Challenges, applications and future directions of precision medicine in prostate cancer – the role of organoids and patient-derived xenografts

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Abstract

Precision medicine is the concept of individualising patient management based on specific tumour characteristics and biology, rather than traditional histological subtypes. The overall aim is to personalise management to individual patients in order to provide the right cancer treatment to the right patient at the right time. While the approach aims to improve clinical outcomes, decrease morbidity and improve survival in men with advanced prostate cancer, its clinical application is in its infancy. In prostate cancer there has been a lack of data identifying potentially targetable alterations or biomarkers indicating response or resistance to therapies. Furthermore, it remains difficult to attain tissue or tumour specific biological material for subsequent analysis. In this paper, we aim to provide a clinically relevant outline of various current precision medicine principles and available evidence on the application and potential for a precision medicine approach in prostate cancer.

Introduction

Prostate cancer (PCa) remains the second highest cause of cancer-related deaths internationally, however management of advanced or metastatic disease is life prolonging but rarely curative (1). “Precision medicine” is a concept which aims to personalise management to individual patients in order to provide the right cancer treatment to the right patient at the right time. Patient management is individualised based on their specific tumour characteristics and biology, rather than traditional histological subtypes. While the precision medicine approach aims to improve clinical outcomes, decrease morbidity and improve survival in men with advanced PCa, its clinical application is in its infancy (2).

A challenge limiting the widespread uptake of precision medicine techniques is the difficulty in attaining tissue or tumour specific biological material (especially bone metastases) for subsequent analysis. Furthermore, identified aberrations may be non-functional or not the main driver mutations in an individual tumour, and thus therapeutic targeting of these aberrations may not produce the expected clinical response. *In vitro* therapeutic screening of patient derived cultures may allow a feasible mechanism by which functional screening of identified aberrations via next generation sequencing (NGS) can occur (Figure 1).

In this paper, we aim to provide a clinically relevant outline of various current precision medicine principles and available evidence on the application and potential for this developing management approach in PCa. First, patient groups who would benefit most from this approach will be identified, followed by discussion of the utility of currently established cell lines, the role of organoids and patient derived xenografts in a precision medicine approach to treating prostate cancer.

Identification of at risk and treatment resistant patients

The benefits of precision medicine will likely be best realised in those patients at highest risk of cancer recurrence and PCa-specific death in order to identify effective therapeutic strategies early or prioritise enrolment to appropriate clinical trials. Furthermore, identifying which men will have biochemical recurrence and which treatment modality would be most effective for them when they recur also remains an important step in precision medicine. Investigations of diagnostic biomarkers for use prior to treatment of localised disease in order to identify these men have been disappointing. In men with biochemical recurrence (BCR), PSA doubling time of less than 3 months, stage T3b or higher, Gleason score 8 or

greater, and time to BCR in less than 3 years are associated with high risk of metastasis and PCa-specific death (3). Similarly, baseline PSA, PSA doubling time and PSA velocity predict development of metastases and overall survival among men with castrate resistant prostate cancer (CRPC) (3). Survival benefits of 3-4 months with taxane-based chemotherapy or second generation antiandrogens, such as enzalutamide or abiraterone, have been observed resulting in these agents traditionally being used in the CRPC setting (3). However resistance to these therapies is observed in up to 30% of cases, thus exposing a significant number of patients to their risks without benefit (4).

In response, a growing body of evidence has been generated in response to the need for clinical samples and subsequent analysis to identify potentially targetable alterations or biomarkers for responses or resistance to therapies in the BCR and metastatic CRPC (mCRPC) disease states (5-7). An established approach has been use of "omic" techniques that correspond to different hierarchical levels of cellular organization, such as transcriptomics (RNA sequencing and analysis), proteomics (studying the protein profile) and metabolomics (analysis of cellular metabolites and products of metabolism in culture) (8). Metabolomics is of direct clinical relevance given choline-based molecular imaging, and studies have mostly focused on biofluids (expressed prostatic fluid, semen, serum) prior to translation to imaging such as magnetic resonance spectroscopic imaging (MRSI) (8-10). Incorporation of MRSI can improve specificity when coupled with high sensitivity of multiparametric MRI used in clinical practice, but remains investigational currently due to limited expertise and longer acquisition times (11). Integration of these 'omics' approaches allows for pathway analysis, to indicate potential loss of tumour suppressor genes in prostate tissues (12).

Large-scale studies using NGS have significantly added to the available knowledge base with further identification of potential therapeutic targets. Robinson et al and the Stand Up To Cancer Prostate Cancer Dream Team (SU2C/PCF) reported DNA and RNA NGS results from 150 patients with mCRPC to show that up to 90% of these patients had actionable aberrations, which was much higher than previously reported in PCa (5). Specifically, aberrations of androgen receptor (AR), ETS genes, TP53, and Phosphatase and tensin homolog (PTEN) were frequent (observed in 40%–60% of cases), while BRCA2, BRCA1, ATM and pathogenic germline aberrations were also higher than previously reported (5). Multiple new genomic alterations were described, including phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha/beta (PIK3CA/B), R-Spondin, B-Raf proto-oncogene/Raf-1

Proto-Oncogene (BRAF/RAF1), APC, β-catenin, and Zinc Finger and BTB Domain Containing 16/Promyelocytic leukaemia zinc finger protein (ZBTB16/PLZF) (5). These results were further confirmed by Pritchard et al. who demonstrated that the incidence of germline mutations in genes mediating DNA-repair processes, such as BRCA among men with metastatic PCa was significantly higher than the incidence among men with localised PCa (13). Expanding on from SU2C/PCF's 150 cohort, 429 patients with mCRPC with longitudinal clinical outcomes, integrating whole-exome, transcriptome, and histologic analysis were analysed (14). The frequency of genomic alterations was similar, particularly with AR, ETS genes, TP53, PTEN and RB1 as the most commonly altered genes (14). Single-nucleotide variants were the most frequently altered genes to be oncogenic, with a high fraction of oncogenic mutations in AR, TP53, PIK3CA, BRCA2, PTEN, APC, and CDK12 (14). Of all the molecular factors examined, alterations in RB1 had the strongest association with poor clinical outcomes (14). This cohort is one of the largest to have undergone genomic analysis to identify mutations, but also clinically representative for men with CRPC receiving standard of care treatment or clinical trial participation (PARP inhibitor, Aurora kinase A inhibitor therapy) with high prevalence of oncogenic mutations, such as TP53, to indicate disease severity at a molecular level.

In identifying these clinically actionable aberrations, this study provided valuable information that could impact treatment decisions for affected individuals. For example, a patient with an AR-V7 splice variant of the androgen receptor (which has been implicated in abiraterone acetate and enzalutamide resistance), could receive greater benefit from alternate, non-androgen dependent therapeutic options, such as up front chemotherapy (15, 16). AR-$V7^{+}$ status in circulating tumour cells has been associated with reduced PSA change, worse progression-free and overall survival when treated with abiraterone or enzalutamide (17). In a small comparative series, AR-$V7^{+}$ status predicted a more favourable PSA response and progression free survival when treated with docetaxel than with abiraterone or enzalutamide (18). Conversely, another variant ARv567 has been reported to predict high sensitivity to docetaxel in patient derived xenografts (PDX) (4). Despite small sample sizes in select clinical cohorts, the reproducible responses across different studies may confer favourable translation of these studies to clinical practice.

Furthermore SPOP, CHD1 and ERG status may be used for segregation, as they predict future AR loss and possibly aggressive cancer development (16, 19). Tumours with loss of RB

on the other hand may require aggressive treatment as RB loss has been shown to increase AR expression. Further applications of this approach could include patients receiving dovitinib (a pan receptor tyrosine kinase (RTK) inhibitor) to counteract gain-of-function mutation in PTEN/AKT. Additionally, tumours with unregulated protein kinase B (AKT) or gain-of- function mutations in AKT may respond to treatment with Everolimus (a mammalian target of rapamycin (mTOR) inhibitor) to inhibit mTOR, a downstream target of AKT (5, 16).

An emerging clinical scenario for this precision approach is in neuroendocrine PCa, which traditionally is a rare but highly aggressive subtype of prostate cancer with a low median survival with a poorly understood molecular and clinical natural history (20, 21). Treatment-induced neuroendocrine PCa is becoming increasingly common clinically, through the use of second line antiandrogens. The ideal management approach is unclear, as it may evolve from pre-existing prostate adenocarcinoma into an AR independent state, thus bypassing most hormone-dependent treatments. However, altered N-myc signaling and aurora kinase A upregulation have recently been implicated in the pathogenesis of neuroendocrine PCa, representing targetable aberrations with ongoing clinical trials (20, 21).

The 100,000 Genomes Project aims to sequence 100,000 genomes from patients affected by rare diseases or cancer. The project completed in 2018 and results are awaiting publication, potentially increasing the understanding of the genomic landscape of prostate cancer (22).

While these large studies have identified new potential targets on the basis of genetic aberrations, it is important to appreciate that these targets may be non-functional or passenger mutations in individuals, and therefore not provide clinical benefit if targeted with specific drugs. Thus *in vitro* therapeutic screening of patient derived cultures, such as with the use of organoids, may be the key to demonstrating functional effect of identified aberrations via NGS prior to treatment in an individual patient. Furthermore, and of clinical relevance, it is important to acknowledge that these studies have mostly focused on men with advanced, often castrate-resistant, disease and these actional aberrations may not be present in primary disease or early biochemical recurrence, thus limiting the applicability in these disease settings.

Patient derived samples and the challenges of *in vitro* investigation

Patent derived samples allow application of methods to recapitulate many of the physiologic tumour characteristics in both *in vitro* and *in vivo* scenarios. The main models used include tumour derived 2D cell culture, patient derived xenografts (PDXs) and more recently 3D "organoid" culture. Each technique has benefits as well as challenges in ease of use, cost, accessibility and reproducibility (Table 1). Historically 2D cancer cell lines have been used for research discovery and drug screening. In these models, cells are isolated from tissues and grown as a single cell monolayer in a culture flask in various growth medias (2, 23, 24). *In vitro* culturing of prostate cancer cells has remained challenging, as demonstrated by the limited number of clinically relevant available PCa *in* vitro cell line models, despite many attempts by various international research groups (25-32). The under-representation of prostate cancer cell lines is due to the difficulty in propagating PCa cells *ex vivo* for extended periods whilst maintaining prostate tumour characteristics (2). The limited variety of prostate cell lines *in vitro* has historically hindered the study and advancement in knowledge of PCa tumour pathogenesis and therapeutic responses of PCa, as well as application of precision medicine techniques.

As most of the readily available cell lines were sourced from metastatic PCa deposits, none of them fully represent the spectrum of PCa disease states encountered clinically, which limits relevance of their investigation. While androgen receptor (AR) signaling has been heavily implicated in PCa development, homeostasis, initiation and progression, many established cell lines lack an intact AR signaling pathway (2, 23). Furthermore, most cell lines lack many of the known genomic aberrations that underlie prostate tumourigenesis for example SPOP mutation, FOXA1 mutations and CDH1 loss (33-36). Although *in vivo* PDX models have been described and are available, their use is limited due to expense, time-frames and technical challenges (23, 37). These limitations in available models has hindered progress in precision medicine techniques and highlights the need for, and importance of, patient-derived cancer-specific cell models that represent contemporary clinical disease to facilitate *in vitro* therapeutic screening and validation with NGS of tumour aberrations.

Characteristics of commercially available PCa cell lines

The commercially available, widely used PCa cell lines were derived from various metastatic sites and show a variety of phenotypes, as outlined in Table 2 and discussed below.

PC-3: The PC-3 cell was derived from a bony metastatic deposit from a 62 year old Caucasian male in 1979 (26) and is one of the most widely utilised PCa cell lines. Despite this however the cell line is atypical of common clinical phenotypes, being both AR and PSA negative, and androgen independent. Furthermore, PC-3 cell line produces osteolytic bone metastasis in vivo, instead of the classical osteoblastic prostatic bone metastases seen most commonly in patients (26, 31). They form lymph node metastases following orthotopic (intraprostatic) inoculation (38).

LNCaP: The LNCaP cell line was derived from a left supraclavicular lymph node metastatic PCa deposit from a 50 year old male in 1977 (25). The cell line is hormone responsive with a mutated AR and expresses PSA (25). It is very slow growing in comparison to PC-3 cells and is less tumorigenic, requiring a higher cell concentration for formation of tumours. They form lymph node metastases following orthotopic inoculation (38).

DU145: The DU145 cell line was derived from a central nervous system metastasis of a 69 year old Caucasian male with PCa and lymphocytic leukaemia at time of parieto-occipital craniotomy (31, 32). The tumour was described to be a moderately differentiated adenocarcinoma. DU145 cells, similar to the PC-3 cell line, do not express AR or PSA, are hormone insensitive and form atypical osteolytic bone metastases (31). They do not form lymph node metastases following orthotopic inoculation (38).

DuCaP: The DuCaP cell line was derived from dura mater tissue taken at autopsy of a 60-year-old Caucasian male with hormone refractory widespread bony metastases and first described in 2001. The line was initially propagated in SCID mice and the resultant patient derived xenograft (PDX) harvested and cultured *in vitro* to generate the cell line (30, 31). DuCaP cells are androgen sensitive and PSA and AR positive.

VCaP: The VCaP cell line was derived from the same patient as the DuCaP line (27, 31), but tissue was harvested from a lumbar vertebral metastasis at autopsy. The tissue was xenografted into SCID mice, and later harvested and converted to *in vitro* culture. VCaP cells express AR and large quantities of PSA (27, 31).

Organoids in prostate cancer and novel media technology

An intrinsic limitation with *in vitro* cell culture is the limited number of passage before cells undergo senescence and stop growing. This is the so-called "Hayflick limit", first described in 1965 (39). The Hayflick limit can be bypassed using artificial immortalisation of cells through reactivation of telomerase and the inactivation of the p53 and RB tumour suppressor pathways (2, 39, 40).

The development of organoid technology and its subsequent utilisation as pre-clinical models of disease began in 2009 following a report by Clevers and colleagues that stem cells resident in the adult intestine proliferate and self-organise *in vitro*. (41-43). The organoid culture technology developed by Clevers and colleagues has been adapted to PCa to allow for the indefinite propagation of both benign and malignant prostate cells without the need for artificial transformation. Furthermore, the culture technology appears to maintain the integrity of the genome without evidence of genetic drift and potentially allows development of new clinically applicable cell lines with a high success rate (2).

Organoids in general, are three-dimensional cell constructs composed of multiple cell types which are believed to originate from stem cells or progenitor cells of the organ of interest (Figure 2). Organoids are capable of self-organisation and differentiation to resemble the morphology and function of their native organ (42, 44-46). For organoids to form, culture media containing target tissue relevant growth factors and tissue containing viable stem cells or progenitor cells are required. These cells from disaggregated tissue are either placed into coated plates or using an extracellular support matrix such as Matrigel™, to allow cells to propagate in a three dimensional manner to form organoids (2, 23). They permit *in vivo* and *in vitro* investigation, and represent one of the latest innovations in the quest for a model to recapitulate the physiologic processes of whole organisms to best model disease progression and therapy resistance (47).

Compared to two-dimensional cultures, organoids have several advantages, including representing near-physiological cellular composition and behaviours. Many organoid cultures are able to maintain genome stability while expanding in culture to allow further analysis for example via drug screening. Compared to similar 3D or near physiological

models, such as PDXs (discussed below), organoids are comparatively simpler and less resource intensive, while more readily affording additional genetic manipulation and analysis than *in vivo* models.

The main limitation of organoids is their derivation from biopsy samples. It is possible that biopsied deposits are heterogenous not representative of the entire spectrum of clones derived from the primary tumour. This may result in potential selection and treatment bias towards specific clones and the role of repeat biopsies and tissue sampling must be considered prior to wide spread clinical use. This limitation is not limited however to just organoids, but also cell lines and PDXs.

Pre-clinical use of organoids in prostate cancer

In 2014 Karthaus and colleagues first described a 3D culture system that supported the long-term expansion of primary mouse and human prostate organoids composed of fully differentiated CK5+ basal and CK8+ luminal cells. They showed that cultured organoids were genetically stable, reconstituted prostate glands in recombination assays and were amenable to experimental manipulation (24). This work was subsequently furthered by Gao, Vela and colleagues from Memorial Sloan Kettering Cancer Centre in New York, in collaboration with the Clevers group. This resulted in the long-term culture of seven prostate cancer organoid lines originating from biopsy specimens and circulating tumour cells (2). Unlike the existing 2D cell lines described above, these organoid cell lines recapitulated the molecular diversity of prostate cancer subtypes, including lines with PTEN loss, TMPRSS2–ERG fusion, TP53, FOXA1, SPOP mutation, SPINK1 overexpression and CHD1 loss not previously replicated in a pre-clinical non PDX model (2). Once established, these lines underwent successful drug testing, to both clinically available treatments such as the antiandrogen enzalutamide used in clinical practice, as well as other experimental and pre-clinical treatments such as PI3-kinase pathway inhibitors and BKM-120 (2).

Organoid cultures can also be used to study interactions and causative mechanisms of prostate cancer pathogenesis. Lee and Park used organoid culture and lentiviral infection of primary human benign prostatatic epithelium to outline the role of N-Myc in

neuroendocrine prostate cancer development (48, 49) Patient derived organoids (PDOs) have also been used to model rare prostate cancer phenotypes (50). This has highlighted the role of key molecular pathways in CRPC-NE pathogenesis. These organoids subsequently underwent drug screening, where concordance of drug response with the tumour genomic profile was identified, including response to AKT inhibition in the presence of PTEN loss. Moreover, using an EZH2 inhibitor in combination with other drugs illustrated potential novel combinations that inhibited organoid growth and which now require further testing in the clinical setting (50).

Patient derived xenografts

Patient-derived xenograft (PDX) models are created by implanting patient derived tumour cells into immunodeficient mice, to allow propagation but prevent tumour rejection by the immune system (51). Solid tumours or cell suspensions derived from solid tumors are placed either subcutaneously, orthotopically (same organ as original tumour), or under the kidney capsule. Subcutaneous implants are technically easier, but do not replicate original tumour microenvironments as orthotopic samples and do not metastasise (51). Implantation under the kidney capsule has been reported to enhance the take rate of tumours due to improved vascularisation but is technically challenging (51). The reliable establishment of PDX cell lines has remained a challenge for investigators as these lines are limited by low take rates and long latency times (37). In 2014, the Movember Foundation launched a Global Action Plan 1 (GAP1) project to support an international collaborative prostate cancer PDX program involving eleven groups with the aim of increasing the global accessibility and availability of PDX lines. A total of 98 lines internationally were collected, including 83 newly derived PDX. The GAP1 series of PDX lines represents the full clinical spectrum of prostate cancer, including androgen-sensitive and castration-resistant primary and metastatic lines. Furthermore, neuroendocrine lines were also generated, demonstrating the clinical relevance and potential utility of PDX as a precision medicine tissue source for drug screening and a platform for pre-clinical models and future therapeutic development (37).

The application of PDXs for laboratory analyses has been limited due to the lack of a culture technology to reproduce significant quantities of prostate PDX-derived *in vitro* cultures. This

limitation had made PDX models less favourable for high-throughput screening, genetic manipulation, and mechanistic analysis studies. These challenges have prompted researchers to explore culture conditions that enable the survival and propagation of prostatic cells *in vitro* (52). To increase the applicability and to allow drug screening and other downstream analysis techniques, groups have attempted the application of organoid technology to allow *in vitro* transformation of PDX *in vivo* tumours (23, 53). Several groups have developed conditions for growing LuCaP PDX-derived cells as organoids to proliferate a sufficient number of them for *in vitro* assays and genetic manipulation prior to reimplantation in vivo (53, 54). Transcriptomic and genomic features were reported to be highly conserved between organoids and the original PDX. Applying this PDX-derived organoid platform concordance has been observed between organoids with BRCA2 mutation and their sensitivity to PARP inhibitors (53).

This potential application was discussed in a recent study where PDX derived organoids were used to study the anti-proliferative effect of HSP90 inhibitors. HSP90 is a chaperone protein that assists proteins, including proteins required for tumour growth and replication to fold properly, stabilises proteins against heat stress, and aids in protein degradation (55). In a high-throughput screening of 15 LuCaP PDX-derived organoids against 110 drugs, which were selected for their potential as therapeutic agents in prostate cancer, they identified ganetespib and onalespib as two of the most broadly active agents that at submicromolar concentrations inhibit HSP90, suggesting potential clinical applications in PCa (55). Armstrong and colleagues have similarly applied techniques to demonstrate that presence of the AR variant ARv567 predicts high sensitivity to docetaxel using a PDX model (4). Finally, Young and colleagues reported establishment and serial passage of cell lines from six LuCaP xenografts using spheroid culture technology (56). These advances may permit establishment and assembling a biobank of patient derived xenografts and even patient derived cell lines and tissues.

Conclusion

A precision medicine approach in PCa has evolved considerably in the last 10 years and application into clinical practice is closer to becoming a reality. Next generation DNA and

RNA sequencing techniques that allow the identification of targetable tumour aberrations can now be coupled with functional *in vitro* therapeutic screening of patient derived cultures. Cell culture conditions, rich in growth factors that allow normal cells to grow and proliferate to enable generation of multicellular prostate organoids, may alter the dependency of cancer cells on driver mutations and oncogenes. Therefore, further studies are crucial to determine which specific culture approaches lead to superior assessment of therapeutic response.

Organoid culture technology can facilitate personalised medicine by enabling high-throughput drug screening for therapeutic effects, development of organoid biobanks and modelling of cancer initiation and the molecular characterisation of tumour phenotypes in order to discover novel biomarkers and therapeutic targets. Clinical trials are necessary to establish the true level of prediction of *in vitro* treatment responses of organoids relative to the individual's actual clinical therapeutic response.

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Table 1. Comparison of the benefits and challenges of potential precision medicine tissue models.

	2D Cell lines	Organoids	PDX
Ease of use	Simple	Complex	Very complex
Cost	Relatively cheap	Expensive	Very expensive
Physiological representation of original tumours	Limited	Some physiological representation	Near physiological
Ability to manipulate cells	Very easily	Good but difficult	Very limited
Ability to drug screen	Yes	Yes	No
Ability of sequence cells/tumours	Yes	Yes	Yes

PDX: Patient derived xenografts

Table 2. Summarising readily available prostate cancer cell lines, in comparison to the novel organoid cell line MSKCC 3 by Gao et al. (2014)

Cell Line	AR	PSA	Androgen Response	Site of Origin
PC-3	Negative	Negative	Independent	Bone
LNCaP	Mutated AR	Positive	Dependent	Lymph Node
DU145	Negative	Negative	Independent	CNS
DuCaP	Positive	Positive	Dependent	Dura
VCaP	Positive	Positive	Dependent	Bone
MSKCC-3	Low Positivity	Low Positivity	Weakly dependent	Lymph Node

AR: androgen receptor, PSA: prostate specific membrane antigen

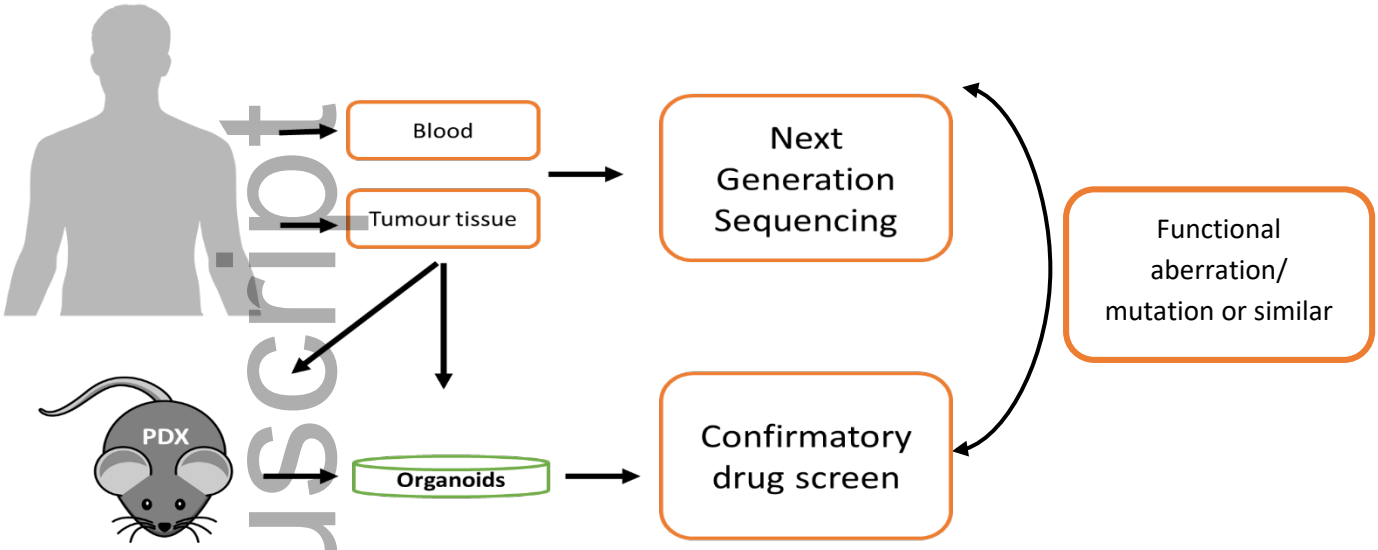

Figure 1. A schematic view of precision medicine in prostate cancer. Patient tissue and blood samples are utilised to identify targetable tumoral aberrations. Concurrent i*n vitro* cell culture is used for confirmatory drug screening to identify silent and active mutations to increase clinical efficacy of treatments. PDX, patient derived xenograft

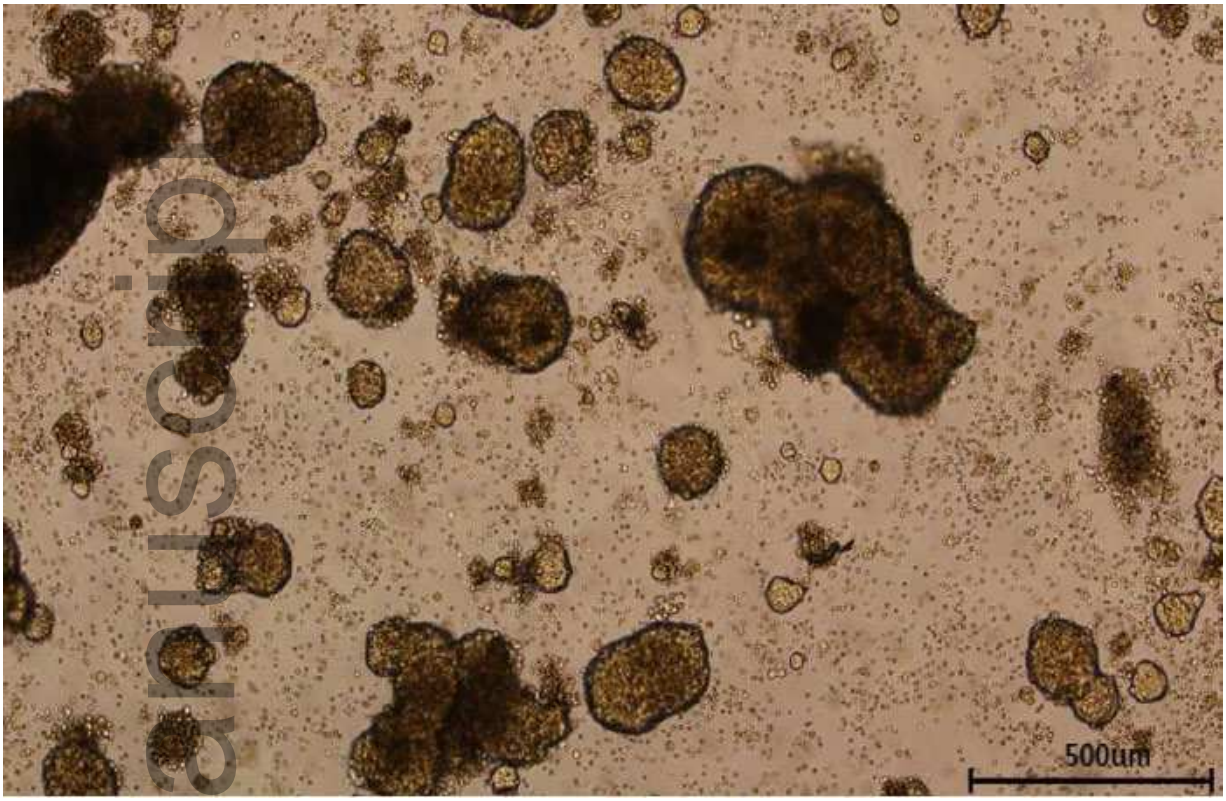

Figure 2. Patient derived organoid culture, derived from excisional lymph node biopsy metastatic prostate cancer-containing lymph node. Bright field image, Scale bar: 500 µm.