Targets of Tumor Found in Liquid Biopsy

Liquid Biopsies: "The Stethoscope For The Next 200 Years"\(^1\)

Mutations in tumor can also be found in the blood\(^2,3\)

\(^1\)Eric Topol, Professor of Genetics, The Wall Street Journal; \(^2\)Sorenson et al. Cancer Epidemiol Biomarkers Prev 1994; \(^3\)Diehl PNAS 2005
Biocept Technology
Biocept offers sensitive & quantitative blood-based methods for the detection and monitoring of clinically actionable cancer biomarkers in both circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA).

This dual platform differentiates Biocept from other liquid biopsy vendors.
Value of CTC and ctDNA

- ctDNA is utilized for mutational tests
  - Quantitative results, measuring volume of mutant copies compared to wild type
  - Optimized to reliably detect \( \approx 7 \) copies of mutant in a background of 14,000 copies of wild-type (0.05%)

- CTCs offer
  - Solid-tissue-type testing, including fluorescence in situ hybridization (FISH) and immunocytochemistry (ICC)
  - Enumeration for monitoring response and progression
Biocept Platform Enables CTC and ctDNA Analysis

**EGFR**
- TARCEVA®
- GILOTRIF®
- IRESSA®
- TAGRISSO®

**KRAS**
- ERBITUX®
- VECTIBIX®

**BRAF**
- ZELBORAF®

**ctDNA Analysis**

**CTC Analysis**

**PD-L1**
- KEYTRUDA®
- OPDIVO®

**HER2**
- HERCEPTIN®
- PERJETA®

**ALK, ROS1**
- XALKORI®
- ZYKADIA®

**ER**
- NOLVADEX®
- Aromatase Inhibitors

KEYWORDS:
- Biocept
- CTC
- ctDNA
- EGFR
- KRAS
- BRAF
- ctDNA Analysis
- CTC Analysis
- TARCEVA®
- GILOTRIF®
- IRESSA®
- TAGRISSO®
- ERBITUX®
- VECTIBIX®
- ZELBORAF®
- KEYTRUDA®
- OPDIVO®
- HERCEPTIN®
- PERJETA®
- XALKORI®
- ZYKADIA®
- NOLVADEX®
- Aromatase Inhibitors
Circulating Tumor Cell Capture

Multiple antibodies bind to different antigens

Antibody Cocktail

Target Cell

Biotinylated secondary antibody

Target Cell

Plasma (55% of total blood)
Buffy Coat leukocytes & platelets (<1% of total blood)
Erythrocytes (45% of total blood)
Traditional CTC Detection

Traditional Definition

- Dapi +
- CD45 –
- CK +

- Not all tumors stain positive for CK

Detection Rates

Positive Detection of ≥1 CTC

Mayer et al. Cancer Genetics, 2011
Biocept Technology Detects More CTCs

Biocept Captures CK-Cells

- >50% of HER2 Positive cells are CK negative, but are clearly tumor cells
- >700 Negative control samples with no CK+ or CK- cells detected

Mayer et al. Cancer Genetics, 2011
What Limits the Sensitive Detection and Quantitation of Mutations?

- Inability to completely suppress wild-type “break through” during amplification
- Amplification errors
- Chemical nucleic acid base damage
Target Selector™ is designed to suppress amplification of wild-type targets, while not suppressing amplification of mutation sequences that differ from wild-type by even a single nucleotide variant (SNV). (The Needle in the Haystack)

Target Selector amplifies mutant sequences while efficiently suppressing wild-type amplification even when the two differ by a single nucleotide.
How the Target Selector Assay Works

WT DNA

Mutation Present

Amp PCR proceeds
Concordance with tissue
Concordance w/ Tissue

Primary Tumor vs. Liquid Biopsy

Metastatic Lesion vs. Liquid Biopsy
Analytical vs Clinical Validation

- Analytical validation demonstrates the accuracy, precision, reproducibility of the test-how well does the test measure what it claims to measure?
- Clinical validation demonstrates the effectiveness of the test-how relevant is the test measurement to the clinical condition?
Biocept's EGFR Target-Selector assays for T790M, L858R, and Del 746-750 have been optimized to reliably detect 7 copies of mutant in a background of 14000 copies of wild-type (1:2000 or 0.05% mixture of mutant in wild-type).
### Biocept PD-L1 Clone 28-8

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>20</td>
</tr>
<tr>
<td>False Negative</td>
<td>0</td>
</tr>
<tr>
<td>False Positive</td>
<td>0</td>
</tr>
<tr>
<td>True Negative</td>
<td>17</td>
</tr>
<tr>
<td>Concordance</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
</tr>
<tr>
<td>NPV</td>
<td>100%</td>
</tr>
</tbody>
</table>

Negative cut-off (H727): 3.9% based on 95% confidence level using BETAINV function: BETAINV(0.95,10,400). 10 = number of false positive plus 1; 400 = number of cells analyzed for each case.
Clinical Validation (EGFR, ER, HER2)

<table>
<thead>
<tr>
<th></th>
<th>EGFR*</th>
<th>HER2^</th>
<th>ER~</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>74</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>82.6%</td>
<td>95%</td>
<td>72%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98%</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>NPV</td>
<td>92.6%</td>
<td>96%</td>
<td>-</td>
</tr>
<tr>
<td>PPV</td>
<td>95%</td>
<td>90%</td>
<td>-</td>
</tr>
<tr>
<td>Concordance</td>
<td>93.2%</td>
<td>93%</td>
<td>79%</td>
</tr>
</tbody>
</table>

* "Highly Sensitive Detection of rare EGFR Mutations with ctDNA using Target-Selector™ Assays"; Vassilios Alexiadis et al.
^ "FISH-based determination of HER2 status in circulating tumor cells isolated with the microfluidic CEE™ platform"; Julie Ann Mayer, et al; Cancer Genetics 2011
~ "Correlation of hormone receptor status between circulating tumor cells, primary tumor, and metastasis in breast cancer patients" K. Kalinsky et al; Clinical Translational Oncology 2015
Clinical applications
Clinical Applications

- Limited Tissue
- Acquired Resistance Mutations
- Heterogeneity
Using Multiplexed Assays of Oncogenic Drivers in Lung Cancers to Select Targeted Drugs

A Patients with an oncogenic driver mutation who did and did not receive targeted therapy, and patients without an oncogenic driver

Survival Probability

Log-rank $P<.001$

Kris et al., JAMA 2014
Physician Experience with Limited Tissue

- Insert Physician specific case study slides here on identified Limited Tissue experience
Right lung biopsy 9/2013 showed adenocarcinoma with EGFR Del19

Started on Tarceva 10/2013 respondend in lung and had CR in brain

Post-progression Tissue Biopsy showed persistent del19, no T790M,

Chemotherapy Started 9/2014

Sent blood to Biocept for ctDNA testing; found T790M pos 11/2014

Enrolled In C01686 Trial at UC Irvine 1/2015

Possible Discordant Result was Shown as True Positive – 56 Yr. Old Female Patient

Re-biosy of tissue Confirmed T790M pos (tissue was required for study enrolment)
Physician Experience with Aquired Resistance
The issue of heterogeneity

Burrell, Nature 2013
Reflex Testing Detects HER2+ Tumor Cells In Patient initially classified as HER2-

OncoCEE-BR Detects HER2+ Tumor Cells

Retesting of new serial primary tumor section Detects HER2+ Tumor cell colony (Primary Tumor Tissue initially reported HER2-)
Physician Experience with Heterogeneity

- Add Physician experience with Heterogeneity
Billing and Reimbursement
Medicare pays hospitals a per-discharge amount for inpatient services pursuant to the inpatient prospective payment system. Similarly, outpatient services are reimbursed on a per-procedure basis under the outpatient prospective payment system. For both inpatients and outpatients, the payment received includes most services provided to the patient.

Payments for inpatient services include all clinical laboratory services as well as TC Services. Clinical laboratory services and TC Services provided to outpatients are separately billable, but the hospital, rather than the independent laboratory, must submit claims for those services to Medicare.
How Can Biocept Help?

- In office draw before or after Hospital stay
- Mobile Phlebotomy
- Get results in 7-10 days with no hold
Biocept Billing

- Biocept bills with established CPT codes
- Biocept is a Medicare Participating Provider
- Biocept is in-network for >140 million patient lives
- Financial Assistance program for uninsured and under-insured patients
• **Financial Assistance Program** – Application has indigent/charity included

** If you are currently receiving treatment from an institutional health care provider such as a hospital or hospice or other care facility (other than your physician), and you are also enrolled in a patient hardship program or charity care program maintained by the that institutional provider, you may submit documentation of that enrollment in place of the Financial Information (we will accept a certification signed by an authorized representative of the institutional provider).

- Can call for pre-approval
• **Self Pay Fee Schedule** and prompt discount 25%
Insurance coverage for testing and coverage for targeted therapies based on results

The average cost of a lung biopsy w/ adverse effects is ~4x higher than a biopsy w/o adverse effects ($37,745 v. $8,869)¹

Adverse effects were reported in 19.3% of patients who underwent a lung biopsy.¹

¹ ASTRO – 2014 Chicago Multidisciplinary Symposium in Thoracic Oncology
## Comparison of different platforms

<table>
<thead>
<tr>
<th>Commercial Test</th>
<th>Detection Method</th>
<th>Analytical Sensitivity</th>
<th>Analytical Specificity</th>
<th>Cost</th>
<th>EGFR</th>
<th>EGFR + ALK</th>
<th>EGFR + ALK + PD-L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guardant 360</td>
<td>NGS</td>
<td>98.6%</td>
<td>99.99%</td>
<td>$5600</td>
<td>$5600</td>
<td>$5600</td>
<td>N/A</td>
</tr>
<tr>
<td>Foundation ACT</td>
<td></td>
<td>&gt;95%</td>
<td>99%</td>
<td>$5800</td>
<td>$5800</td>
<td>$5800</td>
<td>N/A</td>
</tr>
<tr>
<td>Biodesix</td>
<td>Digital PCR</td>
<td>&gt;85%</td>
<td>100%</td>
<td>$1800</td>
<td>$1800</td>
<td>$1800</td>
<td>N/A</td>
</tr>
<tr>
<td>Biocept</td>
<td>Quantitative PCR + Sequencing/FISH/ICC</td>
<td>97%</td>
<td>99.5%</td>
<td>$300-$2100</td>
<td>$329</td>
<td>$1055</td>
<td>$1155</td>
</tr>
<tr>
<td>Trovagene</td>
<td>Quantitative PCR+NGS</td>
<td>93%</td>
<td>99%</td>
<td>$1800</td>
<td>$1800</td>
<td>$1800</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Cost issue - Solved

- Biocept charges only for genes ordered. Example of cost for EGFR including, L858R, del19, T790M
Cost issue - Solved

- Example cost of primary lung panel of markers EGFR, ALK, PD-L1

- Blood based NGS ($5800 GH)
- Solid Tumor NGS ($5800 FM)
- Biocept ($1113)
Using a combination of CTC and ctDNA analysis, Biocept offers sensitive testing of a large number of actionable genetic alterations in our CLIA lab.

Biocept’s liquid biopsy offers ability to evaluate patients with:
- Insufficient tissue at diagnosis
- Potential heterogeneity and sample selection concerns
- Progressing and need for evaluation of resistance mechanisms
- Monitoring needs

Convenience to order disease specific panels or single alteration tests

Simple billing plan, with Medicare coverage
Thank You
Business Overview

Generate genomic test results and send to physicians

Fully integrated, 35,000-square-foot facility

R&D, manufacturing, sample analysis
All targets of tumor essential in Personalized Medicine

- Biocept isolates and analyzes both CTCs and ctDNA from blood

- CTCs are whole cells containing intact genomic material shed from tumors including DNA, RNA and protein

- ctDNA is fragmented DNA shed into the blood as cells die

- CTCs are essential for certain types of testing
  - Protein (PDL1, ER)
  - DNA Amplifications (HER2, Met)
  - DNA Rearrangements (ALK, ROS1)
  - RNA and mRNA based assays

- ctDNA appropriate for mutations that are rarely seen in normal cells (EGFR, BRAF, KRAS)

- Both can be used to monitor patients on therapy, i.e., TKI resistance (T790M)

Crowley et. al., Nature Reviews, 2013
A “cocktail” of antibodies bind CTCs in solution and are then used to drive specific capture in the microchannel.

Hundreds of antibodies were screened across dozens of tumor cell lines to develop optimal antibody “cocktails”.

Highly validated for reproducibility and reliability for the capture and detection of CTCs.

Thousands of samples (patient and controls) have been analyzed in our CLIA Lab.

Pecot et al., Cancer Discovery, December 2011
## Abs in Cocktail

<table>
<thead>
<tr>
<th>Antibody Cocktail (Gene Name)</th>
<th>Target Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>EpCAM (EPCAM)</td>
<td>Epithelial</td>
</tr>
<tr>
<td>Trop-2 (TACSDT2)</td>
<td>Epithelial</td>
</tr>
<tr>
<td>c-Met (Mesenchymal epithelial transition factor)</td>
<td>Epithelial and EMT marker</td>
</tr>
<tr>
<td>Folate binding protein receptor (FOLR1)</td>
<td>Epithelial and Non-epithelial tissues</td>
</tr>
<tr>
<td>N-Cadherin (CDH2)</td>
<td>Mesenchymal, Endothelial, most tissues</td>
</tr>
<tr>
<td>CD318 (CDCP1)</td>
<td>Mesenchymal, stem cell reminiscent</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal stem cells</td>
</tr>
<tr>
<td>Her2 (ERBB2)</td>
<td>Epithelial</td>
</tr>
<tr>
<td>Muc-1 (MUC1)</td>
<td>Epithelial</td>
</tr>
<tr>
<td>EGFR (EGFR)</td>
<td>Epithelial</td>
</tr>
</tbody>
</table>
OncoCEE CTC Testing

Detection, enumeration and molecular analysis all within the patented channel

**Industry-leading accuracy**
- >99% specificity and sensitivity across a broad range of tumor types
- 1 Cell limit of detection

**Captures /Detects more CTCs**
- >50% of HER2+ cells are CK negative, which are missed by competitors
- Antibody capture method captures a broad range of CTCs including Mesenchymal, Epithelial and Stem Cells

**Cells captured in patented clear microfluidic channel**
- Same specimen can be microscopically analyzed for DNA and protein targets for solid tumors, including lung, breast, colon, prostate cancers and melanoma
- Cells can be released for further molecular analysis such as Next Generation Sequencing
Proprietary Target Selector™ Platform for Mutation Analysis

- Blocks amplification of normal DNA without blocking amplification of tumor mutations
- Quantitatively detects rare cancer-related mutations in the presence of large amounts of normal DNA
- >40,000-fold enrichment of mutant targets relative to normal DNA to aid various means of additional analysis, such as NGS
- Can interrogate both DNA and RNA for multiple biomarker targets

Very high accuracy
- >99% specificity, >95% sensitivity with extremely rare mutations*
- 1 mutant copy limit of detection

*At 0.05% mutant allele
### Analytical EGFR Table Summary

**T790M, L858R, Del19 at 0.05%**

(7 copies mutant: 14,000 copies wild-type)

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.05% or 7 mutants in a background of 14,000 WT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>True Positive</strong></td>
<td>TP – 193</td>
<td>FN – 7</td>
</tr>
<tr>
<td><strong>True Negative</strong></td>
<td>FP – 1</td>
<td>TN – 199</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>392/400 (96%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concordance</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.5%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>99%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>97%</td>
</tr>
<tr>
<td>Data Size (N)</td>
<td>400</td>
</tr>
</tbody>
</table>
Mutational Distribution in Lung Cancer by Gene

- **Unknown**: 40%
- **KRAS**: 25%
- **EGFR**: 15%
- **ALK**: 5%
- **HER-2**: 2%
- **BRAF**: 2%
- **MET**: 2%
- **PIK3CA**: 2%
- **MAP2K1**: 1%
- **ROS-1**: 1%
- **RET**: 1%
- **AKT**: 1%
- **NRAS**: 1%

*Plus FGFR1 – 9-13%*
Mechanism of Acquired Resistance to EGFR TKIs

- HER2 amplification (12%)
- BRAF mutation (1%)
- Unknown mechanism (30%)
- T790M (49%)
- \( PIK3CA \) (5%)
- SCLC transformation (14%)