Measurement of SLFN11 protein in circulating tumor cells (CTCs) as a proposed liquid biopsy biomarker to predict response to DNA repair targeted therapies

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Background
- SLFN11 regulates repair mechanisms to DNA damages as well as replication stress and has been investigated as a potential predictive biomarker for response to platinum agents and PARP inhibitors (PARPi).
- Phase II clinical trials in recurrent small cell lung cancer (SCLC) has shown that patients with high SLFN11 expression in tissue biopsies had better survival when treated with PARPi.
- Determining SLFN11 expression could identify patients who might respond to an additional round of inexpensive platinum agents.
- Since recurrent lung biopsies are not common in clinical practice, an Epic Sciences’ CTC-based SLFN11 test could aid these indications.

Materials
- Contrived samples consisting of healthy donor blood, spiked with three PARPi sensitive (DU445, MD-MBA-436 and PCD) and two PARPi resistant (MCF7 and MD-MBA-233) cell line cells were used to establish technical feasibility of the assay.
- SCLC and mCRPC patient samples were used to establish the clinical feasibility of the assay.
- Rabbit Polyclonal anti-SLFN11 antibody was used to assess SLFN11 protein expression.
- Epic single cell genomic platform was used to validate the assay.
- All contrived, SCLC, and mCRPC samples were processed and stained with SLFN11 in a CLIA-like laboratory environment.

Methodology
- The Epic Sciences platform plates all nucleated cells from a blood sample onto microscope slides and stains for DNA (DAPI), WBC lineage marker (CD45) and epithelia cells marker (CK). The platform also allows for an additional biomarker of interest (SLFN11) for the purpose of this study.
- Utilizing automated microscopy CTCs (CK+, DAPI+, CD45+) are identified and the expression of biomarker of interest (SLFN11) was evaluated.
- Epic single-cell genomics methodology was used to profile genome-wide CNV alterations and to identify loss and gain of specific prostate cancer genes.

Technical Feasibility in Cell Line Controls
- PARPi sensitive cell lines DU45, MD-MBA-436 and PCD exhibited a median difference in SLFN11 expression of 19 fold when compared with negative control and 3 fold when compared with PARPi resistant cell lines MCF7, wherein cells were stained using the highest concentration of SLFN11 antibody.
- Each data in the plot (Figure A) represent an independent cell.
- Nucleolus count for SLFN11 was determined as the threshold for the mean intensity for the top 20 primary antibodies of all cell lines contained.

Clinical Feasibility in SCLC & mCRPC
- Inter-Patient SLFN11 protein expression in SCLC and mCRPC

Representative Cell Images
- Nuclear localized SLFN11 expression on single cell and cluster of cells was observed in both SCLC and mCRPC patients.

SLFN11 Does Not Correlate with CK Expression
- Diverse range of CK and SLFN11 protein expression was observed in both SCLC and mCRPC patients. SLFN11 cells had wide range of CK expression.

Single-Cell Genomic Analysis of mCRPC Patients
- Heatmap of CNV profiles from three mCRPC patient samples revealed CNV overlap with high-LTV values.
- Single-cell genomic analysis of randomly selected cells from one mCRPC patient validated the tumor heterogeneity of CTCs revealed using Epic’s SLFN11 assay.
- CTCs showed prostate cancer related gene alterations.

Conclusions
- SLFN11 expression was specifically detected in cell lines and SCLC and mCRPC patient CTCs using Epic Platform.
- Inter- and intra-patient heterogeneous SLFN11 protein expression was observed. Percent of SLFN11 ranged from 0 to 64%.
- PARPi sensitive cell lines, as well as SCLC and mCRPC patients, expressed nuclear localized SLFN11 signal.
- Single cell genomic analysis confirmed tumor origin of SLFN11+ cells in mCRPC.

Six SCLC and six mCRPC patients stained with Epic Sciences’ SLFN11 assay are shown. Patients were selected deliberately to represent the dynamic range observed during the assay technical feasibility development (100% of SLFN11 positive cells).