

Pretreated Patient-derived Xenograft Models: A Key Tool for Developing Innovative Anticancer Therapies



Introduction

Important advances have been made in the field of oncology over the past few decades, with more than 80 new FDA-approved drugs making it to market since 2015 and approximately 20,000 clinical trials underway globally (Webster, 2022). Oncology therapies continue to evolve rapidly, and within the next few years, it is expected that regulators will approve a variety of novel drugs, including MDM2 inhibitors, additional RAS inhibitors, and CEACAM5 targeted therapies (among others).

However, it must be kept in mind that the majority of experimental anticancer agents fail during the late stages of clinical development, after substantial time and expense have been invested (Dowden & Munro, 2019; Seruga, 2015). Drug resistance—either existing before treatment (intrinsic) or generated after therapy (acquired)—is responsible for most relapses of cancer (Wang, 2019), and up to 90% of cancer-related deaths are attributable to drug resistance, relapse, and the resulting ineffectiveness of drug treatments (Gillet, 2011).

Thus, there is an immediate unmet need for preclinical models that can help researchers better understand the mechanisms of drug resistance, especially in the context of modern clinical treatments, so that more effective drugs can be developed.

This white paper discusses how Patient-Derived Xenograft (PDX) models are ideal for preclinical testing of new anticancer agents while emphasizing that these models must evolve to reflect today's current patient populations. Leveraging “next generation” pretreated PDX models—which have been derived directly from tumors of patients who have received known treatments with known treatment responses before tumor collection—has promising applications in developing highly innovative drugs, next generation therapeutics, including precision medicine approaches, and to better understand mechanisms of resistance. Several case studies that highlight the establishment and validation of multiple pretreated PDX models are presented.

Patient-derived Models Bridge the Gap Between Lab and Clinic

Currently, researchers can choose from a variety of patient-relevant tools that can bridge the chasm between the lab and the clinic, including *in vitro* models such as 2D primary cells and 3D patient-derived organoids (PDOs), and *in vivo* models such as PDXs (Urs, 2023) (Figure 1).

While each model has its own set of advantages and disadvantages, patient-derived models tend to have superior clinical translation due to three key strengths:

PDX Advantages

Preservation of parental tumor genomic, molecular, and histopathological features,

- Large collections (biobanks) recapitulate intra- and inter-patient heterogeneity, as well as capture patient diversity across a patient population, and
- Pharmacologically responsive.

Figure 1. Well-characterized patient-derived models such as PDXs and organoids bridge the gap between lab and clinic.



Patient-derived Xenograft Models

PDX models are routinely used in drug screening, biomarker discovery and validation, drug mechanism determinations, tumor biology, and personalized medicine. In fact, since 2016, the US National Cancer Institute (NCI) has replaced the NCI-60 cancer cell line panel with PDX models for drug screening, and more broadly, they have become the 'gold standard' preclinical *in vivo* model due to their high clinical predictivity (Ledford, 2016; Williams, 2018; Xu, 2019; Liu, 2023; Gao, 2015).

Briefly, PDX models are generated by engrafting and passaging patient tumor samples into immunodeficient mice. Importantly, each model maintains the genomic, molecular, and histopathological features of the original patient tumor across different stages, subtypes, and diversified treatment backgrounds, thus behaving as a patient "avatar." Using multiple PDX models can also capture inter- and intra-tumor heterogeneity of the human patient population (Abdolahi, 2022; Liu, 2023) and can be used in "mouse clinical trials" (MCTs) or "HuTrial™." Although PDX models have been shown to be predictive of patient response and used as patient avatars in some applications (Gao, 2015), they can be costly and time consuming to develop and maintain, and thus, they are generally better suited for later-stage validation studies versus early stage large-scale high-throughput applications, which are better suited to *in vitro* models.

While PDX models are important and are continually driving important drug development efforts, it must be recognized that—

PDX Limitation

Long-standing PDX models often fail to capture the realities of today's cancer patients, such as accurately modeling drug resistance and relapse in the context of today's therapies.

For instance, breast cancer models that are estrogen receptor positive (ER+) and resistant to hormone therapy are less applicable today because of the current treatment landscape. The present need is for PDX models exhibiting resistance to newer breast cancer therapies such as CDK4/6 inhibitors, in addition to models recapitulating patient disease subtypes such as those with PIK3CA mutations. Other examples include colon cancers that are refractory to adagrasib (KRAS G12C) or encorafenib (BRAF V600E), and ovarian cancers refractory to mirvetuximan soravtansine. By testing new investigational agents in "next generation" PDX models, researchers have the best chance of creating a differentiated therapy that addresses a significant unmet need.

Meet the Urgent Need for More Clinically Relevant Preclinical Models

"Next generation" PDX models are generated by implanting human tumor fragments obtained from patients previously treated with single drugs or combinations and have relapsed or developed resistance to the treatments. These models offer drug developers the ability to better predict treatment response, enhance the translatability of preclinical data, and help uncover and address resistance mechanisms. Overall, these pretreated models more closely mirror the treatment resistance and relapse patterns observed in today's cancer patients, including responses and resistance to both traditional and newer treatment paradigms such as:

- Hormone therapies,
- Targeted therapies,
- Combination therapies, and
- Immune checkpoint inhibitors.

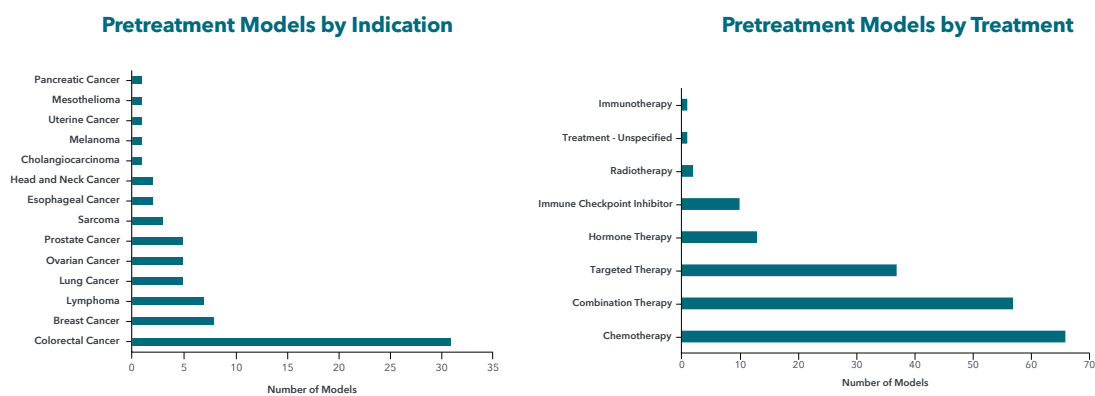


Ideally, these models are annotated with deidentified patient information, treatment histories, and comprehensive molecular characterization. Together, this information is essential to developing innovative therapies and uncovering resistance mechanisms such as therapy-induced target changes or acquisition of other modes of resistance.

Meeting the Need for More Clinically Relevant Preclinical Models

Collections of commercially available PDX models, derived from patients that have relapsed or developed resistance to contemporary standards of care, can be used to optimize preclinical studies to develop innovative therapeutic strategies that are relevant to today's cancer patients ([Crown Bioscience Pretreated PDX Models Factsheet](#)) (Figure 2).

Figure 2. Commercially available pretreated PDX models represent a wide variety of cancer types and treatment histories.



The models from the panel shown in Figure 2 were derived from patients with a range of therapy histories with treatment response or non response—including multiple lines of treatment and current relevant therapies like KRAS inhibitors and immune checkpoint inhibitors. Since the models are developed from tumors sourced from relapsed/refractory populations, each model simulates real-world disease progression and can be used to investigate the underlying determinants of clinical failure and further development of next generation therapeutics. An online database (known as **HuBase™**) allows drug developers to search for models based on indication, treatment histories, mutations, gene expression, etc. Genotypic and phenotypic data such as treatment validation, deidentified patient information, genomic (whole-genome/-exome sequencing), transcriptomic (RNAseq), and proteomic data are also available. Moreover, there is a pretreatment filter that can be used to readily identify pretreated PDX models. The following tables highlight the main characteristics of some pretreated PDX models:

Prostate Cancer: Validated pretreated PDX models derived from patient tumor samples reflecting different cancer subtypes and patient treatment histories (Table 1), where all samples were derived from patients diagnosed by surgical histopathology and immunohistochemical confirmation of prostate markers, including PSA and androgen receptor (AR) expression.



Table 1: Examples of Pretreated Prostate Cancer PDX models.

Model ID	Cancer Type	Patient Treatment History	Comments
PR6511	CRPC	Hormone Therapy, Chemotherapy	TMPRSS-ETS fusion; PTEN loss; Tp53 mutation; RB1 loss; DDR germline mutation
PR6512	CRPC	Hormone Therapy	PI3kCA mutation; AKT mutation; DDR germline mutation
PR6513	HR/mCSPC	Hormone Therapy	ARv7; TMPRSS-ERG fusion; FOXA1 p.A287Rfs Ter33 Tp53 mutation
PR9582	mCRPC	ADT (Lupron/Orchiectomy/flutamide) DES, cytoxan and 5-FU	Experimentally derived castration resistant line; TMPRSS-ERG fusion; PTEN loss; Tp53 mutation
PR9583	ADC	Antiandrogen, DES (Diethylstilbestrol) Given, Mitoxantrone Given	Tp53 mutation
PR9585	mCRPC	ADT (Lupron/flutamide)	Experimentally derived castration resistant line; TMPRSS-ERG fusion; PTEN loss
PR9586	mCRPC	ADT	PTEN loss; FOXA1 p.A353Qfs Ter6; PTEN loss; Tp53 mutation; DDR germline mutation
PR9587	mCRPC	ADT (Lupron, casodex, nilutamide) XRT (pelvis), ketaconazole, Corticosteroid, taxorene (docetaxel), enzalutamide/Xtandi	TMPRSS-ERG fusion; PTEN low/loss; Tp53 loss

Colorectal/Rectal Cancer: Validated pretreated PDX models derived from biopsy samples of patients who received KRAS inhibitors and were either responsive or non-responsive to the treatment (Table 2).

Table 1: Examples of Pretreated Colorectal/Rectal PDX models.

Model ID	Cancer Type	Patient Treatment History	Comments
CR9505	Colorectal	1st: Oxaliplatin 2nd: FOLFIRI + Bevacizumab	KRAS G12D
CR9507	Colorectal	1st: FOLFOX + Avastin 2nd: Xeloda 3rd: FOLFIRI + Avastin	KRAS G12C
CR9508	Colorectal	1st: FOLFOX + Avastin 2nd: XELIRI + Avastin	KRAS G12S
CR9511	Colorectal	1st: FOLFOX 2nd: FOLFIRI	Genetic Results Kras G12D missense
CR9512	Colorectal	1st: FOLFOX 2nd: FOLFIRI 3rd: Avastin	KRAS expression G12D
CR9513	Colorectal	1st: FOLFOX 2nd: FOLFIRI + Bevacizumab	KRAS Q61H
CR9516	Colorectal	1st FOLFOX 2nd FOLFIRI	KRAS G12D, Complete Responses to Investigational + Pembrolizumab – Became Resistant 2 Years Later
CR9519	Colorectal	1st FOLFOX + Avastin 2nd XELIRI + Avastin	KRAS G12S
CR9524	Colorectal	1st Fluorouracil + Oxaliplatin + Bevacizumab 2nd Trifluridine-Tipiracil 3rd FOLFIRI + Ziv-aflibercept 4th Investigational 5th Investigational 6th Investigational + Pembrolizumab	KRAS G12D, Complete Responses to Investigational + Pembrolizumab – Became Resistant 2 Years Later
CR9527	Colorectal	1st FOLFOX, FOLFIRI + 2nd Bevacizumab	KRAS G12D
CR9528	Colorectal	1st FOLFOX 2nd FOLFIRI 3rd Investigational	KRAS G12C; G12 Inhibitor Responder
CR9537	Colorectal	1st FOLFOX 2nd FOLFIRI 3rd Investigational	G12 Inhibitor Responder; Became Resistant, KRAS G12C and Q16H
CR9548	Rectal	1st FOLFOX 2nd FOLFIRI + Avastin 3rd Lonsurf 4th Investigational	KRAS G13D
CR9549	Colorectal	1st FOLFOX + Avastin 2nd FOLFIRI + Avastin 3rd Investigational	KRAS A59T
CR9555	Colorectal	1st FOLFOX + Bevacizumab 2nd FOLFOX + Avastin 3rd Irinotecan 4th Panitumumab, Lonsurf	KRAS p.Q61H
CR9560	Colorectal	1st FOLFOX + Bevacizumab 2nd FOLFOX + Avastin 3rd Irinotecan + Panitumumab 4th Lonsurf 5th Investigational	KRAS p.Q61H



Table 3: Pretreated PDX models of drug resistance (immune checkpoint inhibitors).

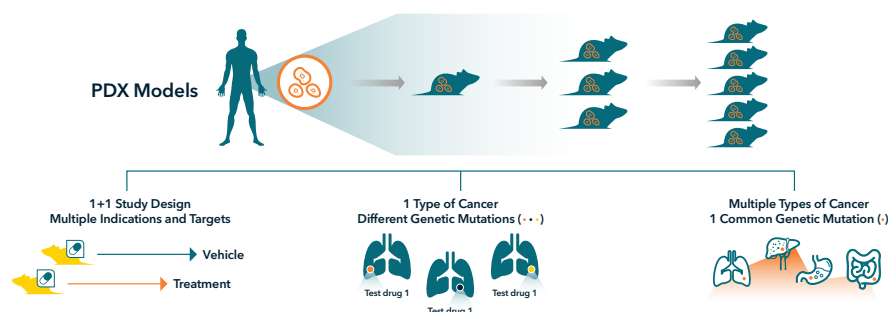
Model ID	Cancer Type	Patient Treatment History	Comments
CR9524	Colorectal	1st Fluorouracil + Oxaliplatin + Bevacizumab 2nd Trifluridine-Tipiracil 3rd FOLFIRI + Ziv-aflibercept 4th Investigational 5th Investigational 6th Investigational + Pembrolizumab	Complete Responses to Investigational + Pembrolizumab - Became Resistant 2 Years Later
CR9537	Colorectal	1st FOLFOX 2nd FOLFIRI 3rd Investigational	G12 Inhibitor Responder; Became Resistant
LU9559	NSCLC	1st Keytruda + Pemetrexed + Carboplatin 2nd Keytruda + Pemetrexed	PD-L1 Positive; Resistant to Pembrolizumab
CR9520*	Anus	1st Fluorouracil + Cisplatin 2nd Carboplatin + Paclitaxel 3rd Fluorouracil + Mitomycin + XRT 4th Nivolumab 5th Fluorouracil + Oxaliplatin	PD-L1 Expression; Non-responder to Nivolumab
LU9536*	NSCLC	1st Opdivo 2nd Investigational + Durvalumab 3rd Investigational 4th Investigational	PD-L1 Positive; Non-responder to Nivolumab
CR9551*	Rectal	1st Fluorouracil + Cisplatin 2nd Carboplatin + Paclitaxel 3rd Fluorouracil + Mitomycin 4th Nivolumab 5th Fluorouracil + Oxaliplatin 6th Investigational x2	PD-L1 Expression; Non-responder to Nivolumab
UT9567*	Endometrial	1st Carboplatin + Taxol 2nd Lenvima + Pembrolizumab 3rd Doxil	FBXO16-NRG1 Chromosomal Rearrangement; PD-L1 Expression

Using Pretreated PDX Models in Mouse Clinical Trials (MCTs)

MCTs (**HuTrials**) are modeled after human clinical trials but in a preclinical setting, using cohorts of PDX models within a randomized, controlled, and statistically powered setting. They can identify responders, partial responders, and non-responders before entering the clinic and aid in the discovery of potential biomarkers of response or non-response. Unlike traditional preclinical mouse model studies, which consist of a small number of models with a large number of subjects, MCTs typically rely on a large number of models with a small number of subjects per arm. Importantly, since each PDX model maintains the pathology of the original patient, they individually behave as patient avatars and can reflect multiple indications and targets. Moreover, as noted earlier, by using a collection of PDX models, the inter- and intra-tumor heterogeneity of the human patient population is captured.

Just like there are multiple possible human study designs, there are variations in MCT designs. Two common choices are 1+1 and 0+1. In a 0+1 study design has no control/comparator arm. It is therefore more similar to a Phase I trial, but drug response must be monitored using criteria specific to the tumor rather than the animal, such as changes in tumor growth and/or size. In contrast, in a 1+1 study design, each PDX model has a control or comparator arm (often standard-of-care treatment), mimicking a human Phase II trial (Figure 3). An advantage of the 1+1 design is that it allows for running a larger study with paired survival analysis and determining a response to a therapeutic agent. A drawback is the larger number of animals needed compared to a 0+1 design. Overall, MCTs provide robust target validation and also allow for exploring resistance mechanisms for non-responsive patients.

Figure 3. Features of a typical MCT (HuTrial) project.



Pretreated PDX Model Establishment and Validation

This section presents case studies highlighting the establishment and validation of several pretreated PDX models (colorectal cancer, prostate cancer, and breast cancer).

Case Study 1: Colorectal PDX Models

Objective:

To evaluate the efficacy of two KRAS G12C inhibitors (AMG510 and MRTX849) in the two PDX models of colorectal cancer with the KRAS G12C mutation, and to compare the results with the clinical outcomes of the original patients.

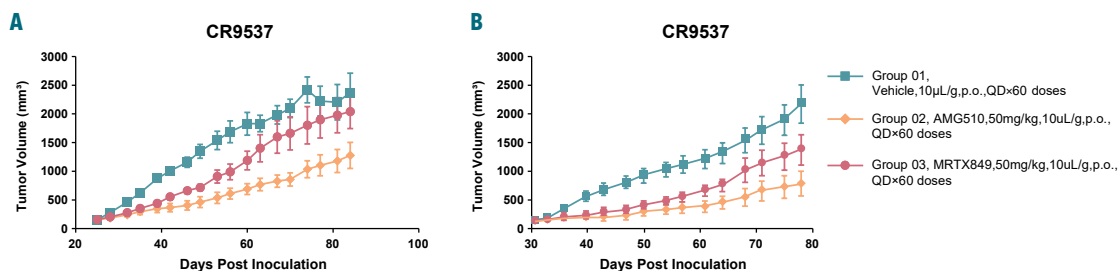
Methods:

- Two PDX models (CR9537 and CR9547) were derived from colorectal cancer patients who had the KRAS G12C mutation and had developed resistance to a KRAS G12C inhibitor.
- Balb/c nude mice were inoculated with tumor samples and then treated with AMG510, MRTX849, or vehicle control. Tumor growth was measured over time and compared with the clinical response of the patients.

Results:

Both PDX models recapitulated the observed clinical treatment response. AMG510 produced a partial response in both models, while MRTX849 was ineffective at blocking tumor growth in both models (Figure 4).

Figure 4: SoC validation of two colorectal pretreated PDX models. A. CR9537 B. CR9547.



Conclusions:

The PDX models accurately reflected the clinical resistance phenotype of the patients and suggest that there may be differences in the potency and specificity of the two KRAS G12C inhibitors.

Expert Insights: Colorectal PDX Model

- This study demonstrates the utility of PDX models in predicting the response of colorectal cancer patients with the KRAS G12C mutation to different KRAS G12C inhibitors. It also provides insights into the mechanisms of resistance to these drugs and the potential strategies to overcome them.
- Such data can help drug developers in designing more effective and personalized treatments for colorectal cancer patients with the KRAS G12C mutation.



Case Study 2: Prostate PDX Models

Objective:

To evaluate the efficacy of enzalutamide and abiraterone, two drugs commonly used for CRPC, in a PDX model derived from a patient who had undergone both chemotherapy and hormone therapy.

Methods:

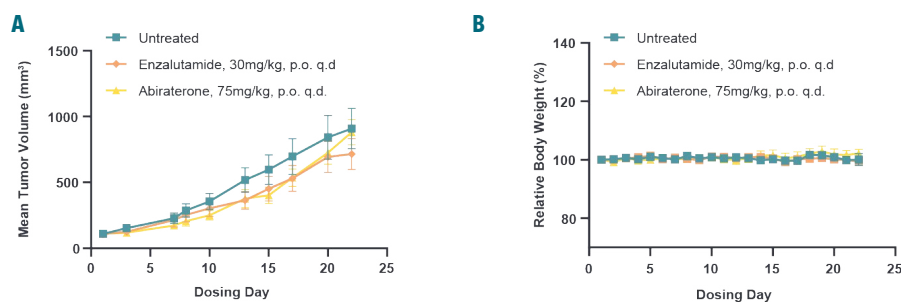
- NSG mice were subcutaneously inoculated with the tumor sample (PR6511) and then treated with either enzalutamide (10 mg/kg/day) or abiraterone (50 mg/kg/day) for 28 days.
- Tumor volume and body weight were measured twice a week. Tumor growth inhibition (TGI) was calculated as the percentage of tumor volume change relative to the untreated group.

Results:

As shown in Figure 5A, as compared to the untreated condition (blue), no significant TGI response was observed following treatment with either enzalutamide (red) or abiraterone (green).

The mean TGI values at day 28 were -9.8% for enzalutamide and -4.6% for abiraterone. No body weight changes were observed across the groups (Figure 5B).

Figure 5: SoC validation of PR6511 prostate cancer PDX model. A. Tumor Volume, and B. Body Weight.



Conclusions:

This study demonstrated that the PR6511 PDX model was resistant to both enzalutamide and abiraterone, suggesting that these drugs may not be effective for patients with CRPC who have received prior chemotherapy and hormone therapy.

Further studies are needed to explore the molecular mechanisms of resistance and identify alternative therapeutic strategies for this patient population.

Expert Insights: Prostate PDX Model

- This study shows that PDX models can be used to test the efficacy of drugs for CRPC in a preclinical setting.
- These data can help inform drug developers that enzalutamide and abiraterone may not be sufficient to treat CRPC patients who have failed previous treatments, and that new drugs or combinations may be needed to overcome resistance.



Case Study 3: Breast Cancer PDX Model

Objective:

To evaluate the drug sensitivity of a PDX model of basal-like HER2-positive breast cancer with BRCA2 mutation, and to identify a potential combination therapy for this tumor subtype.

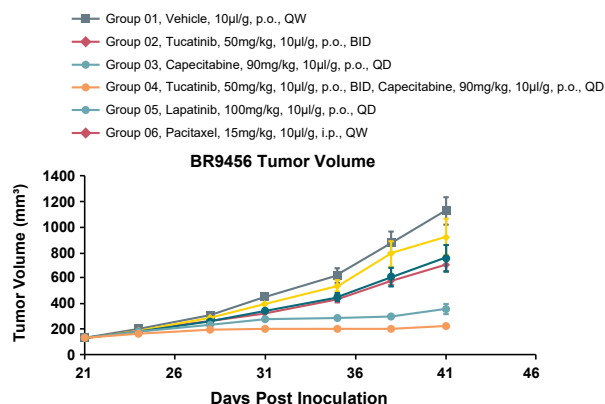
Methods:

- A PDX model was established from a tumor sample of a paclitaxel-treated breast cancer patient who developed a lung metastasis.
- The tumor was characterized by molecular and immunohistochemical (IHC) analysis, and found to be basal-like, ER-negative, PR-negative, HER2-positive, and harboring mutations in BRCA2, TP53, and DNMT1.
- NOD/SCID mice were inoculated with tumor samples and treated with various drugs, including capecitabine, lapatinib, tucatinib, and paclitaxel, alone or in combination. Tumor growth was monitored and compared among different treatment groups.

Results:

The model showed differential drug sensitivity, as shown in Figure 6. Capecitabine monotherapy resulted in significant tumor inhibition, while tucatinib or paclitaxel alone had modest effects. The combination of capecitabine and tucatinib showed the greatest TGI, suggesting a synergistic effect of these two drugs. The results were consistent with the clinical scenario, where the patient had a poor response to paclitaxel, but a good response to capecitabine.

Figure 6: Response of the BR9456 PDX model to a variety of drugs.



Conclusions:

- This study demonstrates the utility of PDX models for preclinical drug testing and personalized medicine.
- The PDX model of basal-like HER2-positive breast cancer with BRCA2 mutation recapitulated the drug sensitivity of the patient and identified a potential combination therapy of capecitabine and tucatinib.
- This finding may have implications for the treatment of other patients with similar tumor characteristics and warrants further validation in clinical trials.



Expert Insights: Breast Cancer PDX Model

- This study shows how a PDX model can help drug developers find effective therapies for challenging tumor subtypes, such as basal-like HER2-positive breast cancer with BRCA2 mutation.
- The study identified a novel combination of capecitabine and tucatinib that may improve the outcomes of these patients, and could be tested in future clinical studies.

Conclusion

PDX models have emerged as the gold standard preclinical platform for basic and translational cancer research, yet these models must continue to evolve alongside clinical practice changes. "Next Generation" pretreated PDX models, derived directly from tumors of patients who have received known treatments with response or non-response data before tumor collection, have promising applications in developing highly innovative drugs, next generation therapeutics including precision medicine approaches, and to better understand mechanisms of resistance. With commercially available models, such as those offered by Crown Bioscience, scientists can effectively address the unmet need for therapies that overcome drug resistance and disease relapse and integrating "next generation" PDX models within MCTs (HuTrials) offers an optimal pathway for oncology drug development.

To view data for specific pretreated models or browse models, register for or log in to Crown Bioscience's free PDX database (HuBase™) at <https://www.crownbio.com/databases/hubase> to view treatment history, available validation (SOC) data, genomic, and proteomic data to help you find the best models for your research needs.

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