Carolina Breast Cancer Study: Lessons Learned

&

MerTK: Potential Therapeutic Target in Breast Cancer and Its Microenvironment

Shelton Earp, Lisa Carey & Chuck Perou • October 5, 2016
Disclosure Information

3rd Annual Gayle Brinkenhoff Breast Cancer Symposium
Shelton Earp

I have the following financial relationships to disclose:
Stockholder in: Meryx Inc.

- and -

I will not discuss off label use and/or investigational use in my presentation.
Breast Cancer Heterogeneity: Multiple Distinct Subtypes

- **Luminal B**: Normal-like?
  - Hormonal pathways dominant

- **Luminal A**: Basal-like

- **Claudin-low**: “Triple negative” (ER, PR, HER2) on clinical assays

- **HER2-enriched**: HER2-driven
Populations to Patients…
And Back Again
3000 breast cancer cases enrolled 2008-2013
44 counties in NC
50% African-American / 50% Caucasian
50% under the age of 50 at diagnosis
CBCS: Benefit of Large Population-Based Studies
150 Publications To Date

Race, Breast Cancer Subtypes, and Survival in the Carolina Breast Cancer Study

Cancer Epidemiology, Biomarkers & Prevention

Determinants of Breast Cancer Treatment Delay Differ for African American and White Women

Clinical Cancer Research

Intrinsic Breast Tumor Subtypes, Race, and Long-Term Survival in the Carolina Breast Cancer Study Clin Cancer Res 2010;16:6100-6110.

American Journal of Epidemiology
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Race, Anthropometric Factors, and Stage at Diagnosis of Breast Cancer

American Journal of Epidemiology
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Hormone-related Factors and Risk of Breast Cancer in Relation to Estrogen Receptor and Progesterone Receptor Status

American Journal of Epidemiology
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Comparative Analysis of Breast Cancer Risk Factors among African-American Women and White Women

The Carolina Breast Cancer Study
CBCS: TNBC Is Elevated Even in Older African Americans: Gene Expression Subtyping

Black women of all ages have higher frequency of basal-like and HER2-enriched cancer
Racial Disparity in Survival within Subtypes (Especially Luminal A)

Persistent disparity despite adjustment for stage, subtype

HR ~ 2 in Luminal A

O’Brien et al, CCR 2010
## Risk Factors for Basal-Like Breast Cancer

**Adjusted ORs (95% CI)**  
N = 1424 cases & 2022 controls

<table>
<thead>
<tr>
<th>Factor</th>
<th>“Luminal A” N=796</th>
<th>Basal-like N=225</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menarche &lt; 13</td>
<td>1.1 (0.9-1.3)</td>
<td>1.4 (1.1-1.9)</td>
</tr>
<tr>
<td>≥ 3 children</td>
<td>0.7 (0.5-0.9)</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>First birth &lt; 26</td>
<td>0.7 (0.5-0.9)</td>
<td>1.9 (1.2-3.2)</td>
</tr>
<tr>
<td>Breastfeeding ≥ 4m</td>
<td>0.9 (0.7-1.1)</td>
<td>0.7 (0.4-0.9)</td>
</tr>
<tr>
<td>Parity ≥ 3 and No breastfeeding</td>
<td>0.7 (0.5-0.9)</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>Waist:Hip &gt; 0.84</td>
<td>1.5 (1.1-1.9)</td>
<td>2.3 (1.4-3.6)</td>
</tr>
</tbody>
</table>

*Millikan et al, BCRT 2008*
ICISS: Integrated Cancer Information Surveillance System

- NC Cancer Registry - ~50,000 cases/year, 2003-onwards, linked to claims data for treatment and outcome (Medicare, Medicaid, BCBSNC)
- Allows study of changes engendered by ACA

Cases Linked to Claims: 80% of NC cancers

Endocrine Adherence Project

- Linking CBCS3 to ICISS
- Adherence from CBCS follow-up questionnaires: who is taking the drugs, who isn’t and why?
- Objective data on prescriptions filled from ICISS

Interventional study

Katie Reeder-Hayes, MD, MBA
Stephanie Wheeler, PhD
Tyro3, Axl, and MerTK
Potential Breast Cancer Immunotherapy Targets

Jacqueline Carrico, Rebecca Cook, Christopher Cummings, David Darr, Kurtis Davies, Deborah DeRyckere, S. Gail Eckhardt, Amanda Hill, Debra Hunter, Kristen Jacobson, William Janzen, Dmitri Kireev, Alisa Lee-Sherick, Jing Liu, Timothy Newton, Susan Sather, Norman Sharpless, Michael Stashko, Lenka Teodorovic, Xiaodong Wang, Weihe Zhang, Albert Zimmerman, and the NCI NeXT/Chemical Biology Consortium Team

Alisha Holtzhausen  Eric Ubil
Shelton Earp, Doug Graham, Stephen Frye  ●  November 2015
Prior to Genome Sequencing: Cloning the Old Way

Cloning tyrosine kinases in bacteria

$^{125}\text{I-}$Staph Protein A binding to anti-p-tyr antibody

Primary

Secondary

Cloning tyrosine kinases in bacteria
Mer Receptor Tyrosine Kinase

- **Normal expression in**
  - Macrophages; Epithelial; Reproductive tissue

- **Physiologic function**
  - Ligands bridge PtdSer and MerTK
    - Apoptotic cells and exosomes activate MerTK
  - Macrophage MerTK:
    - Triggers engulfment
    - Suppresses inflammatory cytokines
    - Polarizes towards an M2 Phenotype
  - Promotes survival not proliferation

- **Function in Neoplastic Disease**
  - Aberrant expression in leukemias and multiple solid tumors
  - Promotes survival and chemoresistance
  - Non-oncogene addiction
  - Immune suppression in the microenvironment
  - Promotes metastases

Mer Over-Expression in Solid and Hematologic Malignancies

- Melanoma
- Glioblastoma
- Gastric Cancer
- Breast Cancer
- NSCLC
- AML

Clin Cancer Res. 12 (2006) 2662-9
J Biol Chem. 289 (2014) 25737-49
Nature Reviews Cancer 14 (2014) 769-785
Oncotarget 6 (2015) 9206-19
Oncogene 32 (2013) 5359-68
Adv Cancer Res. 100 (2008) 35-83
MerTK Expression Increased in a Hormone Dependent Cancer

MerTK significantly overexpressed in metastatic human prostate cancer tissue when compared to benign or localized disease.

MerTK drives prostate bone metastases bone marrow stroma makes Gas6

TAM Family Ligands: A Complex Mix
TAM Family Signaling

Receptor crosstalk and cooperation

AXL  MERTK  TYRO3  FLT3  EGFR  HER2

MEK  p38  PI3K  ERK  AKT

BCL-2  BCL-XL  MCL1  Survivin  PUMA

BAD

Anti-apoptosis and survival

mTOR

Cellular growth and proliferation

SRC family

Invasion and migration

FAK1  RHO  RAC  RALA  MMP9  RALB

NF-κB  CREB  JAK

STAT3  STAT5  STAT6

TWIST  SNAIL  SLUG

EMT  MITF

Cell cycle progression

Cyclin D

ACK1

WWOX

Oncogenic transformation

Tumour growth

Nature Reviews | Cancer
Innate Immunity: Multiple TAM Family Roles

- Efficient clearance of intracellular antigens
  - Macrophage
  - Apoptotic cell debris with PtdSer

- Apoptotic cells polarize macrophages towards M2
  - M1 macrophage
  - GAS6, MERTK
  - M2 macrophage
  - IL-10

- AXL signalling dampens TLR inflammatory response
  - APC
  - SOC51, SOC53
  - TLR

- Activated T cell feedback inhibits innate immunity
  - CD8+
  - PROS1

- TAM signalling inhibits NK cell anti-metastatic effects
  - NK cell
  - AXL or MERTK
  - Lung metastasis

Nature Reviews | Cancer
Innate Immunity
M1 vs. M2 – A Role for MerTK?

“A useful oversimplification”

Biswas and Mantovani *Nature Immunology* 11:889
Immune Infiltrate Is Higher in Basal-Like Breast Cancer

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Basal-like cluster</th>
<th>Luminal cluster</th>
<th>IGG_Cluster</th>
<th>B_Cell_cluster</th>
<th>CD8_cluster</th>
<th>T_Cell_cluster</th>
<th>CD68_cluster</th>
<th>MacTh1_cluster</th>
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- Basal-like cluster
- Luminal cluster
- IGG_Cluster
- B_Cell_cluster
- CD8_cluster
- T_Cell_cluster
- CD68_cluster
- MacTh1_cluster

Breast subtype:
- Basal-like
- HER2-enriched
- Luminal A
- Luminal B
- Normal-like

[Image of tissue section with immune infiltrate]
Inhibition of Tumor Growth in MerTK Knock-out Mice: Due to an Immune Effect?

HYPOTHESIS

Mer TK inhibition promotes M1 phenotype: pro-inflammatory effect
Mer TK activation promotes M2 phenotype: anti-inflammatory effect

Breast

MMTV-PyVmT mammary tumors

Melanoma

B16:F10 intradermal tumors

MerTK is a target in the tumor microenvironment

Rebecca Cook, Doug Graham, Shelley Earp, et al. JCI July 2013
Bone Marrow Transplant from MerTK-/- or MerTK+/+ Mice

MMTV-PyVmT Transgenic Mice:

Tumor growth in MerTK+/+ mice following bone marrow transplants from MerTK+/+ or Mer -/-
MerTK-/- Macrophages in Tumors: Cytokine Profile Turns Angry

4 days after inoculation

IL-10 transcript levels

IL-12 transcript levels

IL-12 serum levels

Serum IL-6 levels

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4 days after inoculation

IL-12 (pg/ml)

IL-6 (pg/ml)

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n.s.

**

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PBS

PyVmT

B16:F10

MerTK^+/+ PBS

MerTK^+/+ PyVmT

MerTK^-/- B16:F10

MerTK^+/+ PBS

MerTK^+/+ PyVmT

MerTK^-/- B16:F10

MerTK^+/+ PBS

MerTK^+/+ PyVmT

MerTK^-/- B16:F10

MerTK^+/+ PyVmT

MerTK^-/- B16:F10

MerTK^-/- PyVmT

MerTK^+/+ B16:F10

MerTK^-/- B16:F10
Tumor Microenvironment & Immunity: A Very Complex Process
Feed Forward, Autocrine Mechanisms?

Rebecca Cook, Doug Graham, Shelley Earp, et al
*JCI in revision*

Gas6 induces IL-10
IL-10 induces Gas6

Zizzo, Cohen and colleagues,
*J Immunology* 2012; 189:3508
MerTK Deletion Inhibits PyMT Tumor Growth & Metastasis: T Cell Dependent

- Prolonged tumor latency and reduced metastatic proliferation
- Increased CD8⁺ T lymphocytes in tumor microenvironment
- CD8 depletion results in abrogation of the effect of MerTK⁻/⁻

Strong biologic data supporting MerTK as a target in the tumor microenvironment
Development of MerTK Inhibitors

2000 Compounds – 6 Years of Work

A great collaboration
Chemistry: Frye, Wang et al., Biology: Earp and Graham labs
The Roles of Perivascular Macrophages in Tumor

Lewis, Harney & Pollard
Cancer Cell 2016;
UNC2025 Efficacy in a Mer-Negative PyMT Orthotopic Model of Breast Cancer

Day 0:
Inject 1x10^6 PyVmt cells into inguinal mammary fatpad

Day 16, 19, 21, 23, 26, 28:
Measure tumor volume

Day 28:
End of study

Day -2 to Day 28:
3 mg/kg UNC2025A or Saline only by oral gavage
UNC2025 Decreases PyMT Tumor Growth \textit{In Vivo}

![Graph showing tumor growth comparison between different treatment groups.](chart.png)

- **Vehicle**
- **3mg/kg bid**
- **50mg/kg qd**

Key:

- `##` p < 0.01 compared to 3mg/kg bid
- `###` p < 0.001 compared to 3mg/kg bid
- `***` p < 0.001 compared to 50mg/kg qd

**n=20, n=10, n=10**

**Tumor Volume (mm³)** as a function of **Days Post-Tumor Injection**.
RNA-Seq

1. Isolate & digest PyMT Tumors
   - Collagenase/DNase treatment & mechanical digestion

2. Tumor Single-Cell Suspension

3. Enrich for MΦ using MACS columns
   - CD11b+ (MΦ) cells
     - Isolate RNA

4. Flow-Thru
   - Enrich for TILs using MACS columns
     - CD8+ Lymphocytes (TILs)
       - Isolate RNA
     - Flow-Thru
Proinflammatory Pathways Are Enriched in UNC2025-Treated TAMs

CXCL10 (IP-10)
CXCL9 (MIG)
CXCL11 (I-TAC)

IL-12
IL-1a
IL-1b

MMU04620
p<0.001
FDR=0.015
Cytotoxic Pathways Are Enriched in UNC2025-Treated CD8+ T cells

Graft vs. Host Disease

CD8+ TIL

CD28

TCR

FasL

Granzyme B

Perforin

IFNg

TUMOR

Apoptosis

MMU05332

p<0.001

FDR=0.042
Targeting MDSCs & Macrophages

- Promoting differentiation – M-MDSCs
  - Vit. A, retinoic acid/ATRA
- Inhibit expansion
  - SCF/Kit, VEGF, MMP9, FLT3L
- Inhibit function
  - Cyclooxygenase - PGE2 - Arg 1
  - Phosphodiesterase2 - iNOS, Arg 1
  - ROS
- Elimination
  - Doxorubicin-cyclophosphamide
  - Gemcitabine
UNC2025 Inhibits MERTK Phosphorylation in Murine Bone Marrow Derived Macrophages

- Graph showing the inhibition of MERTK phosphorylation with UNC2025 at different concentrations.
- IC₅₀ = 24.3 nM
Mer Receptor Tyrosine Kinase

- **Member of TAM family of RTKs**

**Consequences in Neoplastic Disease**
- Promotes survival and chemoresistance
- Non-oncogene addiction
- Immune suppression in the microenvironment
- Promotes metastases

**Next Steps:**

**Immunotherapy**
- Block Innate Immune Checkpoint
- Combine with T Cell Checkpoint Inhibitors

**Conventional Therapy**
- Combine with cytotoxic and targeted agents

**Disaggregate Therapeutic Mechanisms**
Tumor-Elicited MDSCs Express TAM Receptors and Ligands
Decrease in MDSC Immune Suppressive Capabilities in MerTK−/− or UNC2371 Treated Tumor Bearing Mice

B16 Melanoma Syngeneic Model WT Treated with 25 mg/kg UNC2371
UNC2371 + Anti-PD-1 Blocks Pancreatic Tumor Growth

Tumor growth or orthotopically implanted KPC cells was assessed by ultrasound. Treatment was initiated at day 0.

Representative images of orthotopic tumors on control or experimental treatment as measured by ultrasound at day 14 post-injection.
MerTK Inhibition Decreases M2 Macrophages

Stromal MerTK staining in a section of human PDAC.

Decrease in M2-macrophage population in MRX2843-treated PDAC tumors.

UNC2371 decreases M2 macrophage population in pancreatic tumors.
UNC2025 + Radiation Therapy Synergizes in GBM Models

TRP Glioblastoma
GBM Responders vs Non-Responders
Summary

• **TNBC**
  – Higher incidence in African Americans
  – Greater immune infiltration

• **MerTK Action**
  – Ingestion of apoptotic material
  – Altered cytokine response towards M2 away from M1
  – Deletion leads to M1 polarization and potential enhancement of immune anti-tumor responses
  – UNC developed MerTK inhibitors may enhance immunotherapy
Axl\(^{-/-}\), Mer\(^{-/-}\) and Tyro3\(^{-/-}\) MDSCs Reduce T Cell Suppression