Single Cell Analysis is a Multi-Faceted Marketplace

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The focus of this GEN BioMarket Trends Report is the expanding field of single cell analysis (SCA) which is a broad and amorphous space.

The space is composed of technologies on the “front end” for capturing the relevant cellular [sub]-populations and technologies on the “back end” for interrogating the captured cellular [sub]-populations such as for gene expression patterns, epigenetic alterations, proteomic profiles, metabolite profiles.

There are several segments that together constitute the SCA marketplace—many of which are relatively un-developed and somewhat undefined as a segment per se. In this second report on SCA, we focus on Circulating Tumor Cells (CTCs) analysis and utility as a paradigm for the SCA space.

This BioMarket Trends Report on CTC SCA focuses on the qualitative and quantitative trends in this marketplace and alludes to their potential utility as circulating biomarkers.

The field of SCA is broadening to include SCA-based cellular interrogation at the “single cell level”, single cell sequencing, as well as analysis of single cells derived from different anatomical locations.
Market Segmentation, Industry Structure

The Segments of SCA

- CTC Analysis
- Single Cell Sequencing
- FISH
- Flow Cytometry
- Microfluidics Capture of Distinct Cells
- Laser-Capture

Focus of this Report
CTCs Represent a Special Case of SCA

- Prognostic Oncology Biomarkers
- Analysis Happens at the Single Cell Level
- Demonstrated Clinical Utility in Specific Disease Classes
- Circulating Biomarker Class
- CTCs Represent an Excellent Paradigm for the Potential of SCA and Set the Stage for the “Practical Utility” of SCA
CTC Characteristics

• Morphological and Staining Pattern
  – EpCAM+
  – CD45−
  – Cytokeratin 8, 18 or 19 (CK)+
• Appropriate cytoskeleton staining pattern
• Variations in size, shape, variable nuclear-to-cytoplasmic (N/C) tumor tissue
• 4 µm x 4 µm in size
• CTCs represent a random sampling of many phenotypic cell types present in the primary and metastatic tumor deposits
• CTCs can provide real-time information about a patient's current disease state, acting as a “liquid biopsy”
• Diagnostics: Much less invasive than tumor biopsies because they can be detected from a blood draw and don't require surgical intervention
• There is evidence that “sub-populations” of CTCs exist with different molecular features [surface markers such as EpCAM+ or EpCAM−, amongst others]
### Potential Molecular Targets for Detection of CTCs: Possibility to Expand the Market for CTC Testing of Various “CTC Classes” in Different Cancer Sub-types

<table>
<thead>
<tr>
<th>Marker Class</th>
<th>Marker</th>
<th>Function</th>
<th>Enrichment</th>
<th>Detection</th>
<th>Disease Classes where Relevant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Survivin</td>
<td>Apoptosis inhibitor</td>
<td>N/A</td>
<td>qPCR</td>
<td>Breast Cancer</td>
</tr>
<tr>
<td></td>
<td>Telomerase</td>
<td>Telomere extension, inhibition of senescence</td>
<td>N/A</td>
<td>Telomerase PCR ELISA</td>
<td>Advanced prostate cancer patients with undetectable PSA</td>
</tr>
<tr>
<td></td>
<td>KRT19</td>
<td>Potential stem cell marker</td>
<td>N/A</td>
<td>qPCR, ELISPOT, RT-PCR</td>
<td>Advanced NSCLC and breast cancer</td>
</tr>
<tr>
<td>Stem cell-associated</td>
<td>BMI1</td>
<td>Gene regulation at chromatin level</td>
<td>N/A</td>
<td>qPCR</td>
<td>Advanced breast cancer</td>
</tr>
<tr>
<td></td>
<td>EpCAM</td>
<td>Epithelial surface marker</td>
<td>Immunomagnetic enrichment</td>
<td>Immunocytochemistry</td>
<td>Metastatic breast, colon, prostate cancer</td>
</tr>
<tr>
<td></td>
<td>PTEN, BRCA1</td>
<td>Microsatellite instability</td>
<td>Density gradient, immuno-magnetic cell enrichment</td>
<td>Immunocytochemistry, microsatellite PCR</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Therap target</td>
<td>ERBB2</td>
<td>Oncogenic growth factor receptor</td>
<td>Density gradient, immuno-magnetic cell enrichment</td>
<td>Immunocytochemistry, PCR, FISH</td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td>IGFR1</td>
<td>Insulin-like growth factor type 1 receptor</td>
<td>Immunomagnetic (EpCAM) enrichment</td>
<td>Immunocytochemistry</td>
<td>Hormone-refractory prostate cancer</td>
</tr>
<tr>
<td>Multi-marker sets</td>
<td>TERT, KRT19, KRT20, CEA</td>
<td>Various</td>
<td>N/A</td>
<td>RT-PCR</td>
<td>Post-operative colorectal cancer</td>
</tr>
<tr>
<td></td>
<td>TERT, KRT19, CEA, MUC1</td>
<td>Various</td>
<td>N/A</td>
<td>Colorimetric membrane-array method</td>
<td>Gastric cancer and healthy individuals (post-operative marker set)</td>
</tr>
</tbody>
</table>
Major challenge for CTC researchers is the prevailing difficulty of CTC purification that would allow the molecular characterization of CTCs

**Goal of Research Efforts:** Method that is highly sensitive, reproducible and easy to implement in a clinical setting

**Downstream Molecular Analysis of CTCs to Identify Molecular Lesions—Currently an Important Research Area**
Breakout of the Current Marketplace vis-à-vis the Study of CTCs: Image-based Approaches vs. Molecular Approaches

Molecular Approaches [RT-PCR, qPCR, Next-Gen Sequencing, microRNA Analysis] 49%

Image-based Approaches [Immunocytochemistry, CellSearch, Microscopy, EPISOT] 51%

Source: Primary Market Survey of CTC Researchers Worldwide.
### SCA is Much Broader than CTC Analysis Alone

<table>
<thead>
<tr>
<th>Market Segments</th>
<th>Selected Companies/Institutions in the Space Provides a Snapshot of the Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating Tumor Cells/ Rare Cells</td>
<td>Adnagen, Biocept, Cytotrack, Rarecells, Silicon Biosystems, Veridex, Epic Sciences</td>
</tr>
<tr>
<td>DNA Sequencing</td>
<td>IBM, Ion Torrent/Life Technologies, Oxford Nanopore Technologies, Pacific Biosciences,</td>
</tr>
<tr>
<td>Microfluidics</td>
<td>BioNanomatrix, Fluidigm, Fluxion Biosciences, IntegenX, Mathies Lab, Microfluidic ChipShop, RainDance Technologies</td>
</tr>
<tr>
<td>Microscopic Capture</td>
<td>Eppendorf, Hacker Instruments, Ibidi, Leica Microsystems, MMI, Narishige, Zeiss</td>
</tr>
<tr>
<td>Multifaceted</td>
<td>Life Technologies, Miltenyi Biotec, Roche Applied Science</td>
</tr>
<tr>
<td>Others [FISH, etc.]</td>
<td>LBNL, UC Irvine, Biosearch Technologies</td>
</tr>
</tbody>
</table>
Industry Trends and Market Opportunities

• There is an expansion of the Single Cell Analysis field driven by the need to understand individual cellular behavior
• Technology development focusing on cell capture using microfluidics is a driver for the SCA field
• Analysis of CTCs represents a segment of the SCA field and demonstrates the utility of the power of SCA in a “clinically-relevant” discipline → CTC capture and interrogation at a cellular level providing insights into the progression of breast, colorectal or prostate cancer, amongst potentially other cancer classes
• The ability to individually capture and interrogate CTCs on a single cell level is a key driver for the SCA field and there are emerging market opportunities for CTCs as Companion Diagnostics → Liquid Biopsies
• The “collision” of front-end cell capture approaches with back-end analysis approaches will be the drivers of SCA analyses in both research as well as clinical applications
• Single Cell Sequencing and Single Cell Genomics are some of the Drivers of this Field
In an Effort to Bring Together the Key Opinion Leaders in the SCA Space as well as those in the Extracellular Vesicles Space, we’ve Designed this Conference that meshes together Single Cell Analysis, Single Cell Genomics, Analysis of Extracellular Vesicles, as well as Utility/Application of these areas for Research and Biomarker/Diagnostic Development.

Topics include single cell genomics, single cell proteomics, single cell transcriptomics, Technologies for Analysis of Single Cells as well as Extracellular Vesicles, Biomarker Development, and Emerging Trends in the Single Cell and Extracellular Vesicle Spaces.