Cancer cells naturally detach from a primary tumor and can sometimes be detected in the blood of cancer patients. These cells, called circulating tumor cells (CTCs), are extremely rare—there is thought to be one for every one billion normal cells. If they were reliably detectable, CTCs could help physicians diagnose and treat cancer and learn how cancers spread.

The technologies available for detecting CTCs help scientists learn about these cells, but they are not usually sensitive or reliable enough for physicians to use in the clinic to make cancer treatment decisions. This Dream Team, composed of clinicians, bioengineers, and molecular biologists, has developed a novel approach to detecting and isolating CTCs. This approach takes advantage of microscopic fluid dynamics to construct a chip with 100 times the sensitivity of existing technologies.

The CTC-Chip is the size of a business card, has channels with a herringbone design, and is coated with material capable of attaching to CTCs while allowing normal blood cells to flow through unimpeded. It offers unprecedented opportunity to detect tumor cells in patients with early-stage cancer, to genetically characterize tumor cells without an invasive biopsy, and to determine responsiveness to targeted cancer drugs.

The team reported the following progress:

December 2013

- The Dream Team has completed enrollment for their joint phase I clinical trial that compared genetic characteristics of CTCs to those of direct tumor biopsies. Analyses of the samples included testing for EGFR mutations from captured CTCs with next-generation sequencing techniques.
- The Dream Team continued to develop its third-generation device called the iChip. Using a size-based method to eliminate red blood cells, inertial focusing to line up the remaining cells in single file, and magnetic antibodies to isolate CTCs, this device can allow scientists to study CTCs in a variety of new ways. The CTC-iChip was not yet ready for routine operation, or wide distribution. Nonetheless, the Dream Team used CTCs isolated with this new Chip to (i) perform single-cell sequencing, and (ii) establish cultures of CTC-derived cell lines.
June 2013

- The Dream Team enrolled 38 of their target of 40 patients in their joint phase I clinical trial that compared results of genetic analyses in CTCs to those of direct tumor biopsies.
- The Dream Team developed technology to concentrate the CTCs in a much smaller volume of solution that keeps them alive longer than before.
- The Dream Team developed technology to isolate single CTCs in oil droplets. This can enable scientists to sort the CTCs based on different cell characteristics and analyze single cells.

December 2012

- The Dream Team initiated the joint phase I clinical trial stated in the original proposal. CTCs will be tested for EGFR mutations and compared with the results obtained from the tumor biopsy in patients with non-small cell lung cancer who are undergoing a re-biopsy for clinically defined resistance to EGFR inhibitors. Two aims were planned for this clinical study: Demonstrate the feasibility of testing for EGFR mutations from captured CTCs using the latest CTC-Chip and next-generation sequencing techniques; and, describe the agreement of EGFR mutations between the repeat tumor tissue biopsy and DNA/RNA isolated from CTCs captured using the CTC-Chip.
- To explore its role in human cancer, the Dream Team investigated epithelial-mesenchymal transition (EMT) in circulating tumor cells (CTCs) from breast cancer patients. EMT is a biological process that allows an epithelial cell, which normally attaches to surrounding tissue proteins, to enhance its migratory capacity, invasiveness, and resistance to cell death. The Dream Team found that cells rarely expressed mesenchymal and epithelial markers simultaneously in primary tumors, but that in CTCs, mesenchymal markers were found in a higher percentage of cells. Moreover, in some multicellular clusters, all CTCs expressed mesenchymal markers. CTCs were monitored in 11 patients and the data suggest a possible link between the presence of CTCs expressing mesenchymal markers, and disease progression. Additionally, CTCs with mesenchymal marker occurred as both single cells and multicellular clusters, while also expressing genes known to regulate EMT. These data support a role for EMT in breast cancer metastasis -- as a potential biomarker of therapeutic resistance, and as a potential drug target in breast cancer.

June 2012

- The Dream Team has developed the use of triple antibody cell capture (using antibodies specific for EpCAM, EGFR, and HER2), which provides improved capture of lung CTCs. They have also developed a refined method to perform single-molecule RNA sequencing from
nucleic acids extracted from CTCs. This method was applied successfully to pancreatic CTCs and identified an altered signaling pathway in a mouse model of pancreatic cancer. Extension to human pancreatic CTCs confirmed the abnormal signaling is evident in CTCs from approximately 50 percent of cases.

- The Dream Team has completed accrual of a phase II trial for patients with relapse sensitive or refractory small-cell lung carcinoma. The Dream Team found that at baseline, the number of CTCs was not predictive of response to treatment or associated with progression-free and overall survival. However, at the end of cycle 1, an increase in the number of CTCs compared to baseline values was correlated with a response to treatment.

December 2011

- The Dream Team successfully scaled up its efforts to produce the second-generation CTC-Chip. Each collaborator site was trained in the use of these new CTC-Chips and the instruments that process and stain them.
- The Dream Team has started a study to determine a baseline of CTCs found in healthy patients and has accrued 45 of 200 patients.
- The Dream Team started to develop a new array of antibodies to use in the CTC-Chip to enhance CTC capture.
- The Dream Team has accrued 53 of its target 200 patients in a clinical study to determine if preoperative CTCs, and postoperative CTC rate of decline, can predict which noninvasive early prostate cancer will go on to become invasive disease.
- The Dream Team previously reported that they had identified a common gene amplification (HER2) in metastatic breast cancer CTCs. They have followed up on this finding by conducting a study designed to compare the HER2 status of the CTCs with the HER2 status of the primary tumor. This study has enrolled 65 patients. In patients with HER2-negative tumors, 35 percent of CTCs were HER2 amplified. To determine if HER2 status changed over time, 33 patients had a repeat CTC assay performed 3 to 6 weeks after initial analysis. Ten of 12 HER2-amplified patients remained so after retesting. The two patients that became HER2-negative received trastuzumab (Herceptin)-based therapy in the meantime. A total of 17 of 21 HER2-negative patients remained so after retesting.

June 2011

- The Dream Team started enrolling patients in a clinical study of 200 men to determine if preoperative CTCs and postoperative CTC rate of decline can predict which noninvasive early prostate cancer will go on to become invasive disease. In a separate study, they investigated markers of androgen signaling that can be used to guide treatment decisions.
Team Progress Updates

- The Dream Team began using the second-generation CTC-Chip along with multi-color imaging to monitor how tumors respond to targeted therapies used to treat non-small cell lung tumors that have an EGFR or an ALK gene mutation. They have developed assays to detect cancer-specific mutations in DNA taken from the plasma of patients with stage IV disease. They detected mutations in 22 of the 33 samples.
- The Dream Team collaborated with researchers conducting a phase II clinical trial of the drug temozolomide (brand name Temodar) in patients with hard-to-treat small-cell lung cancer. The study showed that, after the first drug cycle, CTCs decreased or remained the same in 92 percent of the patients that showed a response to treatment. High CTC levels were associated with worse overall survival.
- The Dream Team worked to identify new antibodies that can be put on the CTC-Chip so that it can be used to study CTCs in patients with melanoma and glioblastoma, developed a precise way to measure the physical properties of individual CTCs, developed a new protocol for determining the glycan structures on a cell's surface, and began to develop strategies to modify the surface of the CTC-Chip to make it easier to detach CTCs.

December 2010

- The Dream Team extended the pilot study initiated in the first six months to detect Prostate Specific Antigen-positive CTCs in patients with early-stage cancer before and after their surgery. The study found that 60 percent of patients did not have CTCs before their surgery. They also found that, in six of the eight patients that had CTCs before surgery, their CTC levels decline by more than 50 percent within 24 hours following surgery; the other two had a delay in the decline. By three months following surgery, all patients had no detectable CTCs. The Dream Team initiated a clinical trial of 200 patients to determine if pre- and post-surgery CTC levels and rate of decline are predictive of relapse. In addition, the Dream Team is continuing to investigate a second gene, Ki67, which is a biomarker for aggressive cell growth that may be correlated to metastatic prostate cancer.
- The Dream Team investigated whether the CTC-Chip might be beneficial in the treatment of non-small cell lung cancer. They studied blood samples from 32 patients to determine which mutations can be identified using CTCs. They also conducted studies to develop tumor molecular profiles to predict drug resistance. They initiated a study to identify mutations associated with drug resistance that will profile tumor mutations in CTCs in patients before they start treatment, after treatment ends, and at the time of disease progression. The Dream Team collaborated with researchers conducting a phase II clinical trial for 89 patients with hard-to-treat tumors that showed CTCs decreased or remained the same in 89 patients of the patients whose tumors responded to the study drug, and that high CTC levels were associated with worse overall survival.
- The Dream Team previously reported that they had identified in CTCs a common gene amplification (HER2) in metastatic breast cancer. They conducted two studies designed to
follow-up on these preliminary findings, which found that 98 percent of patients with HER2-positive tumors had HER2-positive CTCs and 66 percent of the patients with HER2-negative tumors had HER2-negative CTCs.

June 2010

- The Dream Team initiated a study focused on prostate cancer patients for three reasons: there are unique biomarkers for prostate cancer; metastatic prostate cancer is difficult to biopsy so other approaches are required; and it is difficult to predict whether the cancer will be indolent or aggressive. They developed standard protocols for CTC staining, and showed that CTC levels can be used as a biomarker for tumor response to therapy. They also found that RNA could be collected from CTCs, with subsequent analysis of the RNA showing that a common gene fusion present in approximately half of prostate tumors could be detected.
- A second pilot study developed a new way to visualize a biomarker for aggressive prostate cancer, and this is now being tested on a larger cohort of patients. The Dream Team observed that CTC levels go down after prostate surgery, and is testing a hypothesis that CTC levels can be used to predict the risk for relapse of prostate cancer.
- The Dream Team has designed a second-generation CTC-Chip that is easier to produce, easier to use, cheaper to manufacture, and more functional than the early version. Standard protocols for increased functionality and high-throughput analysis are being developed. The new CTC-Chips are scheduled to be distributed to SU2C institutions in year two of the grant.
- The Dream Team worked on developing technology to understand how the tumor microenvironment influences the growth of CTCs. An Extracellular Matrix Microarray has been designed, built, and characterized that can enable researchers to test cell lines in 800 different conditions. This technology can help investigators test and discover key biological processes that lead to metastases in a variety of cancer types.