

## State of the Research Report

January 2012

Friends,

I would like to review the progress we have made over the past year and the tremendous increase in talent now participating on the project, as well as, the number of new members that will be joining this year. Obviously, we have a long way to go to eliminate breast cancer, but progress is beginning to be seen more rapidly this year. I will review our partnerships and the ongoing projects that are aimed at the elimination of breast cancer and other cancers. Below is a summary of activities, followed by a short description of each one.

- **Publications:**

- *Journal of Immunotherapy*, January 2012
    - [A Novel Dendritic Cell-based Immunization Approach for the Induction of Durable Th1-polarized Anti-HER-2/neu Responses in Women With Early Breast Cancer](#) Koski, Gary K.; Koldovsky, Ursula; Xu, Shuwen; Mick, Rosemarie; Sharma, Anupama; Fitzpatrick, Elizabeth; Weinstein, Susan; Nisenbaum, Harvey; Levine, Bruce L.; Fox, Kevin; Zhang, Paul; Czerniecki, Brian J. *Journal of Immunotherapy*. 35(1):54-65, January 2012. doi: 10.1097/CJI.0b013e318235f512
  - *Cancer*, Journal of American Cancer Society –will be published in next few months
  - Cancer Prevention Book, chapter about Vaccines in Breast Cancer Prevention –published later in 2012
  - Two manuscripts with the George Coukos Group
    - [PLoS One](#). 2011;6(12):e28732. Epub 2011 Dec 14. **Day-4 myeloid dendritic cells pulsed with whole tumor lysate are highly immunogenic and elicit potent anti-tumor responses.** [Chiang CL](#), [Hagemann AR](#), [Leskowitz R](#), [Mick R](#), [Garrabrant T](#), [Czerniecki BJ](#), [Kandalaft LE](#), [Powell DJ Jr](#), [Coukos G](#).
    - **Chiang CL, Maier DA, Kandalaft LE, Brennan AL, Lanitis E, Ye Q, Levine BL, Powell DJ Jr, Coukos G: Optimizing the parameters for clinical scale production of high IL-12 secreting dendritic cells pulsed with oxidized whole tumor cell lysate.** *J Translat Med* 9(1):198, 2011.
  - Finishing manuscript about freezing the vaccine
- **DCIS Second Trial**
  - **Other new HER Family Targets**
  - **HER-3 Immune Response**
  - **Vaccines for Early Invasive Breast Cancer**
  - **Vaccines for Patients with Large HER-2<sup>POS</sup> Breast Cancer Following Chemotherapy and Herceptin**
  - **Prevention Programs for BRCA Mutation Carriers**
  - **Prime- Boost Vaccines**
  - **Melanoma Vaccines**

- **Compassionate Use Vaccines for Metastatic Pancreatic Cancer, Triple Negative, and HER-2<sup>pos</sup> Breast Cancer**

#### **Publications:**

We have published two papers this year from our first DCIS trial, alluding to its tremendous success. One was published in the *Journal of Immunotherapy* in January 2012, and the second will be coming out in *Cancer* within the next couple of months. **Shayna Showalter, our Breast Fellow**, and I had put together a chapter about vaccines for a cancer prevention book to be published shortly. Together with the **George Coukos Group**, we have published two manuscripts demonstrating that the modifications of our dendritic cell vaccine can be used when pulsed with ovarian cancer cells for personalized vaccines. **Elizabeth Fitzpatrick, who is our director of vaccine production**, is completing a manuscript showing we can successfully freeze our activated dendritic cell vaccines and they retain their activity. This is very important because it means vaccines can be mass produced from patients anywhere and shipped. All of these are major accomplishments in the past year.

**In Layman's Terms: We have been busy scientifically and continue to receive the support of peer-review with our scientific writings.**

#### **DCIS Second Trial:**

We are continuing this trial with around 26 patients accrued and we have modified the trial for patients that are estrogen receptor positive to receive anti-estrogen therapy with vaccines. Our trial was highlighted on the CBS Evening News (11/16/2011). The patient highlighted was the first with our combination anti-estrogen vaccine component and had no disease left at surgery. Immune response rates in this trial are close to 90%, a remarkable outcome. We are continuing the trial through around 54 patients. With the data we have collected we are anticipating applying to the NIH for renewal to expand the anti-estrogen therapy with the vaccine group into Phase II and for those that are estrogen negative HER-2<sup>pos</sup> add trastuzumab (Herceptin) with vaccines. We anticipate these maneuvers will continue to help us get to complete responses (meaning no disease left at surgery) for all these patients, eliminating long courses of anti-estrogen therapy (5 years), and reducing the need for radiation therapy and mastectomy rates. We are confident that the vaccines will help reduce recurrence rates and new breast cancers. **Elizabeth Fitzpatrick and Shuwen Xu, MD** continue to provide the engine for these studies.

**In Layman's Terms: This trial is still accumulating patients rapidly and is heading toward our goal of 50 patients. We are combining vaccines with a brief course of Tamoxifen or Letrozole (aromatase inhibitors) for patients with estrogen positive HER-2 positive DCIS: An exciting combination to block two pathways and increase the number of complete responses.**

### **Other new HER Family Targets:**

**Holly Graves, a Penn surgery resident**, has been performing pathology staining in the lab in patients with DCIS with HER-3, HER-4, HER-1 and survivin. She has made the observation that survivin expression in DCIS increases with grade, and although not associated with invasion, is a good immunologic target independent of HER-Family expression. She is about to submit a paper with **Dr. Paul Zhang, in Pathology**, describing the role of survivin in DCIS. It is known that this molecule may play a role in recurrence. Holly is also making critical findings with HER-3 and is showing it is expressed in a large number of DCIS lesions. This is great news as it confirms our suspicion that other HER family members are involved in early breast cancer and invasive breast cancer, and is responsible for resistance to some of the breast cancer recurrences following Herceptin. Holly has shown HER-3 expression remains in some of our HER-2 vaccine patients on residual DCIS and thus is prime for targeting. Holly is looking at the association between HER-3 expression and the development of invasive breast cancer in DCIS and it appears to be highly associated like HER-2! Anti-HER-3 vaccines and antibodies would be primed to prevent breast cancer invasion and not only in HER-2 cancers. Data is accumulating that many patients' breast cancers are HER-3 positive and would be a target for personalized therapy. Herceptin treated patients often fail in the brain (meaning the treatment fails and the cancer moves into the brain) and HER-3 expression is associated with metastatic brain tumors. Others have found HER-3 expression in melanoma, lung cancer, brain cancer, and colon cancer, suggesting a critical target for attack. HER-1 is expressed on a minority of breast cancers, generally triple negative, but is another target of the family. We are trying to convince our pathologists to assess these molecules in all of our breast cancer patients for profiling and therapy decisions. Holly is also looking at other biomarkers in DCIS and including some of these biomarkers in melanoma.

**In Layman's Terms: We are identifying the role of HER-2's sister protein in early breast cancer so that we can effectively target these and develop other vaccines to help with resistance and recurrence to anti-estrogen and Herceptin therapy. This is important in preventing recurrence.**

### **HER-3 Immune Response:**

**Sara Matthews, a Penn surgery resident**, joined the lab in July to identify peptides from HER-3, survivin, and HER-1 that can be developed and used for our next generation of vaccines. She has started with HER-3 and made amazing progress. She has analyzed the protein and designed peptides for CD8 T cells. She has already identified two of these peptides to which patients can react! This is the first demonstration of peptides from HER-3 that can be used for targeting HER-3. She is now testing tumor cells to find HER-3 expression so as to use these CD8 T cells to determine whether they can recognize and kill HER-3 expressing cancers. She has done the same with CD4 peptides and will be testing those shortly. She has really settled in well and will test normal donors for HER-3 activation as well. She will continue with HER-1 and survivin. This is exciting because we are going to be able to rapidly add these peptide targets to vaccines for patients with breast cancers and melanoma. Other tumors such as

ovarian cancer, colon cancer, pancreatic cancer, and lung cancer are also targets for these HER-3 vaccines.

**In Layman's Terms: We are already beginning to develop the peptides that can be used to target HER-3, attacking a critical path in cancer development in estrogen positive, HER-2 positive and triple negative breast cancer. This work is the prelude to bringing this into the clinic very shortly.**

#### **Vaccines for Early Invasive Breast Cancer:**

Inspired by one of our vaccine patients, we are committed to use our HER-2 dendritic cell vaccine in combination with Herceptin (3 doses) for patients with early HER-2<sup>pos</sup> breast cancer. This would be the first trial for these patients without the use of chemotherapy. **PURE IMMUNOTHERAPY! Dr Carol Tweed, a medical oncologist**, is supporting and developing this protocol. We are applying for funding for this trial. All patients will have T1a T1b HER-2<sup>pos</sup> ( $\leq 1$  cm) breast cancer. This may be the beginning of the end for chemotherapy.

#### **Vaccines for Patients with Large HER-2<sup>pos</sup> Breast Cancer Following Chemotherapy and Herceptin:**

We are developing a breast cancer vaccine with HER-2 and HER-3 to give patients after chemotherapy and Herceptin to help prevent recurrence. **Dr. Angela DeMichele, in Medical Oncology**, is helping to develop clinical trials that can be used for these patients. The HER-3 vaccine we are developing will be critical in this trial. **Rachel Yang, a medical student**, is looking at the immune responses that develop in these patients from chemotherapy and Herceptin. This study will be open in February and we will be recruiting patients to give us blood to see whether the development of a cellular immune response is associated with preventing recurrence. Anyone in this category we need your blood help us out.

**We are hopeful that we will be able to get the Abramson Cancer Center to support a Translational Center of Excellence for Secondary Prevention of Breast Cancer. A lot of breast cancer patients fear the recurrences that can develop even many years after eliminating the primary disease. I suggest you support this cause by letting the Abramson Cancer Center Director Chi Van Dang and Hospital Administration Ralph Mueller know that you support and demand such a novel program.**

**In Layman's Terms: A late recurrence is something all breast cancer patients fear. We are developing the tools to predict immune response in patients treated with Herceptin as to who will and will not recur, and use our vaccines for those that are likely to recur to prevent a late recurrence. We want to develop a center for breast cancer patients devoted to determining and reducing their risk of recurrence without having to wait 10 or 15 years until recurrence happens! This would be the first center of its kind in cancer care. I suggest you support this cause by letting the Abramson Cancer Center Director Chi Van Dang and Hospital Administration Ralph Muller know that you support and demand such a novel program.**

### **Prevention Programs for BRCA Mutation Carriers:**

**Rachel Yang, a medical student**, is spending a year with us evaluating the DCIS and Breast Cancer patients with HER-family members that are mutation carriers as a first step to assessing whether our HER family dendritic cell vaccines can be utilized for these patients that often have a 70% lifetime risk of breast cancer. If these patients have HER-1, HER-2 or HER-3 expression on their tumors then it would make sense that this would be the ideal group in which to test a breast cancer prevention vaccine. **Dr. Susan Domcheck, the Director of the High Risk Screening Genetic Clinic** at Rowan Breast Center, is assisting with this project.

**In Layman's Terms: We are identifying targets in patients that have inherited the breast cancer genes to offer them vaccines for prevention as alternative to bilateral mastectomies. That's a good thing: who at 20 or 30 should face bilateral mastectomies?**

### **Prime-Boost Vaccines:**

**Dr. Gary Koski, our colleague at Kent State**, is busy at work identifying the peptides that Herceptin and pertuzumab recognize so that we can add these peptides to our current vaccines so that patients can make their own Herceptin and pertuzumab. We will also identify peptides that can be used to generate antibodies against HER-3 and HER-1. This is exciting and will be used with our early breast cancer vaccine trials and our late adjuvant HER-2, HER-3 Vaccines. This data is moving along very nicely. This is our next big addition to these vaccines.

**In Layman's Terms: We can teach patients' own immune systems to make their own Herceptin and pertuzumab for lifelong surveillance against recurrence.**

### **Melanoma Vaccines:**

**Jessica Cintolo, a Penn Surgery Resident** who started in July, is working in a mouse model and in human melanoma to assess whether vaccines can be developed against the melanoma protein BRAF and HER-3. She has a model of melanoma development in the mouse that is caused by BRAF. Working with our colleagues at Wistar Institute, she is studying anti-BRAF vaccines and combinations of BRAF kinase medications and vaccines in melanoma therapy. Jessica, with Holly Graves, is assessing HER-3 expression in patients receiving BRAF kinase drugs to determine whether HER-3 vaccines combined with BRAF will be useful to eliminate metastatic melanoma. Jessica has the mouse models approved and is purchasing mice for breeding while assessing the HER-3 expression in patients with metastatic melanoma.

**In Layman's Terms: Our vaccine program can be used to combat melanoma that has spread and try to reduce deaths in this deadly disease. We will combine it with the new melanoma targeted therapy to eliminate remaining cells that often become resistant to drug therapy.**

## **Compassionate Use Vaccines for Metastatic Pancreatic Cancer, Triple Negative, and HER-2<sup>pos</sup> Breast Cancer:**

We have lost some good friends to pancreatic cancer and metastatic breast cancer and therefore are bringing in some of our new peptides against HER-1, survivin, and Mesothelin against these metastatic cancers. Unfortunately, we have only CD8 peptides for these antigens thus far, so we will use our HER-2 backbone. However this means all the patients being treated have to be HLA A2 haplotype to be able to derive any benefit. This type of study is impossible to fund because of the nature of the vaccine and the stage of patients, but we are happy that Pennies in Action will supply money so that these patients can receive the vaccine. The trial is with the FDA and the IRB. Currently we anticipate being able to provide vaccines for patients by late February or early March. Each of these treatments cost about \$5,000-\$10,000 per patient.

**In Layman's Terms: We can offer some hope to those that have metastatic breast cancer (triple negative or HER-2 positive) or those with metastatic pancreatic cancer to try to get their immune response rolling after they have finished chemotherapies. Pennies in Action is supporting this endeavor completely as no scientific funding agency would take on a task like this. We will get Phase I data as a result with new targets.**

## **Conclusion**

As can be seen, there is a lot of activity ongoing in the group. We have three others joining the lab this spring and summer. As a result of the Henle Foundation support, we have been able to provide a new two year research fellowship experience. This year, starting later in the spring, **Eric Berk** will join the lab from the University of Pittsburgh. Eric will complete his PhD in March in dendritic cell biology with **Dr. Pawel Kallinski**. He will study T cell dendritic cell interactions. **Kathryn Lee, a Penn Surgery resident**, will join us in July and **Megan Fracol, a medical student at Penn**, will join us in the summer for eight weeks and study whether our T cells and antibodies from vaccinated patients can prevent in 3D models Breast Cancer Invasion. Megan has learned Penn selected her project above all other students for national competition. We are also pretty sure that **Dr. Robert Roses will be returning from MD Anderson to the faculty at Penn** and will take a leading role in our Vaccine Projects as well. Robert was a major contributor when he was a resident. We take this opportunity to thank all of our donors and sponsors for both their inspirational and financial support. We hope to bring this annual report to our friends yearly so they can see where we are going. We are indebted to Pennies in Action for the purchase of new incubators, a refrigerator and a microscope for performing our pathology work. Our numbers of young scientists are growing and we are continuing to gain traction. We expect to continue the growth and accomplishments of this group as we report in 2013.

A healthy and Blessed New Year to all our friends,

Brian J Czerniecki, MD, PhD