Carolina Breast Cancer Study: Lessons Learned
&
MerTK: Potential Therapeutic Target in Breast Cancer and Its Microenvironment
October 5, 2016

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Melissa Troester & Andy Olshan
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

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

- HER2-enriched
  - HER2-driven
Populations to Patients… And Back Again
Carolina Breast Cancer Study Phase 3
The Jeanne Hopkins Lucas Breast Cancer Study

3000 breast cancer cases enrolled 2008-2013
44 counties in NC
50% African-American / 50% Caucasian
50% under the age of 50 at diagnosis

JEANNE HOPKINS LUCAS
1935-2007
CBCS: Benefit of Large Population-Based Studies

- Race
- Survival
- Age
- Intrinsic Subtype
- Radiation
- Electromagnetic Fields
- Pesticides
- Nutrition
- Breast-feeding
- Menopause
- Risk
- GWAS
- Lifestyle
- Hormones
- Antidepressants
- Physical Activity
- Menarche
- Parity
- NSAIDS
- Anthropometrics
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)

Whites (11% 5 year)

African Americans (17% 5 year)

Persistent disparity despite adjustment for stage, subtype

Luminal A Breast Cancer Survival by Race

Basal-like Breast Cancer Survival by Race

HR: 1.9 (1.3, 2.9)  p= 0.039

HR: 1.3 (0.7, 2.3)  p= 0.3

O’Brien et al, CCR 2010
Breast Cancer Risk Factors, Revisited

<table>
<thead>
<tr>
<th>Factor</th>
<th>ER+ HER2-</th>
<th>Triple Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early menstruation</td>
<td>-</td>
<td>40% ↑</td>
</tr>
<tr>
<td>&gt; 2 children</td>
<td>30% ↓</td>
<td>90% ↑</td>
</tr>
<tr>
<td>Having children young</td>
<td>30% ↓</td>
<td>90% ↑</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>-</td>
<td>30% ↓</td>
</tr>
<tr>
<td>Obesity</td>
<td>50% ↑</td>
<td>130% ↑</td>
</tr>
</tbody>
</table>

Lifestyle modifications may be really important in preventing triple negative disease.
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

Primary

Secondary

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

Cloning tyrosine kinases in bacteria
Mer Receptor Tyrosine Kinase

- **Member of TAM family of RTKs**

**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
TAM Family Ligands: A Complex Mix
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

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
  - M2 macrophage
  - IL-12
  - IL-10

- AXL signalling dampens TLR inflammatory response
  - APC
  - AXL
  - SOCS1
  - SOCS3
  - TLR

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

- TAM signalling inhibits NK cell anti-metastatic effects
  - NK cell
  - AXL or MERTK
  - Lung metastasis
Innate Immunity
M1 vs. M2 – A Role for MerTK?

“A useful oversimplification”

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

<table>
<thead>
<tr>
<th></th>
<th>MerTK^{+/+} N=17</th>
<th>MerTK^{+/-} N=15</th>
<th>MerTK^{-/-} N=17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent tumor-free</td>
<td></td>
<td></td>
<td>P &lt; 0.0001</td>
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Melanoma

B16:F10 intradermal tumors

<table>
<thead>
<tr>
<th></th>
<th>MerTK^{+/+} N=10</th>
<th>MerTK^{+/-} N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent tumor-free</td>
<td></td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

# lung mets per mouse

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 -/-

Rebecca Cook, Doug Graham, Shelley Earp, et al. JCI 2013
MerTK-/- Macrophages in Tumors: Cytokine Profile Turns Angry
Tumor Microenvironment & Immunity: A Very Complex Process
Feed Forward, Autocrine Mechanisms?

GAS6

relative transcript levels

MerTK+/+ PBS
MerTK+/+ PyVmT
MerTK+/+ B16-F10
MerTK+/- PBS
MerTK+/- PyVmT
MerTK+/- B16-F10

n.s.

Gas6 induces IL-10
IL-10 induces Gas6

Zizzo, Cohen and colleagues,
*J Immunology 2012; 189:3508*

Rebecca Cook, Doug Graham, Shelley Earp, et al
*JCI in revision*
The Roles of Perivascular Macrophages in Tumor

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

Day 0:
Inject $1 \times 10^6$ PyVM T 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 *In Vivo*

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

Days Post-Tumor Injection

Tumor Volume (mm³)

- **##** 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
Proinflammatory Pathways Are Enriched in UNC2025-Treated TAMs

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

TLR

IRAK

TRAF6

MYD88

TAK1

TAB 1/2

IKB

NF κB

MKK4/7

STAT1

NF κB

Fos

Jun

IL-12

IL-1a

IL-1b

MMU04620

p<0.001

FDR=0.015
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

Gabrilovich & Nagaraj
UNC2025 Inhibits MERTK Phosphorylation in Murine Bone Marrow Derived Macrophages

![Graph and western blot images showing the inhibition of MERTK phosphorylation by UNC2025 at different concentrations. The graph displays the IC₅₀ value for UNC2025 as 24.3 nM.]
Tumor-Elicited MDSCs Express TAM Receptors and Ligands

![Graphs showing expression levels of receptors and ligands in Monocytic and Granulocytic MDSCs]
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

IDO RNA Expression

iNOS RNA Expression

Arginase RNA Expression
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
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.
UNC2025 + Radiation Therapy Synergizes in GBM Models

TRP Glioblastoma

- **Control**
- **UNC2025**
- **XRT**
- **UNC2025 + XRT**

Percent survival vs. Days
Mer Receptor Tyrosine Kinase

- **Physiologic function feeds Neoplasia**
  - Ligands bridge PtdSer and MerTK
    - Apoptotic cells and exosomes activate MerTK
  - Macrophage MerTK:
    - Suppresses inflammatory cytokines
    - Polarizes towards an M2 Phenotype

**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**
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