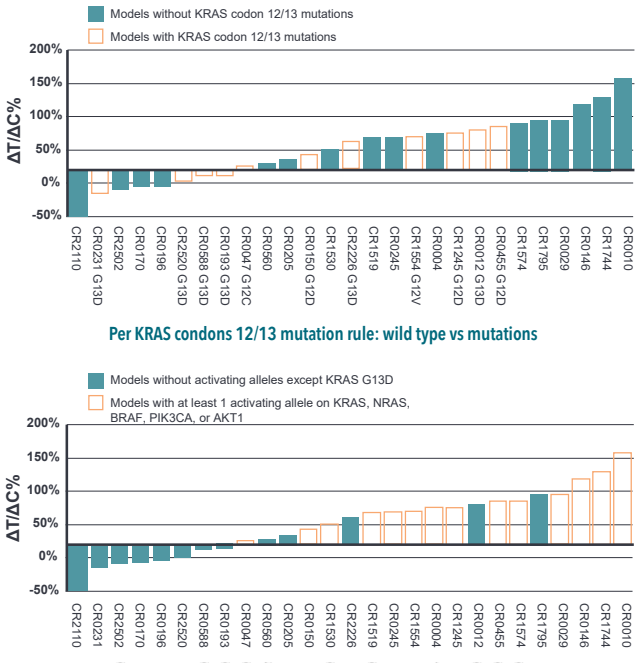
Case Study 2: Evaluating Response to Cetuximab in Colorectal Cancer to Validate a Ras Pathway Signature Score

Cetuximab development has had a major impact on the treatment of colorectal cancer (CRC), which is a common and highly metastatic cancer type. The use of cetuximab in this indication is contraindicated for patients harboring KRAS mutations; however, a retrospective analysis of patients from several, separate clinical trials involving cetuximab revealed differential response outcomes with regard to KRAS mutation status (G13D versus other mutations). The study showed that patients with G13D KRAS mutation did still respond to cetuximab therapy[15].

Therefore, a target-driven MCT was performed to prospectively explore this KRAS restriction, using a random cohort of 27 EGFR expressing CRC PDX models[13]. **Figure 13** shows treatment response data, with the PDX models first segregated by the presence of absence of KRAS codon 12/13 mutation. Each group of models is scattered across responders or non-responders. However, if the PDX are segregated by models without activating mutation (wild-type) except KRAS G13D versus at least one activating mutation on KRAS, NRAS, AKT1, PIK3CA, and BRAF, the results are grouped into responder and non-responder populations.

This confirms the retrospective clinical analysis – that KRAS G13D mutations do not confer resistance to cetuximab. We then investigated if the published Loboda RAS pathway signature correlated with response to cetuximab in EGFR+ CRC PDX models. The Loboda RAS pathway signature is based on the relative expression levels of 147 genes, and may be more predictive of RAS pathway activity than KRAS mutation status. Retrospective analysis has also shown some correlation with cetuximab efficacy in treated mCRC patients. We therefore assessed the RAS pathway signature scores of 25 CRC PDXs treated with cetuximab. We found that KRAS 12/13 mutants and wild type models had similar patterns of RAS signature score, and that there was no statistical difference between their means, suggesting that KRAS 12/13 mutation status has insignificant correlation with RAS signature score. We then compared cetuximab response (ΔT/ΔC) with RAS pathway score and observed a tight correlation - Pearson's correlation of r = 0.59 with p value = 0.0018 **(Figure 14)**[14]. We also observed close correlations for the response of KRAS 12/13 wild type and mutant models with RAS signature score **(Figure 14)**. Of note, 6 out of 15 KRAS 12/13 wild type PDXs had positive RAS signature scores and were associated with poor response. This implies that mechanisms other than KRAS 12/13 activating mutations can also upregulate RAS signaling, consistent with earlier results. Similarly, 4 out of 10 KRAS 12/13 mutants have negative scores and are associated with a certain degree of cetuximab sensitiveness.

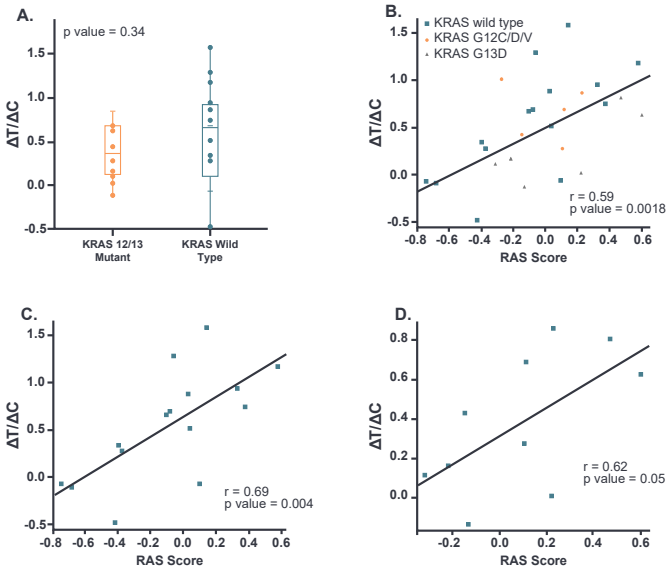
Figure 13: *In Vivo* CRC PDX Model Response to Cetuximab



Per KRAS condons 12/13 mutation rule: wild type vs mutations

Figure 14: Ras pathway Activity Correlation with Cetuximab Response

A: RAS signature score distribution in KRAS 12/13 mutants vs wild type models.
B-D: Correlation between RAS signature score and TGI by cetuximab ΔT/ΔC in all PDX models; 15 KRAS wild type models; 10 KRAS 12/13 mutant models.



Case Study 3: Identifying a Subset of Small Cell Lung Cancer PDX Models Sensitive to the PARP Inhibitor Niraparib

Small cell lung cancer (SCLC) is an aggressive form of disease that accounts for approximately 15% of all lung cancers. SCLC is characterized by rapid growth and early development of metastases, and while patients are initially highly responsive to treatment, relapse commonly occurs within months. Historically, efforts at characterizing the molecular underpinning of SCLC have lagged behind those of non-small cell lung cancer (NSCLC), and the current treatment paradigm is dominated by platinum based chemotherapy regimens. Unlike NSCLC treatment, newer targeted therapies have shown little impact on SoC in SCLC or patient survival.

The first-in-class PARP inhibitor Lynparza™ was approved by the US Food and Drug Administration (FDA) in 2014 for patients with deleterious germline BRCA mutated advanced ovarian cancer following previous treatment with chemotherapy[16]. Early 2016 saw a Breakthrough Therapy designation follow for treatment of BRCA1/2 or ATM gene mutated metastatic castration resistant prostate cancer (mCRPC) following taxane-based chemotherapy and at least one newer hormonal agent[16]. Due to the role of PARP in DNA repair, PARP inhibitors were originally developed as chemo- and radio-potentiators, and *in vitro* data have shown that PARP inhibitors may be beneficial in tumors relying upon mechanisms of DNA repair for survival, including SCLC[17,18].

To test this hypothesis, a cohort of 31 SCLC PDX models from Crown's PDX collection were recruited to PDX mouse clinical trial to evaluate an orally active PARP inhibitor niraparib (which was subsequently approved for the treatment of patients with ovarian, fallopian or primary peritoneal cancer)[18].

To mimic the niraparib maintenance therapy previously used in the clinic, niraparib monotherapy followed a single cycle of cisplatin plus etoposide in the mouse clinical trial. Initially an N of 1 design was used – with one animal per model receiving SoC treatment, and a partner animal receiving SoC followed by niraparib[18].

A range of responses were observed **(Figure 15)**, which included models that were either:

1. resistant to both cisplatin/etoposide and niraparib;
2. sensitive to both cisplatin/etoposide and niraparib or
3. sensitive to cisplatin/etoposide but resistant to niraparib

From the cohort, 6/31 (19%) had a robust response (>75%) to niraparib following SoC, with all of these models also being sensitive to SoC. A moderate response (>50%) was observed in 13/31 (41%) of the models and a minimal response (<25% to niraparib following SoC was observed in 15/31 (48%) of the models **(Figure 16)**[18].

Six responsive models were chosen for confirmatory efficacy studies, utilizing n=5 animals per group. Five out of the six models reproduced the screening results, supporting the N of 1 design as an effective means of evaluating therapeutics in Hu**Prime** SCLC models[19].

Whole exome sequencing was performed on untreated tumor samples from six highly sensitive and four resistant models to identify biomarkers predictive of response. Sequencing was performed via Illumina's Nextera Rapid Capture Exome Kit on Illumina HiSeq (140x mean coverage). Gene expression analysis was performed on 10 SCLC PDX models on the HTG EdgeSeq Oncology Biomarker Panel (HTG Molecular, Tuscon, AZ). Preliminary findings revealed genes with variants more prevalent in niraparib sensitive (i.e. NADK, TMEM14B) or resistant models (i.e. CBX4, MAP3K4). Expression values of approximately 2,500 genes were subjected to Gene Set Enrichment Analysis (GSEA). The top cancer hallmark gene set enriched in the niraparib-resistant group is the MYC target set (FDR q-value = 0.0001; **Figure 17)**[18].

Figure 15: Example Range of Responses to SoC and SoC + Niraparib

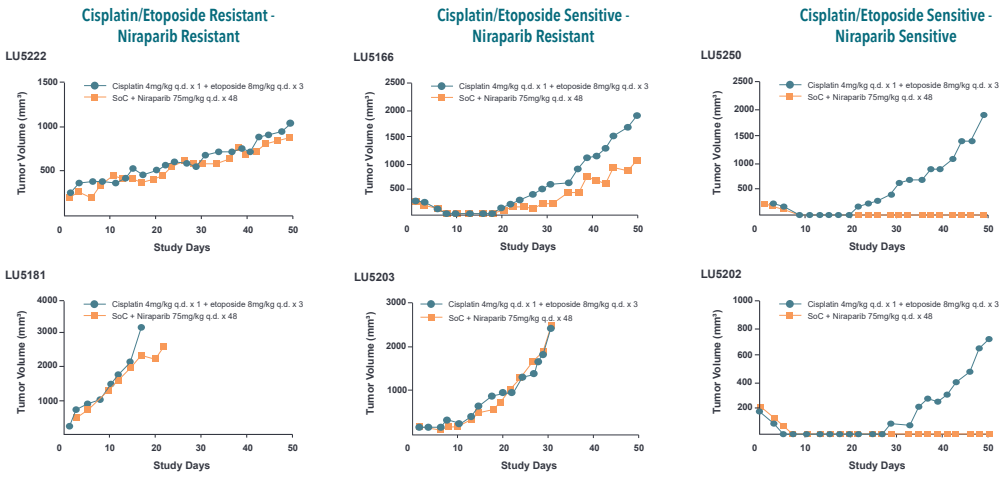
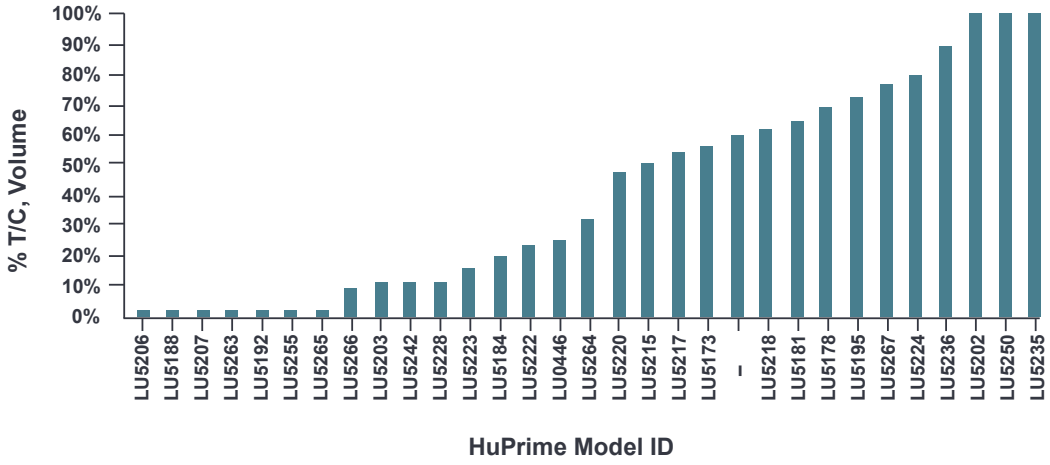


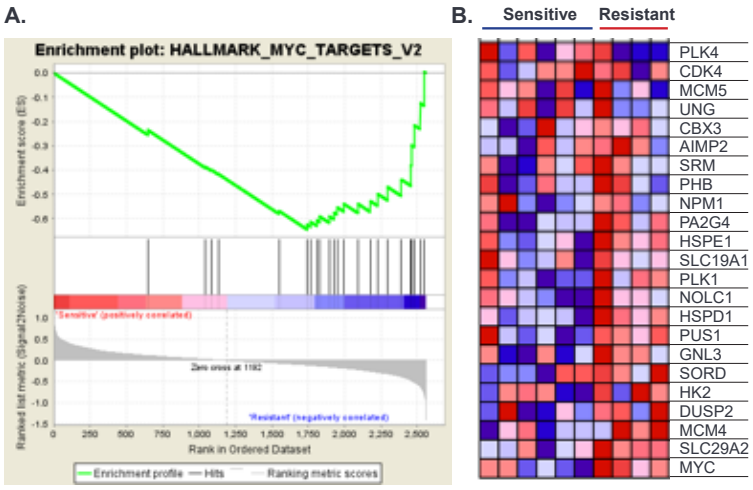
Figure 16: Summary of *In Vivo* HuPrime SCLC Model Response to Cisplatin and Etoposide Treatment followed by Niraparib Maintenance Therapy



For each model: one animal dosed with chemotherapy to mimic front line SoC (Day 1: cisplatin 4mg/kg; Days 1 through 3: etoposide 8mg/kg); one animal dosed with the same representative SoC regimen followed by niraparib 75mg/kg maintenance regimen (dosing from Day 8, q.d. x 48)[18].

Figure 17: Example Range of Responses to SoC and SoC + Niraparib

A: Expression values of ~2,500 genes resulting from this assay were subjected to Gene Set Enrichment Analysis (GSEA)(2).
B: Heat map of core enrichment genes in hallmark myc targets-V2 gene set(2).



Summary

Increasing the efficiency and translation of preclinical to clinical research in oncology is essential to reduce drug attrition rates by improving clinical prediction, patient stratification and biomarker discovery.

PDX MCTs provide valuable insights into clinical response when designed, executed and analyzed appropriately. Crown Bioscience offers a comprehensive Translational Oncology Platform to improve molecule selection and identify patients who will benefit most from a treatment regimen by leveraging the world's largest commercial collection of well-characterized PDX models and biomarker discovery capabilities.

Our extensive experience and knowledge has been applied in our step by step guide to MCTs to identify:

1. The optimal design of each MCT
2. Which models treatment arms and number of subjects to include
3. The approach to efficiently execute and manage the study
4. The most robust analysis to interrogate and unlock the power of MCT data
5. How to translate the data into clinically relevant actions of MCT data

The clinical predictivity of PDX MCT models is changing the way preclinical data is viewed, how drug discovery programs are progressed, and how clinical trials are designed. Crown Bioscience is leading the way in making these models accessible, affordable, and translatable.

References

[1] BIO Industry Analysis. (2021). Clinical Development Success Rates 2011-2020.

[2] Gillet, *et al*. (2013). The Clinical Relevance of Cancer Cell Lines. JNCI: Journal of the National Cancer Institute, 105(7), 452-458.

[3] Hidalgo, *et al*. (2014). Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discovery, 4, 998-1013.

[4] Bertotti, *et al*. (2011). A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discovery, 1, 508-523.

[5] Corcoran, *et al*. (2015). Combined BRAF and MEK inhibition with Dabrafenib and Trametinib in BRAF V600-mutant colorectal cancer. Journal of Clinical Oncology, 33(34), 4023-4031.

[6] Guo, *et al*. (2016). Molecular pathology of patient tumors, patient-derived xenografts and cancer cell lines. Cancer Research, 76(16), 4619-4626.

[7] Gao, *et al*. (2015). High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nature Medicine, 21(11), 1318-1325.

[8] Guo, *et al*. (2019). The design, analysis and application of mouse clinical trials in oncology drug development. BMC Cancer, 19(1), 718.

[9] Zhang, L., *et al*. (2013). A subset of gastric cancers with EGFR amplification and overexpression respond to cetuximab therapy. Scientific Reports, 3, 2992.

[10] Mao, *et al*. (2023). Statistical Assessment of Drug Synergy from *In Vivo* Combination Studies Using Mouse Tumor Models. Cancer Research Communications, 3(10), 2146-2157.

[11] Zhang, *et al*. (2014). Predictive biomarkers for the efficacy of cetuximab combined with cisplatin and capecitabine in advanced gastric or esophagogastric junction adenocarcinoma: a prospective multicenter phase 2 trial. Med Oncol, 31(10), 226.

[12] Lordick, *et al*. (2013). Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. The Lancet Oncology, 14(6), 490-499.

[13] Chen, *et al*. (2015). A set of defined oncogenic mutation alleles seems to better predict the response to cetuximab in CRC patient-derived xenograft than KRAS 12/13 mutations. Oncotarget, 6(38), 40815-40821.

[14] Guo, *et al*. (2016). Cetuximab response in CRC patient-derived xenografts seems predicted by an expression based RAS pathway signature. Oncotarget, 7, 50575-50581.

[15] De Roock, F., *et al*. (2010). Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *The Journal of the American Medical Association*, 304, 1812-1820.

[16] Kim G, Pazdur R., *et al*. (2015). FDA Approval Summary: Olaparib Monotherapy in Patients with Deleterious Germline BRCA-Mutated Advanced Ovarian Cancer Treated with Three or More Lines of Chemotherapy. Clin Cancer Res. 2015 Oct 1;21(19):4257-61.

[17] Byers, L. A., Wang, J., Nilsson, M. B., *et al*. (2012). Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. Cancer Discovery, 2(9), 798-811.

[18] Wang, Y., Ricono, J., Admunson, K., *et al*. (2014). Niraparib, a selective PARP 1/2 inhibitor, is efficacious in pre-clinical models of small-cell lung cancer [abstract]. In: Proceedings of 26th EORTC-NCI-AACR Symposium on Molecular Targets and Therapeutics. European Journal of Cancer, 50(Supplement 6), Abstract nr 239.

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