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## Cancer: A Global Concern that Demands New Detection Technologies

**Steven A. Soper**

University of North Carolina, Chapel Hill

There are over 100 cancer-related diseases and it is becoming clear that the standard-of-care for managing cancer diseases, which involves physical examinations and assessment of a patient's symptoms and/or various imaging modalities, is not keeping pace with the expanding nature of these diseases. For example, while the death rate (per 100,000 individuals) has decreased by 39% over the time span from 1991 to 2015 for cardiovascular diseases, over this same time period, the death rate for the cancer diseases has only seen a 21% drop. Worldwide, in 2014 there were >13 million new cancer cases and 70% of all cancer deaths occurred in low to middle income countries; cancer is not restricted to developed countries only. In the US alone, there were 580,350 cancer deaths and 1.66 million new cases. The (US) National Cancer Institute estimates that cancer care costs were \$125 billion in 2010 and are project to be \$200B by 2020! It is abundantly clear that the future will require new paradigms for managing all cancer-related diseases, such as molecular diagnostics/prognostics.

Cancer is particularly well-suited to this notion of detection and characterization by identifying unique molecular signatures because of the complexity of the disease as a whole and the clear need to align therapeutic strategies against the specific mechanism driving the growth and spread of the cancer.<sup>1</sup> This is because cancer is effectively a broad family of more than 100 unique conditions as mentioned above and collectively characterized by the presence of cell populations that undergo uncontrolled division that displays the potential to invade other tissues. Discovery of a tumor or lesion by traditional macro-scale diagnostic strategies is inadequate for understanding the actual condition a patient is suffering from, which is required for identifying therapeutic options that might offer relief. This is the reason cancer is specifically highlighted in President Barack Obama's (US) Precision Medicine Initiative, which is based on the concept that “prevention and treatment strategies...take individual variability into account.<sup>1</sup>”

Research efforts have yielded extraordinary progress in understanding the broad complexity of cancer as 60+ years of molecular biology research has provided a clearer understanding of how complex and intimately intertwined these conditions are in our cellular systems.<sup>2</sup> Renowned geneticist and Nobel laureate Sydney Brenner (and one of the original founders of the field of molecular biology) is credited with declaring, “Progress in science results from new techniques, new discoveries and new ideas; probably in that order.<sup>3</sup>”

Cancer research has benefitted immensely from the development of new tools that allow for interrogating biology at the sub-cellular level. Examples include high-throughput genomic sequencing technologies that emerged as a result of the Human Genome Project and drove

the development and outcomes of the nearly completed Cancer Genome Atlas initiative. Radical improvements in protein screening via mass spectrometry instrumentation emerging around the same time has allowed for identification of many (if not most) proteins in a sample, giving researchers a large population of targets for both basic research and clinical applications, new contrast agents and imaging tools that offer *in situ* identification and enumeration of specific drivers of cancer in collected cells at extraordinarily high resolution, and new modeling approaches that allow for *ex vivo* growth and analysis of a patient's own cancer cells to allow for more detailed profiling and characterization of the unique condition the patient has and even for screening potential therapeutic options to identify an optimal treatment strategy.

Research efforts have identified molecular signatures for a number of cancers and many of the technologies used to discover those are being employed to make clinical molecular diagnoses a fast growing option for many patients. Genomic assays like Agendia's MammaPrint and Genomic Health's Oncotype DX first became available in 2005 and involve detection of specific gene mutations using RT-PCR that was developed in the 1980's and 1990's. While RT-PCR still serves as an important tool for sensitive quantitation of these relatively rare transcripts revealing a key gene mutation signature, microarray technologies developed in the early 2000s, and more recently advanced genome sequencing technologies, are clear platform alternatives for detecting these signatures. Painstaking clinical research efforts have provided patients and their doctors with important tools, but these research efforts and clinical realities have also underscored important insufficiencies with these approaches and new molecular detection technologies are still needed to reduce the cost of carrying out such assays and simplifying their operation to allow potential implementation at the point-of-care (PoC; this is defined here broadly speaking – PoC can involve testing by a technician at a doctor's office and not necessarily a home-based test).

Fundamental causes and prominent risk factors remain an open question for many cancers and the multidisciplinary field of molecular epidemiology has emerged to explore this question using molecular analysis tools to study families and populations.<sup>4</sup> Such research is important to identify groups or individuals at greater risk of the disease so that effective screening and prevention strategies may be devised. The technologies driving research in this area often involve capabilities for high-throughput analysis of samples from relatively large cohorts. Furthermore, techniques that can either tolerate substantial variability in sample quality and quantity or otherwise offer a means for minimizing such variances are extremely important.<sup>5</sup>

The evidence for early-stage factors contributing to tumorigenesis potentially present across the genome and across the analyte population of each cancer cell is particularly complex. Panning through this immensity with the resolution required to pick up subtle aberrations of potential interest is challenging to say the least and to pursue this from each sample in a sizeable cohort requires maximizing efficiency and expediency at every step.

Further efforts are thus required to translate basic research findings into clinically useful diagnostics and at the same time, new innovations are still needed to allow for studying cancer biology at the immense level of complexity of the disease, as much of the cancer

puzzle has yet to be fully understood. Thus, the work reported in this themed issue of *Analyst* is timely. Many contributions contained in this issues report on state-of-the-art analytical instrumentation, methods and biomarkers that will assist in managing the entire array of cancer diseases and generate potential PoC technologies so that the most intricate assays can service low to middle income countries, even rural areas in developed countries, that do not have access to high standard-of-care cancer centers with their extensive portfolio of imaging equipment.

As a final general note, the management of cancer-related diseases is not just hinged on early detection of the disease, but other facets of disease progression such as patient stratification (which patient should receive which form of treatment), response to therapy, disease recurrence and assisting in the discovery of new therapeutics (technology serving as a companion diagnostic), which can dramatically drop the development time on pulling new and life-saving drugs through the FDA approval process.

As a matter of clarity, early detection, which is extremely important because cancer survival rates are much improved when detecting a cancer at an early stage of development, still requires aggressive new strategies (existing tests for screening are fraught with issues, such as PSA levels of prostate cancer). Early detection generally implies identifying a growing malignancy before the patient begins to exhibit symptoms, but this leaves open the possibility for many cancers that the condition has already progressed to the point that available therapeutic options offer only low chances for success. To improve public health outcomes, a more appropriate definition for early detection would involve detecting a lesion with malignant potential before it is beyond clinical control capabilities. Note that this means both positively identifying the malignant potential as well as finding it early enough that promising treatments are available; preferably targeted treatments with minimal side effects. This definition is subject to both improvements in detection strategies as well as improvements in treatment capabilities. For the purposes of this themed issue, the articles primarily focus on detection capabilities for cancer biomarkers.

The compilation of articles comprising this themed issue consists of a variety of review articles (mini-reviews and critical reviews) and innovative research articles (regular papers and communications). To introduce the readers to the compositional content of this exciting themed issue, we will in this introductory section briefly introduce the various articles appearing in this issue. We have divided the articles (both research reports and reviews) into the following topical areas: (1) Biomarkers; (2) imaging; and (3) platform technologies.

Biomarkers consist of a variety of molecular entities, such as those that are cell-bound including proteins, DNA and RNA, and markers that are not found in the tumor cell. These biomarkers can be located either at the primary tumor and secured using a biopsy or more recently, those that are found in circulation (*i.e.*, peripheral blood), also known as liquid biopsies. Liquid biopsies are attractive formats for securing biomarkers because of the less invasive nature of securing these markers and the fact that they can be more representative of the primary and metastatic sites as well. Indeed, liquid biopsies, which consists of circulating tumor cells (CTCs), extracellular vesicles, proteins, microRNA and cell free DNA (cfDNA), are becoming very popular and a plethora of new technologies are evolving

to secure these biomarkers. Cho and her team provided a minireview on emerging techniques for the analysis of extracellular vesicles, such as exosomes, including an overview of their clinical significance for the management of many cancer-related diseases and new technologies for their isolation. Cho and team also discussed tools for extracellular vesicle isolation. Issadore and co-authors presented a minireview on the isolation and detection of both exosomes and microvesicles using micro- and nanotechnology and the application of the technologies to monitor and diagnose cancer. Jung and co-authors provided a review on tools for the isolation and enrichment of circulating markers for cancer screening, detection and diagnostics. In their review, they not only survey current technologies for the analysis of circulating biomarkers, but also describe future directions for cancer screening, detection and diagnostics. Finally, in the area of liquid biopsies, Wong and team presented a minireview on analytical methods to determine the mutational status of key oncogenic genes, such as *KRAS* and *EGFR*, from body fluids. Clinical applications of these technologies were also presented.

Santangelo *et al.* provided a tutorial review on messenger RNA (mRNA) and RNA binding proteins and their role in the tumorigenic process. In addition, the authors reviewed methodologies to study RNA/protein interactions in particular, peptide-modified RNA imaging probes combined with proximity ligation and rolling circular amplification.

In their critical review, Dr. Weihong Tan *et al.* discussed aspects of cancer biomarker discovery using aptamers as reporters of certain proteins associated with many cancer-related diseases. Tan *et al.* also discussed the selection of new aptamers from protein targets using SELEX.

On another front, Allbritton and team presented a research paper on the discovery of peptide substrates for studying intracellularly the ubiquitin proteasome system, which is a new target system being used for certain chemotherapeutic agents, such as the proteasome inhibitors Bortezomib and Carfilzomb for the treatment of multiple myeloma. They used both high performance liquid chromatography and gel electrophoresis to determine viable peptide substrates. Finally, Yang *et al.* generated a molecular beacon for the detection of DNA methyltransferase (MTase) activity, which can produce abnormal methylation patterns in genomic DNA. The allosteric molecular beacon was able to detect MTase activity by clipping of the molecular beacon following methylation using a restriction enzyme (DpnI) releasing an aptamer containing a fluorophore that can bind to streptavidin-coated beads; non-clipped molecular beacons can bind to streptavidin but showed very weak fluorescence.

For imaging, three reviews appeared covering such areas as infrared spectral cytopathology, mid-infrared biomedical spectroscopy and Raman imaging. Old *et al.* reviewed micro-spectroscopy using infrared to interrogate the compositional nature of various cells, including tumor cells. The combination of micro-spectroscopy using infrared imaging and multi-variate analysis to detect abnormalities in exfoliated tumor cells was also reviewed. Hughes and Baker reviewed the use of mid-infrared spectroscopy from a patient perspective: Insights into the applications of mid-IR for interrogating patients with non-specific disease symptoms. Finally, Evans *et al.* reviewed the use of Raman spectroscopy for the non-invasive detection and diagnosis of cancer from an *in vivo*, *ex vivo* and *in vitro* perspective.

The authors also provided future perspectives on the use of Raman spectroscopy in terms of clinical translation.

Martin and team provided a new research report on using ATR-FTIR spectroscopy coupled to chemometric analysis to distinguish between normal, borderline and malignant ovarian tissue as a possible technique for patient classification that can have ramifications on the course of treatment for these patients. Their data indicated the ability to segregate the tissue type using ATR-FTIR spectroscopy, which can mitigate risk of either over- or under-treatment of ovarian cancer patients. Nazari and Muddiman discussed the use of infrared matrix-assisted laser desorption electrospray mass spectrometry (IR-MALDESI). Using IR-MALDESI, the authors were able to distinguish, via disease-specific metabolite and lipid signatures, diseased from normal tissue of ovarian hen tissue slices. Discussion of spectral accuracy and sulfur counting to improve the confidence in the assay were provided as well. Goormaghtigh *et al.* presented original research on the use of IR imaging of breast cancer tissues. Using principle component analysis, the researchers reported the ability to distinguish between normal and diseased tissue.

Finally, Stone and co-authors presented a research article on the use of mid-infrared imaging spectroscopy for monitoring histopathological features in cells comprising colon tissues in a non-destructive and non-labeling approach. The authors report high-resolution imaging (<5  $\mu\text{m}$ ) using a conventional FTIR microscope retrofitted with novel high magnification optics; the authors show the ability to image sub-cellular structures. Using their high-resolution microscope, mucin rich goblet cell features were observed in the colonic tissue.

Platform technologies are devices used to “detect” certain biomarkers secured from a clinical sample. These platforms can be used to enrich certain biomarkers from clinical samples such as blood or urine or simply detect the biomarker of interest directly within the clinical sample. These devices consist of microfluidics or biosensors.

Lim and coworkers provided a critical review in this themed issue discussing the use of microfluidics for basic research and clinical applications in the area of oncology. In their review, the advantages of microfluidics for clinical applications including the fast processing time, small sampling volume, high sensitivity and full process automation were noted. The authors also include a discussion concerning the use of microfluidics for detection, diagnosis, prognosis and drug screening as well as the future of this platform technology in oncology. Pappas provided a review on the use of microfluidics for the analysis of cancer cells. Specifically, microfluidics used to culture cells and single cancer cell analyses all enabled by the use of microfluidics.

A number of research papers appear in this themed issue discussing the use of microfluidics for a variety of cancer discovery and clinical applications. For example, Soper *et al.* presented a microfluidic assay for the detection of minimum residual disease (MRD) in acute myeloid leukemia (AML). In their work, three plastic-based microfluidic devices, each consisting of an array of sinusoidal channels decorated with antibodies targeting circulating leukemic cells (CLCs) from the peripheral blood, could be used to search for MRD and relapse in AML patients much earlier than traditional assays, which use PCR or flow

cytometry. In addition, the assay could secure results using peripheral blood and not requiring a bone marrow biopsy. Chae and co-workers developed a thin (<30 nm) Si membrane to detect bladder cancer cells in the urine of patients. The microfluidic device could produce wrinkle patterns in the Si membrane while normal cells did not generate such a wrinkle pattern. In the 5 bladder cancer patients tested, all produced the required wrinkle pattern while normal cells did not. Lockett and his team generated a paper-based device to provide real-time monitoring of cancer cell chemotaxis. A regular sheet of paper was used with wax printed on the paper to define the required fluidic pattern. In the manuscript, the invasiveness of cancer cells cultured in the presence/absence of oxygen gradients were carried out.

Fan and Zhang reported on the generation of a microfluidic device for the selection of circulating tumor cells (CTCs) from peripheral blood using fibrin-coated microchannels. Brady *et al.* also reported on a microfluidic device for the analysis of CTCs in patients with small cell lung cancer. The system used the Parsortix system, which separated CTCs based on size differences between normal blood cells and the CTCs. For small cell lung cancer, they reported that 83% of the captured CTCs were cytokeratin positive, indicating that the Parsortix system enriched EpCAM negative CTCs as well.

Revzin and team reported on a microfluidic consisting of compartments (~7000; volume = 20 pL) produced for monitoring the activity of single cells. Using their system, they were able to monitor the release of inflammatory cytokine IFN- $\gamma$  and exosomes from single immune cells and cancer cells, respectively. Gardner and co-workers reported on a microfluidic flow chamber for carrying out laser tweezer Raman spectroscopy on single cancer cells. The device was configured with a dual chamber to allow for the automated capture and analysis of the captured cells using a non-destructive readout format (*i.e.*, Raman spectroscopy). Results were presented on the analysis of live epithelial prostate cells and lymphocytes.

For biosensor-based technology platforms for cancer diagnostics, Rusling *et al.* presented a review of electrochemistry sensors for multiplexed detection of protein biomarkers using immunoassays. In their review article, they mentioned two major challenges for cancer diagnostics addressed by these biosensors including discovering the panel of biomarkers needed to secure proper clinical results and also, detection of the markers with high sensitivity and specificity. Finally, they addressed the potential use of electrochemical biosensors for PoC measurements. Masson and co-authors reviewed nanobiosensors for the analysis therapeutic drug monitoring that required frequent testing.

The themed issue included three reports on the use of biosensors for cancer cell analysis. For example, Zhu and co-authors reported on the use of an electrochemical-TUNEL method to detect apoptotic cells. The sensor consisted of a carbon nanotube containing recognition elements for cell capture followed by labeling the quantum dots for the electrochemical stripping analysis. Masson *et al.* reported a nanobiosensor for monitoring methotrexate using a plasmon-coupling assay. Free methotrexate and folic acid Au nanoparticles competed with human dihydrofolate reductase functionalized Au nanoparticles. Rapid and sensitive detection of methotrexate was determined using the localized surface plasmon resonance

readout. Lawrence and team discussed their results on a grating coupled SPR microarray for protein and cell analysis from breast cancer xenografts. Antibodies to the targeted proteins were printed on the grating coupled SPR surface. The authors were able to detect CD24, CD44, CD326, CD133 and CD49b simultaneously using the grating coupled SPR sensor.

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