Conflict of Interest

• Marker Therapeutics, Inc.
  • Cancer Vaccines and T Cell Therapy – Houston Tx
  • Scientific Advisory Board (unpaid)
  • Several Patent Licensing Agreements (Mayo)

• Kiromic, Inc.
  • Cancer Vaccines – Lubbock, TX
  • Scientific Advisory Board (Stock)

• Antigen Express, Inc.
  • Cancer Vaccines – Cambridge, MA
  • Scientific Advisory Board (Paid)

• Macrogenics, Inc.
  • Biologics – Bethesda, MD
  • Grant funding
Breast Cancer

Worldwide: 1950K cases/year, 650K deaths/year

USA BREAST: 240K cases/year, 40K deaths/year
USA OVARIAN: 22K cases/year, 14K deaths/year

Estimated USA Breast Cancer Costs: $180,000,000,000
1% of the GDP
The adaptive immune system in the body’s drug making machinery

CD4 “helper” T cells
- Inflammation (macrophages and neutrophils)
- Antibodies
- Induce/Enhance cytotoxic T cells
- Immune-surveillance
- Epitope-spreading

CD8 “cytolytic” T cells
- Tumor lysis

B cells
- Antibodies
- Signaling
- ADCC
- Complement

12 million unique T and B cells per teaspoon of blood
Differentiation of the adaptive immune response

Physical Triggers of Immune Response:
- Infections
  - Bacterial, viral
  - Fungal, parasitic
- Toxins
  - Exogenous
  - Endogenous
- Food peptides
- Allergens
- Medications
- Auto antigens

Antigen Presenting Cells

Th17
- Extracellular bacteria (skin, lining of intestine)
- Fungi
- Autoimmunity

Th1
- Cell-mediated immunity and inflammation
- Intracellular pathogens
  - Viruses, bacteria
- Autoimmunity
- Inflammation

Th1
- IL-2
- IFN-γ
- TNF-α

Th0
- Naïve T cell

Th0:
- Naïve T cells
- Helper T cells
- Regulatory T cells
- Interleukin
- TGF-β
- IL-35
- IL-10

TGF-β: Tumor necrosis factor-alpha
IFN-γ: Interferon-gamma
TGF-β: Transforming growth factor-beta

Treg
- Immune tolerance
- Lymphocyte homeostasis
- Regulation of immune responses

Th2
- Antibody-mediated immunity
- Extracellular parasites
- Asthma, allergy

IL-4
IL-5
IL-6
IL-10
IL-13

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Immune-based approaches for cancer

- Cancer vaccines
- Monoclonal and other antibodies
- Adoptive T cell therapies
- Immune checkpoint blockade and reversal of immune suppression
Vaccination is used to heighten the sensitivity of the immune system to tumor antigens.
The Immune System Naturally Responds to Breast Cancer – The T Cell Response is Associated with Improved Survival

Patients with Breast Cancer Demonstrate Elevated T cell and Antibody Immunity to Several Tumor Antigens

- Disis et al., 2000, *Breast Cancer Research and Treatment*
- Kalli et al., 2008, *Cancer Research*
- Karyampudi et al., 2013, *Plos One*
- Krempski, et al. 2011, *Journal of Immunology*
- Knutson, et al., 2006, *Journal of Clinical Oncology*

**HER-2 Breast Cancer – 10 Year Survival Analysis**

- $T_{fh}$
- CXCL3
- $T_{H1}$

Immune suppression in the cancer microenvironment blocks anti-tumor immunity

Cancer Res, 2016
Cancer Immunol and Immunother, 2015
Cancer Immunol Res, 2014
Plos One, 2013
Plos One, 2011
J Immunol 2013
J Immunol 2009
J Immunol 2006
Nature Med 2004
Nature Med 2003
Target neoantigen choices for a cancer vaccine

- Microbial neoantigens
- Amino acid mutation neoantigens
- Frameshift / fusion neoantigens
- Splicing variant neoantigens
- Indel neoantigens
- Nonmutated ‘self’ antigens (subdominant neoantigens)
Overexpressed self proteins as a source of tumor neoantigens

Normal Cell

Tumor Cell

MHC

Dominant Epitope

Subdominant Epitope

MHC

Dominant Epitope

Subdominant Epitope
Normal healthy HER2+ cardiomyocytes are not recognized by HER2 neoepitope specific T cells
Early generation HER2 vaccines

- ECD Vaccine
- ICD Vaccine (Phase I/II)
- HLA-A2 Vaccine (Phase II)
- E75 Vaccine

Knutson KL, et al., JCI 2001
Disis ML, et al., JCO, 2002
Knutson KL, et al., Clin Cancer Res, 2002
HER2 vaccines to protect against disease recurrence in breast cancer

Ag-Specific Vaccines for Prevention of Recurrence

- **JCI 2001**
- **JCO, 2002**
- **Clin Cancer Res, 2002**
- **Clin Cancer Res, 2010**
- **CII, 2010**
- **JCO, 2007**
- **J Clin Immunol, 2004**
- **JCO, 2004**
- **Blood, 2004**
Development of immunity to vaccine is associated with reduced relapse.

Increased response – reduced relapse.

Increased Presence of AE37-specific T cells (Month 0 → 6)

Recurrence above the median: 1/40 (2.5%)
Recurrence below the median: 11/83 (13.3%)

Mean Δ 2988 cpm ± 593

P = 0.05

Courtesy of Eric von Hofe
Vaccine Prolongs Remission in Triple-Negative Breast Cancer

SAN FRANCISCO -- Treatment with a novel peptide vaccine appeared to delay disease recurrence in triple-negative breast cancer (TNBC) patients with low HER2 expression, a subgroup analysis of a phase II trial found.

At a median follow-up of 26.1 months, disease recurrence occurred in 7.5% of TNBC patients who received nelipepimut-S (NeuVax) compared with 26.7% in the control arm (HR 0.26, 95% CI 0.08-0.81, \( P = 0.01 \)), reported Guy T. Clifton, MD, of San Antonio Military Medical Center in Texas.

"We think the results are intriguing in light of what we now understand as far as triple-negative breast cancer being a more immunogenic subtype of breast cancer that's more responsive to immunotherapy," he said during his presentation here at the ASCO-SITC Clinical Immuno-Oncology Symposium.

In the NeuVax and control arms, respectively, rates of disease-free survival (DFS) among the 97 TNBC patients were:

- 92.6% versus 70.2% at 24 months
- 82.3% versus 70.2% at 36 months
Human MHC Locus

DRB1*0101, DRB1*0301
DRB1*0401, DRB1*0404
DRB1*0405, DRB1*0701
DRB1*0802, DRB1*0901
DRB1*1101, DRB1*1201
DRB1*1302, DRB1*1501
DRB3*0101, DRB4*0101
DRB5*0101
## Binding of predicted HER2 neoantigens to purified HLA-DR

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Peptide Name</th>
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<tbody>
<tr>
<td>NLELYLPTNASLSF</td>
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<tr>
<td>LTYLPTNASLSFLQD</td>
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<td>HQVRVQPLQRRLIV</td>
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</tr>
</tbody>
</table>

*Position of N-terminal amino acid; ND=not determined; Peptides that constitute degenerate pool are in bold

**IC₅₀ nM to purified HLA**

**Karyampudi, Cancer Res, 2010**
Detection of pre-existent immunity

Karyampudi et. al., Clin Cancer Res. 2010

DRB1*0101, DRB1*0301
DRB1*0401, DRB1*0404
DRB1*0405, DRB1*0701
DRB1*0802, DRB1*0901
DRB1*1101, DRB1*1201
DRB1*1302, DRB1*1501
DRB3*0101, DRB4*0101
DRB5*0101
Vaccine induces immunity to naturally processed antigens

Knutson et. al., 2019 under review
Majority of patients can be vaccinated
Generation of durable HER2-specific T cells in majority of patients with resected HER2 breast cancer

Knutson et. al., 2019 under review

Norton, Breast Cancer Res Treat, 2018
Knutson, Cancer Res 2016
Taylor, Clin Cancer Res, 2007
BC170530: Phase II resected advanced HER2+ breast cancer

Stage II-III HER2+ BC N=380

Neoadjuvant HP Chemotherapy

Surgery

1. ARM 1
   pCR N=190
   HP maintenance q 3 wk 1 year
   Follow up

2. ARM 2
   TDM-1 maintenance + Placebo
   Follow up

3. ARM 3
   TDM-1 maintenance + H2NVAC
   Follow up

Tissue collection prior to neoadjuvant chemotherapy (NAC) and at time of the surgery.

Research blood draw prior to NAC and at the time of surgery. For patients with pCR, research blood draw will be collected on cycle 1 of maintenance with trastuzumab and pertuzumab (HP), 30 days and 24 months after completion of HP. For patients without pCR, who will be randomized, research blood draw will be collected on cycle 1, 4, 7 of maintenance HP as well as 30 days, 3, 12, and 24 months after completion of HP.

Placebo vaccination every 4 weeks for 6 cycles.

HER2 vaccination every 4 weeks for 6 cycles.
Spontaneous immunity to the folate receptor alpha in cancer patients

Knutson, K. L. et al. JCO; 24:4254-4261 2006
Folate receptor alpha peptide vaccine generates immunity in breast and ovarian cancer patients

Kalli, Block *Clin Cancer Res*, 2018
BC141410: FRa Vaccination to Prevent Progression of Triple Negative Breast Cancer

FRa is preferentially overexpressed in TNBC

- Multicenter Phase II Trial to Test Whether Vaccine Prevents Recurrence in Patients Diagnosed and Treated for TNBC

Stages IIb/III TNBC

Convention al Therapy

Placebo N=93

Vaccine N=187

IL-17 association with improved survival in ovarian cancer

Kryczek et al., JI 2011
Th17-inducing vaccines generate Th1 and Th17 immunity

Block, 2017, Unpublished Observations, SPORE P8
The generation of antibody immunity is associated with improved survival.

Antigen-specific antibodies (µg/ml)

- FR30: p=0.28
- FR56: p=0.71
- FR76: p=0.002
- FR113: p<0.0001
- FR238: p<0.0001
- FRα: p=0.001

Time (weeks)

- No Recurrence
- Recurrence

Block, 2017, Unpublished Observations, SPORE P8
Neoantigen discovery bioinformatics pipelines

- Maximized discovery of insertional and deletional neoepitopes
- Identification of MHC class I and class II
- Minimized cross-reactivity
- Optimized HLA genotyping
Breast cancer appears to be enriched in the type of neoantigens that are highly immunogenic.
Neoantigens are largely private making every product different
Mutation rates in different types of breast cancer

- **Neoantigen Load and Race**
  - Asian
  - Black Race
  - White

- **Neoantigen Load and BRCA Subtype**
  - Basal
  - Her2
  - LumA
  - LumB
  - Normal

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Mutation rates in different types of breast cancer

![Box plot showing neoantigen load and tumor stage]

- **Neoantigen Load and Tumor Stage**
  - Tumor Stage: i, ii, iii, iia, iib, iib, iia, iv, x
  - Log2 Neoantigen Load: 0, 5, 10, 15, 20

The box plot illustrates the distribution of neoantigen load across different tumor stages.
Neoantigen-based trial
The Checkpoint Blockade Revolution

Lymphomation.org
Immune checkpoint blockade for TNBC

A Progression-free Survival in the Intention-to-Treat Population

B Progression-free Survival in the PD-L1-Positive Subgroup

C Overall Survival in the Intention-to-Treat Population

D Overall Survival in the PD-L1-Positive Subgroup
Combination therapy results in complete regression and sustained progression free survival

~75% Complete Regression Rate

Combination therapy results higher infiltration of memory effector T cells

Goals

• To develop a vaccine that targets all three major subsets of breast cancer
• To develop a vaccine that reduces the incidence of breast cancer
• To develop a vaccine that prevents death from breast cancer
• To develop a safe and cost-effective vaccine
The mammary gland has a mucosal immune system
• HER2/neu (185 kDa) (OC 30%)
  • Cell surface growth factor receptor.
  • Angiogenesis, proliferation, embryonic development.
  • Expressed in majority of breast cancers and amplified in 20%.
  • Associated with aggressive behavior.

• MAGE3 (34 kDa) (OC 100%)
  • Limited to placental trophoblast cells and germ cells of the testes.
  • Function is not known.
  • Expressed in ~50% of breast cancers.

• MUC1 (225-500 kDa) (OC 95%)
  • Large membrane glycosylated protein – lubrication/hydration.
  • Overexpressed and aberrantly glycosylated in 90% of breast cancer.

• Survivin (16 kDa) (OC 85%)
  • Anti-apoptosis protein.
  • Extensive expression in fetal and embryonic development. Not expressed in normal differentiated cells.
  • Expressed in more than 90% of breast cancer.

• Mammaglobin A (10 kDa) (OC ?)
  • Secretory protein of unknown function.
  • Very limited expression in normal healthy tissue and expressed 10 fold-higher in 40-80% of breast cancers.

• hTERT (126 kDa) (OC 100%)
  • Main protein component of the telomerase enzyme, an enzyme that maintains the length of chromosomes.
  • Not expressed in dividing cells but overexpressed in more than 90% of breast cancer.

1) To develop a vaccine that targets all three major subsets of breast cancer
2) To develop a vaccine that reduces the incidence of breast cancer
3) To develop a vaccine that prevents death from breast cancer
4) To develop a safe and cost-effective vaccine
<table>
<thead>
<tr>
<th>Product</th>
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<th>Preclinical</th>
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<th>Phase 2</th>
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<tr>
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<td>FR DC Vaccine</td>
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<td>FR</td>
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<td>FR</td>
<td>Platinum-Sensitive Ovarian Cancer (Fast Track)</td>
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<td>Prophylactic</td>
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</table>
Conclusions

- More needs to be done in the disease free period to boost host immunity against cancers at high risk for relapse.
- Vaccines can be developed that target aberrantly expressed proteins. Useful for preventing disease recurrence?
- Repolarizing immune response may improve outcomes.
- Checkpoint activity appears to be limited for TNBC but may be improved by inclusion of vaccines.

Breast Cancer
Typical Tx Disease Course

Window of opportunity to boost host immune defenses

Tumor volume

Dx/surg Chemo Observe Asymptomatic recurrence Clinical recurrence

Months

3,500

35
Acknowledgements

Mayo


VGTI FL

Lavakumar Karyampudi, Ph.D.       Patrick Yeramian, M.D. Ph.D.       Richard Jove, Ph.D.       Kathleen Kemp       Shaun White, M.A.

NBCC

Frank Calzone, Ph.D.       Sylvia Formenti, M.D.       Alan Welm, Ph.D.       Fran Visco, J.D.

Financial support

National Breast Cancer Coalition
VGTI FL
K01 100764
R01 113861
R01 152045
Mayo Ovarian Cancer SPORE
Mayo Breast Cancer SPORE
Mayo Comp Cancer Center
Komen Foundation
Mayo CTSA
MOCA
VaxOnco
TapImmune
Andersen Foundation
Cancurables
National Breast Cancer Coalition
Department of Defense BCRP
Department of Defense OCRP

Other

Raphael Clynes, M.D. Ph.D.  Columbia University
Martin Cannon, Ph.D. University of Arkansas
Nora Disis, M.D. UW
Mac Cheever, M.D. UW
Doug McNeel, M.D. Ph.D.  Uwisc
Glynn Wilson, Ph.D. Tapimmune
Eric von Hofe, Ph.D. Antigen Express