HUB ORGANOIDS

Accelerating oncology drug discovery with HUB Organoids™

Reduce oncology drug attrition rates with the only technology that brings a *"patient in the lab®"*

WHITE PAPER

INTRODUCTION

Oncology drug attrition rates are significantly higher than for other therapeutic areas. This is partly attributed to the poor translatability of current preclinical models and to their suboptimal application in drug development programs.

Patient-derived xenografts (PDX) have been widely adopted over the last two decades for highly predictive *in vivo* therapeutic testing in translational research, as they preserve key patient tumor features and the response to certain treatments. PDX, however, can be costly and time consuming to develop and are not amenable to large scale screens encompassing multiple test agents or combinations across several models in parallel.

New patient-relevant models that can be utilized early in the drug development process (e.g. more clinically relevant *in vitro* platforms) are urgently needed to better identify target patient populations and improve anticancer agent success rate. Cell lines currently used for *in vitro* investigations are poorly reflective of original disease and maintained in a 2D environment, but are routinely used in the early stages of drug development.

Tumor organoids are a novel 3D *in vitro* system that, similarly to PDX, preserve the morphological, genomic, and pathophysiological identity of their corresponding *in vivo* tumor by showing similar pharmacological profiles and treatment response. Organoids can be generated from tumors directly from a patient´s biopsy or resection, and sufficiently expanded for large scale screens without losing the original tumor identity, demonstrating high predictive power and great potential to revolutionize the drug discovery workflow.

THE NEED FOR EARLY STAGE PATIENT-RELEVANT MODELS

Since the US National Cancer Institute (NCI) announced the phasing out its NCI-60[1] panel of cancer cell lines for drug screening purposes in favor of a PDX repository, numerous libraries of PDX models have been established representing the diversity and heterogeneity of the patient population. PDX are widely adopted *in vivo* models in preclinical drug discovery and are more patient relevant than conventional xenografts, as original tumor morphology and genetic make-up are preserved. Patient tumors are never manipulated *in vitro* when establishing PDX models, which supports the maintenance of original patient tumor heterogeneity.

PDX models have impacted drug development in many different ways. Their most prominent use has been as patient surrogate models, which are enrolled in Phase II-like mouse clinical trials to identify responder populations and predictive biomarkers. The power of PDX in mouse clinical trials is highlighted in high profile scientific publications[2] demonstrating the feasibility of using these models as patient "avatars" to predict response.

Mouse clinical trials are run as prospective studies, with study design varying to focus on either a specific cancer mutation across various cancer indications or a tissue specific cancer type. Typically, these studies take a population approach and require a fairly large cohort of mice to produce statistically relevant predictions of agent efficacy. N of 1 studies are also run where 1 animal is enrolled in the treatment and 1 in the control arm but these require large numbers of different models of the same cancer type to produce meaningful data.

Co-clinical trial approaches have been trialed where PDX are generated and run at the same time, with the same treatment as the patient in the clinic. However, the applicability of this approach to a wide patient population has been challenging due to the time taken to establish PDX, associated costs, and ethical concerns around animal use.

Overall, PDX are a powerful preclinical *in vivo* platform with proven predictive value but can be costly and time consuming to develop, as well as not being optimally positioned to perform large scales screens. Due to a lack of functional immunity, PDX also have limited immuno-oncology applications.

To overcome PDX challenges, a new patient-relevant system is needed that can produce fast results to replace and reduce the need for *in vivo* testing. Tumor organoids provide such an *in vitro* platform, preserving key tumor genetic and morphological characteristics and proving predictive of response (Figure 1). To exploit the full benefits of tumor organoids in early drug development, it's important to thoroughly understand this new technology and key features it offers.

Figure 1. A comparison between PDO and PDX

	Patient-derived organoids (PDO)	Patient-derived xenografts (PDX)
Development time	4-8 weeks	6-8 months
Initiation success	Moderate	Moderate
Cost	$$	$$$$
Throughput	High/moderate	Low
Standardization	High	Moderate

WHAT ARE ORGANOIDS?

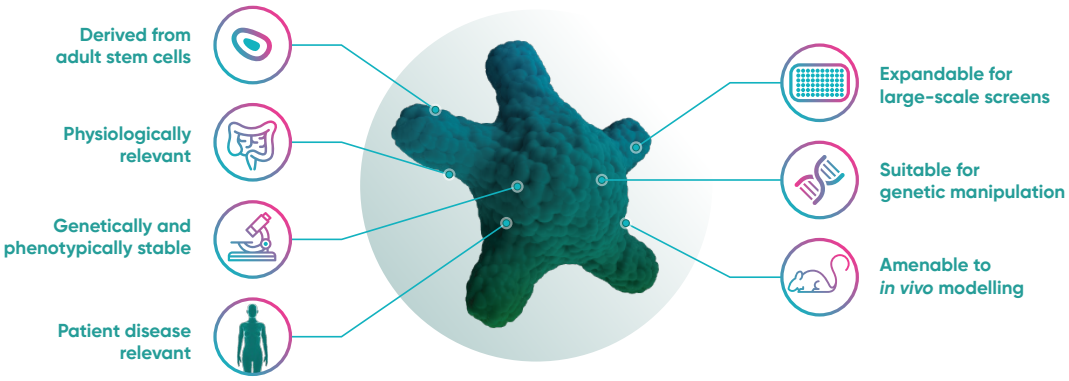
Organoids are innovative microscopic 3D structures derived from stem cells, providing "mini-organs in a dish". They are a robust self-organizing *in vitro* model, preserving the genomic, physiological and multicellular identity of the corresponding *in vivo* tissue. Organoid key characteristics include:

- Self-organization which parallels the parental organ's intrinsic organizing principles
- Presence of multiple differentiated cell types
- Retaining the identity of the organ being modeled (both stem cells and their progeny)
- Some physiological aspect of the organ's specialized function.

Organoids are derived from either multipotent organ-specific adult stem cells (ASCs), pluripotent stem cells (PSCs) (i.e. embryonic stem cells, or ESCs), or induced PSCs (iPSCs). The resulting organoids can be composed exclusively of epithelial cells or both epithelial and mesenchymal cell types, respectively. Specific culture conditions allowing the establishment of *in vitro* epithelial intestinal organoids from single organ-specific stem cells was defined in a seminal publication[3] by the Clevers lab. These ground-breaking organoid protocols have now been refined to allow the growth of mini organs from virtually any epithelial tissue, directly from

patient resections or biopsies, without the need for isolating single adult stem cells. The resulting organoids are also knows as patient-derived organoids (PDOs) or HUB Organoids. Their key features are summarized in Figure 2.

Figure 2. Key features of HUB Organoids



HUB Organoids are more quickly established than PSC-derived organoids as they don't require additional reprogramming and differentiation steps, or passage from spheroid aggregates to Matrigel®. As *in vitro* systems, they can also be established much faster than *in vivo* models and, by harnessing the adult stem cells present in most epithelial organs, they can be propagated and kept in long term culture without compromising their identity, therefore providing a quick and easily scalable "mini-organ" system.

In the context of precision oncology, PDO provide enhaced patient relevance, whereas the more complex and indirect route of engineering PSCs to derive tumor organoids, would implicate the loss of the original patient genetic make-up.

On the other hand, PSC-derived organoids are valuable tools to simulate the epithelial-mesenchymal interaction and to establish organoids from tissues with negligible self-renewal capacity, such as parts of the central nervous system[4, 5], heart muscle[6, 7] or the glomeruli of kidneys[8].

ORGANOIDS PREDICT CLINICAL RESPONSE: A PATIENT IN THE LAB

ASC-derived organoids can be developed from healthy as well as diseased tissue, including cancer lesions.

To leverage the pioneering scientific discoveries from the Clevers lab and further develop organoid technology for large scale applications the Hubrecht Institute, the University Medical Center Utrecht, and the Royal Netherlands Academy of Arts and Sciences (KNAW) founded a new entity called **HUB Organoids (HUB)**. HUB has developed large biobanks of PDOs from many different epithelial organ types such as breast, colon, and pancreas, including both healthy and diseased organoids.

HUB Organoid Technology is the only available technology to have relevance for the study of cancer as, unlike PSC-derived organoids which use embryonic stem cells as starting material, PDO utilize fresh patient tumors as a source of stem cells.

Given their high establishment rate compared to other patient-derived *in vitro* systems, large libraries or biobanks of organoids can be generated from multiple different cancer types and patients to capture the heterogeneity observed in the patient population. This provides a robust and predictive patient-relevant *in vitro* platform or *"patient in the lab"* with a greater economy of scale than *in vivo* studies.

Recent independent studies have shown the predictive value of HUB Organoid Technology for gastric and colorectal cancer patients. These findings published in *Science*[9] and further highlighted in a *News* article by *The Scientist*[10] demonstrate that organoids can be generated from patient biopsies with a very high success rate (>70%). When organoids were tested for response to treatment, they were able to predict lack of response in patients in all cases. Even more strikingly, in almost 90% of cases, organoids predicted a positive response from patients in the clinic, therefore demonstrating the translational utility of *in vitro* PDO for screening candidate treatments and highlighting the potential of this disruptive technology.

ORGANOIDS FOR ONCOLOGY PRECISION MEDICINE

Cancers are highly variable in terms of stage, genetic background, and molecular behaviors, therefore tailoring individual therapies to a patient's genetic profile has been recently pursued as an avenue to try and improve success rates in cancer treatment.

Genome guided therapies are already being applied to cancer patients, however, conservative estimates place the percentage of cancer patients with advanced metastatic disease who benefit from these targeted therapies at only 7%[11]. This limited success rate can be attributed to a number of reasons like narrow patient eligibility, prohibitive costs, as well as poor predictivity of genome-guided drug discovery alone.

Modeling the clinical variability at the preclinical stage to inform clinical decisions is especially difficult using standard cancer models. PDO are uniquely well-suited to capture the intratumor and interpatient clinical heterogeneity[12-13] thanks to being derived from fresh patient tissue without prior manipulation and to preserving the original cancer features indefinitely once in culture. Large biobanks of breast, colorectal, pancreatic, ovarian, bladder, and head and neck cancer organoids[13-17] have therefore been created in the attempt to replicate the clinical heterogeneity in the lab.

These living biobanks reflect the histopathological and genetic profiles of the host, making them powerful avatars to interrogate the efficacies of treatments for different cancer subtypes and patient populations (Figure 3). PDO can be used to inform multiple stages of the drug development pipeline, from initial compound screening to target validation and clinical trials. PDO can reflect a greater range of patient backgrounds, thus identifying therapeutics which may be effective but had been rejected using standard preclinical cancer models.

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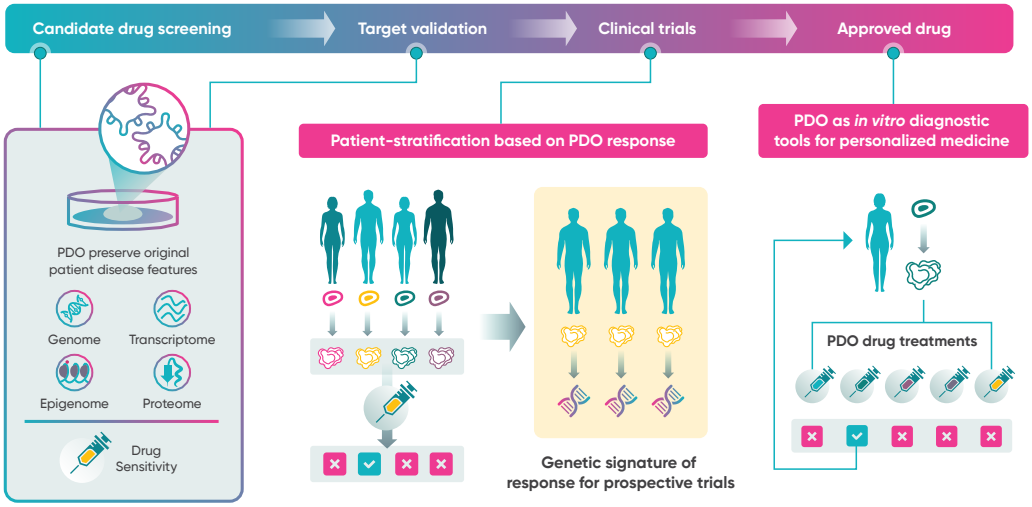
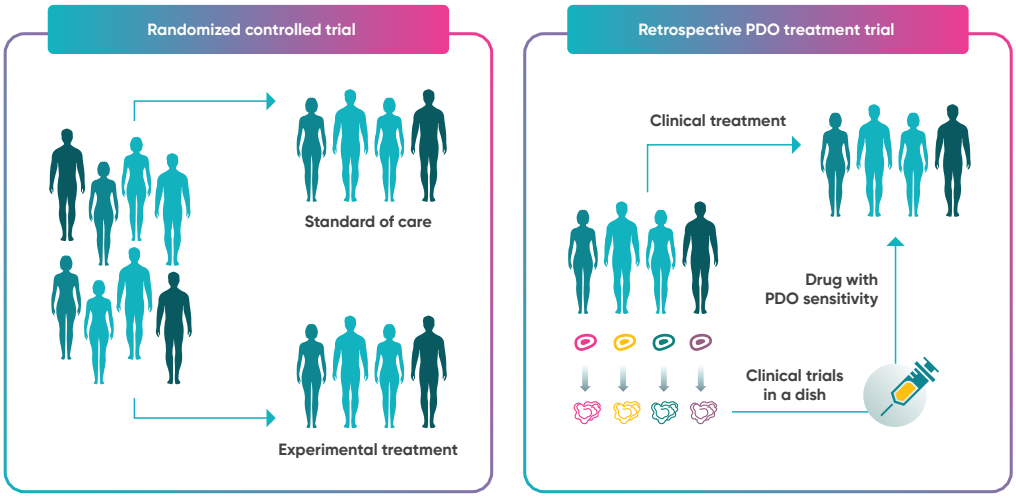
Figure 3. HUB Organoids in drug development



PDO-based retrospective studies (Figure 4), or ***"clinical trials in a dish"***, have been used to establish the validity of PDO-guided therapy in the clinic and have reproduced known associations between specific genetic mutations and sensitivity to targeted therapies[18-19]. These results suggest that a PDO-guided therapeutic decision-making integrated with a genetically tailored approach could outperform genome-guided therapies alone, thanks to providing a more physiological and patient-relevant environment for drug screening. This can ultimately lead to the identification of more robust genomic targets with greater clinical relevance.

Figure 4. Restrospective studies have been used to establish the validity of PDO-guided therapies in clinic



OVERCOMING THE COMPLEXITY OF ORGANOID SCREENING

The *in vitro* nature of organoid technology lowers the barrier for scientists to start using patient-derived models in their lab.

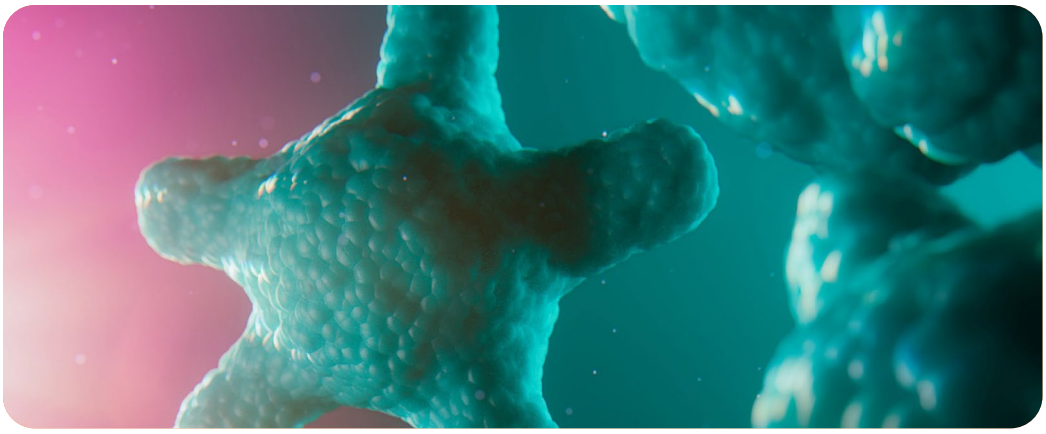
The collective knowledge and experience of HUB and the Clevers group has developed robust protocols to enable this technology to be applied to many different organs and diseases. The precise control of culture conditions and organoid manipulation are essential for successful and reproducible organoid establishment and expansion. This in depth knowledge of cellular pathways and biological processes represents HUB Organoid Technology itself and it is a key part of the strong and broad patent portfolio of HUB.

In addition to specializing in new organoid model and assay development for their customers, HUB offers drug screening services which allow to apply HUB Organoid Technology to any drug development program. Alternatively, HUB provides licenses to pharma and biotech companies wanting to set up an organoid platform in their labs using HUB Organoid Technology, which will also allow them to receive extensive support to establish the technical and scientific depth of understanding required to successful apply the models.

SUMMARY

In summary, patient-derived models such as PDX and HUB Organoids are more clinically relevant than conventional models as they preserve key genomic features and histopathology of original patients and represent the heterogeneity and diversity of the patient population. Additionally, organoids offer the scalability of an *in vitro* system which makes them better suited to large scale drug screens compared to *in vivo* models.

Precision medicine, whereby patients are treated depending on the genetic make-up of their tumor, represents a promising avenue but requires preclinical models that faithfully recapitulate the patient genetic signature to investigate new treatment options and improve the success rate of these strategies. HUB Organoids are highly predictive of patient response and enable to perform clinical trials in a dish, which can be run agnostically to identify biomarkers of response or based on a genetically tailored approach to evaluate the efficacy of a new treatment strategy. These type of studies allow to more accurately predict patient response, therefore leading to better clinical candidates. This in turn results in more cost effective drug development programs and, ultimately, patient benefit.



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