CTCs are isolated, enumerated (#/ml) & phenotyped

Phenotyping Pancreatic Cancer CTCs As Biomarkers For RX-3117 Clinical Trials
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Background:
RX-3117 is an oral, small molecule nucleoside analogue prodrug being used in combination with nab-paclitaxel in a Ph2 clinical trial for pancreatic cancer. RX-3117 can be transported intracellularly by SLC29A1 (hENT1) and is converted to an active agent by the cancer-enriched enzyme, uridine-cytidine kinase 2 (UCK2) for incorporation into RNA and DNA. This leads to cancer cell apoptosis and epigenetic effects. We developed an approach to enumerate and phenotype circulating tumor cells (CTCs) from pancreatic cancer subjects by quantitative immunofluorescence (QIF) to assess whether CTC numbers, phenotypic features and/or early apoptotic responses to therapy predict or presage clinical response.

Methods:
QIF staining parameters were developed with cancer cell lines sensitive and resistant to RX-3117 utilizing monoclonal antibodies to hENT1 and UCK2. The staining methods were then applied to CTCs isolated from 10 ml anticoagulated blood by dielectrophoretic (DEP) properties that differentiate cancer cells and normal PBMCs using the ApoStream device (ApoCell, TX). In addition to the drug-related markers, CTCs were stained for a panel of either epithelial or mesenchymal markers, CD45 and nuclei (DAPI). Phenotyped CTCs were binned into 6 categories defined by whether they were EPI+/EMT- or EPI-/EMT+. The percentage of cells in each category also positive for hENT1 or UCK2 was determined as was the mean fluorescence intensity of the marker+ sub-population. In addition, assessment of hENT1 and UCK2 expression was performed by qRT-PCR utilizing mRNA isolated from isolated CTCs, the buffy coat from spun blood and in plasma.

Conclusions:
- CTCs can be readily isolated, enumerated and characterized from PDAC patients by the ApoStream technology.
- In a pilot study of 5 PDAC subjects, CTC numbers ranged from 100 to 20,000 / ml independent of phenotyping.
- CTCs can be phenotyped by expression of epithelial or mesenchymal markers and presence or absence of CD45.
- Assessment of biomarkers relevant to the RX-3117 MOA - hENT1 (SLC29A) and UCK2 - can be quantified by immunofluorescence.
  - The percentages of UCK2+ cells were higher in EPI+/EMT+ subsets than EPI-/EMT- subsets.
  - The percentages of hENT1+ cells were generally low in all of the subsets.
- This approach is now being used to monitor the response of PDAC patients in a Phase 2 trial of RX-3117 in combination with nab-paclitaxel (NCT03189914).

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