Analysis of circulating cell-free DNA identifies multi-clonal heterogeneity of *BRCA2* reversion mutations associated with resistance to PARP inhibitors

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Background
Approximately 20% of metastatic prostate cancers harbor inactivating mutations in genes required for DNA repair, most frequently affecting double strand DNA repair by homologous recombination (HRR) such as *BRCA2*. Cells lacking HRR must repair double-strand DNA breaks through more error-prone forms of DNA repair such as non-homologous end joining. HRR-deficient tumors show increased sensitivity to drugs such as platinum salts, or inhibitors of the DNA repair protein PARP1 (PARPi) that induce double-strand breaks by stalling replication forks and causing replication fork collapse. In ovarian or breast cancers, resistance to the PARPi olaparib has been associated somatic reversion mutations that reactivate a non-functional HRR gene. Whether similar mechanisms operate in prostate cancer, and could be detected in liquid biopsies, is unclear.

Methods
We analyzed DNA in patients with metastatic CRPC obtained from solid tumor biopsies and circulating cell-free DNA (cfDNA), comparing samples obtained before PARPi treatment and after PARPi resistance.

Results
Multiple PARPi reversion mutations were identified in each patient in samples obtained after the onset of PARPi resistance. Analysis of circulating cell-free DNA revealed reversion mutation heterogeneity not discernable from a single solid tumor biopsy and potentially allows monitoring for the emergence of PARPi resistance.

Conclusions
We demonstrated the use of cfDNA to identify heterogeneous *BRCA2* reversion mutations as a mechanism of resistance to PARPi in patients with metastatic prostate cancer. Furthermore, we show PARPi resistance is highly multi-clonal, and that cfDNA allows monitoring for PARPi resistance.

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