The INSIDE Study: Innovative Preclinical Models for Precision Medicine in Advanced Prostate Cancer

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ABSTRACT

Introduction and Objectives: Despite progress in prostate cancer (PC) therapies, treatment resistance and low response rates remain major clinical challenges. PC is genomically heterogeneous, with DNA damage repair (DDR) gene mutations present in 10–19% of primary cases and up to 27% in metastatic castration-resistant PC (mCRPC). While *BRCA* mutations predict PARP inhibitor (PARPi) response in mCRPC, their role in high-risk, locally advanced PC is unclear, and the impact of non-BRCA DDR alterations is poorly understood. In the era of precision medicine, there is a need for experimental models that faithfully represent tumor heterogeneity. Patient-derived 3D models are emerging as valuable tools to address this gap. This study aims to identify novel actionable vulnerabilities in DDR pathways and assess the clinical impact of DDR alterations beyond BRCA in high-risk advanced PC. **Methods:** The INSIDE study is a prospective trial led by the Candiolo Cancer Institute, FPO-IRCCS with San Luigi Hospital, focusing on DDR gene mutations in high-risk advanced PC. As of March 2025, 68 patients have been enrolled. Tumor samples, together with blood samples, are collected to generate patient-derived organoids (PC-PDOs) for histological, genomic, and pharmacological profiling. Whole exome sequencing (WES) and TruSight Oncology 500 HRD (TSO500-HRD) are used to analyze genomic variability and genomic instability scores (GIS), offering insights into DDR-related therapeutic targets. **Results:** We developed a pipeline to establish PC-PDOs from tumor tissue, achieving a 77% derivation success rate. Notably, 18% of these organoids were suitable for pharmacological screening, an achievement rarely demonstrated for models directly derived from patient tissue, underscoring the translational value of our approach. Preliminary findings suggest DDR effectors such as DNA-PK, ATM, and ATR as promising pharmacological targets. Using TSO500- HRD, GIS was assessed in both patient samples and PDOs, revealing potential sensitivity to PARPi in cases beyond BRCA mutations, including specific missense variants in homologous recombination genes. **Conclusions:** The INSIDE study confirms the feasibility of using PDOs to model DDR alterations in high-risk advanced PC. These findings support the integration of genomic and pharmacological data to guide precision therapies, underlining the importance of comprehensive DDR profiling to expand treatment options for PC patients.

AIMS

To genomically profile prostate cancer patients' samples

To validate the clinical impact of DDR alterations

Translational aims:

- To generate patient-derived preclinical models (prostate cancer organoids, PDOs)
- To identify novel therapeutic targets and strategies (focused on DDR) for prostate cancer treatment

RESULTS

INSIDE: Identification of Genomic Screening Pathways in Cancer Patients With DNA Repair Alterations	
Principal investigators of the study:	Prof. Sabrina Arena, Dr. Pasquale Rescigno
EudraCT number:	NCT06334809
Promotor:	Fondazione del Piemonte per l'Oncologia
Study type:	Prospective observational
Coordinating centre:	Fondazione del Piemonte per l'Oncologia
Satellite centre:	AOU San Luigi Gonzaga
Status of the study:	Recruiting
Study web:	https://clinicaltrials.gov/study/NCT06334809?cond=Prostate%20Cancer&term=INSIDE&rank=1

Study cohorts:

Cohort A: high risk localized PC

Cohort B: de novo metastatic hormone sensitive PC

Cohort C: metastatic castration resistant PC

Fig.1 General information about the INSIDE study and patient cohort characterization.
The clinical study began with first patient enrollment in March 2023 at the coordinating center. The satellite center joined the study in July 2023. Patient recruitment is still ongoing. The study includes three main patient cohorts.

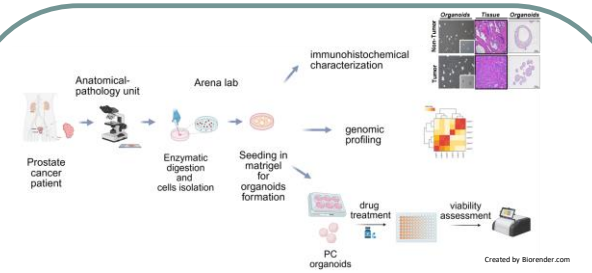


Fig.2 Workflow for generation, characterization and pharmacological screening of PDOs.
Patient-derived organoids (PDOs) are established directly from prostate cancer (PC) tissue obtained during radical prostatectomy. All samples are first evaluated by a specialized pathologist and subsequently processed in the laboratory. Organoids are generated, biobanked, and later used for detailed characterization and, when suitable, pharmacological screening. For drug testing, PDOs are seeded in 96-well plates, treated with selected compounds, and after 7 days, cell viability is measured using the CellTiter-Glo assay (Promega).

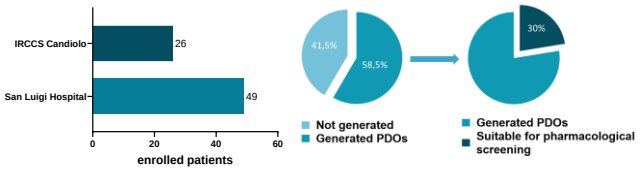


Fig.3 Enrolled patients in the INSIDE study
A total of 75 patients from cohort A have been enrolled in the INSIDE study. Samples from 66 patients were eligible for organoid derivation. The overall derivation efficiency is 58.5%, and 30% of the generated PDOs are suitable for pharmacological screening (last update: 13/08/2025).

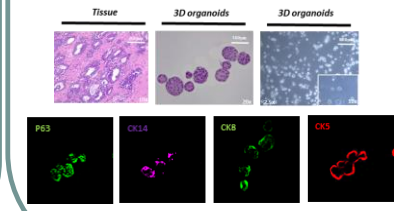


Fig. 4 Characterization of PC PDOs. Representative images of bright-field microscopy, immunohistochemistry (H&E staining), and immunofluorescence analysis of PC PDOs. Markers include p63 (basal cells), CK14 (prostate basal epithelial cells), CK8 (prostate luminal epithelial cells), and CK5 (prostate basal epithelial cells).

Homologous recombination deficiency (HRD) in cancer

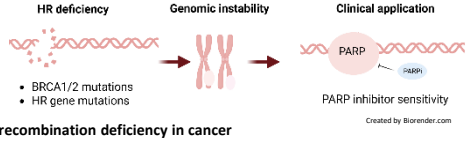
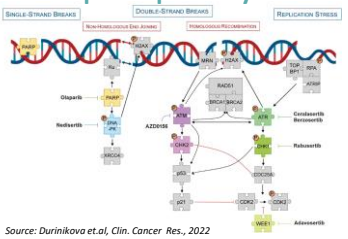


Fig.5 Homologous recombination deficiency in cancer
Homologous recombination deficiency (HRD) is characterized by a reduced or lost ability of cells to repair DNA double-strand breaks through the homologous recombination (HR) repair pathway. HRD frequently results from mutations or epigenetic alterations in HR repair genes such as BRCA1, BRCA2, or ATM. This deficiency causes genomic instability, driving tumor development and progression. In HRD tumors, inhibition of PARP leads to the accumulation of DNA damage, making PARP inhibitors (PARPi) a therapeutic option.

Therapeutic targets in the DNA damage response pathway



Source: Durinikova et.al, Clin. Cancer Res., 2022

Fig.6 Therapeutic targets in the DNA damage response pathway
DNA damage response (DDR) pathways are necessary for maintaining genomic stability and cell survival. Somatic alterations in DDR genes have been identified in approximately 10-19% of primary PCs, increasing to 23–27% in metastatic cases. DDR inhibitors (DDRi), which disrupt DNA repair mechanisms in tumor cells, have therefore emerged as promising potential therapeutic options for PC.

Genomic Instability Score, genetic profiling and response to PARPi

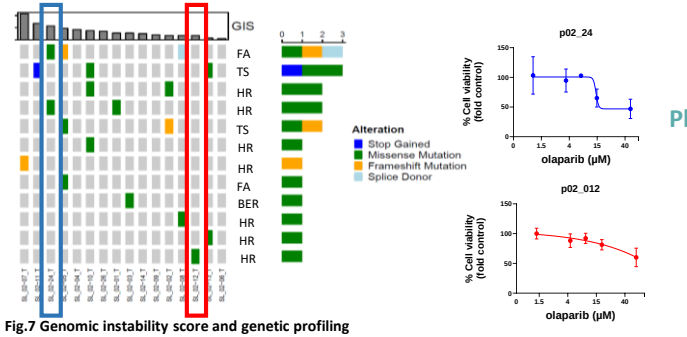


Fig.7 Genomic instability score and genetic profiling
An initial set of samples was analyzed using the TruSight Oncology 500 HRD (TSO500) assay to determine the genomic instability score (GIS) as a marker of homologous recombination deficiency (HRD). Our results revealed variable GIS across the cohort. In some cases, GIS correlated with pharmacological response to olaparib: sample p02_24, with a GIS of 23, showed increased sensitivity to olaparib, whereas sample p02_12, with a GIS of 7, exhibited lower responsiveness. Abbreviations: FA – Fanconi anemia; TS – tumor suppressor; HR – homologous recombination repair; BER – base excision repair.

Pharmacological screening of PC PDOs with DDRi

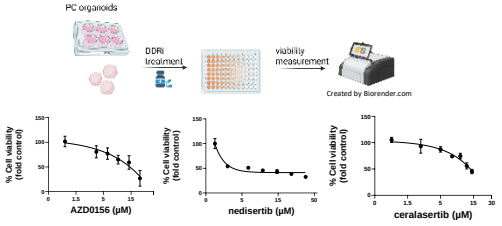


Fig.8 Pharmacological screening of PC PDOs
Pharmacological testing of selected PC PDOs with DNA damage response inhibitors revealed marked sensitivity to the DNA-PK inhibitor nedisertib. In contrast, the PDOs exhibited lower sensitivity to the ATM inhibitor AZD0156 and the ATR inhibitor ceralasertib.

CONCLUSIONS

The INSIDE study demonstrates that patient-derived organoids (PDOs) can effectively model DDR alterations in high-risk, advanced prostate cancer. These results highlight the value of integrating genomic and pharmacological data to guide precision therapies and emphasize the importance of comprehensive DDR profiling to broaden treatment options for patients.