Exploring new drugs, new pathways, and new combinations for the treatment of metastatic breast cancer

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Disclosures/COI

- Entinostat was provided by Syndax Pharmaceuticals
- No other disclosures

Outline

- Objectives
- Overview & significance
- Rationale
- Review of current research
- Ongoing projects
- Future directions
Objectives:

1. Give an overview of other novel immune therapy combinations to improve efficacy of treatment in breast cancer
2. Discuss importance of immunotherapy in the treatment of metastatic breast cancer
3. Discuss the added benefits of epigenetic modulators combined with immunotherapy in breast cancer

Metastatic Breast Cancer

Global Analysis 2005-2015:
- 0.5 million deaths globally/year
- Modest improvements in outcomes
- Inadequate patient education
- Caregiver needs overlooked
- Lack of focus on quality of life
- Substantial economic burden
- United global effort required

Goals of Therapy:
- Cure (rare)
- Prolong progression-free (PFS) and overall survival (OS)
- Symptom control
- Prevent complications
- Improvement in quality of life
**Immune Response in Breast Cancer**

- Key in determining response to standard therapy and long-term survival

Tumor elimination $\rightarrow$ Equilibrium $\rightarrow$ Tumor escape

<table>
<thead>
<tr>
<th>T helper type 1 TME</th>
<th>T helper type 2 TME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute Inflammation</strong></td>
<td><strong>Chronic Inflammation</strong></td>
</tr>
<tr>
<td>M1 macrophages, DCs, NK, CD4+CD8</td>
<td>M2 macrophages, MDSCs, Tregs</td>
</tr>
<tr>
<td>- T cells, IL-2, INFγ, IL-12, TNF, PD-L1 upregulation</td>
<td>Bregs, TAF, IL-4, IL-6, IL-10, TGFβ</td>
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**Significance:**

- Despite significant advances in chemotherapy and targeted therapy breast cancer is a prevalent and often life threatening disease
- Immune checkpoint inhibitors have led to durable responses in some cancers.
- Rational design of clinical trials guided by preclinical findings will identify ways to improve response rates to prolong the lives of patients with breast cancer and limit toxicity due to standard therapies

**Breast Cancer Subtypes**

- All Breast Cancer
- ER+ 65%–75%
- HER2+ 10%–20%

Carey et al. JCO 2016, Metzger-Filho et al. JCO 2012, Courtesy Antonio Wolff
Breast Cancer Subtypes
Novel drugs & Pathways:

All Breast Cancer

ER+
65%–75%
HER2+
15%–20%

CDKi:
palbociclib, ribociclib, abemaciclib

PI3Ki:
buparlisib, pirtalib, pilaralisib, alpefilast, tasefilast

mTOR:
evansimus, temsirolimus

HDACi:
Entinostat, vorinostat

Breast Cancer Subtypes
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PARPi:
Olaparib, talazoparib, veliparib, niraparib,rucaparib

Other:
Glembatumumab vedotin, lirilumab, pembrolizumab

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What we know:
- Consistent PFS advantage regardless of agent (MONALEESA-3), line of therapy, menopausal status (MONALEESA-7), clinico-path features (Gao J. ASCO 2018)
- Numerical improvement OS (not sig) 2nd line setting (PALOMA3)
- Consider schedule, toxicity profile, quality of life when prescribing CDKi backbone

What we are working on:
- Other biomarkers of response: Currently, ER only robust predictive biomarker
- Best patient populations to target: Do all need (upfront)?
- CDKi after CDKi (no data) (NCT02738866, NCT03147287)
- Role in other breast cancer subtypes (NCT02947885, NCT01320592)
- Combination strategies? +PI3K? immune?

### CDK inhibitors

**Drug Class** | **Drug Name** | **Select Phase 3 Trials**
---|---|---
Pan CDK inhibitors | Buparlisib | BELLE2, BELLE3* |
 | Pictilisib | |
 | Pilaralisib | |
Isoform specific | Alpelisib | SOLAR1 |
 | Tasselisib | SANDPIPER |
PI3K inhibitor | GSK2636771 | |

*Seeleke J. Lancet Onc 2017
Campone M. EJC 2018
Di Leo. Lancet Onc 2018

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**HDAC inhibitors**

- Considered broad re-programmers that lead to generalized changes in the epigenome.
- Focus is now on targeted inhibitors of certain isoforms of HDACs to increase efficacy and decrease side effects — i.e. Bromodomain and extraterminal inhibitors, CDK9i
- Current clinical uses:
  - Exemestane +/- Entinostat in Patients With Recurrent locally advanced or metastatic HR+ Breast Cancer (Phase 3)
  - Entinostat, Nivolumab, and Ipilimumab in HER2+ mBC (Phase IIa)
  - Entinostat, Nivolumab, and Ipilimumab in unresectable, locally Advanced or HER2-Negative mBC (Phase 2)

**PARP inhibitors**

- FDA approval 2018: Olaparib (OlympiAD trial)
  - in patients with deleterious or suspected deleterious BRCA1 or BRCA2 germline BRCA mutations and who have undergone platinum-based therapy for metastatic breast cancer.
- FDA approval 2018: Talazoparib (EMBRACA trial)
  - for the treatment of adult patients with deleterious or suspected deleterious germline BRCA1 or BRCA2 mutations and who have undergone platinum-based therapy for metastatic breast cancer.

**What about immunotherapy?**

- Brown = CD8+ T cells
- TNBC
- HER2+
- ER+
Overview of immune checkpoint inhibitors

- None FDA approved for breast cancer
- α-CTLA4 (Tremelimumab & Ipilimumab)
  - Tremelimumab + exemestane (26 pts) → 42% stable disease x12wks
  - Neoadjuvant Ipilimumab +/- cryoablation (12 pts) → Induced T₉₁ cytokines, proliferating CD4 and CD8 T cells, and increased TIL.
  - Many ongoing
- α-PD1/α-PD-L1 (Nivolumab & Pembrolizumab, Avelumab & Atezolizumab)
  - Many completed and ongoing

### Study Antibody | Target | Combination | Subtype | Patients | ORR
--- | --- | --- | --- | --- | ---
JAVELIN Avelumab PD-L1 Single agent All | 168 | 4.8%
  - PD-L1+ all | 13 | 33.3%
  - TNBC | 58 | 8.6%
  - PD-L1+ TNBC | 9 | 44.4%
  - PD-L1- TNBC | 39 | 2.6%
KEYNOTE-012 Pembrolizumab PD-1 Single agent PD-L1+ TNBC | 27 | 18.5%
KEYNOTE-086 (Cohort A) Single agent TNBC | 170 | 4.7%
  - PD-L1+ TNBC | 105 | 4.8%
  - PD-L1- TNBC | 64 | 4.7%
KEYNOTE-086 (Cohort B) Single agent TNBC | 112 | 10%
  - PD-L1+ TNBC | 71 | 13%
  - PD-L1- TNBC | 37 | 5%
mpassion130 Atezolizumab PD-L1 Nab-paclitaxel PD-L1+ TNBC | 32 | 4.4%

Immunotherapy in HER2+ disease

### Intrinsic immune properties of HER2 directed therapy:
- Trastuzumab— intrinsic immune modulating activity via antibody-dependent cellular cytotoxicity (ADCC) and promotion of HER2 directed T cell response
- TDM1— modulates DC activity and further augments immune priming

### Pre-clinical data:
- Trastuzumab augments activity of cell based vaccine
- αHER2 + αPD1 augments clearance of HER2+ tumors
- TDM1 + αCTLA4 and αPD1 prolongs survival in 90% HER2+ mice

### Trials underway:
- NCT02924883 atezolizumab + TDM1 in HER2+ mBC
- NCT02129556 (PANACEA) pembrolizumab + trastuzumab in trastuzumab resistant, PD-L1+ mBC

Rationale

• Tumor infiltrating lymphocytes and changes in gene expression indicative of immune activation are associated with improved outcomes in HER2+ and TNBCs

• Immunosuppressive tumor microenvironment (TME) indicative of the need to sensitize tumors to immunotherapy

How can we change the landscape?

Review of current research
Hypothesis: combinations of immune checkpoint inhibitors with epigenetic and targeted therapies will transform non-immunogenic breast tumors.

Epigenetic modulators as an immune priming agent

- Promotes type I interferon response and restores TH1 cytokines and chemokines
**Methods:**

1. Neu/N - A tolerogenic model of transplantable NT2.5 breast cancer, FVB/NJ background

**Results:** Addition of entinostat to ICI's significantly improves survival and slows tumor progression

**Drugs used:**

- Entinostat (ENT)
- Class 1 HDACs
- Immune checkpoint inhibitors (ICIs)
  - Anti-PD-1 (RPM1-14 BioXCell)
  - Anti-CTLA-4 (Clone 9H10 BioXCell)
ENT + ICIs significantly increases infiltration of G-MDSCs into the tumor microenvironment and inhibits their immunosuppressive functions. ENT + ICIs promotes infiltration of activated cytotoxic CD8+ effector T cells.
ENT + ICI significantly alters gene expression profiles involved in myeloid function

### Treatment

<table>
<thead>
<tr>
<th>KEYS Pathway</th>
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<th>P-value</th>
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<tbody>
<tr>
<td>ENT vs. ENT</td>
<td>+ a-CTLA-4</td>
<td>ErbB</td>
<td>0.00083</td>
</tr>
<tr>
<td>ENT vs. ENT</td>
<td>+ a-CTLA-4</td>
<td>VEGF</td>
<td>0.03325</td>
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<td>ENT vs. ENT</td>
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<td>0.01336</td>
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**Roussos Torres ET et al. Cancer Immunology Research 10/2018**

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<tr>
<td>Vehicle vs. ENT</td>
<td>ErbB</td>
<td>0.00939</td>
<td>Raf1, Areg, p21, Egf, Hbegf</td>
</tr>
<tr>
<td>Vehicle vs. ENT + a-PD-1</td>
<td>ErbB</td>
<td>0.05165</td>
<td>Raf1, Areg, p21, Egf, Hbegf</td>
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**Roussos Torres ET et al. Cancer Immunology Research 10/2018**

### STAT3 drives production of Arg-1

**Adapted from: Yen BL et al., Stem Cell Reports 2013**
Combination ENT + checkpoint inhibition alters arginase production via STAT3

Ongoing projects & future directions

1. Determine the mechanism driving response to ICIs and epigenetic modulation in HER2 positive and TNBC.

2. Evaluate the role of HDAC inhibitors in regulating STAT3 in pro-carcinogenic inflammation.
Project 1

Determine the mechanism driving response to ICIs and epigenetic modulation in HER2 positive and TNBC

Goal of preclinical studies

- Use mouse models and in vitro studies to determine the mechanism behind response in primary and metastatic HER2+ and TNBC

Goal of clinical studies:

- Determine if mechanisms identified in the mouse models can be validated in biological samples collected from patients with advanced breast cancer who have received ENT in combination with nivolumab + ipilimumab

Preclinical experiments underway

- Single cell RNA-sequencing of intratumoral MDSCs and bulk tumors from treated and untreated mice.
  - Determine entinostat’s effect on tumor cells
  - Identify other immune cell types affected by entinostat
  - Preliminary scRNA-seq of MDSCs shows over 1300 genes differentially regulated in untreated vs. ENT treated MDSCs
- Identify target genes and pathways that will help identify response mechanisms and define new breast cancer subtypes that may respond to ICIs
Primary Objectives
• Safety and tolerability of entinostat and nivolumab +/- ipilimumab in patients with advanced solid tumors and HER2-negative breast cancer
• Determine the recommended phase 2 dose (RP2D)

Secondary Objectives
• Evaluate whether entinostat alone or in combination with nivolumab and ipilimumab results in an increase effector T cells (Teff): regulatory T cell (Treg) ratio in tumor biopsies compared to baseline
• Assess antitumor activity (RECIST and irRC)
• Manuscript currently in preparation

Trial being held across 4 sites: JHU, City of Hope, Yale, University of Pittsburgh

Clinical correlates underway CTEP (NCI 9844):

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• Additional immune and epigenetic correlates currently being planned

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Clinical correlates underway CTEP (NCI 9844):

Patient on trial with adenoid cystic carcinoma:

0 = Baseline
1 = 2 weeks post run-in with ENT
2 = 8 weeks post 6 wks with combined therapy

Images: Torres ET & Connolly R et al. (Unpublished data)
Preliminary data: IHC for CD8+ T cells increases post treatment

0 = Baseline
1 = 2 weeks post run-in with ENT
2 = 8 weeks post tx with combined therapy

24 CD8 cells/mm²
9 CD8 cells/mm²
3325 CD8 cells/mm²

Patient on trial with rectal cancer: Roussos Torres ET & Connolly R et al. (Unpublished data)

Manuscript in preparation includes:
• All clinical findings
• Immunohistochemistry for CD8, FoxP3

Planned correlates include:
• Bulk RNA sequencing from biopsies of all patients enrolled with breast cancer (~25)
• Bulk RNA sequencing of responders vs. non-responders
• Evaluation of circulating tumor DNA to evaluate

Project 2

Evaluate the role of HDAC inhibitors in regulating STAT3 in pro-carcinogenic inflammation.
Establishing *in vitro* MDSC model

- Obtained J774M cell derived from sorting of CD11b+ Gr1+ cells from J774A macrophage line
- Cells suppress T cell proliferation and produce similar immunosuppressive factors as MDSCs
- Cells respond to entinostat in a way similar to *ex vivo* G-MDSCs:

![Graph showing response of J774M and ex vivo G-MDSCs to entinostat](image)

Entinostat inhibits IL6-induced STAT3 phosphorylation in J774M cells

- ![Graph showing phosphorylation levels of STAT3](image)

Planned experiments for preclinical studies:

- Perform ChiP-Seq on treated and untreated J774M cells and *ex vivo* MDSCs to detect changes in STAT3 promoter binding caused by treatment

Planned experiments for clinical studies:

- Immunohistochemistry for STAT3/ pSTAT3, other important markers using biological samples collected from patients with advanced breast cancer (same cohort from aim 1).
- Look for gene targets and pathways identified in cell lines in Bulk-RNA seq results from patient samples
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