

Developing a tissue immunofluorescence-based assay to identify homologous recombination deficient prostate cancers towards patient stratification for treatment

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Background: Metastatic prostate cancer is enriched for somatic and germline DNA repair gene alterations; among them, tumors with homologous recombination repair (HRR) deficiencies are a target for PARP inhibitors and platinum based chemotherapies. Clinically qualifying the predictive value for each individual biomarker (mutation/gene) is challenging; we hypothesize that an IF-based test assessing γ H2AX (marker of DNA damage) and RAD51 (marker of HRR function) could be applied to FFPE tumor biopsy samples to stratify patients for precision medicine strategies.

Methods: An IF-based test initially developed in breast cancer patient-derived xenograft models and then validated in breast cancer biopsies (Cruz et al, Ann Onc 2018) is now being optimized for its use in FFPE prostate cancer primary and metastatic biopsies. RAD51 was scored as the % of cells in S/G1 cell cycle phase showing at least 5 RAD51 nuclear foci. In order to consider a sample evaluable, at least 50 geminin positive cells were assessed and the % of positive cells for γ H2AX nuclear foci had to be over 25%. We here present the pilot phase of this ongoing study, including primary and metastatic tumor biopsies from patients who underwent parallel NGS characterization using a capture-based customised panel, and discuss the clinical qualification plan.

Results: First, we tested a cohort of samples from primary prostate tumor biopsies from patients who later developed mCRPC. In this pilot cohort, enriched for patients with HRR mutations, 5/5 patients with somatic or germline BRCA2 mutations or homozygous deletions presented high levels of γ H2AX and low levels of RAD51 foci, indicating HRR deficient status; in addition, 2/12 samples with not know HRR mutations presented low RAD51 levels (further NGS tests are ongoing). One liver metastasis biopsy from a patient with a germline *BRCA2* mutation acquired after secondary resistance to carboplatin showed high levels of RAD51 foci, in keeping with HRR function restoring and a *BRCA2* reversion mutation was detected by NGS. Correlation of NGS data with different cut-off levels for the RAD51 assay were explored. Clinical qualification plans include retrospective correlation analysis with NGS/clinical outcome data for PARP inhibitors and implementation as prospective stratification tool for a multicentre investigator-initiated clinical trial in Spain with carboplatin (BioChip), funded by a DoD Impact Award.

Conclusions: An IF-based test could help identifying patients with HRR defective prostate cancer to optimize precision medicine strategies, overcoming part of the challenges associated to NGS testing in FFPE samples; analytical validation is ongoing and clinical qualification plan would include implementing the assay in clinical trials.

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