Molecular Heterogeneity of Triple Negative Breast Cancer

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Estimated Cancer Deaths per year in the USA

Women 270,290

26% Lung & bronchus
10% Breast Luminal
9% Colon & rectum
7% Pancreas
5% Ovary

5% Triple-Negative BC (ER-, PR-, HER2-)

4% Non-Hodgkin lymphoma
3% Leukemia
3% Uterine corpus
2% Liver & intrahepatic bile duct
2% Brain/Other nervous system
24% All other sites
Basal-like subtype

1. 10-25% of tumors
2. distinct cell type of origin
3. >80% TP53 mutant
4. BRCA1 associated
5. highly proliferative (RB null)
6. Typically ER-, PR-, and HER2-not amplified (Triple-negative), so treatment options are limited - mostly chemotherapy only

HER2
- CRYAB
- TCF4
- Frizzled 7
- c-KIT
- Keratin 5
- Keratin 17
- P-Cadherin

Luminal

Proliferation
Molecular Characterization of Basal-Like and Non-Basal-Like Triple-Negative Breast Cancers.
Prat et al., The Oncologist, 2013 (PMID:23404817)
Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies.
Lehmann et al., JCI, 2011 (PMID:21633166)

- 6 subtypes were identified by analyzing global gene expression patterns within TNBC only
  - Basal 1: cell cycle
  - Basal 2: growth factor signaling
  - Immunomodulatory: immune cells
  - Mesenchymal
  - Mesenchymal Stem Like
  - Luminal Androgen Receptor (LAR)
  - No Class / Unclassified
The updated TNBCtyper removes the Immunomodulatory Group, and the Mesenchymal Stem-Like Group.

“We used histopathological quantification and laser-capture microdissection to determine that transcripts in the previously described immunomodulatory (IM) and mesenchymal stem-like (MSL) subtypes were contributed from infiltrating lymphocytes and tumor-associated stromal cells, respectively. Therefore, we refined TNBC molecular subtypes from six (TNBCtype) into four (TNBCtype-4)”. 
Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer.

Burstein, Tsimelzon, Poage, Covington, Contreras, Fuqua, Savage, Osborne, Hilsenbeck, Chang, Mills, Lau, and Brown. Clinical Cancer Research, 2015 (PMID:25208879)

1-LAR: Luminal Androgen Receptor
2-MES: Mesenchymal
3-BLIS: Basal-like Immune Suppressed
4-BLIA: Basal-like Immune Activation
The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups.
Curtis et al., Nature 2012 (PMID:22522925)
TCGA Breast mRNAseq Data
1101 tumors and 97 normals

177 TNBC

Collaborative bioinformatics analysis of TCGA data with:

Katie Hoadley and Chris Fan of University of North Carolina
= (PAM50 + Claudin-low subtyping)

Brian Lehmann and Jennifer Pietenpol of Vanderbilt University = (TNBCtype-4)

Powel Brown (MDACC) and Susan Hilsenbeck
(Baylor College of Medicine) = (MDA/BCM TNBC-4)

Carlos Caldas and Oscar Rueda of
University of Cambridge = (IntClust)
TCGA Breast mRNAseq Data
1101 tumors and 97 normals
177 TNBC

PAM50 + Claudin-low

IntClust

MDA/BCM TNBC-4

TNBCtype-4
### TCGA Breast mRNAseq Data

1101 tumors and 97 normals  
177 TNBC

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#### TNBC Type-4 Vanderbilt

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#### TNBC-4 MDA/BCM

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#### TNBC Type-4 Vanderbilt

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#### IntClust from Cambridge

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How can Triple Negative Breast Cancers be stratified?

- **Luminal/AR**: 20-30%
  - **Luminal A+B**
  - **HER2-Enriched**

- **Basal**: 70-80%
  - **Claudin-low / Mesenchymal**
  - **Basal-like**

Possible AR Dependency

- **Lapatinib-Sensitivity**
- **Chemo-Sensitivity**
Molecular Characterization of Basal-Like and Non-Basal-Like Triple-Negative Breast Cancers.
Prat et al., The Oncologist, 2013 (PMID:23404817)
Study Schema (MDV3100-11)
A Phase 2, Single Arm, Open Label, Multicenter Study of the Clinical Activity and Safety of Enzalutamide in Patients With Advanced, Androgen Receptor Positive, Triple Negative Breast Cancer
ClinicalTrials.gov Identifier:NCT01889238

Eligibility
- "AR positive" advanced TNBC*
- ECOG-PS ≤ 1
- Any number of prior therapies permissible
- Evaluable bone-only disease allowed
- No CNS metastases
- Sufficient tissue to enable biomarker discovery

Endpoints
- Primary
  - CBR16
- Other Key Endpoints
  - CBR24
  - Response rate
  - PFS
  - OS
  - Safety
  - AR biomarker discovery

Treatment
Enzalutamide 160 mg/day orally

Stage 1
- ≥ 3 of 26 Evaluable have CBR16
- "Go" to Stage 2

Stage 2
- ≥ 9 of 62 Evaluable have CBR16
- Rejection of H₀

Statistical Considerations
- 85% power to detect true CBR16 = 8% tested against 1-sided alternative (CBR16 ≥ 20%); alpha = 5%

Overall Survival (OS) from the Phase 2 Study of Enzalutamide, an Androgen Receptor (AR) Signaling Inhibitor, in AR+ Advanced Triple-Negative Breast Cancer (aTNBC)

Javier Cortes, John Crown, Ahmad Awada, Peter Schmid, Luca Gianni, Laura Garcia-Estevez, Noelia Martinez-Janez, Stephen Chan, Joyce L. Steinberg, Martha Blaney, Iulia Cristina Tudor, Hirdesh Uppal, Amy Peterson, Kathy Miller, Denise A. Yardley, Clifford A. Hudis, Tiffany A. Traina

Javier Cortes et al. ECCO 2015
Median Treatment Duration
8 weeks (range 1–81)

Median Treatment Duration
15 weeks (range 1–79)

CBR 16: Clinical benefit rate (CR + PR + SD for ≥16 weeks)
CBR 24: Clinical benefit rate (CR + PR + SD for ≥24 weeks)

Data cutoff 01 July 2015. * Censoring applies PFS = progression free survival, AR = androgen receptor

Javier Cortes et al. ECCO 2015
Prognostic Value of Intrinsic Subtypes in Hormone Receptor–Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib

Prat et al., JAMA Oncology 2016 (PMID:27281556)

HR+/ HER2-negative

Clinically HER2-negative, HER2-E subtype benefit from lapatinib
How can Triple Negative Breast Cancers be stratified?

- **Luminal/AR**
  - Luminal A+B
  - HER2-Enriched
  - 20-30%

- **Basal**
  - Claudin-low / Mesenchymal
  - Basal-like
  - 70-80%

Possible AR Dependency

- Lapatinib-Sensitivity
- Chemo-Sensitivity

Low – Immune – High
Gene Expression or TILs

Proliferation

Chemo-Sensitivity
Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific Breast and Ovarian Cancer. Iglesia et al., Clinical Cancer Research 2014. PMID:24916698
Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific Breast and Ovarian Cancer. Iglesia et al., Clinical Cancer Research 2014. PMID:24916698

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**TCGA Basal-like Breast, IGHV4-39**

- **Overall Survival (%)**
  - high
  - low
  - p=0.0336
  - years: 0 1 2 3 4 5

**TCGA Basal-like Breast, IGHV1-8 Expression**

- **Overall Survival (%)**
  - high
  - low
  - p=0.032
  - years: 0 1 2 3 4 5
Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98.


J Clin Oncol. 2013 (PMID: 23341518)
Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer:
Phase Ib KEYNOTE-012 Study,
Nanda et al., JCO 2016 (PMID:27138582)
Multivariate Analysis of Subtype and Gene Expression Signatures Predictive of Pathologic Complete Response (pCR) in Triple-Negative Breast Cancer (TNBC): CALGB 40603 (Alliance)

Katherine A. Hoadley¹, Terry Hyslop², Cheng Fan¹, Donald A. Berry³, Olwen Hahn⁴, Sara M. Tolaney⁵, William M. Sikov⁶, Charles M. Perou¹, Lisa A. Carey¹

Stage II-III TNBC 2x2 Randomization

- Paclitaxel 80 mg/m² weekly x 12
- ddAC x 4
- Paclitaxel 80 mg/m² weekly x 12
- Bevacizumab 10 mg/kg every 2 weeks x 9
- Paclitaxel 80 mg/m² weekly x 12
- Carboplatin AUC 6 every 3 weeks x 4
- Paclitaxel 80 mg/m² weekly x 12
- Bevacizumab 10 mg/kg every 2 weeks x 9
- Carboplatin AUC 6 every 3 weeks x 4

Surgery**

XRT⁶

No Adjuvant Systemic Treatment Planned⁸

Research Biopsies

*Research Biopsies if residual tumor
& MD discretion

pCRRate by Arm and Subtype

San Antonio Breast Cancer Symposium, December 6-10, 2016

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Conclusions

- We achieved moderate predictive ability in all TNBC and within the Basal-like subset (see poster). While low, there are few predictive markers in TNBC and this will help us understand the biology underlying response/resistance.

- Future work includes performing DNA sequencing on this cohort to determine mutated genes, mutational signatures and estimate copy number alterations that will be added to the modeling approach.
Triple Negative Breast Cancer Conclusions

1. TNBC genomic subtyping tools are concordant with each other and identify common features including Basal, Luminal/AR, Immune High, and Mesenchymal.

2. TNBCs are often chemotherapy sensitive, with features including Basal vs not (cell type), the presence of immune cells (microenvironment), a number of DNA-based features (tumor genetics).

3. Many promising anti-TNBC drugs exist and are being tested including: chemotherapeutics, AR antagonists, lapatinib for HER2E, epigenetic inhibitors, and immune system modulating agents.
UNC Collaborators
Charles Perou
Michael Iglesia
Shelley Earp, Ned Sharpless (LCCC)
Melissa Troester (UNC, Epidemiology)
Steve Marron, Andrew Nobel (Statistics)
Lisa Carey, Neil Hayes, Carey Anders, Jon Serody, Ben Vincent, Hy Muss (Oncology)
Joel Parker, Sai Balu and members of the LCCC
Bioinformatics Group
Corbin Jones and members of the High Throughput Sequencing Facility (HTSF)

TNBC Comparison Analysis
Chris Fan and Chuck Perou
University of North Carolina

Brian Lehmann and Jennifer Pietenpol (TNBCtype-4)
Vanderbilt University

Powel Brown and Susan Hilsenbeck (MDA/BCM TNBC-4)
MD Anderson Cancer Center and Baylor College of Medicine

Carlos Caldas and Oscar Rueda (IntClust)
University of Cambridge

Aleix Prat
University of Barcelona

The Cancer Genome Atlas Network
Alliance Clinical Trials Network
Terry Hyslop

Funding:
NCI
Komen Career Catalyst Grant