Jeffrey Epstein, Science Philanthropist, and The American Cancer Society Tackle Genetic Resistance to Drugs.

There are two common dilemmas in the treatment of cancer today: the first is that many therapies, including chemotherapies, radiation and other drug regimens, can debilitate healthy cells and tissue, to the point of killing the person before defeating the cancer. The second problem is that many cancer cells, responding to a prevention drug, can quickly mutate to become immune and more resilient.

Due to recent advances in circulating tumor cell technology, conducted by Dr. Daniel Haber, Director at the Massachusetts General Hospital Cancer Center and Dr. Mehmet Toner, director of the Center for BioMicroElectroMechanical Systems and supported by The American Association of Cancer Research, the American Cancer Society and the Jeffrey Epstein VI Foundation, this second problem of genetic resilience is being confronted head on.

Over the last few years, Dr. Haber and Dr. Mehmet Toner have developed a simple blood test to detect circulating cancer cells (CTC's). Using a microfluidic chip, the test isolates cancer cells in the blood and allows them to be purified to analyze their genetic structure. Although many challenges remain in the test, the advantages have already made a huge impact on the treatment of cancer. To date, the test has identified more than 1,200 cancer-causing genetic mutations, the largest collection in the world. The findings have led to a host of cancer specific targeted therapies including the use of reversible and irreversible inhibitors, which have been highly effective in tumor reduction. For instance, Dr. Haber's team recently found that gastric adenocarcinomas, stemming from high-level amplification of the growth factor receptor gene *c-MET,* only respond to novel inhibitors of the MET tyrosine kinase, leading to the initiation of a genotype-directed clinical trial. The test can also help identify the specific mutation within the cell that makes it cancerous. "Even though a cancer cell may have hundreds of mutations, some cancers are wired in such a way that a particular mutation drives the cancer," Haber said. Furthermore, as more genotype mutations are identified, commonalities arise, facilitating the search and categorization of genetic aberrations.

Critically though, the CTC test also addresses the major problem of secondary and tertiary genetic mutation to treatment. For while targeted inhibitors can be highly effective in tumor shrinkage, almost all cancer cells quickly mutate to be resistant, reversing tumor reduction within six to eight months. Furthermore, resistance becomes highly effective from the slightest evolution. For example, approximately half of non-small cell lung cancer cases with mutations to EGFR TK inhibitors, resistance came from a single mutation of *T790M* within the EGFR kinase domain. Indeed, the bulkier methionine residue at position *T790M* hinders interaction with the inhibitor, preventing binding to the EGFR kinase domain while preserving catalytic activity. An analogous mutation (T315I) in the BCR-

ABL fusion kinase in chronic myelogenous leukemia cells renders them resistant to ABL kinase inhibitors, gleevec and dasatinib.

By extracting cancer cells in a CTC test however, a patient can be analyzed in *real time*, meaning a preliminary genetic analysis to determine the first line of treatment and then a secondary treatment, a couple months later, based on any secondary mutations. In fact, since the first line of treatment can be tested on the patient's cells *in vitro*—and relatively quickly—any secondary or tertiary mutations detected in the cell culture, can be treated preemptively as part of the first line of attack, as a cocktail with the primary treatment or in immediate sequence.

By using the microfluidic test, Dr. Haber's team has a growing catalog of secondary and tertiary mutations and has shown how several irreversible inhibitors produce significant, if not permanent anti-tumor activity on a variety of secondary mutations such as the EGFR receptor double mutation, L858R/T790M. Several of these irreversible inhibitors, namely HKI-272, EKB-569, BIBW2992, and PF00299804, are currently undergoing clinical testing; however, none of them have yet received approval by FDA.

Technically, the CTC microfluidic chip test works by taking only 10 milliliters of blood, containing about 80 billion cells. Magnetic beads on the chip are coated with antibodies that bind to both EpCAM positive and EpCAM negative cells (epithelial cell adhesion molecules), a common marker present on CTCs originating from epithelial cancers. The binding of antibodies, makes the CTC cells detectable and can they can then be extracted via purification.

The toxic effect of targeted therapies are significantly less than standard chemotherapy drugs, however toxicity is still a major hurdle. Inhibitors, though increasingly mutant-receptor specific, can still negatively impact healthy receptors throughout the body, leading to heart disease, gastrointestinal damage and the development of other cancers. "One of the many advantages the CTC test, is that treatment will not only be increasingly specific to the mutation driving the cancer," Jeffrey Epstein noted, whose foundation supports cutting edge medical and science research around the world, "but doses can be closely minimized to tumor reduction and secondary treatments can be given in tandem or immediately thereafter."