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

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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

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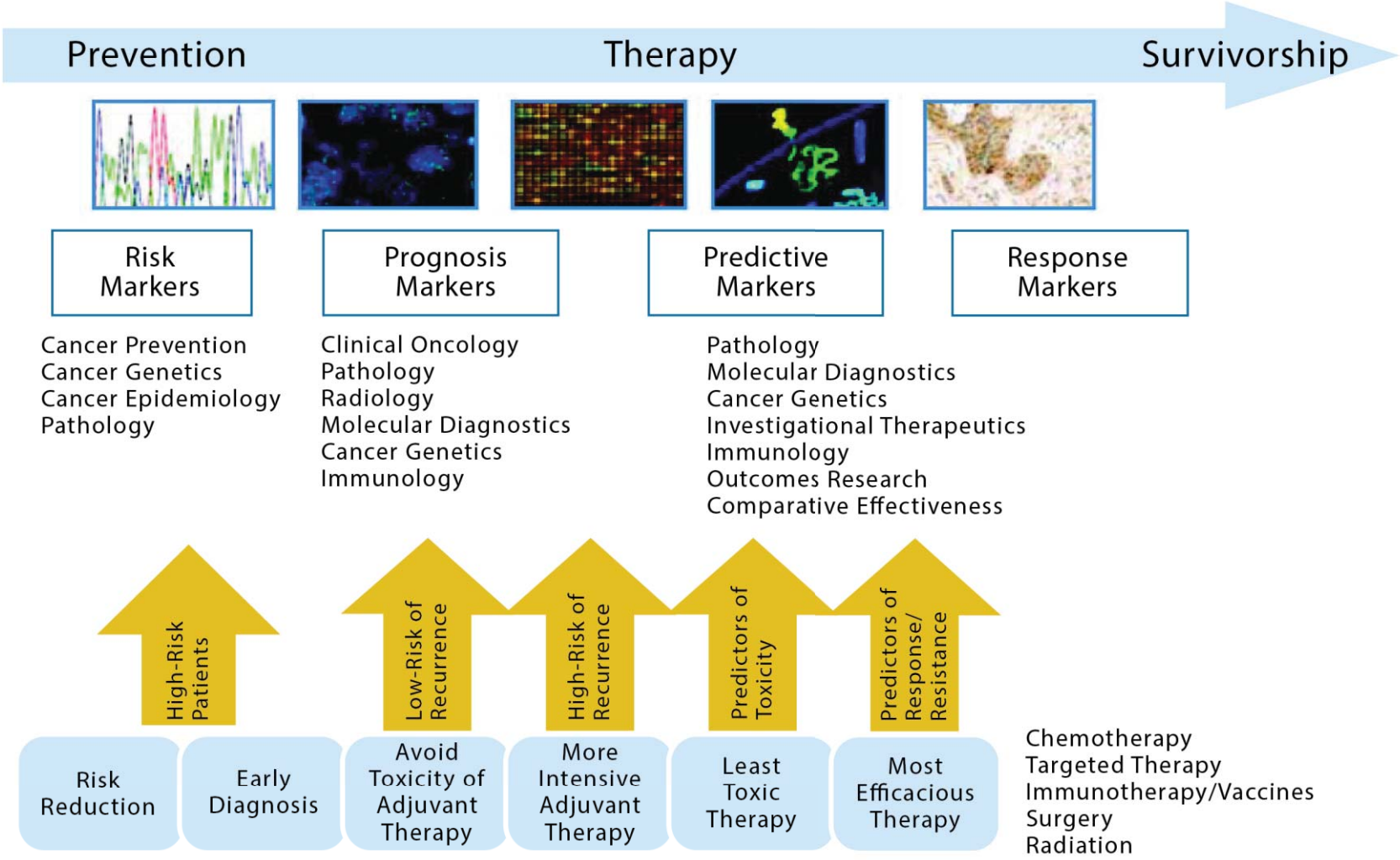
Personalized Cancer Care Continuum



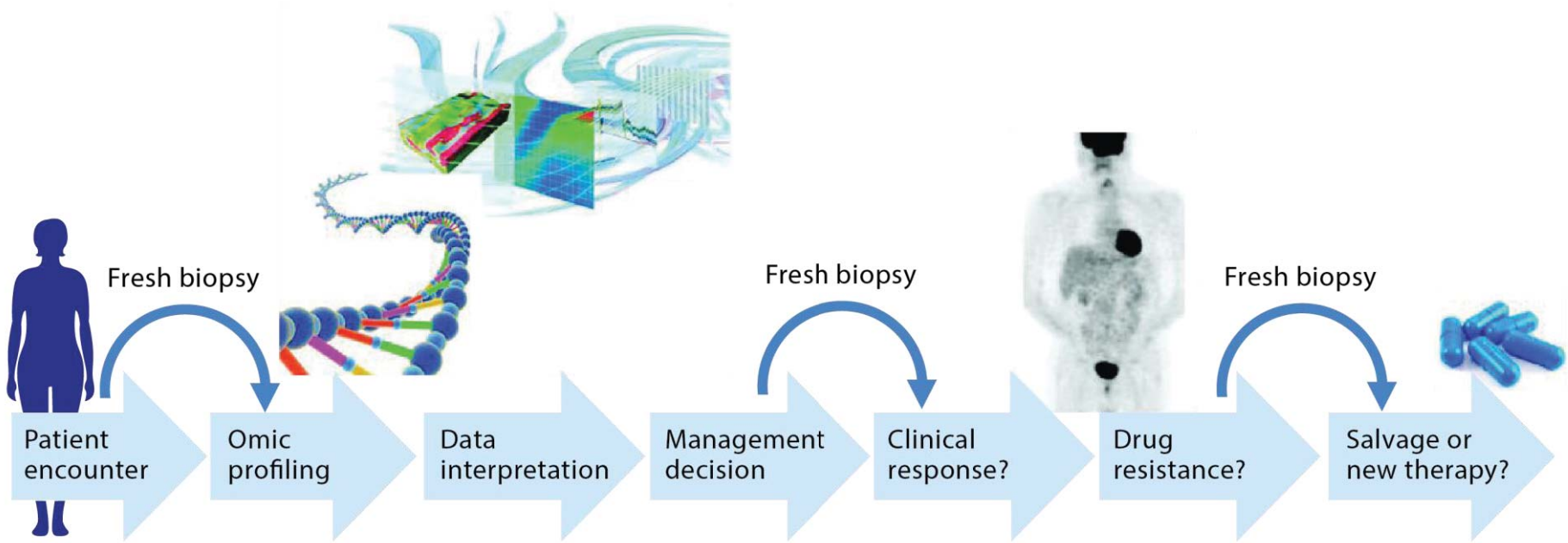
MAYO CLINIC

Meric-Bernstam F, et al. *J Clin Oncol.* 2013;31(15):1849-1857.

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



- Aggressive/ metastatic tumors
- Distinctive characteristics

v

- 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
- Pharmaco-dynamic analyses

- Molecular mechanisms/ correlates
- Integration with preclinical studies

Inform novel therapeutic trials or therapeutic combinations

MAYO CLINIC

Garraway LA. *J Clin Oncol.* 2013;31(15):1806-1814.

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)

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Mayo Clinic
Jacksonville, FL

POLL QUESTION:

Have you ever ordered CTC testing?

Answer Choices:

- Yes
- No

POLL QUESTION:

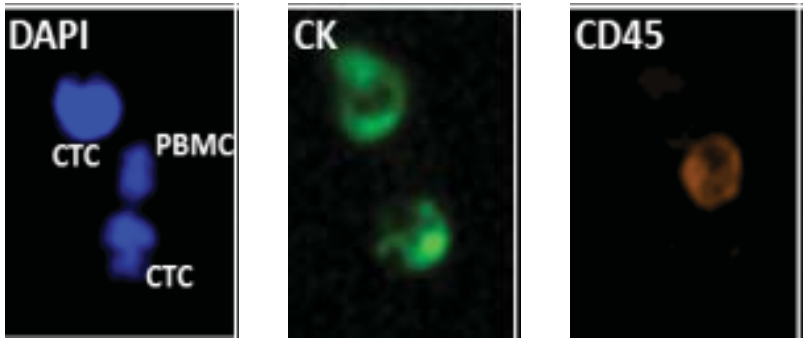
When do you think CTCs will become an important part of the clinical decision-making process in breast cancer?

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.

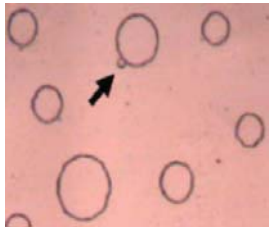
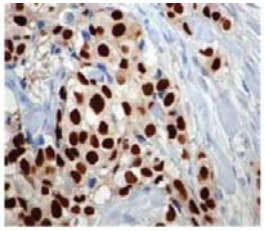
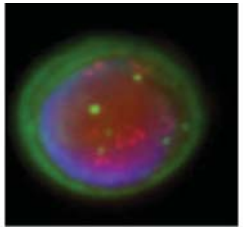
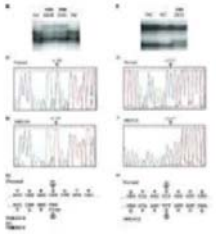
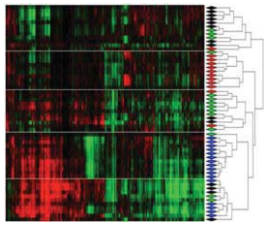
How Can CTCs Be Used?

- 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
				

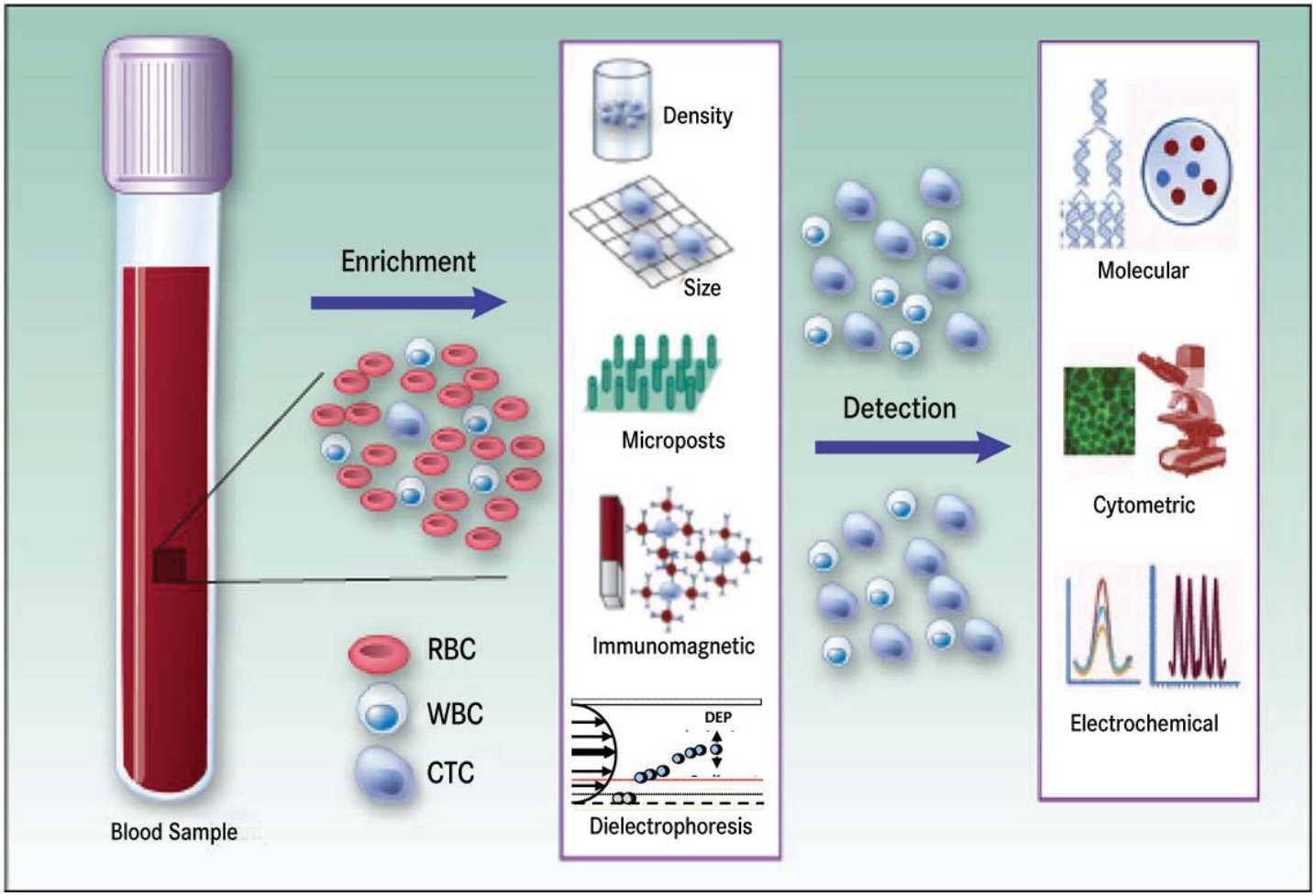
First-Generation CTC Enrichment Technologies

- 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]

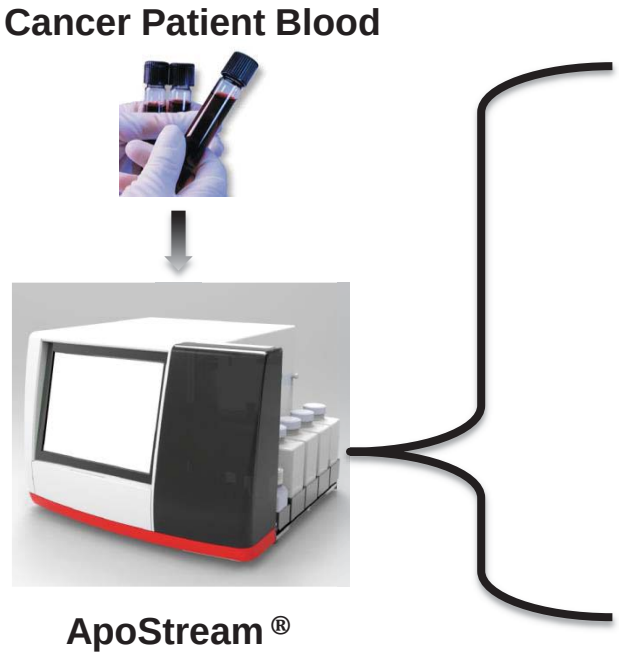
- Key limitations of CELLSEARCH:
  - ≥ 5 CTCs/7.5 mL detected in only 17%-41% of blood samples from Stage IV cancer patients[2]
  - Limited to epithelial CTCs
  - Low target cell recovery and purity
  - Captured cells are fixed and not viable

[1]CellSearch Circulating Tumor Cell Test Received FDA Clearance in January 2004
[2]Allard, WJ, et al. *Clin Cancer Res*, 2004; 10:6897-6904.

Several Second-Generation CTC Recovery Methods Under Development



Going Beyond CTC Enumeration



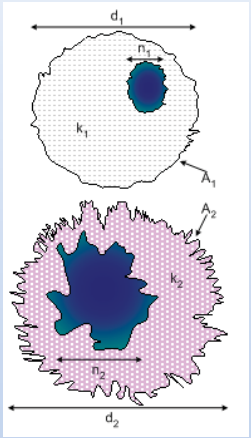
- **High CTC recovery—**facilitating downstream characterization, including protein or gene expression analysis, mutation/translocation detection via NGS and single-cell sequencing, and pharmacodynamic studies
- **Isolation of viable cells—**enabling cell culture and patient-derived xenograft models
- **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

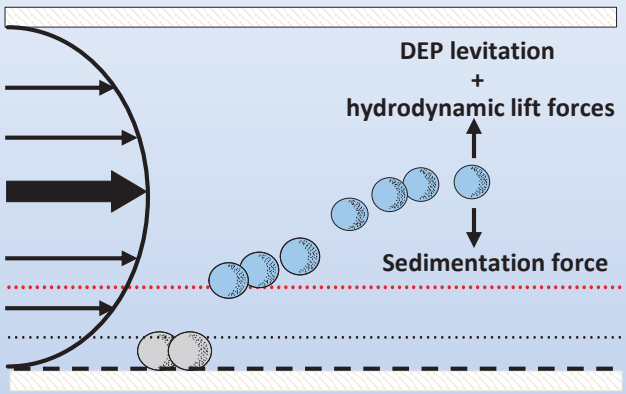
Normal cell

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.

Micro-fluidics

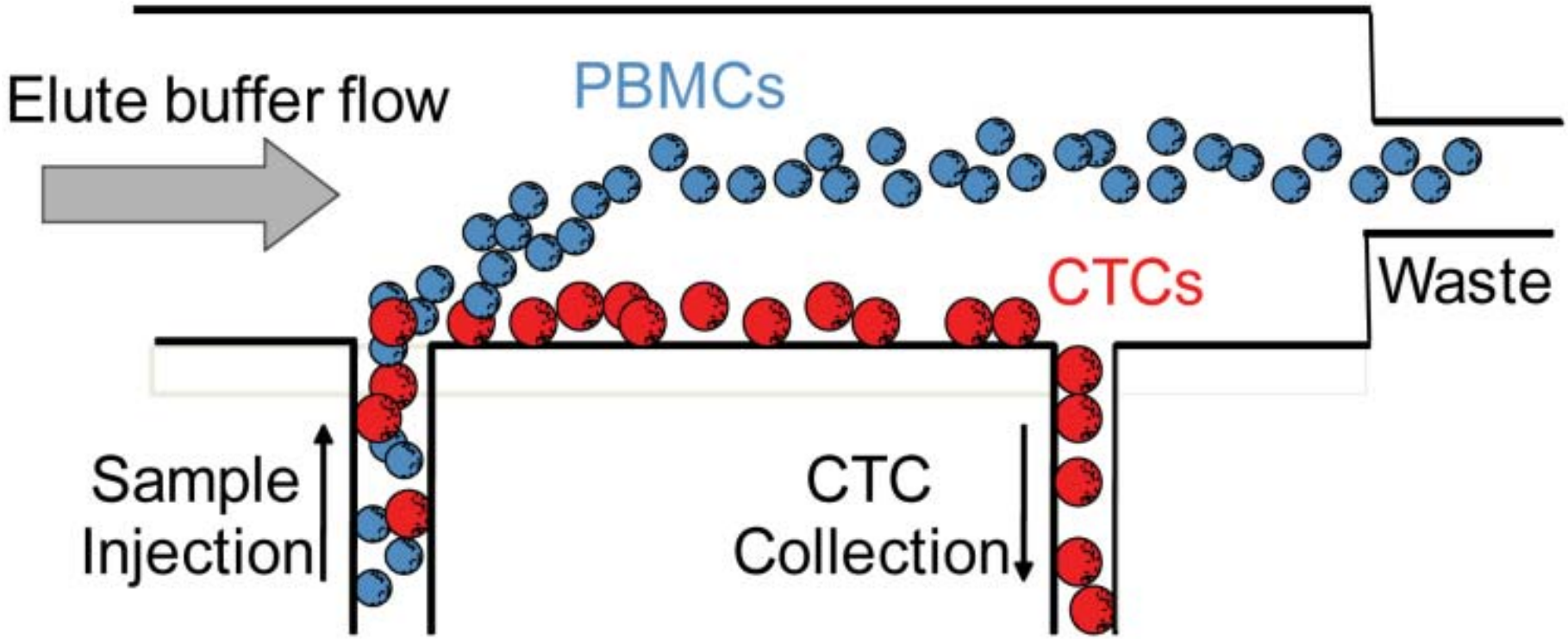


The force balance and levitation of various cells at different heights

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.

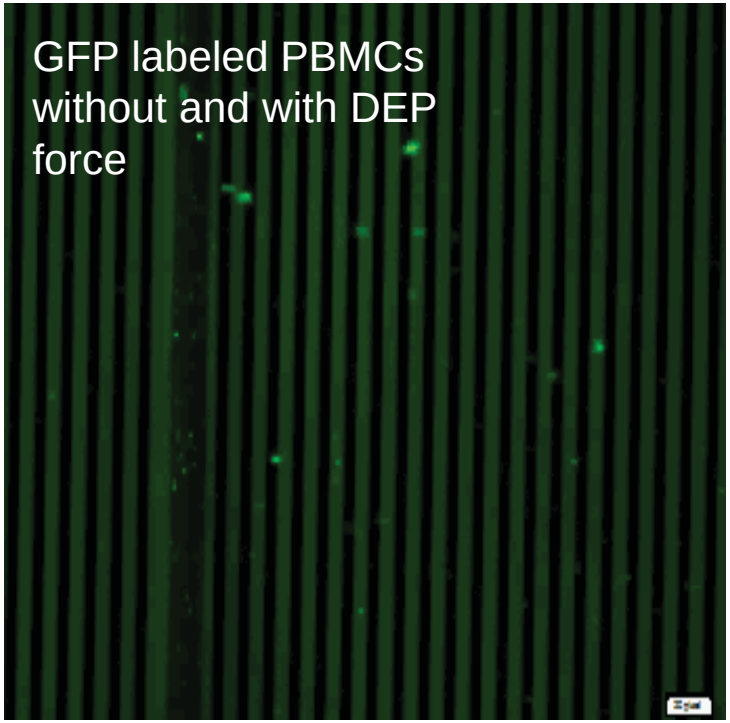
Gupta V, etal. Biomicrofluidics 6, 024133 (2012).

ApoStream® Technology: Theory of Operation

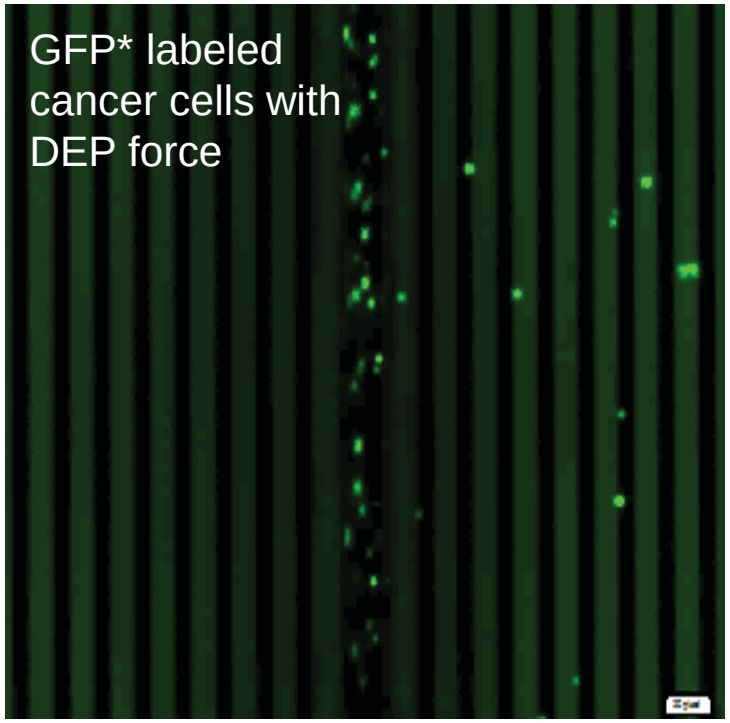


ApoStream®: Separation of CTCs from Blood Cells Based on Dielectrophoresis (DEP) Frequency

PBMCs



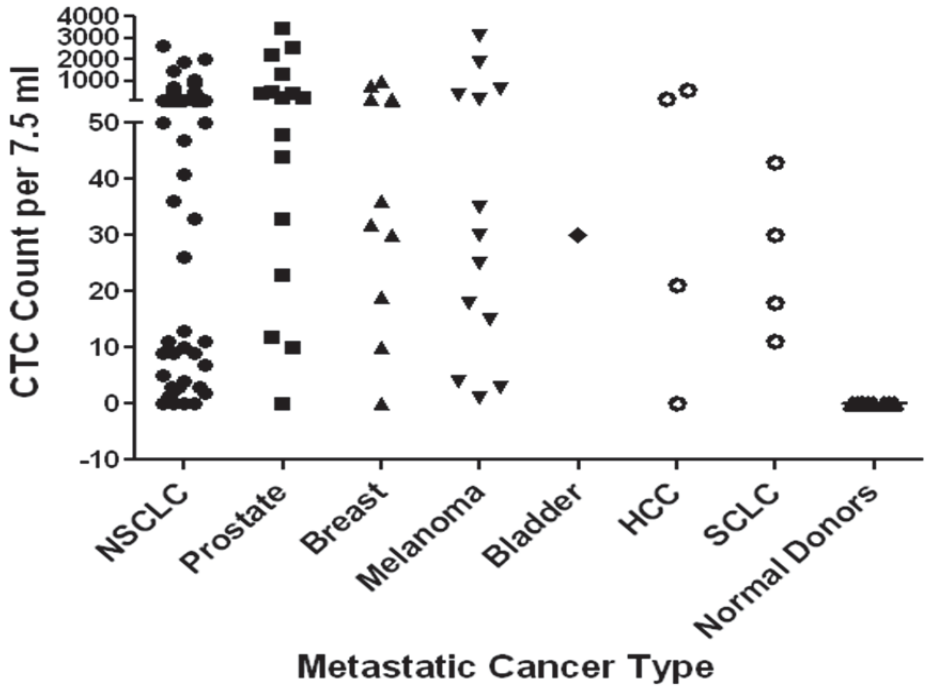
Cancer Cells



*GFP = Green Fluorescent Protein

Gupta V, Jafferji I, Garza M, et al. *Biomicrofluidics*. 2012;6(2):24133.

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



Enumeration

Metastatic Cancer Type	*# of Patients*	*Mean ± SD*	*% Patients with CTC >0*
NSCLC	66	287	94
Prostate	16	721	94
Melanoma	13	486	100
Breast	10	203	100
Bladder	1	30	100

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

Gupta V, Jafferji I, Garza M, et al. *Cancer Res* 72 (Suppl 8), Apr 2012. Abstract 2374.

ApoStream®: High Recovery and Viable CTCs

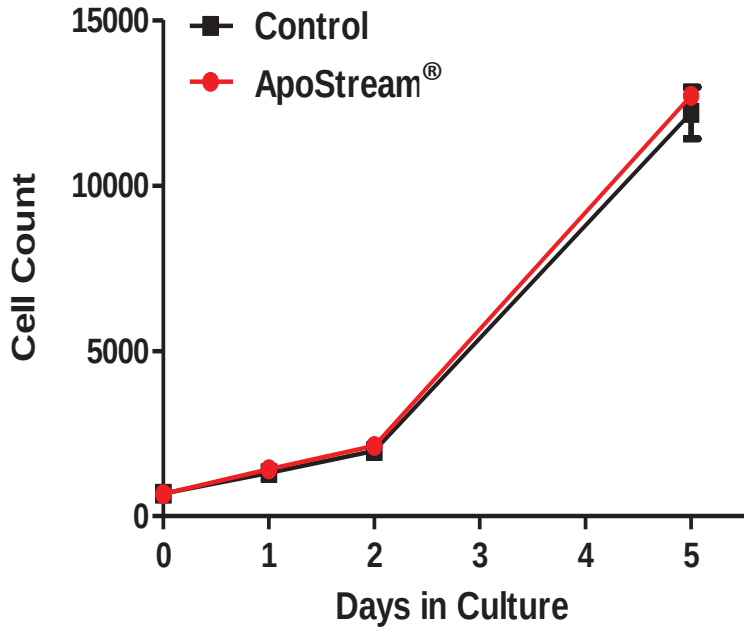
Enumeration

Metastatic Breast Cancer (CK+CD45-DAPI+ CTCs per 7.5 mL of blood)[1]		
Patient No.	CELLSEARCH®	ApoStream®
1	0	81
2	0	241
3	0	40
4	0	71
5	0	41
6	2	149
7	0	10

Anderes K, et al. *Cancer Res* 73, 2013, abstract P1-0405.

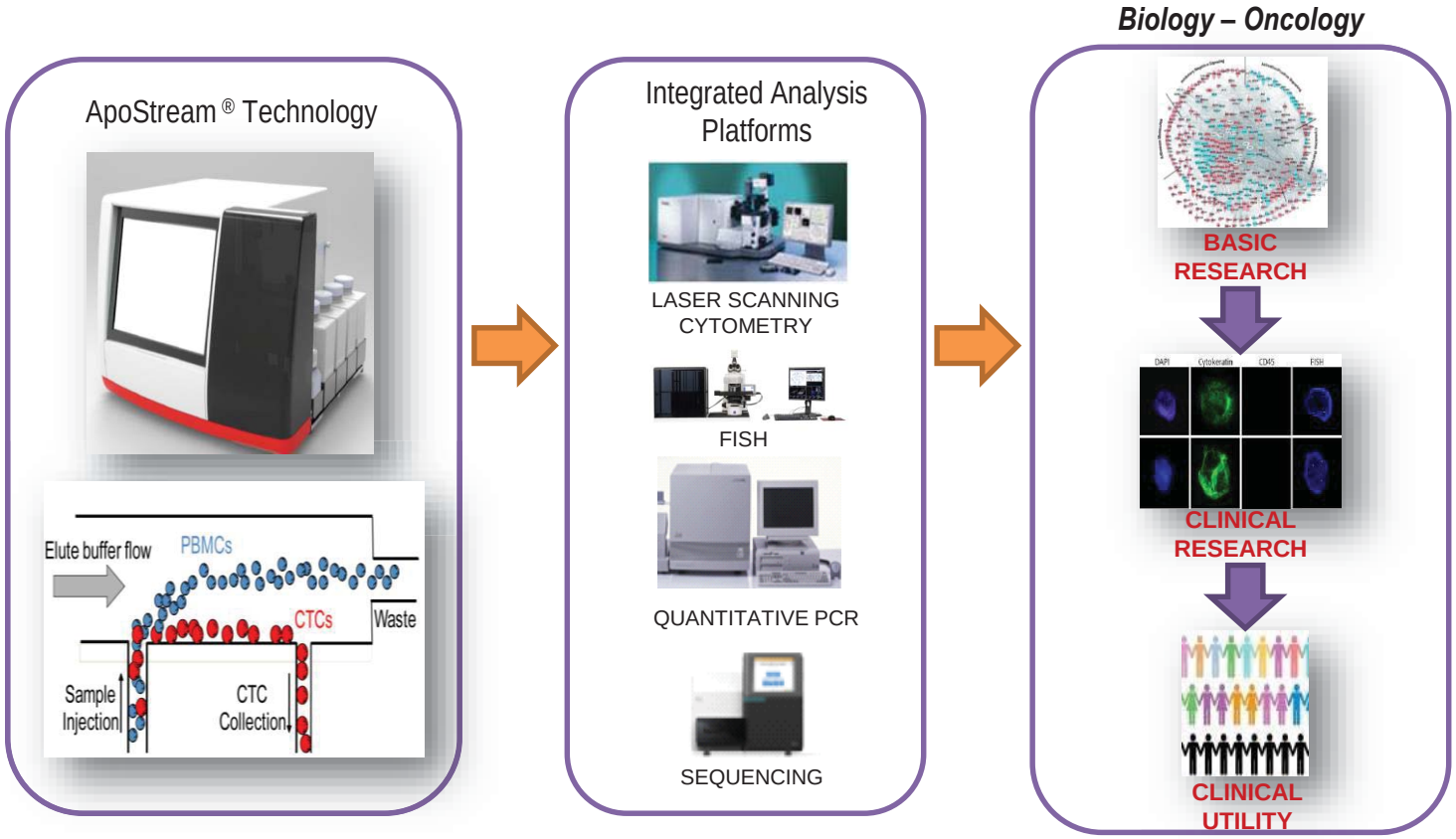
Viability

ApoStream®-recovered SKOV3 cancer cells retain exponential growth characteristics[2]



Gupta V, et al. *Biomicrofluidics* 6, 024133 (2012); doi: 10.1063/1.

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



CTC Applications in Breast Cancer

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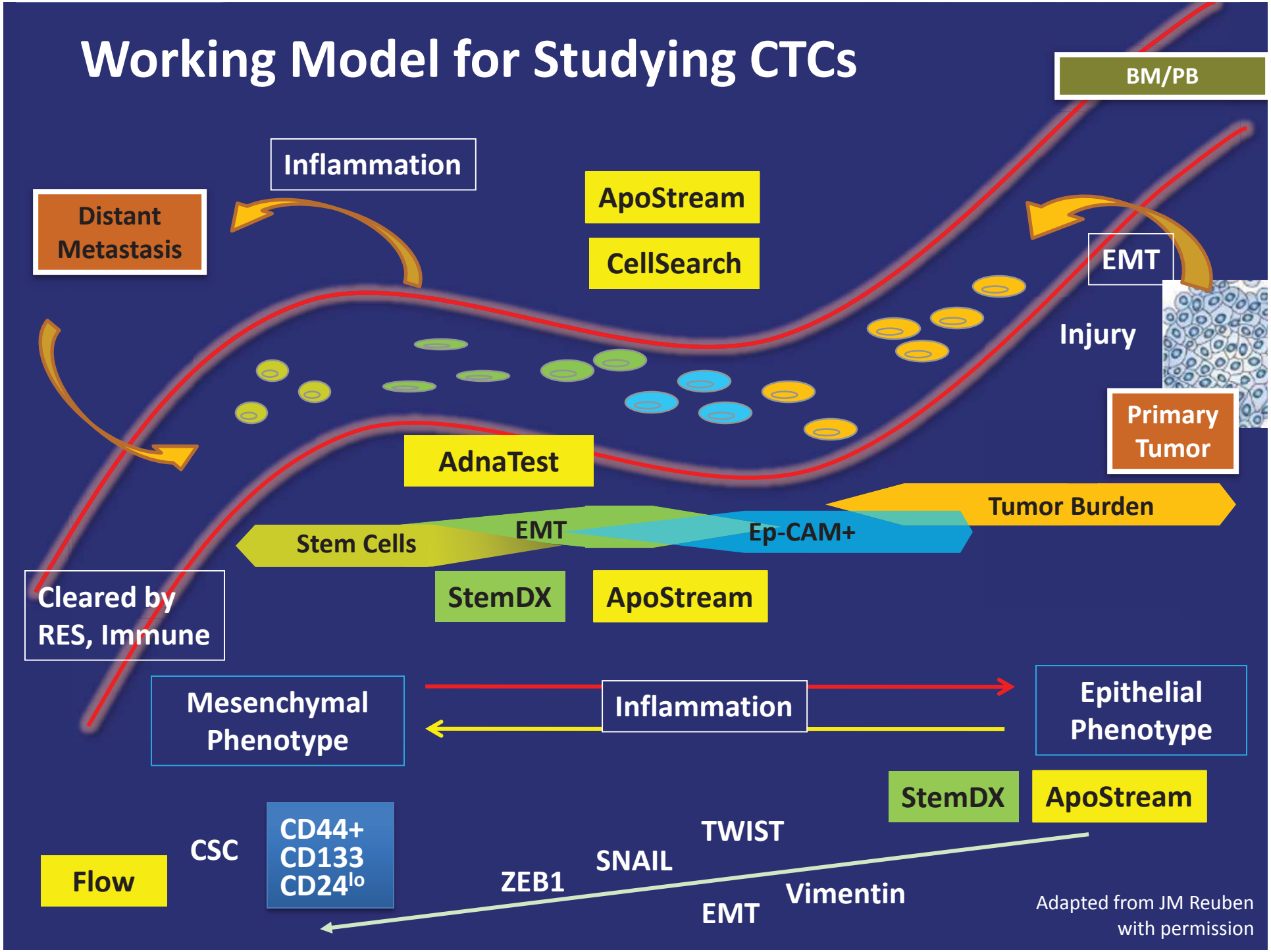
Working Model for Studying CTCs

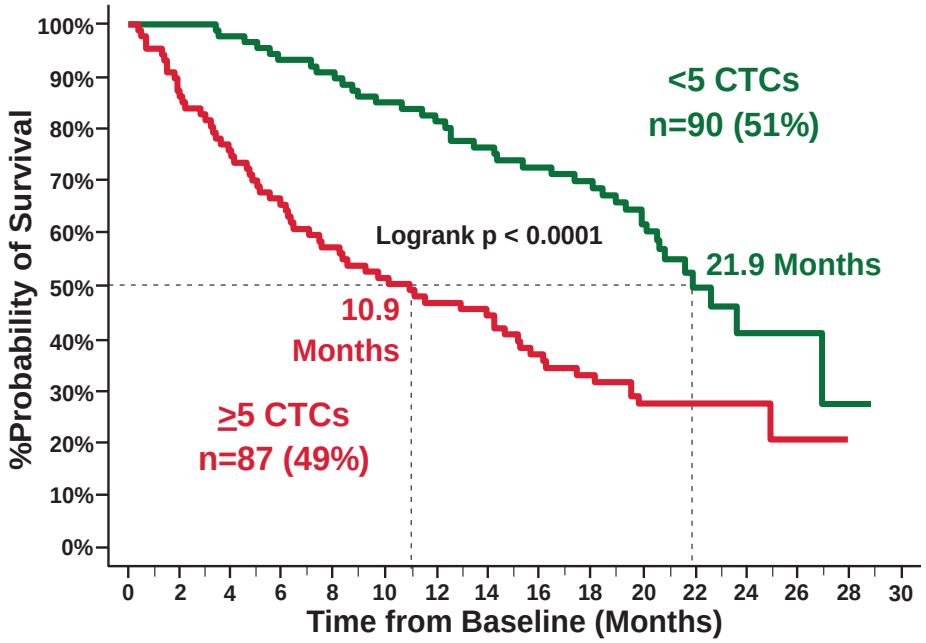


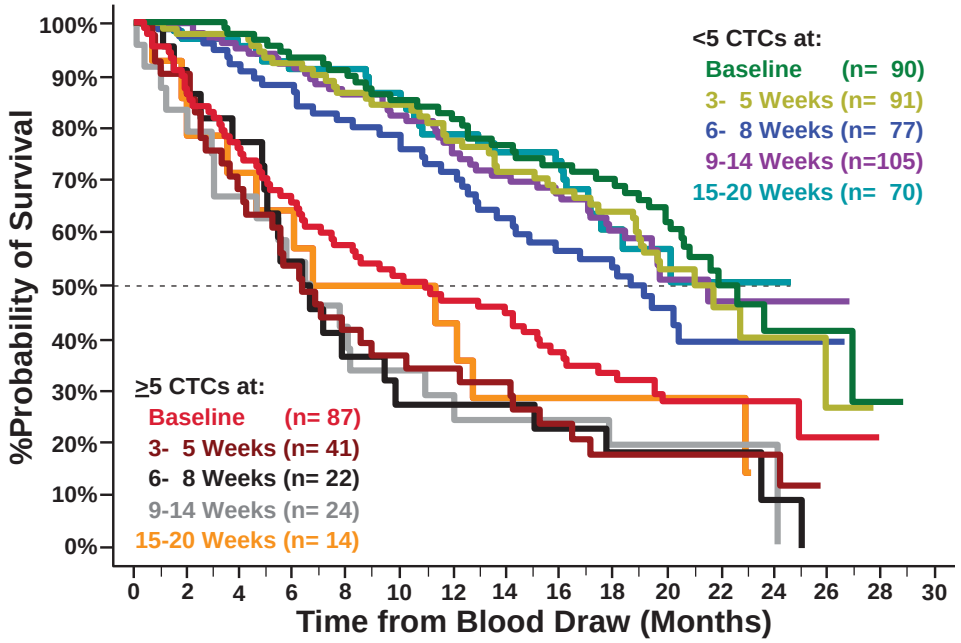
Clinical Application of CTCs

First Generation - Enumeration

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

Overall Survival





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Adapted from Cristofanilli, ...Reuben, et al.
N Engl J Med 2004;351:781-791
Hayes DF et al. *Clin Cancer Res*, 12:4218-24, 2006

Other Clinical Uses of CTCs

- Bone metastasis
- Widely metastatic HR positive disease

	No Bone Involvement	Bone	Bone & Other Sites
N	58	28	108
Mean ± SD	3.3 ± 8.7	52.7 ± 91.2	69.2 ± 206.4
Median	1	13	8.5
% ≥5 CTC	16%	69%	58%

De Giorgi U, et al. *Ann Oncol*. 2010;21(1):33-39.

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?

Adapted from JM Reuben
with permission

SWOG S0500:
CTC (CELLSEARCH) Did Not Predict Response to Therapy

CTCs drawn at baseline prior to first-line chemotherapy

CTC < 5

CTC ≥ 5

Arm A (n=276)

Monitor for PFS & OS

Eligible for other first-line chemo therapy trials

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

CTC < 5

CTC ≥ 5

Arm B (n=163)

Maintain first-line chemotherapy until progression

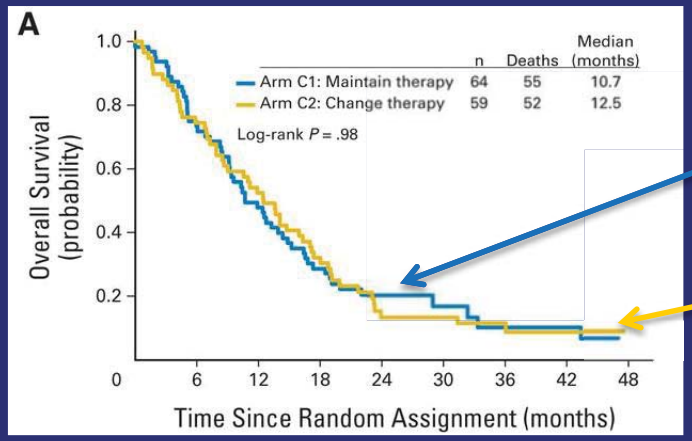
123 pt randomized 1° endpoint OS

Arm C1 (n=64)

Maintain first-line chemotherapy

Arm C2 (n=59)

Switch to alternate therapy



Follow-up

Imaging & CTCs midway and at progression

Smerage JB, et al, *J Clin Onc*. 2014;32(31):3483-3489

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

Trial	Randomization	Patient Population	CTC Parameter for Treatment Prediction	Primary Objective	Trial Number Accrual Date "N" Cases
STIC CTC METABREAST (France)	Clinical choice vs CTC driven choice of chemotherapy vs hormonotherapy	MBC, HR+, HER2-	CTC count (≥5 CTC/7.5 mL vs <5 CTC/7.5 mL)	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).	NCT0 1710605 Preliminary Analysis SABCS 2013
CirCe01 (France)	CTC-driven choice of chemotherapy	MBC, HR+, HER2-, third-line chemotherapy	CTC count (≥5 CTC/7.5 mL vs <5 CTC/7.5 mL)	Overall survival	NCT01349842 Jan 2018
Treat CTC (Europe)	Trastuzumab vs observation	HER2-non-amplified **primary** breast cancer with ≥1 CTC/15 mL PB after completion of (neo-)adjuvant chemotherapy and surgery	CTC count (≥1 CTC/15 mL of blood vs <1 CTC/7.5 mL)	CTC detection rate at week 18	NCT01548677 April 2017 N = 2175
DETECT III (Germany)	Standard therapy or standard therapy plus lapatinib	MBC, 1 to 3 lines of previous chemotherapy, HER2-	HER2+ CTC/7.5 mL of blood	Progression-free survival	NCT01619111 March 2018 N = 228
COMETI P2 (USA, Canada)	No randomization	MBC HR+, HER2-	Expression of ER, Bcl-2, HER2, Ki67 on CTC	Progression-free survival	NCT01701050 June 2015 N = 200

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

Primary Tumor	Total Patients	Patients with HER2-CTC	Patients with HER2+CRC*	Discordance
HER2+*	45	1	44	2.3%
HER2-	30	20	10	33.3%

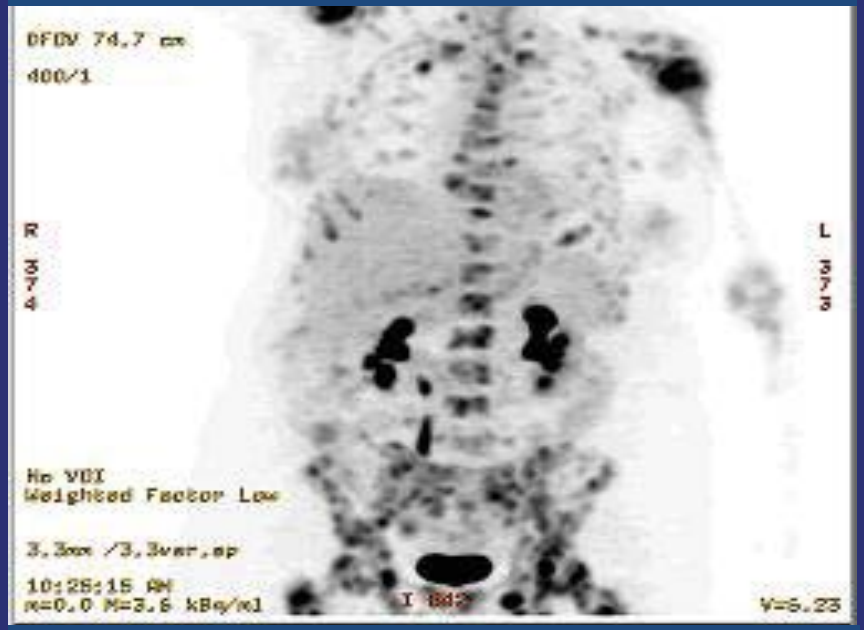
*HER2+ defined as the ratio of HER2/CEP17>2.0 by FISH

Flores LM, et al. *Br J Cancer*. 2010;102(10):1495-1502.

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

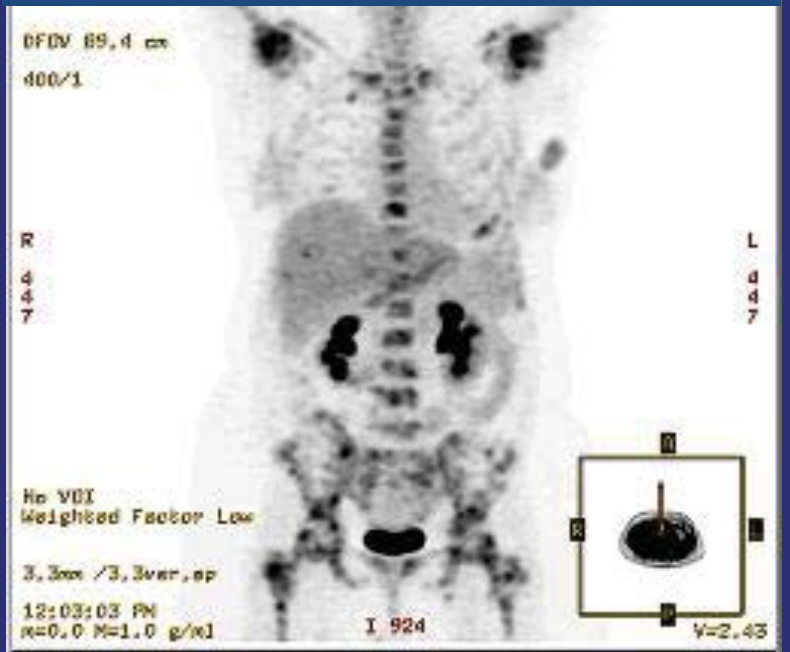
900 CTCs = ER-, HER2+



Hormonal Therapies
4th line CHT

CTCs = 400

Trastuzumab Therapy



CTCs = 95

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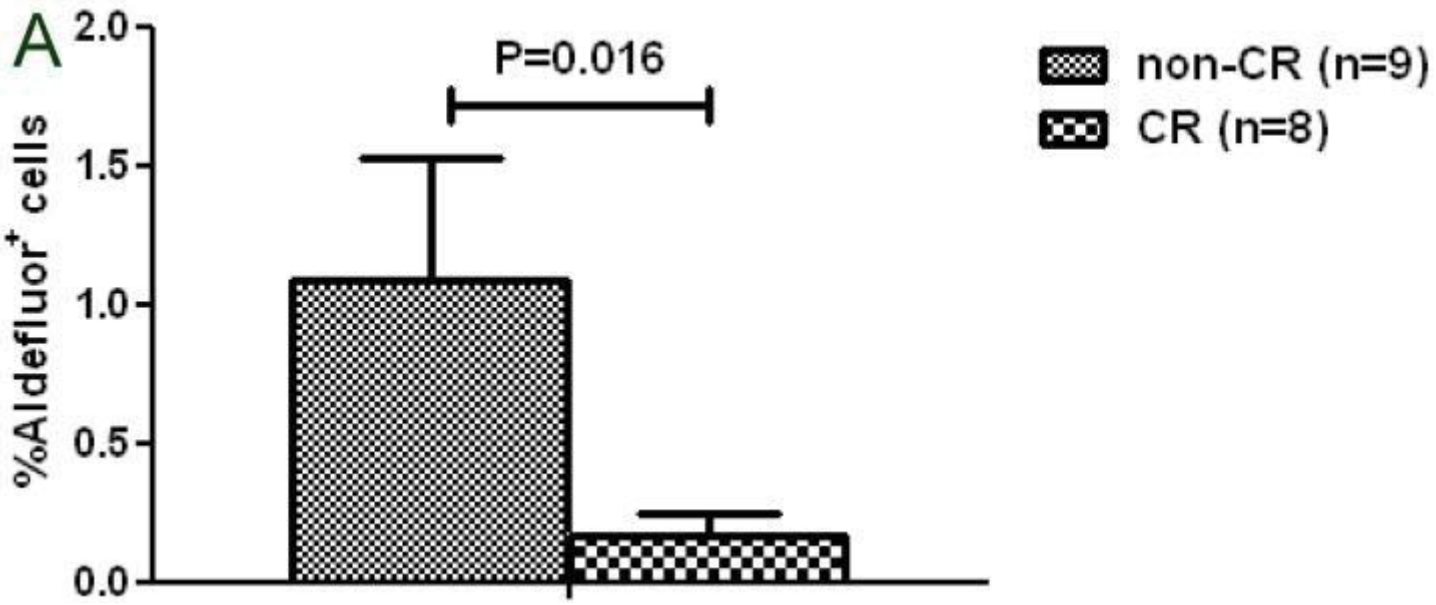
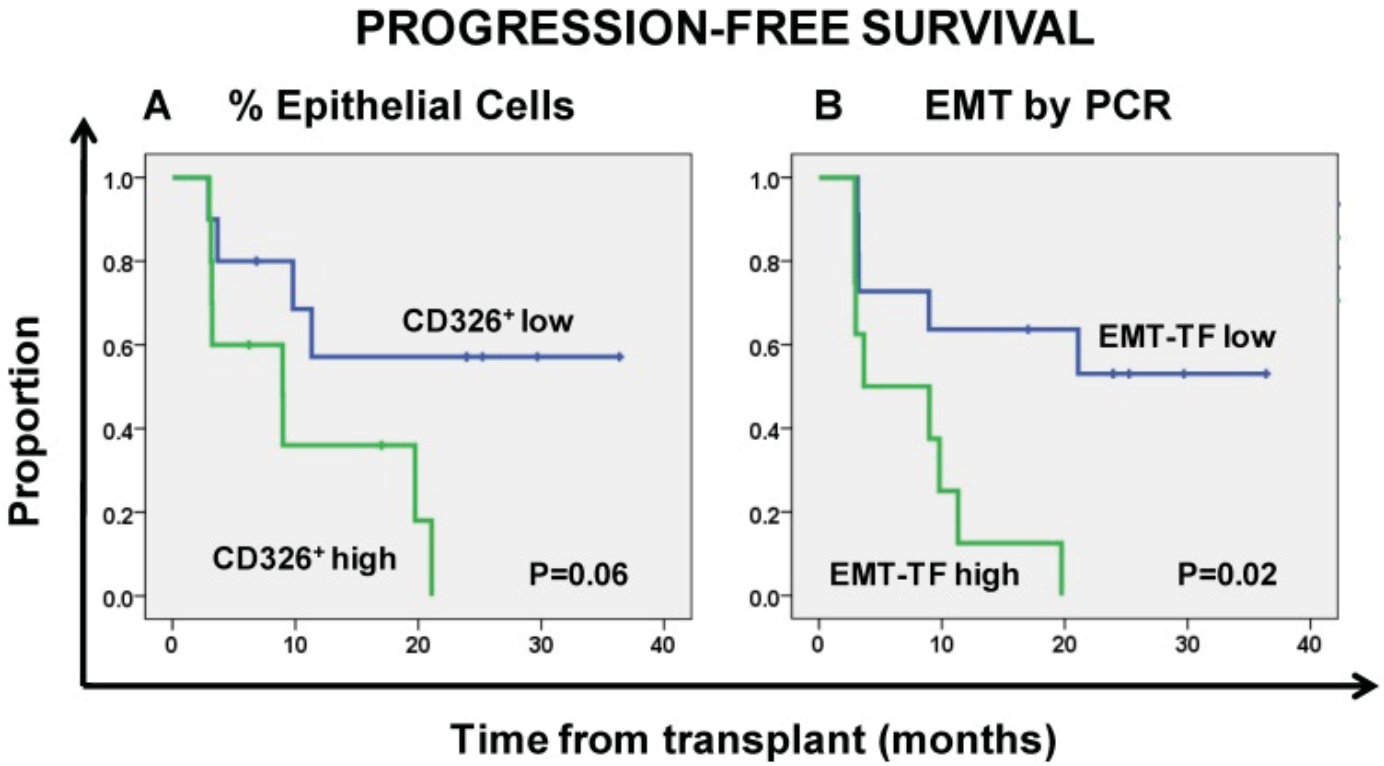
High % Cancer Stem Cells in Apheresis Product is Associated with Non-CR in MBC Undergoing Autologous Stem Cell Transplant



EMT-CTC in Apheresis Product of MBC Undergoing Autologous Stem Cell Transplant: Decreased PFS



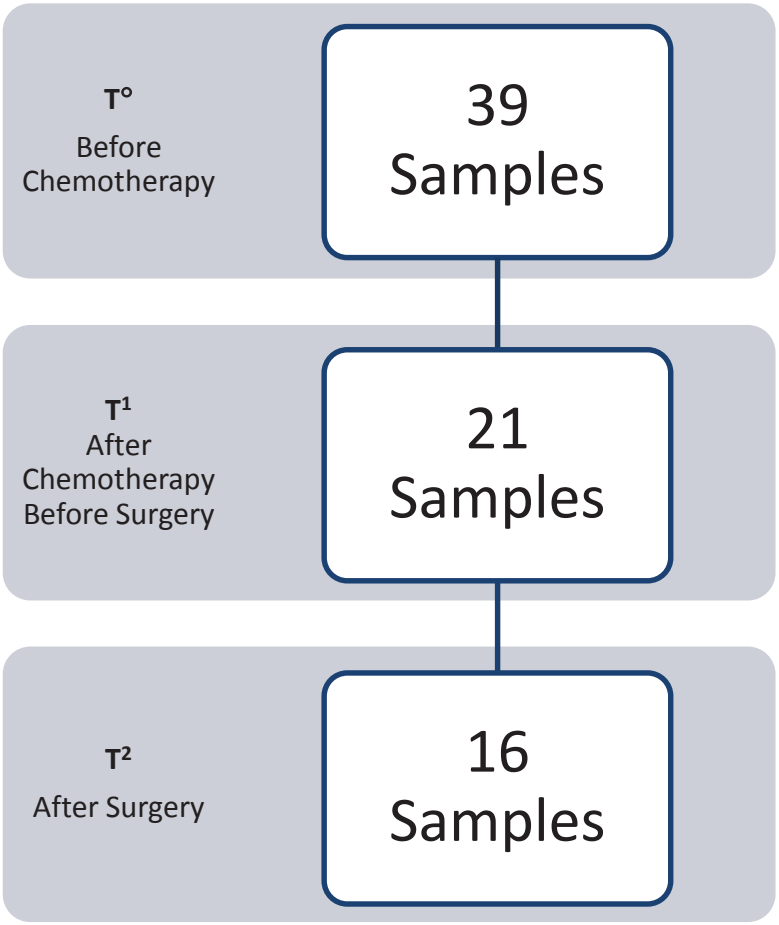
Neoadjuvant Chemotherapy Enriches EMT Genes

	N	Positive > 1 EMT Gene	*P* Value
No NACT	47	12.8% (n=6)	
NACT	20	35.0% (n=7)	0.047
Pathologic CR (pCR)	6	16.6% (n=1)	
No pCR	14	42.9% (n=8)	0.3

Reuben, JM et al, ASCO 2009, Chang, J et al, JNCI 2008

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

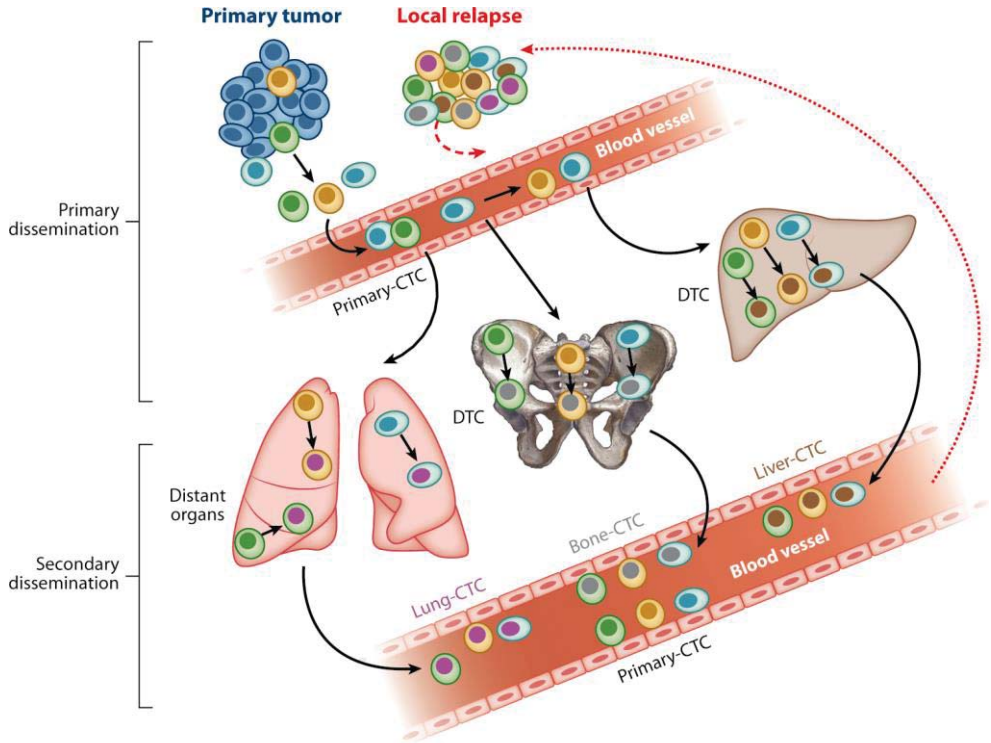


Further information will be presented on Friday in Poster Session P4-01-10.

- **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)



Alix-Panabières C, et al. *Annu Rev Med*. 2012;63:199-215.
Reproduced with permission of Annual Reviews, http://www.annualreviews.org.

POLL QUESTION:

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

Answer Choices:

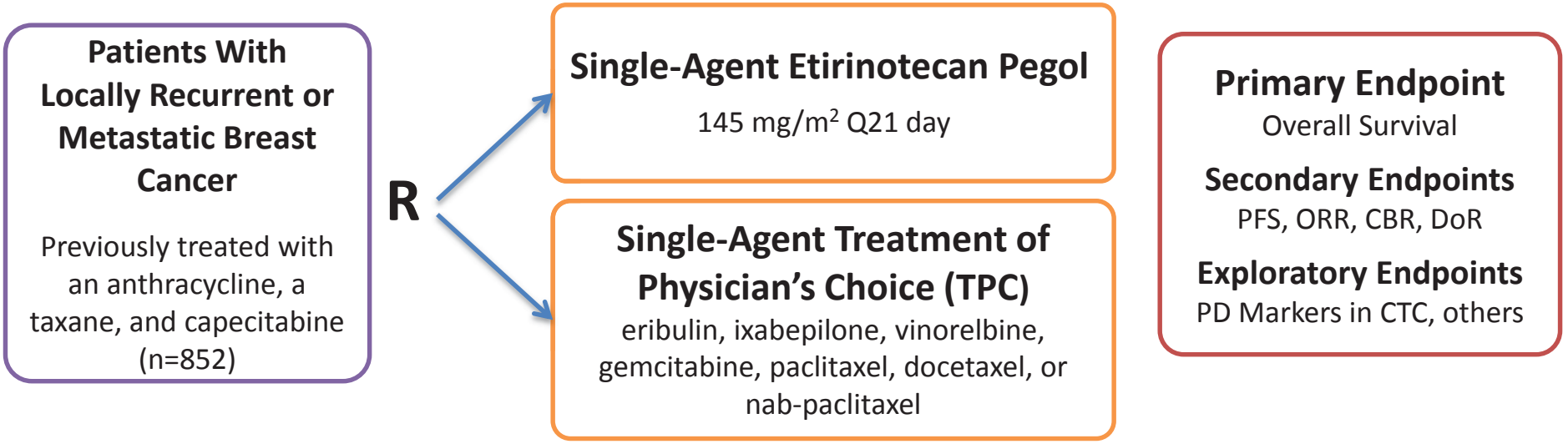
- Now
- 1-5 years
- 6-10 years
- 10 years+

BEACON: An Example of a Modern Study Incorporating Latest CTC Technology

Edith A. Perez, MD
Mayo Clinic
Jacksonville, FL

MAYO CLINIC

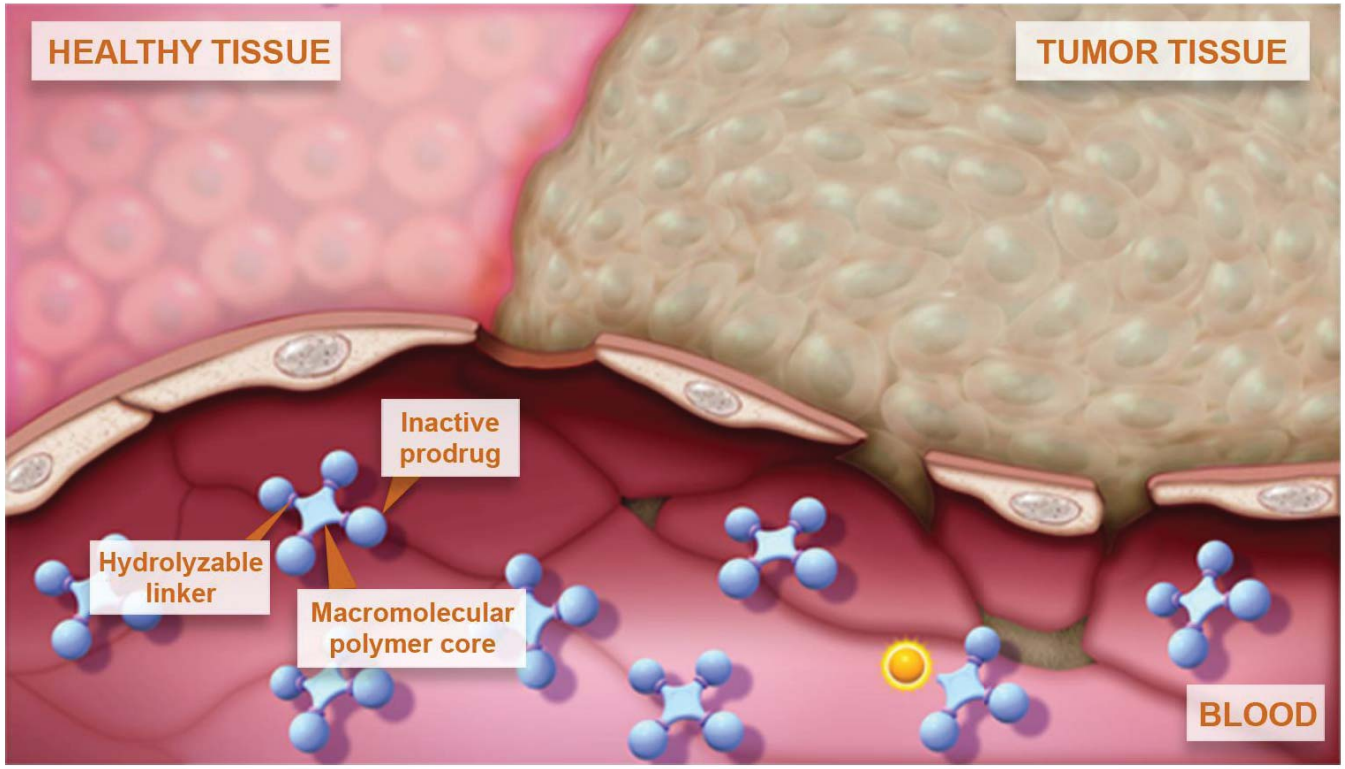
BEACON Phase 3 Registration Study of Etirinotecan Pegol in Metastatic Breast Cancer



- 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

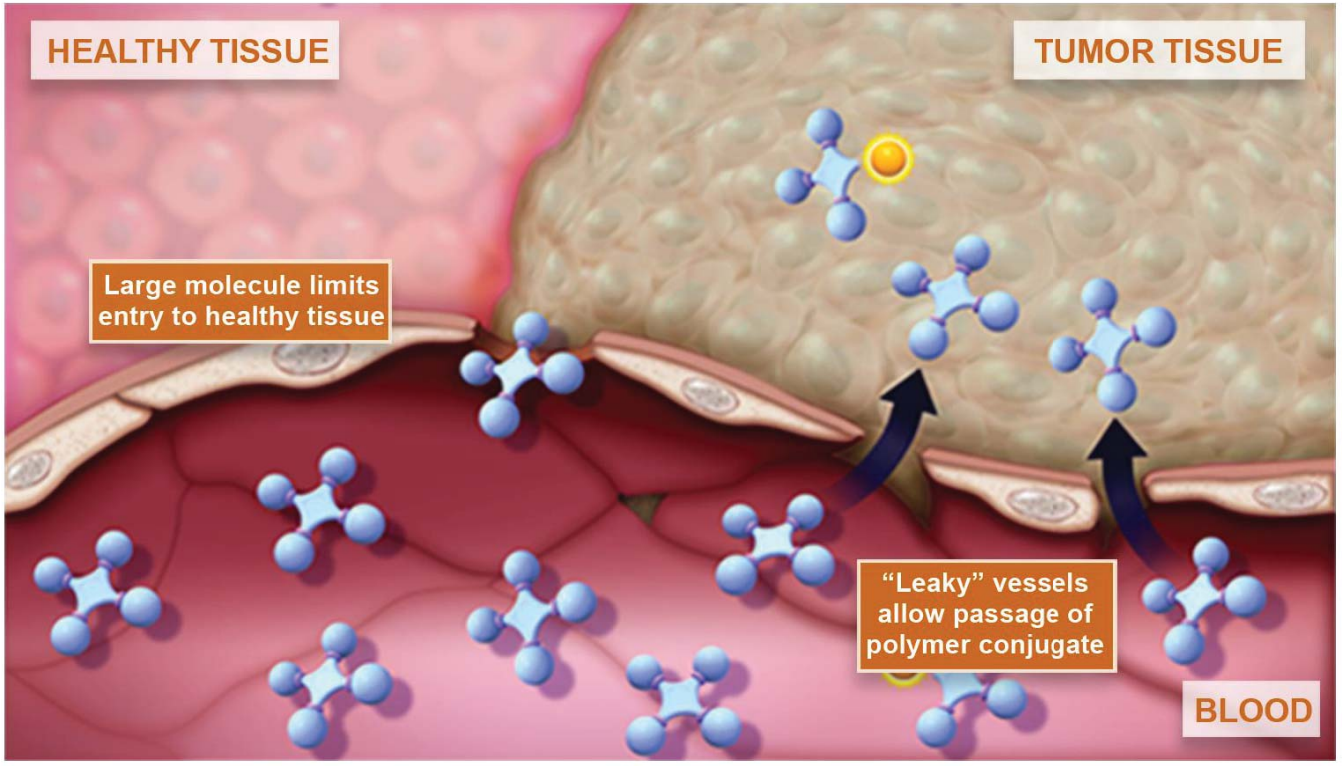
Etirinotecan Pegol: Targeting Tumor Tissue

Cytotoxic small molecules are attached to a unique macromolecular polymer core using hydrolyzable linkers to target disease tissue.



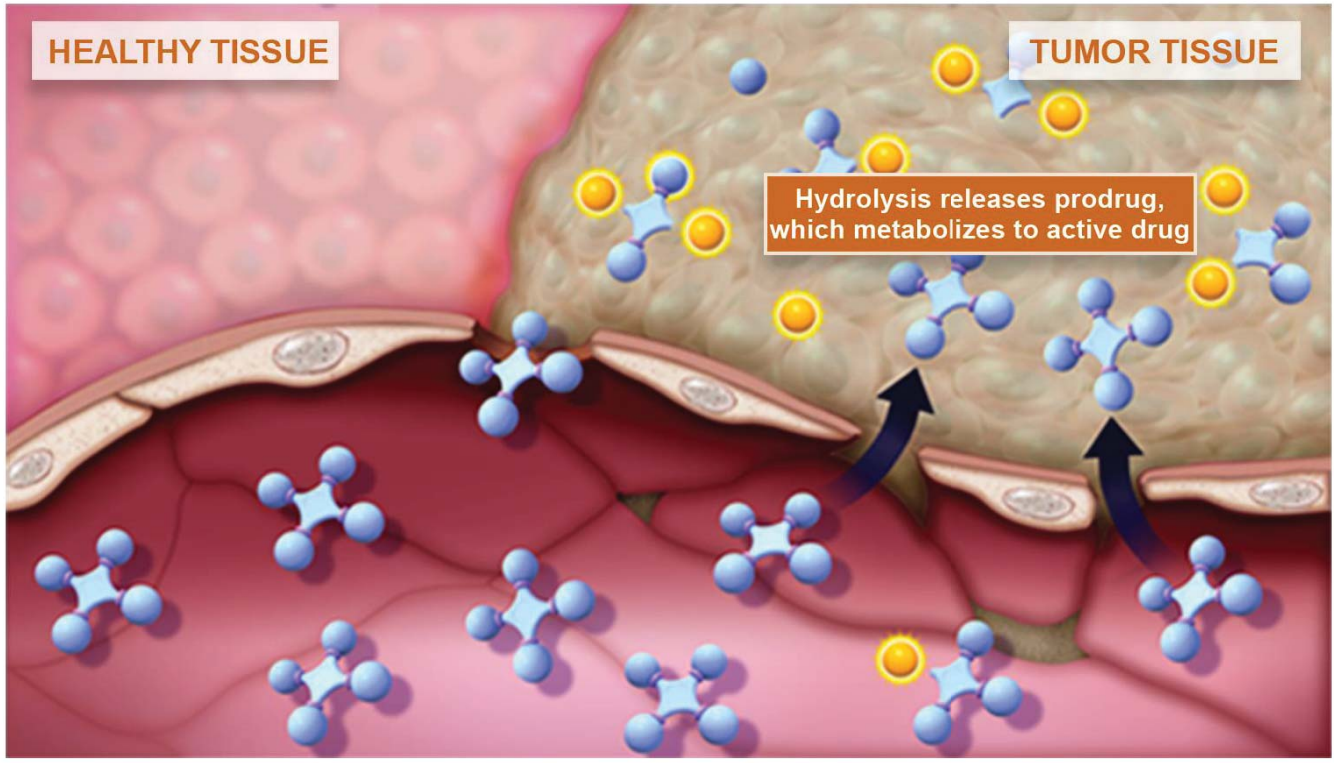
Etirinotecan Pegol: Targeting Tumor Tissue

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.



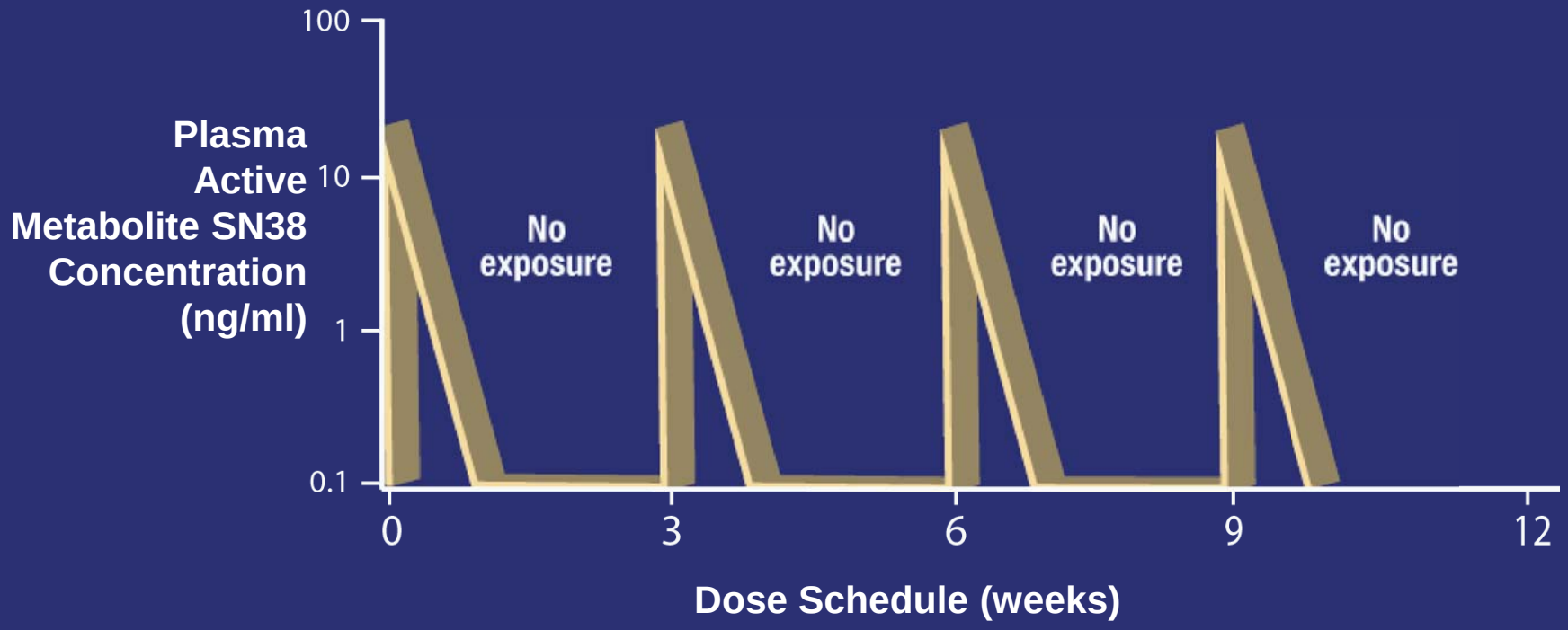
Etirinotecan Pegol: Targeting Tumor Tissue

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.



Irinotecan: Poor Pharmacokinetic (PK) Profile

First-generation topoisomerase I inhibitors have a high initial peak concentration and short half-life

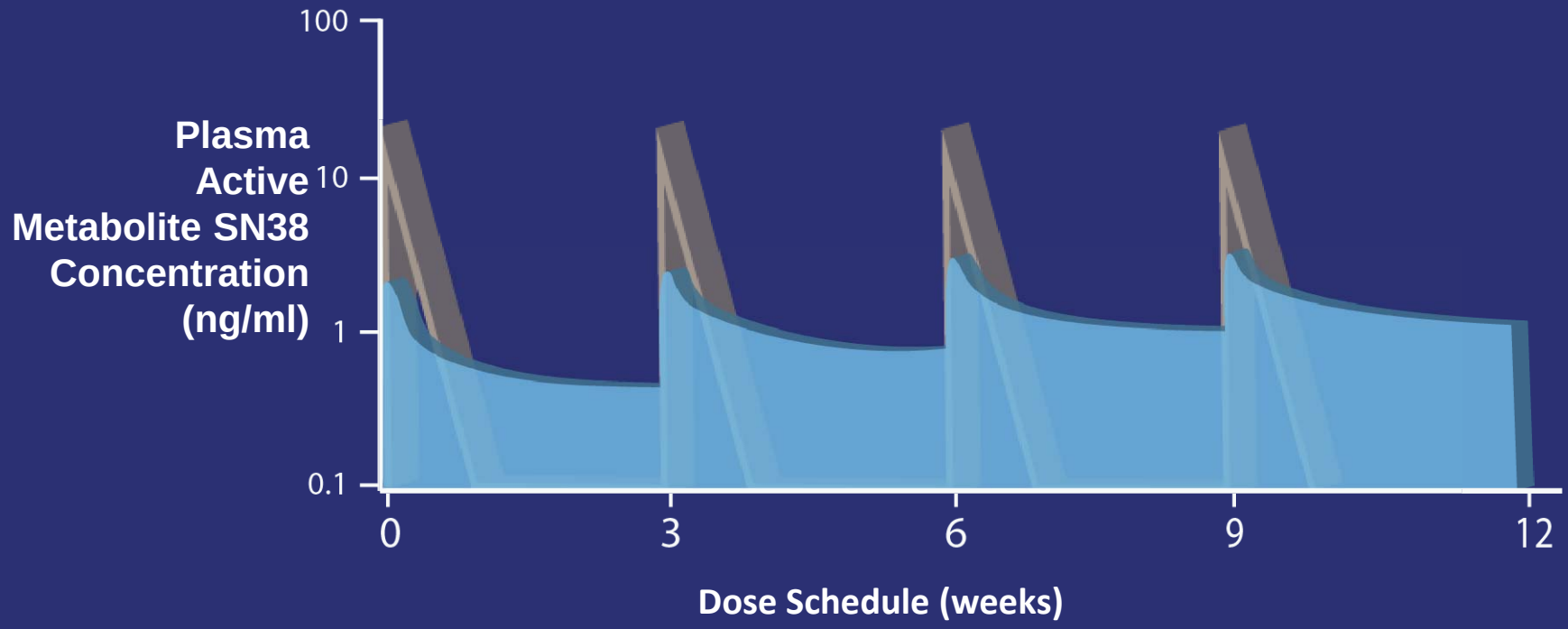


MAYO CLINIC

Jameson et al. *Clin Cancer Res* 2013, 19, 268-278.

Etirinotecan Pegol: Sustained PK Profile

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



MAYO CLINIC

Jameson et al. *Clin Cancer Res* 2013, 19, 268-278.

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-

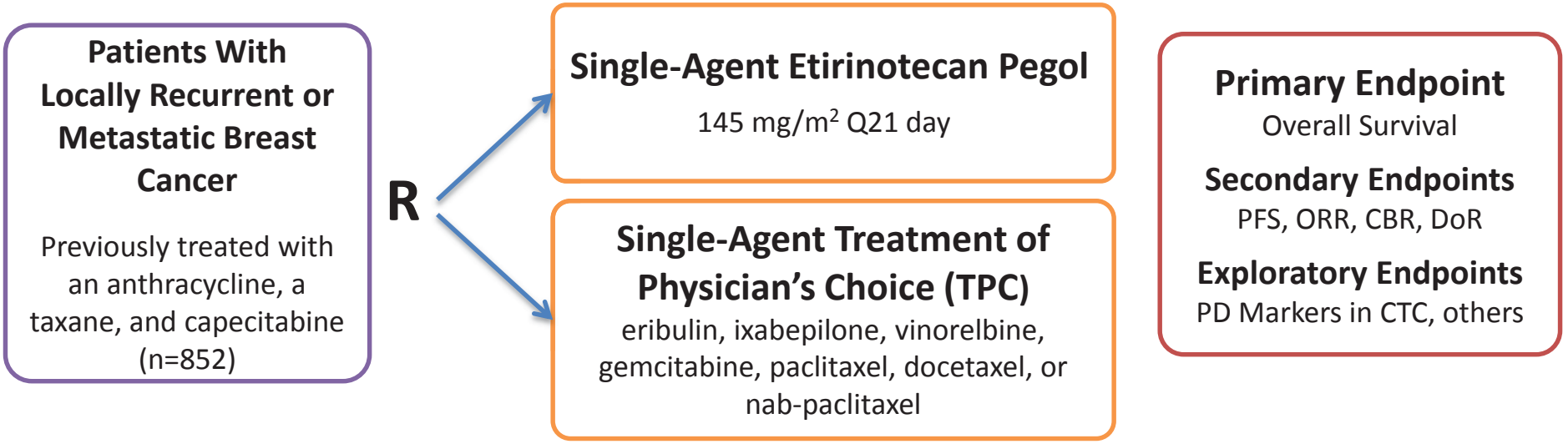
Awada et al., *Lancet Oncology* 2013, 14(2), 1216–1225.

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

Awada et al., *Lancet Oncology* 2013, 14(2), 1216–1225.

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



- 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

Collection of CTCs was Successfully Incorporated in the Phase 3 BEACON Study

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

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