Circulating Tumor Cell Opportunity – Prostate Cancer Detection

September 2009
Overview

- Circulating tumor cells (CTCs) detection is receiving tremendous interest in both oncology research and practitioner communities.
- CTCs can provide early-detection, prognosis and disease monitoring for a broad range of cancers.
- Veridex’s (J&J) CellSearch has built awareness and sponsored studies establishing clinical utility and has helped to drive reimbursement.
- However, Veridex technology has limitations making it difficult to widely commercialize the system.
- The Ikonisys platform provides superior signal detection and imaging that allows accurate and efficient CTC screening and analysis for wide commercial use.
Ikonisys: An Emerging Best-in-Class CTC Platform

- Ikonisys’s CellOptics is a next-generation, best-in-class, platform for CTC detection
- The platform is a hardware / software combination built upon the Ikonoscope
- The platform provides high quality CTC imaging that allows pathologists and oncologists to observe morphology and dysregulated chromosomal structures
- The technology does not require an expert technician and can provide unambiguous answers on CTC presence in less than ten minutes
- The Ikonisys platform will facilitate rapid market growth by standardizing and automating CTC detection technology
The Market Opportunity
Current Cancer Diagnostic Methods

Current methods for diagnosis of primary tumors and metastatic disease have inherent limitations....

- **Pathology**
  - Evaluation of primary tumor and lymph nodes (Grading and Staging)
  - Only value in initial staging and grading
  - No ability to monitor disease

- **Imaging**
  - MRI; X-Ray; CT Scan; Bone Scan
  - Can be expensive
  - Lack sensitivity and specificity
  - Rarely useful for early detection

- **Blood Tests**
  - CA15.3; CA125; CA19.9; PSA; CEA
  - Lack sensitivity and specificity
  - Only PSA used for early detection

Clear medical need for a new approach
The Potential of Circulating Tumor Cells

The ability to detect, quantify and analyze tumor cells in the blood of cancer patients represents a new paradigm in cancer diagnostics that opens up a world of possibilities.

- Circulating Tumor Cell (CTC) analysis can be applied to all major cancers (Breast, Colorectal, Ovarian, Prostate and Lung)

- From a routine blood draw, it is possible to:
  - Determine the presence or absence of CTCs
  - Quantify the number of CTCs in a given volume of blood
  - Characterize the Tumor Cells through biochemical marker analysis

- Clinical applications:
  - Earlier detection of cancer and risk assessment
  - Guidance of therapeutic choices and prediction of treatment benefit
  - Monitoring patient progression during treatment
CTCs are a Topic of Tremendous Scientific Interest

- CTCs, a major focus at recent AACR and ASCO meetings
  - Focus on prognostic value of CTCs
  - Focus on use of CTCs as surrogate marker for cancer progression in prostate
- Pubmed, the scientific portal, now includes about 15,000 articles with the phrase “circulating tumor cells”
- Today, research on the role and benefits of CTCs is in explosive growth
Examples of Recent Studies

- Panteleakou et al. (MOLMED, 2009): Detection of Circulating Tumor Cells in Prostate Cancer Patients: Methodological Pitfalls and Clinical Relevance
- DeBono, Scher et al. (Clin Cancer Res, 2008) Circulating tumor cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data
- DeBono, Scher et al. (Clin Cancer Res, 2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer
- Budd et al. (Clin Cancer Res, 2006): Less variability in predicting survival of breast cancer patients from CTC counts than from radiological methods
- Cristofanelli et al. (NEJM, 2004): CTC count is a strong prognostic for breast cancer survival
Sizing the Market Potential

- **Pricing**
  - Medicare reimbursement: $312
  - Estimated Lab revenue/test: >$400
  - Price/test assumption: $175

- **Test Volume (Monitoring)**
  - US: 10MM Tests
  - World: 25MM Tests

- **Addressable Market**
  - Monitoring (World):
    - 25MM Tests @ $175/Test = >$4B
  - Earlier Detection:
    - 200MM Tests @ $175/Test = >$35B

<table>
<thead>
<tr>
<th>US Market</th>
<th>Monitoring</th>
<th>Earlier Detection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monitoring</td>
<td>2.5</td>
<td>40.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Earlier Detection</td>
<td>0.2</td>
<td>50.0</td>
<td>40.0</td>
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Over $35bn Global Market Opportunity
oncoFISH® prostate
Circulating Tumor Cells

\textit{oncoFISH® prostate}

- CTC based
- Early detection
- Analysis and guidance
- Disease monitoring
Why CTC Detection for Prostate Cancer?

CTC detection has a number of potential applications in patients with prostate cancer:

- Early-stage diagnostics
- Assessment of prognosis
- Recurrence after definitive local treatment
- Response to therapy
- Non-invasive tumor sampling procedure in patients with metastases to assess target modulation by drugs
Prostate Cancer

PSA

| 10 ng/ml | 4 - 10 ng/ml | 2 - 4 ng/ml | 0 - 2 ng/ml |

Biopsy

- Up to 75% false positive diagnoses
- 25% positive for cancer

oncoFISH® prostate will:

- Increase the specificity of PSA for screening
- Be used to monitor disease recurrence

25 million tests per year in the US - 40-50 million worldwide

An elevated PSA leads to...

1-1.5 million biopsies per year
How Does it Work?

Enrichment

**Isolation of CTCs**
Physical enrichment using a cell-size filter allows fast and efficient isolation of epithelial cells with minimum manipulation and breakage, thereby maintaining cellular integrity.

Labeling

**Antibody Staining**
Five different combinations of signals including antibodies and FISH

**Signal Amplification**
Signal amplification enables the combination of immunostaining with FISH, while keeping fluorescence signal intensity.

Detection/Analysis

**Robotic Fluorescence Microscopy using Ikoniscope**
Accurate and efficient identification, quantification and image analysis using advanced and proprietary software and hardware.

Possible to remove cells for further analysis.
CellOptics: Enrichment

- Ikonisys uses physical filtering to obtain a good starting sample.
- Physical filtering retains large cells including almost all CTCs while excluding normal erythrocytes.
- The Nucleopore (Whatman) track-etched polycarbonate membrane filter (8.0 mm pore size, 25mm diameter) was found to be the best fit.
- A patent-pending system is under-development for standardization and commercialization of this filtration process.
- Our method is focused on achieving consistently high CTC yields in the filtration process.
- Other players in the CTC space are solely focused on better enrichment technology. We have the ability to incorporate all of these new technologies into our platform.
CellOptics: Labeling

- Labeling helps finding a subpopulation of cells that has as high an abundance of CTCs as possible
- Ikonisys uses up to five different combinations of signals including antibodies (e.g., anti-EpCam, anti-PSA, anti-cytokeratin) and FISH
- Ikonisys has pending IP on using antibodies and FISH in combination
- We believe in optimizing the labeling step based upon the cell types being detected
- For example, we are studying the use of PSA and PSMA antibodies for prostate CTC detection but for broad CTC detection prefer EpCAM and cytokeratin antibodies
CellOptics: Detection

- The Ikonisys CellOptics platform provides *better signal detection and imaging* that allows accurate and efficient CTC identification.

- This is where we are likely to have a long-term edge in the market.

- Differentiated features using the Ikoniscope include:
  - Advanced software-based automation providing low-to-high magnification for imaging and analysis.
  - High throughput attributable to highly automated sample processing.
  - Superior optics than the FDA-approved CTC detection system.

- This allows the user to obtain numerous, high quality CTC images for better research and clinical applications.
The Ikonisys Edge...

...Sensitive and Accurate Detection

• Existing systems tend to either capture cell fragments (false positives) or fail to capture CTCs (false negatives)
• The Ikonisys platform provides superior and accurate detection
• The Ikonisys immunolabeling and high-quality images allows sensitive interpretation, monitoring and analysis
These CTCs were isolated from prostate cancer patients and immunostained with Cam5.2 (Cy5) and AUA1 or PSA (green) in Walter Bodmer’s lab. Researchers are able to obtain very good images with attendant information on morphological cell characteristics through the Ikoniscope.

Compare to a Veridex image above with lower resolution and clarity.
Informal discussions with Veridex users indicate:
- Interpretation time is typically two hours
- Substantial technical expertise and experience is required

The typical time to interpret Ikoniscope images is about ten minutes because images are clear

No technical expertise is necessary

The CellOptics system is therefore, far more practical for widespread use in CTC detection

As a result, we have much larger market potential than the current Veridex system.
Project Status

- Pilot study using Ikoniscope first generation system has been completed
  - Positive CTC detection results – Bodmer et al. study

- Second generation system with increased sensitivity of CTC detection has been developed

- We have rolled out the new system to three research sites for use and testing

- Preliminary feedback is extremely positive, implying clear advantage over existing systems
“Circulating tumor cells in the blood promise to be a very valuable source of material for early indication of the presence of a cancer. Early detection of a cancer, or of its recurrence, at a stage when it is much more likely to be successfully treatable, is a most important approach to dealing with cancer. The use of the Ikonisys technology opens up exciting new opportunities for the early detection of cancer and I am very happy to be able to collaborate with Ikonisys using their novel automated microscope to develop such new approaches...”

Sir Walter Bodmer  
PhD, FRCPath, FRS  
Oxford University

Dr. Johann S. de Bono  
Royal Marsden Hospital

Dr. Howard Scher  
Memorial Sloan Kettering
Pilot Study - Bodmer et al.

*Demonstrates that the Ikoniscope is a relatively simple and rapid procedure for the clear-cut identification of CTCs*

- Bodmer’s pilot study used the Ikoniscope to detect CTCs in peripheral blood
  - No positive cells were detected in 11 healthy controls, and CTCs were detected in 23/25 colorectal, 10/10 prostate and 4/4 ovarian cancer patients
  - This shows a high degree of sensitivity and specificity in the Ikoniscope CTC detection system
  - Less than one epithelial cell per milliliter of blood could be detected, and through FISH could identify chromosomal abnormalities in these cells
Bodmer’s approach involved:

- Spiking experiments with cancer cell lines to estimate recovery yield
- Enrichment was performed either by density gradient centrifugation using Lymphoprep or by filtration
- CTCs were labeled with monoclonal antibodies against cytokeratins 7/8 and either AUA1 (against EpCam) or anti-PSA
- The slides were analyzed with the Ikoniscope robotic fluorescence microscope imaging system
Ikonisys’ CTC System

- Today

- Filtration sub-system
  - Filter holder: prototype and production design complete
  - Pump system: prototype complete

- Reagent sub-system
  - Multiple antibodies and FISH probe protocol: complete
  - Disease Monitoring Reagent Kit: initial kit validated (EpCAM, Cytokeratins, CD45)

- Ikoniisoft CTC discovery software
  - Initial version complete
Project Plan

- The Ikonisys platform has best-in-class detection technology, superior enrichment method and advanced labeling tools
- Our technology is stable and highly impactful
- Continued labeling development:
  - Study markers beyond EpCAM for prostate including PSMA and PSA
  - Current system accommodates up to three antibody labels per use
- Our goal is to roll-out to the research market in 2009 and 2010 while validating the utility of oncoFISH prostate for an FDA approval
  - Potential 510k comparison to Veridex
  - Or PMA for screening in early prostate population
Regulatory Timeline

2009
- Rollout of the CTC system to 10+ research sites
- Research use only of the prostate test
- Experimentation with a variety of protocols for cell enrichment and detection

2010
- Finalization of a CTC detection protocol in prostate
- Launch an LDT Test
- Initiation of PMA studies

2011
- PMA approval for OncoFISH Prostate
- Commercial introduction
Our Objective and Opportunity for Partnership

- Demonstrate the central role of the Ikonisys system in CTC detection and analysis
- Bring products based on the Ikonisys CTC system to market
- Develop the current prototype system into one, or more, broad, clinically validated tests
  - System finalization
    - Filtration system
    - Ikoniscope (H/W & S/W) – from input of expert users
  - Prostate
  - Screening
  - Companion diagnostics
  - Disease Monitoring (with Veridex as predicate)
- Other: Breast; Colorectal; Ovarian; Lung
Future Potential Applications in Clinical Settings

- Patient has a high PSA but not a positive biopsy
  - Order an OncoFISH prostate test
  - Provides a check on prostate tumor status
  - Risk assessment
- Patient has a positive biopsy
  - Want more precise information than Gleason scores and PSA
  - CTC is an important status check and prognostic test
- Patient in treatment
  - Guidance for treatment method
  - Monitor treatment success
Comparison of OncoFISH prostate to Other CTC Detection Methods
### Ikonisys vs. Veridex: Aspects of CTC Analysis

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<thead>
<tr>
<th>Characteristics</th>
<th>CellSearch</th>
<th>CellOptics</th>
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<tbody>
<tr>
<td><strong>Sample Volume</strong></td>
<td>7.5 ml</td>
<td>7.5 ml</td>
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<tr>
<td><strong>Enrichment</strong></td>
<td>Immunomagnetic: anti-Epcam-antibody</td>
<td>Physical enrichment using a cell size filter</td>
</tr>
<tr>
<td><strong>Labeling</strong></td>
<td>Cells labeled with anti-cytokeratin and anti-CD45 antibodies to distinguish between epithelial cells and leukocytes</td>
<td>Ability to use 5 different combinations of antibodies as well as FISH</td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>Rudimentary microscopy system; semi-automated fluorescence-only</td>
<td>“Complete” optical microscopy; fully automated</td>
</tr>
<tr>
<td><strong>Image Analysis</strong></td>
<td>Limited</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Sample Processing</strong></td>
<td>Automated</td>
<td>Automated</td>
</tr>
<tr>
<td><strong>Batch Size</strong></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><strong>Overall Throughput Rate</strong></td>
<td>16 per day</td>
<td>&gt; 100 per day</td>
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- Ikonisys’ platform holds significant advantages in many aspects of CTC analysis as compared to the current market CTC detection leader, Veridex’s CellSearch.
Veridex’s EpCAM Challenge

There are inherent weaknesses in Veridex’s choice of EpCAM for tumor cell enrichment...

- **Veridex Method**
  - FDA-cleared
  - Veridex is a Division of J&J
  - Offered by Quest Diagnostics
  - But....
    - Dependent upon expression of the EpCAM cell surface antigen
- **Limitations:**
  - Some cancers down regulate EpCAM – e.g., no EpCAM on melanoma cells
  - Some cells have EpCAM antigens masked
  - Only 70% of prostate cancer cells express EpCAM
- **Results in risk of not capturing all relevant cells**

- **Ikonisys’ methods based on PHYSICAL enrichment**
Veridex’s “Debris” Challenge

There are inherent weaknesses in Veridex’s choice of immunomagnetics for tumor cell enrichment...

- The Veridex method involves attachment of magnetic particles to antibodies that bind to EpCAM which is over-expressed on many cancer cells.
- Veridex’ use of erythrocytic lysis tends to break many cells resulting in large amounts of cell fragments in a sample.
- These fragments have EpCAM sites that bind to the magnetic particles.
- As a result, the Veridex method requires laborious focus of a pathologist who has to find intact cancer cells in a soup of fragments.
- It can take over two hours to get a reasonable cell count from a sample using the Veridex technology.
- One study by Bogen et.al. (2008) found that lysis-based CTC detection levels were unacceptably low.
Other Technologies in Development

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<tbody>
<tr>
<td>Ikonisys</td>
<td>Uses the Ikoniscope for automated FISH based testing of CTCs using an 8 micron filter for physical enrichment, labeling with EpCAM, cytokeratin and 3 other stains.</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Ikonisys has a very promising CTC detection platform in development. This platform may be compatible with a number of other new technologies.</td>
</tr>
<tr>
<td>AdnaGen</td>
<td>Has several CTC tests in development that employ Veridex technology to locate cells. They then examine the gene profiles of these cells using RT-PCR.</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>This company's technology suffers from all of the problems associated with the Veridex technology.</td>
</tr>
<tr>
<td>Amnis</td>
<td>Has created a single instrument called the ImageStream which facilitates high speed flow cytometry with a high resolution microscope. This can be used for detection of CTCs.</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>The Amnis technology is not useful for CTC detection as it does not use FISH and thus only permits examination of gross cell morphologic features.</td>
</tr>
<tr>
<td>AVIVA</td>
<td>Microfiltration filter developed by Richard Cote of USC. Finds cells by size. Has shown ability to filter out CTCs without lysis method. Recently presented at 2009 AACR conference.</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Pantelakou et.al. (2009) note that this method avoids use of cell lysis but results in low numbers of collected CTCs. No advantage from automated microscopy.</td>
</tr>
<tr>
<td>BIOCEPT</td>
<td>Biocept employs a microfluidics-based device to detect CTCs using EpCAM antibodies. This company’s technology is similar to that of Cellective DX.</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Like Cellective, this is a very time consuming method that is trying to optimize the enrichment phase of CTC detection.</td>
</tr>
<tr>
<td>CELLective Dx</td>
<td>Has licensed technology from Massachusetts General Hospital that uses microfluidics and EpCAM antibodies to detect CTCs. Backed by Mohr Davidow Ventures.</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>This method is very time consuming and focused on EpCAM only. Hard to alter design to accommodate other antibodies for tumor cell detection. No automation.</td>
</tr>
<tr>
<td>CytoTrack</td>
<td>Is a Danish company that has developed a high speed system for detection of CTCs using a disk with variable scanning speeds and FISH signals.</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>This high speed method is promising but does not permit imaging with microscopy and is subject to the same enrichment problems facing Veridex.</td>
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</table>

There are some promising alternatives to Veridex in development. However, none of these methods combine Ikonisys’ leading enrichment technology with superior labeling and detection.
Ikonisys, Inc.

Next Generation Diagnostics

www.ikonisys.com
203.776.0791