Advancements Utilizing Circulating Tumor Cell Technology to Predict Outcomes in Patients With Breast Cancer

December 10, 2014
San Antonio, Texas
Edith A. Perez, MD

Deputy Director at Large, Mayo Clinic Cancer Center
Director, Breast Cancer Translational Genomics Program
Serene M. and Frances C. Durling Professor of Medicine
Division of Hematology/Oncology and Cancer Biology
Group Vice Chair, Alliance for Clinical Trials in Oncology
Mayo Clinic
Jacksonville, FL
Naota T. Ueno, MD, PhD, FACP
Professor, Breast Medical Oncology
Nylene Eckles Distinguished Professor in Breast Cancer Research
Chief, Section of Translational Breast Cancer Research
Executive Director, Morgan Welch Inflammatory Breast Cancer Research Program and Clinic
The University of Texas MD Anderson Cancer Center
Houston, TX
Today’s Learning Objectives

- Update the concept of personalized medicine
- Review the advantages and limitations of technologies for CTC capture and enumeration
- Understand where CTCs fit into the current treatment paradigm and where the future is headed
- Learn about the Phase 3 BEACON study exploratory CTC endpoint for outcomes with etirinotecan pegol
Personalized Medicine

Edith A. Perez, MD
Mayo Clinic
Jacksonville, FL
Personalized Cancer Care Continuum

Prevention
- Risk Markers
  - Cancer Prevention
  - Cancer Genetics
  - Cancer Epidemiology
  - Pathology

Therapy
- Prognosis Markers
  - Clinical Oncology
  - Pathology
  - Radiology
  - Molecular Diagnostics
- Predictive Markers
  - Cancer Genetics
  - Investigational Therapeutics
  - Immunology
  - Outcome Research
  - Comparative Effectiveness

Survivorship
- Response Markers
  - Pathology
  - Molecular Diagnostics
  - Cancer Genetics
  - Immunology

- High-Risk Patients
  - Risk Reduction
  - Early Diagnosis
  - Avoid Toxicity of Adjuvant Therapy
  - More Intensive Adjuvant Therapy
  - Least Toxic Therapy
  - Most Efficacious Therapy

- Low-Risk of Recurrence
- High-Risk of Recurrence
- Predictors of Toxicity
- Predictors of Response/Resistance

Chemotherapy
- Targeted Therapy
- Immunotherapy/Vaccines
- Surgery
- Radiation

Reprinted with permission. © 2013 American Society of Clinical Oncology. All rights reserved.
Considerations

- Biology
  - Heterogeneity of cancer genomes and proteomes
  - Epigenetics
  - Cancer stem cells
  - Cancer cell mutations

- Therapy
  - Immunotherapy
  - New cytotoxics
  - Targeted agents

- Imaging and circulating markers
Omics-Driven Cancer Medicine

Fresh biopsy

Patient encounter

Omic profiling

Data interpretation

Management decision

Clinical response?

Drug resistance?

Salvage or new therapy?

- Aggressive/metastatic tumors
- Distinctive characteristics
- Enterprise-wide

“Cutting-edge” and emerging technologies

- Integrative heuristic algorithms
- Focused experimental validation?

- Evaluation committee
- Framework for decision making
- Hypothesis-driven phase I trials
- Mechanism-based clinical studies

- Registry studies
- Pharmacodynamic analyses

- Molecular mechanisms/correlates
- Integration with preclinical studies

Inform novel therapeutic trials or therapeutic combinations

State of the Art Management of Breast Cancer: Personalized Medicine

Risk reduction, early and advanced breast cancer strategies

- Utilization of optimal standards
- Application of systems biology to personalized breast cancer therapy
  - Identifying and validating molecular markers
  - Understanding molecular crosstalk and bypass mechanisms
  - Early predictors of outcome
Circulating Tumor Cells (CTCs)

Edith A. Perez, MD
Mayo Clinic
Jacksonville, FL
POLL QUESTION:
Have you ever ordered CTC testing?

Answer Choices:
• Yes
• No
POLL QUESTION:
When do you think CTCs will become an important part of the clinical decision-making process in breast cancer?

Answer Choices:
• Now
• 1-5 years
• 6-10 years
• 10 years+
What Are Circulating Tumor Cells?

- First described in a woman with mBC almost 150 years ago\(^1\)
- Circulating tumor cells (CTCs) are cancer cells shed from either the primary tumor or its metastases that circulate in the peripheral blood
  - Traditionally defined as having an intact, viable nucleus, presence of cytokeratins and absence of CD45, large size, and irregular shape

- Newer CTC isolation techniques increase sensitivity and allow for the expansion of the phenotypic definition and molecular characterization

\(^1\)Ashworth, TR. *Australian Medical Journal*, 1869, 14:146-147.
How Can CTCs Be Used?

- Liquid biopsy/noninvasive tumor sampling
- Early diagnosis
- Surrogate marker in clinical trials
- Monitor evolution of disease over time
- Monitor response to treatment
- Potential for molecular and genomic profiling of CTCs
Ideal CTC Test Components

CTCs Detected in a Majority of Metastatic Patients

Comprehensive Characterization

- CTC Enumeration
- Protein Marker Analysis (e.g. ER)
- Gene Amplification (e.g. HER2)
- Mutation Analysis (e.g. EGFR)
- RNA Expression Profiling
First-Generation CTC Enrichment Technologies

- Existing CTC testing platform is CELLSEARCH® by Veridex/J&J
  - Provides CTC enumeration
  - FDA cleared for 3 cancer types (breast, CRC, and prostate) limited to metastatic patients\(^1\)

- Key limitations of CELLSEARCH:
  - \(>5\) CTCs/7.5 mL detected in only 17%-41% of blood samples from Stage IV cancer patients\(^2\)
  - Limited to epithelial CTCs
  - Low target cell recovery and purity
  - Captured cells are fixed and not viable

\(^1\)CellSearch Circulating Tumor Cell Test Received FDA Clearance in January 2004
Several Second-Generation CTC Recovery Methods Under Development

Going Beyond CTC Enumeration

- **High CTC recovery**—facilitating downstream characterization, including protein or gene expression analysis, mutation/translocation detection via NGS and single-cell sequencing, and pharmacodynamic studies
- **Isolation of viable cells**—enabling cell culture and patient-derived xenograft models
- **Universal enrichment of additional cell subsets (stem cells, EMT)**—independent of antigen expression levels or the requirement for predetermined antibody labeling
ApoStream® Technology: Theory of Operation

DEP

Micro-fluidics

Normal cell

Tumor cell

Dielectric properties (polarizability) of cells are dependant upon cell diameter, membrane area, density, conductivity and volume. Inherent differences in morphology of CTCs and normal cells result in different polarisation charges when exposed to an AC electric current.

Cell levitation is controlled by balancing DEP, hydrodynamic and sedimentation forces. CTCs are collected from the bottom of the flow chamber while the other cells flow into a waste collection port.

ApoStream® Technology: Theory of Operation

Elute buffer flow

PBMCs

CTCs

Waste

Sample Injection

CTC Collection
ApoStream®: Separation of CTCs from Blood Cells Based on Dielectrophoresis (DEP) Frequency

PBMCs

- GFP labeled PBMCs without and with DEP force

Cancer Cells

- GFP* labeled cancer cells with DEP force

*GFP = Green Fluorescent Protein

ApoStream®: High CTC Recovery From Patients With Various Cancer Types

ApoStream® system was able to isolate high number of CTCs in >90% of patients from lung, prostate, breast, melanoma, and bladder cancer patient blood.

<table>
<thead>
<tr>
<th>Metastatic Cancer Type</th>
<th># of Patients</th>
<th>Mean ± SD</th>
<th>% Patients with CTC &gt;0</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>66</td>
<td>287</td>
<td>94</td>
</tr>
<tr>
<td>Prostate</td>
<td>16</td>
<td>721</td>
<td>94</td>
</tr>
<tr>
<td>Melanoma</td>
<td>13</td>
<td>486</td>
<td>100</td>
</tr>
<tr>
<td>Breast</td>
<td>10</td>
<td>203</td>
<td>100</td>
</tr>
<tr>
<td>Bladder</td>
<td>1</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

ApoStream®: High Recovery and Viable CTCs

**Enumeration**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>CELLSEARCH®</th>
<th>ApoStream®</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>241</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>149</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Metastatic Breast Cancer (CK+CD45-DAPI+ CTCs per 7.5 mL of blood)\(^1\)

**Viability**

ApoStream\(^\circledR\)-recovered SKOV3 cancer cells retain exponential growth characteristics\(^2\)

---


ApoStream®: Enabling Molecular and Biological Investigation of Rare Cell Subsets (Stem Cells, CTC, EMT, MET)
CTC Applications in Breast Cancer

Naoto T. Ueno, MD, PhD, FACP
MD Anderson Cancer Center
Houston, TX
Working Model for Studying CTCs

- Distant Metastasis
- Inflammation
- ApoStream
- CellSearch
- AdnaTest
- Primary Tumor
- BM/PB
- EMT
- Injury
- Tumor Burden
- Stem Cells
- EMT Vimentin
- Ep-CAM+
- TWIST
- SNAIL
- ZEB1
- CD44+
- CD133
- CD24
- CSC
- Flow
- Cleared by RES, Immune

Adapted from JM Reuben with permission
Clinical Application of CTCs

First Generation - Enumeration

- Prognostic value of CTCs enumeration (CellSearch®) in solid tumors
- Predictive value of CTCs enumeration (CellSearch®) in solid tumors

Overall Survival

Other Clinical Uses of CTCs

- Bone metastasis
- Widely metastatic HR positive disease

<table>
<thead>
<tr>
<th></th>
<th>No Bone Involvement</th>
<th>Bone</th>
<th>Bone &amp; Other Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>58</td>
<td>28</td>
<td>108</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.3 ± 8.7</td>
<td>52.7 ± 91.2</td>
<td>69.2 ± 206.4</td>
</tr>
<tr>
<td>Median</td>
<td>1</td>
<td>13</td>
<td>8.5</td>
</tr>
<tr>
<td>% &gt;5 CTC</td>
<td>16%</td>
<td>69%</td>
<td>58%</td>
</tr>
</tbody>
</table>
Research/Clinical Questions

Q1. Can we affect prognosis by changing therapy in patients with persistent elevated CTCs?

Q2. What is the implication of biomarkers in CTCs vs. tissue for therapy?

Q3. What is the role of Cancer Stem Cells (CSCs) and EMT-CTC in clinical outcomes?
SWOG S0500:
CTC (CELLSEARCH) Did Not Predict Response to Therapy

CTCs drawn at baseline prior to first-line chemotherapy

CTC < 5

Arm A (n=276)
Monitor for PFS & OS
Eligible for other first-line chemotherapy trials

CTC ≥ 5

Arm B (n=163)
Maintain first-line chemotherapy until progression

CTCs drawn 3 weeks after 1st dose of chemotherapy (n=319)

CTC < 5

Arm C1 (n=64)
Maintain first-line chemotherapy

CTC ≥ 5

Arm C2 (n=59)
Switch to alternate therapy

123 pt randomized 1° endpoint OS

Follow-up
Imaging & CTCs midway and at progression

# Ongoing Trials Testing Impact of CTC (CELLSEARCH) on Treatment Decision Making Process

<table>
<thead>
<tr>
<th>Trial</th>
<th>Randomization</th>
<th>Patient Population</th>
<th>CTC Parameter for Treatment Prediction</th>
<th>Primary Objective</th>
<th>Trial Number Accrual Date “N” Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>STIC CTC METABREAST (France)</td>
<td>Clinical choice vs CTC driven choice of chemotherapy vs hormonotherapy</td>
<td>MBC, HR+, HER2-</td>
<td>CTC count (≥5 CTC/7.5 mL vs &lt;5 CTC/7.5 mL)</td>
<td>Non-inferiority of the CTC arm for PFS (primary clinical end point) and a superiority of the CTC arm for the medico-economics study (co-primary end point).</td>
<td>NCT0 1710605 Preliminary Analysis SABCS 2013</td>
</tr>
<tr>
<td>CirCe01 (France)</td>
<td>CTC-driven choice of chemotherapy</td>
<td>MBC, HR+, HER2-, third-line chemotherapy</td>
<td>CTC count (≥5 CTC/7.5 mL vs &lt;5 CTC/7.5 mL)</td>
<td>Overall survival</td>
<td>NCT01349842 Jan 2018</td>
</tr>
<tr>
<td>Treat CTC (Europe)</td>
<td>Trastuzumab vs observation</td>
<td>HER2-non-amplified primary breast cancer with ≥1 CTC/15 mL PB after completion of (neo-)adjuvant chemotherapy and surgery</td>
<td>CTC count (≥1 CTC/15 mL of blood vs &lt;1 CTC/7.5 mL)</td>
<td>CTC detection rate at week 18</td>
<td>NCT01548677 April 2017 N = 2175</td>
</tr>
<tr>
<td>DETECT III (Germany)</td>
<td>Standard therapy or standard therapy plus lapatinib</td>
<td>MBC, 1 to 3 lines of previous chemotherapy, HER2-</td>
<td>HER2+ CTC/7.5 mL of blood</td>
<td>Progression-free survival</td>
<td>NCT01619111 March 2018 N = 228</td>
</tr>
<tr>
<td>COMETI P2 (USA, Canada)</td>
<td>No randomization</td>
<td>MBC HR+, HER2-</td>
<td>Expression of ER, Bcl-2, HER2, Ki67 on CTC</td>
<td>Progression-free survival</td>
<td>NCT01701050 June 2015 N = 200</td>
</tr>
</tbody>
</table>
**CTCs and biomarkers, implication for therapy?**

Patients with HER2- primary tumors are more likely to have discordance between primary tumor and CTCs than patients with HER2+ primary tumors

<table>
<thead>
<tr>
<th>Primary Tumor</th>
<th>Total Patients</th>
<th>Patients with HER2-CTC</th>
<th>Patients with HER2+CRC*</th>
<th>Discordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2+*</td>
<td>45</td>
<td>1</td>
<td>44</td>
<td>2.3%</td>
</tr>
<tr>
<td>HER2-</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

*HER2+ defined as the ratio of HER2/CEP17>2.0 by FISH*
Metastatic Breast Cancer (MBC) With Discordant HER2 Amplification in Tumor and CTCs had a **Metabolic** Response with a Reduction in CTCs With Trastuzumab Therapy

- **PT and MT= ER+, HER-2 neg**
- **900 CTCs = ER-, HER2+**

High % Cancer Stem Cells in Apheresis Product is Associated with Non-CR in MBC Undergoing Autologous Stem Cell Transplant
EMT-CTC in Apheresis Product of MBC Undergoing Autologous Stem Cell Transplant: Decreased PFS

PROGRESSION-FREE SURVIVAL

A  % Epithelial Cells

CD326\(^{+}\) low

CD326\(^{+}\) high  P=0.06

Time from transplant (months)

B  EMT by PCR

EMT-TF low

EMT-TF high  P=0.02

Neoadjuvant Chemotherapy Enriches EMT Genes

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Positive &gt; 1 EMT Gene</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No NACT</td>
<td>47</td>
<td>12.8% (n=6)</td>
<td></td>
</tr>
<tr>
<td>NACT</td>
<td>20</td>
<td>35.0% (n=7)</td>
<td>0.047</td>
</tr>
<tr>
<td>Pathologic CR (pCR)</td>
<td>6</td>
<td>16.6% (n=1)</td>
<td></td>
</tr>
<tr>
<td>No pCR</td>
<td>14</td>
<td>42.9% (n=8)</td>
<td>0.3</td>
</tr>
</tbody>
</table>
EMT-CTC by ApoStream®

- CTCs were also stained with additional markers and examined on a laser scanning cytometer to measure protein expression levels of epithelial (EpCAM, E-cadherin), mesenchymal (β-catenin, vimentin) and CSC-markers (CD44, CD24).
- pCR status after preoperative treatment was obtained to correlate baseline CTCs and marker expression with treatment response.
Further information will be presented on Friday in Poster Session P4-01-10.

- **Presenter:** F. Le Du
- **Poster Number:** P4-01-10
- **Title:** Predictive impact of circulating tumor cells with an epithelial-to-mesenchymal transition phenotype in patients with primary breast cancer treated with primary systemic therapy
- **Date and Time:** Friday, December 12th from 7:30 AM – 9:00 AM
- **Location:** Halls A-B
Next Generation Research: Liquid Biopsy

- Circulating tumor cells (CTCs) in blood
- Disseminated tumor cells (DTCs) in BM
- Circulating tumor DNA (ctDNA)

POLL QUESTION:
When do you think CTCs will become an important part of the clinical decision-making process in breast cancer?

Answer Choices:
• Now
• 1-5 years
• 6-10 years
• 10 years+
BEACON: An Example of a Modern Study Incorporating Latest CTC Technology

Edith A. Perez, MD
Mayo Clinic
Jacksonville, FL
BEACON Phase 3 Registration Study of Etirinotecan Pegol in Metastatic Breast Cancer

- **Patients With Locally Recurrent or Metastatic Breast Cancer**
  - Previously treated with an anthracycline, a taxane, and capecitabine (n=852)

- **Single-Agent Etirinotecan Pegol**
  - 145 mg/m² Q21 day

- **Single-Agent Treatment of Physician’s Choice (TPC)**
  - eribulin, ixabepilone, vinorelbine, gemcitabine, paclitaxel, docetaxel, or nab-paclitaxel

- **Primary Endpoint**
  - Overall Survival

- **Secondary Endpoints**
  - PFS, ORR, CBR, DoR

- **Exploratory Endpoints**
  - PD Markers in CTC, others

- **Key Points**
  - Agreement with FDA and EMA on study design
  - Granted Fast Track status by the FDA for MBC
  - Global enrollment completed ahead of schedule in August 2013
  - Top-line survival data expected early 2015
Etirinotecan Pegol: Targeting Tumor Tissue

Cytotoxic small molecules are attached to a unique macromolecular polymer core using hydrolyzable linkers to target disease tissue.
Etirinotecan Pegol: Targeting Tumor Tissue

Etirinotecan pegol has been optimally sized so that it penetrates the leaky tumor vasculature more readily than normal vasculature, concentrating and trapping the drug in the tumor tissue.
Etirinotecan Pegol: Targeting Tumor Tissue

The linkers are hydrolyzed over time by specific mechanisms which may be enzymatic or pH-driven within the body, continuously freeing active drug within the tumor tissue and in the plasma.
First-generation topoisomerase I inhibitors have a high initial peak concentration and short half-life.

Etirinotecan Pegol: Sustained PK Profile

Etirinotecan pegol's design results in a lower initial peak concentration of active topoisomerase I inhibitor in the blood.

Etirinotecan Pegol: Metastatic Breast Cancer
Phase 2 Results

- Single-agent NKTR-102 demonstrated a 29% ORR in heavily pretreated (median 2 prior lines of therapy) advanced metastatic breast cancer
  - PFS: 4.7 months
  - Median OS: 10.3 months
  - Progression-free at 6 months: 35.5%
- ORR was maintained in heavily pretreated and poor-prognosis subsets
  - A/T/C pretreated: 33%
  - Triple-negative: 33%
  - Visceral disease: 30%
- Activity in the 3 main subtypes: TNBC, HER2+, HER2-
Etirinotecan Pegol: Metastatic Breast Cancer
Phase 2 Results

- Most common grade 3/4 toxicity was diarrhea (21%)
  - Typically occurring after approximately 3 months of therapy for both schedules

- 21-day schedule better tolerated and more efficacious
  - ORR: 29%; PFS: 5.6 months, OS: 13.1 months
  - Selected for Phase 3 BEACON study

BEACON Phase 3 Registration Study of Etirinotecan Pegol in Metastatic Breast Cancer

**Patients With Locally Recurrent or Metastatic Breast Cancer**
Previously treated with an anthracycline, a taxane, and capecitabine (n=852)

**Single-Agent Etirinotecan Pegol**
145 mg/m² Q21 day

**Single-Agent Treatment of Physician’s Choice (TPC)**
eribulin, ixabepilone, vinorelbine, gemcitabine, paclitaxel, docetaxel, or nab-paclitaxel

**Primary Endpoint**
Overall Survival

**Secondary Endpoints**
PFS, ORR, CBR, DoR

**Exploratory Endpoints**
PD Markers in CTC, others

- Agreement with FDA and EMA on study design
- Granted Fast Track status by the FDA for MBC
- Global enrollment completed ahead of schedule in August 2013
- Top-line survival data expected early 2015
Rationale:

- Challenges of using tumor biopsy in a Phase 3 trial
- CTCs are an attractive, minimally invasive alternative to tumor biopsies
- Longitudinal assessment of target-specific biomarkers possible

80% of the 852 BEACON patients (n=665) participated in the CTC substudy and provided serial blood samples for CTC analysis.
Further characterization of BEACON CTC baseline samples will be presented tomorrow in Poster Session P3-10-03.

- **Presenter**: Perez EA
- **Poster Number**: P3-10-03
- **Title**: Etirinotecan pegol target-specific pharmacodynamic biomarkers in circulating tumor cells from patients with metastatic breast cancer in the Phase 3 BEACON study.
- **Date and Time**: Thursday, December 11th from 5:00 PM - 7:00 PM
- **Location**: Halls A-B
Molecular Profiling of CTCs Was Successfully Achieved in the Phase 3 BEACON Study

- CTC detection rate using ApoStream® was high:
  - CTCs detected in >95% of baseline samples
  - Median number of CTCs/7.5 mL was ~500

- High CTC harvest enabled assessment of etirinotecan pegol target-specific pharmacodynamic biomarkers.
  - Top1, Top2, γH2Ax, Rad51, Ki67, ABCG2

- BEACON efficacy and safety results are expected in early 2015, which will allow analysis of baseline CTC data and change of CTC data over time with patient outcome.
Take-Home Messages
Take-Home Messages

- CTC technology has improved in the last few years
  - Potentially enabling early detection and treatment intervention
- Molecular profiling and characterization now possible with CTC technology
- Personalized medicine is evolving to include CTC research
POLL QUESTION:
Would you use CTC tests routinely in your practice if they were actionable/predictive of therapy for specific agents?

Answer Choices:
• Yes
• No
Questions?
Advancements Utilizing Circulating Tumor Cell Technology to Predict Outcomes in Patients With Breast Cancer

December 10, 2014
San Antonio, Texas