Circulating tumor cell monitoring, isolation and culture from a patient with metastatic triple negative breast cancer for drug screening and creation of a patient-derived xenograft model

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Background
Enumeration, phenotyping, and single cell genomics of circulating tumor cells (CTCs) provide information to guide cancer therapy. In some instances, functional analysis in vitro, or in vivo patient-derived xenografts (PDXs) are possible. We used a density-based rare cell separation and analysis system to collect CTCs from the blood of a patient with metastatic triple-negative breast cancer (TNBC) for in vitro culture, high-throughput drug screening, and to generate a PDX model.

Methods
Patient was enrolled in the ITOMIC-001 study (University of Washington). After informed consent, CTCs were evaluated prior to initial cisplatin treatment and tracked longitudinally using the AccuCyte-CytoFinder system (RareCyte). Samples containing high numbers of CTCs were placed into 3 different culture media. Cells grown in culture were tested against a panel of anti-cancer drugs and injected into mice to form a PDX model.

Results
Nine CTC evaluations were performed over 9.5 months. CTCs were verified by expression of epithelial (cytokeratin and/or EpCAM) and nuclear stains without CD45 expression. After initial treatment with cisplatin, the CTC count rose, consistent with the lack of a clinical response, and decreased after treatment with a CD44/6 inhibitor and then an anti-gpNMB. At 9 months, CTC count rose shortly before her death. At autopsy there was massive infiltration of the liver and pulmonary vasculature by tumor cells. Cultures in all media showed initial growth, but only one (RPMI + 10% serum) was sustained. CTC cell line has grown continually in culture for nearly 1.5 years. Aliquots of this cell line have been frozen and thawed with no noticeable effect on cell growth. 6 million cells were harvested and a drug screen using 160 anti-cancer agents was performed. The CTC line showed sensitivity to several agents. Approximately 350,000 cells were injected into each abdominal fat pad of an immunodeficient mouse. One palpable tumor was observed after 34 days. Tumor was about 3x2 mm. It was sectioned into 4 pieces and each piece was transplanted into the abdominal fat pad of 2 different mice. Mice were euthanized after 5 months and inspected for tumor growth. No tumors were found.

Conclusions
Using a density-based rare cell collection system, we have established a CTC cell line from a TNBC patient with extremely high CTC counts. The line was used to perform a screen for agents active against the tumor cells and to create a PDX model. As in other technology, smaller number of CTCs may be effectively cultured and thus allow this approach to be used in clinical time to find effective drug regimens for individualized cancer therapy.

Significance
Although this patient had extremely high CTC numbers and by the time the drug screen and PDX models were generated she had passed away, this approach could be used to find a personalized treatment that is effective even if no a-priori information exists that would predict response to said treatment.