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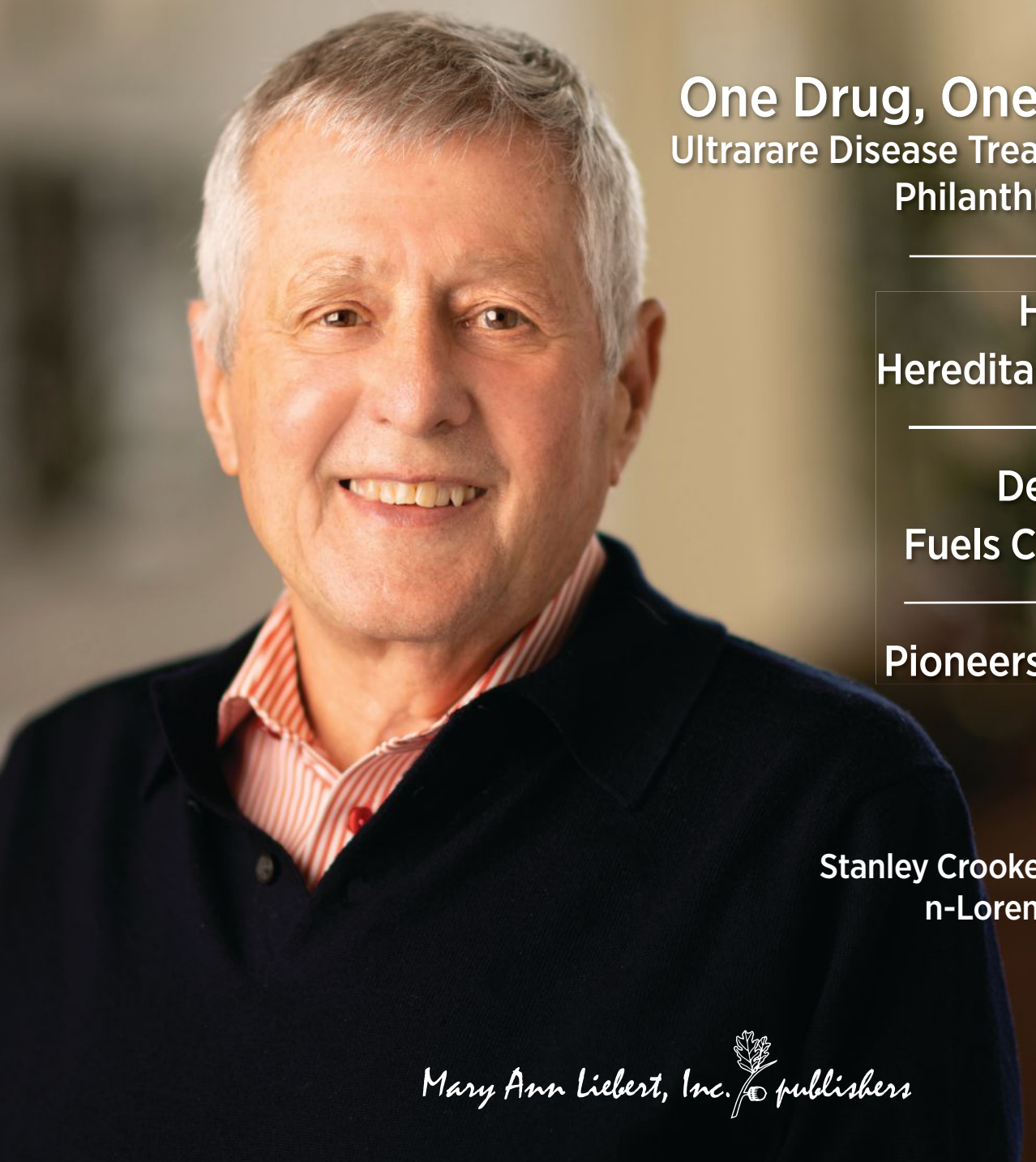
Clinical OMICs

Molecular Diagnostics in Precision Medicine



One Drug, One Patient

Ultrarare Disease Treatments Get Philanthropic Boost

Hunting for Hereditary Cancers

Deeper Data Fuels Clinical PRS

Pioneers Under 40

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Technology that Captures Circulating Tumors Cells, Along with Their Potential

Circulating tumor cells (CTCs) hold great potential as a tool for monitoring tumor growth and response to treatment, but capturing these rare tumor cells from liquid biopsies can be difficult.

Many of the existing single-cell analysis systems struggle to efficiently capture CTCs because they rely solely on epitopes for detection and capture. By using this approach, these systems require a preexisting knowledge of the rare cell population and, because it is unlikely that all CTCs from the same tumor will have the same epitopes, only a fraction of the available CTCs are captured.

A recently published peer-reviewed study in *Translational Oncology* showed that researchers can overcome these challenges with rare cell capture—if the right technology is used.[1]

A Novel Approach

Bruce Patterson, M.D., CEO of IncellDx, a single-cell diagnostic company, and his colleagues were evaluating paired blood and tissue samples from patients with non–small-cell lung cancer (NSCLC) and wanted to know what factors in the primary tumor were predictive of CTCs and presumably metastasis.

"If you knew at that moment that you took a core biopsy from the primary tumor, that the tumor was going to metastasize, that would be a huge step forward," Patterson said.

To answer this, they captured and analyzed CTCs from peripheral blood samples from ten patients using the Celselect Slide Technology on the Genesis System, an automated, single-cell isolation platform manufactured by Celsee, Inc. a Bio-Rad Laboratories company. The system enables whole blood and other liquid biopsies to be processed without additional manipulation of the samples which, in other methods, can lead to CTC loss.

Containing 56,400 microchambers, the Celselect Slide works with the Genesis System to uniquely capture rare cells, like CTCs, based on their size. As the liquid sample flows through the patented microfluidic slide, tumor cells, which are relatively large, become trapped in the microchambers. Meanwhile, smaller cells, like red blood cells and while blood cells, escape. Any white blood cells captured can be stained and eliminated in downstream analysis.

The novel approach of capturing cells based on size instead of epitope presence makes the system highly versatile: Cells from many different types of cancers, not just those of epithelial cell lineage, can be captured,[2] as well as cells expressing multiple lineages[3] or undergoing phenotypical change like an epithelial-to-mesenchymal transition (EMT). Evidence suggests that EMT allows the cells to travel to distant parts of the body and metastasize.

A study showcased the versatility of the Celselect Slide Technology when a heterogenous set of cell lines—which included a mesenchymal cell line—were spiked into blood samples from healthy patients and more than 80% of cells on average were reproducibly captured.[4]

Adding to the open nature of the system is that researchers can customize the antibodies used to identify and characterize the captured CTCs, which when added to the other advantages of the system, make for a highly sensitive technology.

A study confirmed this by showing that the Genesis System was more sensitive than the CellSearch system.[2] Specifically, among 18 blood samples from patients with metastatic prostate cancer that were analyzed in the study, 17 samples—or 94%—were found to have CTCs using the Celselect Slide Technology. The CellSearch system found CTCs in only 11 samples, or 61%. Also, the slides captured significantly higher CTC counts than the CellSearch system in most samples.

"We found [the Genesis System] to be the most sensitive system on the market," said Patterson.

Catch—and Release

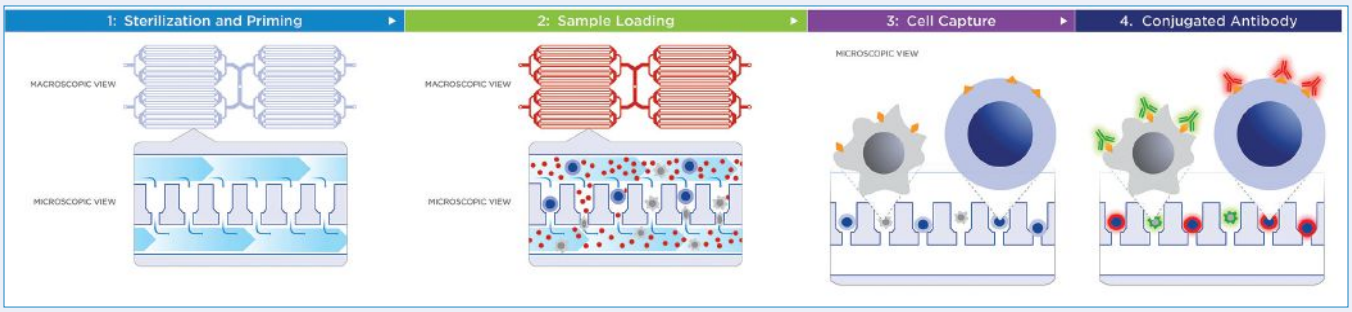
Before Patterson and colleagues could evaluate patient blood samples for CTCs, they needed to test their CTC method by spiking 4 mL of normal blood with cells from a PD-L1 positive lung cancer cell line. Cells were captured and stained with a custom set of biomarkers: CD45 (which is expressed on white blood cells), cytokeratin (which is expressed on epithelial cells), and PD-L1 (which is expressed in high amounts on certain cancer cells). Customization of the biomarkers enabled the researchers to efficiently identify their target cells, and only those cells.

The Genesis System successfully captured 90% of the tumor cells spiked into the normal blood and reproducibly detected as few as five tumor cells, confirming the analytical performance of the CTC method the researchers planned to use.

With the goal of being able to predict metastasis, CTCs in the blood samples from ten patients with NSCLC were then enumerated and stained to further characterize their phenotype and genotype and compared with patient tumor samples, which were evaluated using high-parameter flow cytometry.

"This technology pushed us way forward in terms of determining which one of those tumor cell clones is the metastatic clone," said Patterson.

Ultimately, a cell signature that predicted metastasis was identified—T regulatory cells that expressed CD4, CD103, FoxP3, and CCR5—as well as potential therapeutic strategies. Patterson explained that there are several investigational CCR5 inhibitors in development, and by targeting CCR5, there is the potential to not only treat the primary tumor but also prevent metastasis because the cells that are being targeted are the ones contributing to metastasis.



CTCs captured on the Celselect Slide with the Genesis System can also be returned to the researcher in a viable state and enriched for many downstream applications. The retrieved cells can be analyzed using a range of techniques, such as next-generation sequencing, fluorescence in situ hybridization, and molecular profiling, as well as cultured and expanded for further study.

In fact, a study showcased this feature when researchers successfully retrieved prostate cancer cells that were captured using Celselect Slide and further analyzed them using PCR amplicon sequencing.[5]

The next step for Dr. Patterson and his colleagues is to use the system for enrichment and genetically sequence retrieved CTCs. This will enable them to compare the genetics of the CTCs with the genetics of the primary tumor and potentially reveal whether different therapeutics are needed to treat the metastasis versus the primary tumor.

Broad Applications

The applications for which CTCs are being captured and studied extend beyond monitoring a patient's tumor growth and response to treatment. Using single-cell analysis systems, researchers can also capture CTCs to study their potential in early cancer detection, achieve more accurate staging of disease, get a better understanding of tumor heterogeneity and disease progression, and deliver on the promise of precision medicine by tailoring treatments to individual patients.

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https://bit.ly/3vjHYgc**

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