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

Luisa E Fernandez, Angel E Dago, Robin Richardson, Rhett Jiles, Lincy Chu, Ryon Graf, Yipeng Wang, Mark Landers, Ryan Dittamore

Epic Sciences, 9381 Judicial Drive, Suite 200, San Diego, CA 92121 ● epicsciences.com

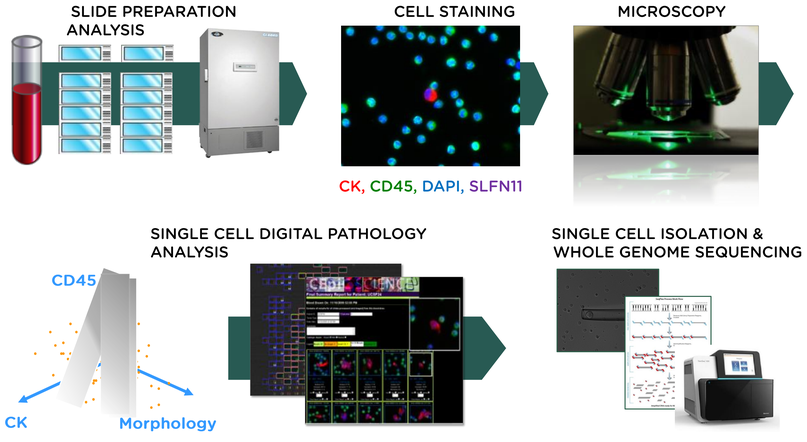
Background

- 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

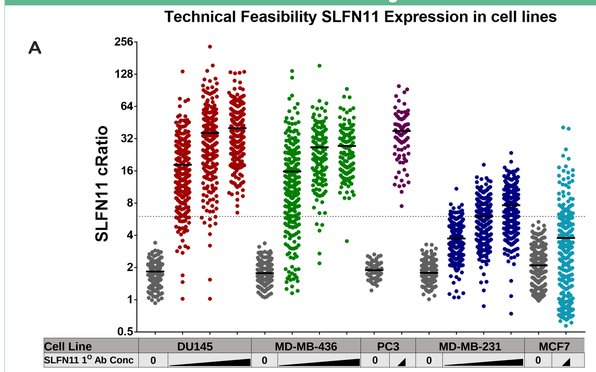
- Contrived samples consisting of healthy donor blood, spiked with three PARPi sensitive (DU145, MD-MBA-436 and PC3) and two PARPi resistant (MCF-7 and MD-MBA-231) cell line cells were used to stablish technical feasibility of the assay.
- SCLC and mCRPC patient samples were used to stablish the clinical 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



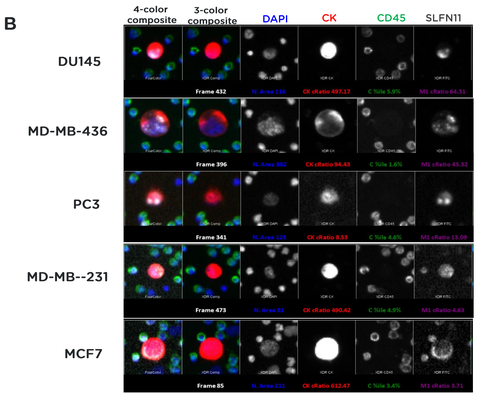
- The EPIC Sciences platform plates all nucleated cells from a blood sample onto microscope slides and stains for DNA (DAPI), WBC linage marker (CD45) and epithelia cells marker (CK). The platform also allows to for an additional biomarker of interest (SLFN11 for the purpose of this study).
- Utilizing 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 DU145, MD-MBA-436 and PC3 exhibited a median difference in SLFN11 expression of 19-fold when compared with negative control and 9-fold when compare with PARPi resistant cell line MCF-7, when cells were stained using the highest concentration of SLFN11 antibody.

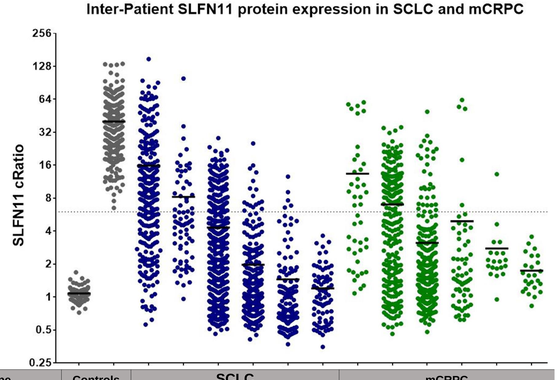
- Each dots in the plot (Figure A) represents an independent cell
- Positivity cutoff for SLFN11 was determine as three-times the mean intensity for the no primary control (0ug/mL of primary antibody) of all cell lines combined



- PARPi sensitive cell lines express nuclear-specific localized SLFN11 signal, visualized in sub nuclear focal bodies in the case of MD-MBA-436 and PC3 cell lines.
- PAPRi resistant cell line MD-MBA-231 showed a low non-nuclear expression of SLFN11. BRAC1-wt PARPi resistant MCF-7 does not express SLFN11.

* SLFN11 and CK expression is reported as a ratio of the expression in the cell of interest compare with the expression of the cells in the surroundings

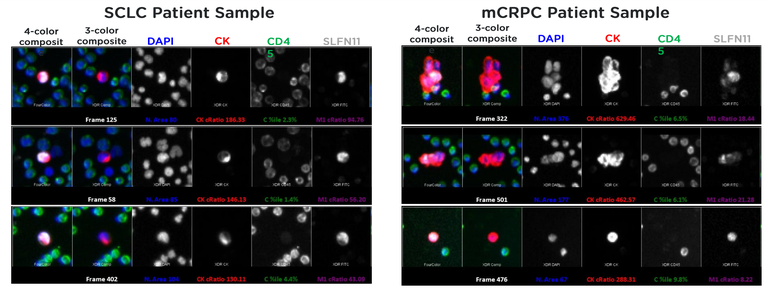
Clinical Feasibility in SCLC & mCRPC



Cancer Type	Controls		SCLC						mCRPC					
Patient	Negative	Positive	SCLC-1	SCLC-2	SCLC-3	SCLC-4	SCLC-5	SCLC-6	mCRPC-1	mCRPC-2	mCRPC-3	mCRPC-4	mCRPC-5	mCRPC-6
SLFN11 Positive cells (%)	0	100	64	40	25	8	4	0	53	44	10	8	5	0

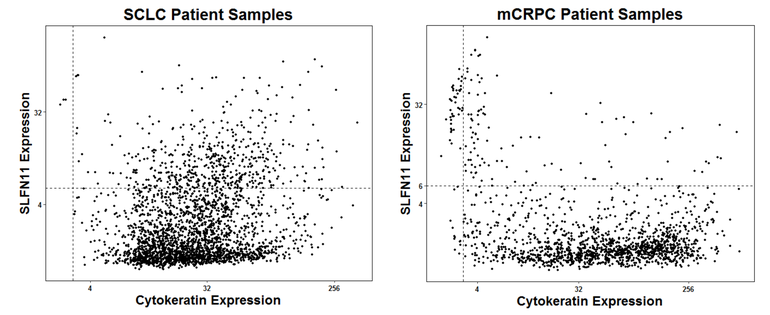
Six SCLC and six mCRPC patients stained with EPIC Sciences' SLFN11 assay are shown. Patients were selected deliberately to represent the dynamic range observed during the assay clinical feasibility development (0-60% of SLFN11 positive cells).

Representative Cell Images



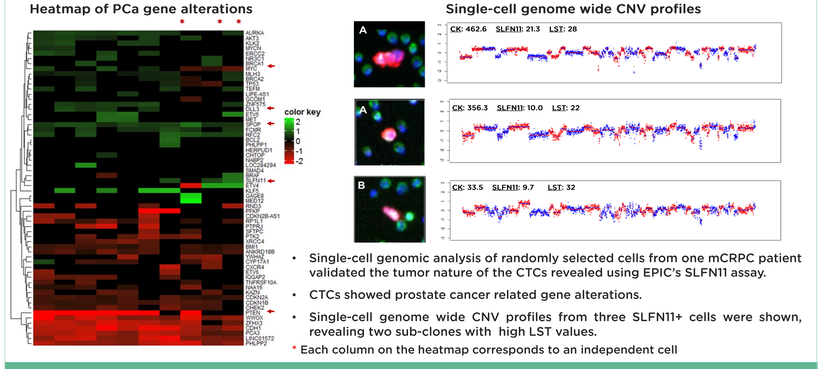
Nuclear localized SLFN11 expression on single cell and cluster of cells was observed in both SCLC and mCRPC patients.

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



- Single-cell genomic analysis of randomly selected cells from one mCRPC patient validated the tumor nature of the CTCs revealed using EPIC's SLFN11 assay.
- CTCs showed prostate cancer related gene alterations.
- Single-cell genome wide CNV profiles from three SLFN11+ cells were shown, revealing two sub-clones with high LST values.

* Each column on the heatmap corresponds to an independent cell

Conclusions

- SLFN11 expression was specifically detected in cell lines and SCLC and mCRPC patients 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.