341d Detection of Rare Cancer Cell in Peripheral Blood

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Detection of circulating tumor cells (CTC) in body fluids has a significant potential in diagnosis and treatment decisions (Braun et al. NEJM 342:525-533; 2000). Aim of this research is to develop a highly sensitive, reliable, and potentially quantitative detection method for rare cancer cells. Both traditional immunocytochemistry and molecular analysis technology, such as RT-PCR, have a significant limitation: accuracy/efficiency significant improves as frequency of the rare cell increases above 1 cancer cell per 105 normal cells. Consequently, it is highly desirable to perform one or more enrichment steps before these detection methods are applied.

We are developing enrichment protocols to enrich rare cancer cells in human peripheral blood by depletion of normal cells using either density separation or red cell lysis, flow-through, magnetic cell separation, followed by membrane filtration or complete cell lysis for molecular analysis.

To optimize/quantify the performance of these protocols, a human cancer cell line, Detroit-562, was cultured and spiked into human blood. CD45 antigens were targeted to impart magnetophoretic mobility on “normal” leukocytes and a magnetic, flow through, high-throughput Quadrupole Magnetic cell Sorter (QMS) was used to deplete magnetically labeled leukocytes.

By optimizing the QMS operation parameters, as well as the other steps in the process, we want to maximize the cancer cell recovery and log depletion of “normal” leukocytes. Currently, we are routinely able to deplete approximately 98% of the leukocytes with a recovery of the cancer cells of 50%. We subsequently perform a number of detection technologies including RT-PCR and real-time PCR. A variety of examples will be presented on the ability of this methodology to improve the sensitivity of detection.