Therapeutic Discovery for Castration Resistant Prostate Cancer: PDX Modelling Brings New Options to the Table

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Keywords: abiraterone; androgen receptor; castration-resistant prostate cancer; enzalutamide; patient-derived xenograft; ribosome

Word count: 1000

Licensed treatment options for metastatic castration resistant prostate cancer (CRPC) now include hormonal interventions (abiraterone, enzalutamide), chemotherapy (docetaxel, cabazitaxel), the radiopharmaceutical radium-223 and, in the US, immunotherapy (sipuleucel-T). However, median survival remains inadequate at 2-3 years from castrate resistance [1]. Within this issue of European Urology, Lawrence et al [2] describe a labour intensive, but data and opportunity rich, approach to discovery of novel treatment options, by exploiting a suite of CRPC models, centred on patient derived xenografts (PDX).

Four PDX models were selected from two CRPC patients, derived from dural (x2), inguinal lymph node and lung metastases harvested at rapid autopsy (from 109 samples from 29 patients, yielding 10 serially transplantable PDXs). These recapitulated genomic features of the original samples across multiple PDX generations and grew in castrate hosts, although three also retained androgen responsiveness. They also exhibited inter- and intra-patient genomic diversity reflecting CRPC intra-tumoural heterogeneity [3]. Complex mechanisms of androgen receptor (AR) associated resistance to hormonal therapy existed, to which both were extensively exposed. These included *AR* genomic structural rearrangements in one patient associated with co-expression of full length and variant AR forms (ARv567es). From the other patient, one PDX contained gain of function *AR* mutations including a previously documented T878A and a novel C687Y mutation, conferring gain of function agonist properties to flutamide, hydroxylflutamide and dexamethasone, and partial enzalutamide resistance. By contrast, this patient's other PDX displayed genomic and gene expression characteristics consistent with an AR null neuroendocrine phenotype.

These models were then utilised for 'rapid' evaluation of therapeutic options in both tumour explants and organoids on enriched 'identified core pathways' based on RNA-seq data and druggable targets. *MYC* pathway gains were the most frequently enriched. This was targeted through bromodomain and extra terminal (BET) protein inhibition (iBET151, JQ1) to deplete c-MYC levels

and, in addition, ribosome biogenesis through RNA polymerase I transcription inhibition (CX-5461) and pan-PIM kinase inhibition (CX-6258). Other targeted pathways were AR response (enzalutamide, galeterone), cell cycle (CDK4/6 inhibitor ribociclib) and DNA repair (PARP inhibitor talazoparib, cisplatin). Consistent with genomic derangements suggesting hormonal therapy resistance, a lack of homologous recombination (HR) defect, and loss or *RB1*, there was therapeutic resistance to AR directed therapy, PARP inhibition, cisplatin and ribociclib. However, treatment responsiveness existed in transcriptomic, PDX and explant models, to targeting of MYC signalling, and particularly ribosome biogenesis and function through CX-5461 and CX-6258. Response occurred in each PDX, despite their genomic diversity.

Ribosome biogenesis and nucleolar function are central to suppressing p53 activation following oncogenic stress and their derangement is characteristic of MYC driven cancers. PIM kinases activate MYC signalling and mRNA translation in many cancers. As a result, ribosome biogenesis is proposed as a potential therapeutic target in various cancers, including prostate cancer, and now supported by the work presented in this issue, either through inhibition of RNA polymerase I transcription leading to p53 activation (e.g., through agents like CX-5461), or through PIM1 inhibition (e.g., through agents like CX-6258) [4-6]. Phase I clinical evaluation of CX-5461 in advanced haematologic cancers reported initial tolerability and on-target drug effect of Pol1 transcriptional inhibition [7]. A further phase I/II trial in breast cancer is also recruiting currently (NCT02719977). It is worth noting however that CX-5461 activity may be as a G-quadruplex DNA stabilizer, with specific toxicity in cancer cells with HR and non-homologous end joining DNA repair deficient cancers, or through activation of ATM/ATR19 or targeting mammalian target of rapamycin-related signalling pathways [8-10]. Thus, the mechanism of efficacy with this specific agent might conceivably be broader than ribosome biogenesis.

A PDX centred approach to therapeutic discovery provides opportunity to develop complex models from which we can attempt to draw insights. Conceptually these could provide a more clinically relevant model compared, for example, to cell line experiments or genomic or gene expression analyses of tumour samples. However, there are some potential challenges also. The drop off from 109 samples/29 patients to 4 PDXs/2 patients in this work is substantial, and likely reflects the complexities of generating and maintaining such models with scalability a challenge. The authors focussed on these particular PDXs to represent aggressive tumours and thus a 'high threshold' for pre-clinical evaluation of novel therapeutic options. However, a converse perspective is that having exhausted conventional, and some historical, treatment options their cancers almost certainly had heavily treatment induced genomic change. Arguably, in 2018, we need most to develop therapeutic options to reflect the point of emergence of CRPC, or earlier, rather than at extensively pre-treated death. Although described as 'enzalutamide and abiraterone resistant' (one patient received both), each patient was also exposed to multiple AR targeted drugs (both received each of bicalutamide, nilutamide, stilboestrol and dexamethasone, one received cyproterone also), that are arguably no longer components of modern therapy but may induce specific AR activating point mutations, and chemotherapy (both received docetaxel, one cabazitaxel). Furthermore, whilst CRPC may be a disease of nodal and visceral metastases, it remains commonly dominated by bony metastatic disease and often solely this site. These are points that future application of a PDX model program should ideally aim to address to attempt to optimise the unique benefits that this methodology appears able to add to other options for CRPC therapeutics discovery.

Notwithstanding these points, I believe these data warrant extension of clinical evaluation of ribosome directed therapy to CRPC. One immediate question for clinical development in CRPC would be identification of a patient selection biomarker for ribosome directed therapy. p53 status and markers of MYC driven cancer progression are perhaps starting options. The data here perhaps suggest that fresh tumour sampling might be required, but that this might be complicated through

intra-patient tumour heterogeneity. These are significant challenges for any drug development program with competing concerns over scientific rigour and pragmatism.

This work highlights the unique opportunities and complexities of a PDX model centred approach to novel CRPC therapeutic discovery. These would not replace other methodologies, but they would appear to provide unique complementation. This opportunity should be embraced to improve on the currently inadequate treatment options for CRPC.

References

[1] James ND, Spears MR, Clarke NW, et al. Survival with newly diagnosed metastatic prostate cancer in the "docetaxel era": Data from 917 patients in the control arm of the STAMPEDE trial (MRC PR08, CRUK/06/019). Eur Urol 2015;67:1028-38.

[2] Lawrence MG, Obinata D, Sandhu S, et al. Patient-derived models of abiraterone- and enzalutamide-resistant prostate cancer reveal sensitivity to ribosome-directed therapy. Euro Urol (in press).

[3] Gundem G, Van Loo P, Kremeyer B, et al. The evolutionary history of lethal metastatic prostate cancer. Nature 2015;520:353-7.

[4] Padmanabhan A, Gosc EB, Bieberich CJ. Stabilization of the prostate-specific tumor suppressor NKX3.1 by the oncogenic protein kinase Pim-1 in prostate cancer cells. J Cell Biochem 2013;114:1050-7.

[5] Rebello RJ, Kusnadi E, Cameron DP, et al. The Dual Inhibition of RNA Pol I Transcription and PIM Kinase as a New Therapeutic Approach to Treat Advanced Prostate Cancer. Clin Cancer Res 2016;22:5539-52.

[6] Woods SJ, Hannan KM, Pearson RB, Hannan RD. The nucleolus as a fundamental regulator of the p53 response and a new target for cancer therapy. Biochim Biophys Acta 2015;1849:821-9.

[7] Harrison SJ, Khot A, Brajanovski N, et al. A phase 1, open-label, dose escalation, safety, PK and PD study of a first in class Pol1 inhibitor (CX-5461) in patients with advanced hematologic malignancies (HM). J Clin Oncol 2015;33:e22212-e.

[8] Li L, Li Y, Zhao J, Fan S, Wang L, Li X. CX-5461 induces autophagy and inhibits tumor growth via mammalian target of rapamycin-related signaling pathways in osteosarcoma. Onco Targets Ther 2016;9:5985-97.

[9] Negi SS, Brown P. rRNA synthesis inhibitor, CX-5461, activates ATM/ATR pathway in acute lymphoblastic leukemia, arrests cells in G2 phase and induces apoptosis. Oncotarget 2015;6:18094-104.

[10] Xu H, Di Antonio M, McKinney S, et al. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. Nat Commun 2017;8:14432.