

Establishing an automated high-throughput assay that supports the growth of prostate cancer organoids

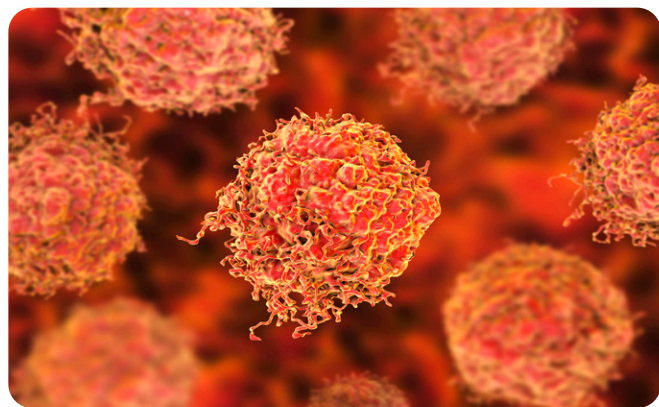
Introduction

Prostate cancer is one of the leading causes of cancer-related deaths in men, second only to lung cancer.¹ The standard of care for patients diagnosed with advanced and metastatic prostate cancer is Androgen Deprivation Therapy (ADT), sometimes in combination with other Androgen Receptor (AR)-directed treatments or taxane-based chemotherapy. However, 10–20% of cases progress after androgen deprivation to a more aggressive disease stage known as Castration-Resistant Prostate Cancer (CRPC).² Unfortunately, various mechanisms of resistance emerge in CRPC, including enrichment of genomic alterations associated with poor prognosis, aberrations that maintain the activation of the AR pathway, and phenotypic transformation into AR-null and neuroendocrine phenotypes.³

Patient-derived tumor models

Two of the most common ways that cancer tissue is used in preclinical research is as Patient-Derived Xenografts (PDXs) and organoids, both of which have distinct advantages and disadvantages for preclinical testing. Once these models are established, they provide a renewable source of patient tumor cells that can be exploited for multiple research purposes. However, it can often be challenging to establish PDXs and organoids from fresh prostate cancer tissue.

PDX models are established by transferring patient tumor fragments into immunodeficient mice. This allows tumor growth and subsequent transplantation into secondary recipient mice. PDXs often maintain the cellular and



histopathological structures of the original tumors and are a robust and low throughput way to model human tumors *in vivo*. Organoids are three-Dimensional (3D) cultures of prostate cancer cells embedded within an extracellular matrix. These cultures closely recapitulate the genetic/epigenetic diversity and morphological features of the original tumors. One of the major advantages of organoids is their scalability, making them particularly useful for screening large numbers of compounds or comprehensive dose responses of individual compounds, which would not be feasible with *in vivo* PDX experiments.

High-throughput assay development

In a recent study, a group of Australian researchers successfully developed an automated, high-throughput assay that enabled the growth, treatment, and analysis of organoids grown from prostate cancer PDXs.³ The team believes that coupling this approach with other

patient-derived models will expand the capacity and rigor of preclinical testing of new treatments before they enter clinical trials.

In the first phase of their study, the researchers acquired prostate cancer tissue from patients to establish serially transplantable PDXs. PDX tissue was then digested, and cells were seeded at a density of 1×10^5 cells per 40 μL of Matrigel in 24-well plates. Once the organoids were established, they were robotically reseeded in Matrigel in 384-well plates at 10 μL of 35% to 80% Matrigel using the JanusG3 liquid handling robot with a cooled stage. Although automation of 3D organoid imaging pipelines with liquid handling and robotics has been reported by other research groups, the organoids in those assays were layered on top of the gel rather than fully embedded within the 3D extracellular matrices. In the present study, the JanusG3 enabled the team to automatically dispense a complete organoid/Matrigel suspension.

The researchers initially supposed that Matrigel couldn't be kept cold on a robot deck and that it would be too viscous for them to manage all of the pipetting steps. However, the use of a cold block helped overcome these inherent challenges and enabled them to test multiple concentrations of Matrigel. This meant they could rapidly optimize the organoid growth conditions. "It was much quicker than we expected, and the reproducibility is fantastic," said Kaylene J. Simpson, who is based at the Peter MacCallum Cancer Centre in Melbourne and one of the study authors. "We keep the same tips because it has already aspirated an amount of Matrigel, so we don't have any wastage."

Drug compound treatment and imaging

Next, the researchers identified robust readouts for measuring drug responses based on talazoparib treatment and then applied the high-throughput imaging assay to a pilot compound screen. For this, the organoids were grown for eight days to develop larger structures and then treated with drug compounds every two to three days until day 21.

A total of 42 compounds were tested at three concentrations of compound stock solution in 100% DMSO (0.1, 1, and 10 μM) in technical duplicates on each plate, along with six controls. The drug stock plates were hydrated with organoid media and the compounds added to two biological replicate cell plates per organoid type using the Sciclone ALH 3000 robot. The researchers used live-cell imaging with brightfield microscopy to monitor the morphology of organoids before, during, and after the drug treatment. On day 21, the organoids were stained with Hoechst and imaged with fluorescence microscopy and metabolic activity was measured using a luminescent cell viability assay.

Following analysis of their results, the researchers concluded that their study demonstrates the ability to automate preclinical testing with prostate cancer organoids with diverse phenotypes. Writing in *SLAS Discovery*, they said: "We have shown that using a combination of plate-reader and imaging-based parameters can generate robust readouts. Our approach can be used to quantify changes in the growth of heterogeneous 3D cultures to candidate drugs or compound libraries and across whole wells or specific subpopulations of organoids."

References

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2. Kirby M, Hirst C, Crawford E. Characterising the castration-resistant prostate cancer population: a systematic review. *International Journal of Clinical Practice*. 2011;65(11):1180-1192.
3. Choo N, Ramm S, Luu J, Winter J, Selth L, Dwyer A et al. High-Throughput Imaging Assay for Drug Screening of 3D Prostate Cancer Organoids. *SLAS Discovery*. 2021;26(9):1107-1124.

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