LION HEART

Grantees for 2023

Developing CAR-mast cell therapy for breast cancers

Principal Investigator: Xiaolei Su, PhD. Assistant Professor of Cell Biology, Yale School of Medicine

Co-Investigators: Sidi Chen, PhD, Associate Professor of Genetics and Neurosurgery, Yale School of Medicine Christopher Kepley, Associate Professor of Nanoscience, University North Carolina Greensboro

New high-impact cancer therapies harness the patient's immune system to attack their cancers. Combination therapies including immune checkpoint inhibitors that release restraints on anti-tumor responses of T cells have moderate efficacy in breast cancer and have been approved by the FDA. Another new approach is Chimeric antigen receptor-T (CAR-T) therapy, in which a patient's T cells are engineered to recognize and attack specific molecules expressed by cancers. While CAR-T therapies have been extraordinarily successful for controlling some blood cancers, they have not shown much utility on solid tumors including breast cancer. Reasons include challenges in maintaining stable T cell localization at tumors, the generally immune suppressive neighborhood of tumors, and exhaustion of activated T cells. T cells are only one cellular component of effective immune responses, and recent findings indicate that another type of immune cells, mast cells, can inhibit tumor growth as well. Hence Dr. Su plans to open a new area of research by engineering mast cells to recognize tumor cells and alert other immune cells including the patient's T and Natural Killer cells so that a coordinated anti-tumor response will be mounted. Use of CAR-mast cells should circumvent some of the practical problems leading to failure of CAR-T therapies, especially as mast cells reside in sites for long periods of time, and can be expected to continue to produce factors for prolonged recruitment of T cells and other immune cells to the tumors and maintenance of a more immune-active microenvironment local to the tumor cells. Moreover, it is expected that the CAR-mast cells engineered to recognize will collaborate with the patient's T cells and natural killer cells to provide a powerful and coordinated anti-tumor immune response. The technical approach uses the same general strategy as used for CAR-T production, but include an innovative screen for optimal signaling domains to be fused with the tumor targeting domains that bind HER2 or GD2 proteins upregulated in breast cancers. Dr. Su anticipates that working with mast cells will circumvent known issues limiting success of CAR-T in breast cancer.

This exciting proposal builds on Dr. Su's leadership investigation of immune cell signaling. Drs. Chen and Kepley add expertise in genome editing and mast cell biology, respectively. Success will introduce a new mode of therapy (CAR-mast) into the immune modulation armamentarium that will advance efficacy of adoptive cell therapies in breast cancer and are expected to have major impact in combination with immune checkpoint therapies.

Development of chimeric antigen receptor (CAR) T cells targeting MET in metastatic triple negative breast cancer

Principal Investigator: Po-Han Chen, MD, PhD Instructor in Hematopathology, Dept. of Pathology, Yale School of Medicine
Co-Investigator: Samuel Katz, MD, PhD Associate Professor of Pathology, Yale School of Medicine
Collaborator: Qin Yan, PhD Professor of Pathology, Yale School of Medicine

Chimeric antigen receptor (CAR)- T cell therapies, in which a patient's T cells are engineered to recognize cancer cells, have been successful for controlling of some blood cancers, but are ineffectual against solid tumors such as breast cancers. The reasons include limited choice of targeting molecules in breast cancer and early exhaustion of T cell populations that do find their tumor target. Dr. Chen will engineer T cells to express an antibody targeting them to MET, a protein that is overexpressed in triple negative breast cancers using state-of-the-art CAR-T production methods and is also a therapeutic target for drug resistance. The fact that

MET has been targeted in other contexts for cancer therapy means that there is some information on expected toxicity profiles that will accelerate translation to the clinic if the method is effective in model systems. T cells targeted to MET-expressing breast cancers are expected to kill tumor cells through the "extrinsic" cell death pathway. Dr. Chen will further test the hypothesis that the lethality of these engineered T cells will synergize in combination with cell death agonists that sensitize tumor cells to killing through this mechanism and have some efficacy as TNBC monotherapies.

This funding will not only support an innovative new CAR-T targeting approach but will further help launch Dr. Chen as an independent junior scientist who has specialized training in immuno-oncology. Dr. Katz provides expertise through his own experience in mechanisms of cell death and design and production of CAR-T cells, and Dr. Yan contributes experience in cell culture and animal modeling to understand and treat breast cancer.

Investigating a novel epigenetic mechanism in breast cancer

Principal Investigator: Andrew Xiao, Ph.D. Associate Professor of Genetics, Yale School of Medicine

Epigenetic regulators trigger cancer development and resistance to therapies through two general mechanisms. First, they can operate much like DNA mutations in imposing stable changes that activate or suppress expression of individual genes. For example, extinguishing expression of a tumor suppressor gene will have the same impact as mutating that gene, since both mechanisms prevent the gene product from doing its job. Secondly, epigenetic regulators jointly regulate many genes, and can even switch cells across to different general states or identities. This can promote or interfere with the ability of tumor cells to evolve and eventually escape the effects of cancer therapies. Histones are proteins that package the entire genome and are important mediators of epigenetic regulation. There are several forms of histones, each of which has specialized functions. One of the poorly characterized histone variant is highly expressed in subsets of breast cancers. Dr. Xiao's laboratory has shown that this histone variant forms a complex with a major breast cancer tumor suppressor, where it presumably alters the ability of the tumor suppressor to modulate expression of target genes and processes including cell division and stem-like cell properties. The plan is to investigate the involvement of this histone variant in recruitment of the tumor suppressor to its DNA target sites, and the impact on regulation of its well-characterized targets. Since this tumor suppressor gene is only one of many genes that may be affected by this histone variant. Aim2 will evaluate differences in DNA bound sites of this histone variant across breast cancer subtypes and determine the impact of this histone variant on breast cancer phenotypes including

progression, stemness, and invasiveness. This work will be done using patient-derived xenograft models that retain high fidelity to the tumor of origin.

Dr. Xiao is a leader in developmental epigenetics with an established cancer research program, but new to breast cancer research. Success of this pioneering research on this histone variant will advance understanding of a poorly understood category of epigenetic regulators that has been linked to breast cancer, and may deepen understanding of a significant breast cancer tumor suppressor. This work may in the long run lead to new therapeutic approaches to control breast cancer.

Grantees for 2022

Immunoproteomics of targeted protein degradation - $50,000

Principal Investigator

Stacy A. Malaker, Ph.D. Assistant Professor Department of Chemistry Yale University

Co-Investigator

Craig M. Crews, Ph.D.
American Cancer Society Professor
John C. Malone Professor of Molecular, Cellular, and Developmental Biology Department of Chemistry, Department of Pharmacology

Co-Investigator Dr. Crews invented a new form of anti-cancer therapy called PROTACs. A drug that binds to proteins marking cancer cells is joined to another molecule that targets that protein for destruction. The target protein is eliminated by being cut up into small protein fragments. The proposal takes advantage of the fact that these fragments are unique to the targeted cancer cell and may be recognized by the immune system. For example, PROTAC drugs that bind to the estrogen receptor are now in clinical trials. They could potentially have two kinds of therapeutic impact. First, they would promote destruction of the estrogen receptor which is driving the cancer. Second, fragments derived by destruction of the estrogen receptor may be presented on the surface of the cancer cells. This may incite a T cell immune response that would help control the cancer. Aim 1 of the proposal will determine if such immune responses occur and which protein fragments are most important, using a protein marker that is often overexpressed in breast cancer. If so, this information will be used to guide development of second generation PROTACs optimized to provoke immune attack. The second Aim takes a different approach to exploiting this phenomenon. Many cancer cells overexpress the cell surface receptor MET, which confers resistance to targeted agents such as Herceptin/Trastuzumab by promoting cancer growth. In principle, a PROTAC targeting MET for degradation would both inactivate MET and also cause cell surface expression of some of the MET fragments. These fragments will be identified. Antibodies will be produced that bind to the surface form of these fragments. Finally, these antibodies will be coupled to a second set of antibodies that bind to immune cells. These double or "bispecific" antibodies can be used as drugs that physically connect immune cells to tumor cells with high MET, leading to an anti-tumor immune response.

Quoting Professor, David F. Stern, Ph.D., Professor Dept of Pathology, and Vice Chair of Basic and Translational Services, Yale Cancer Center, and the Chair of the Lion Heart Advisory Committee"

"This innovative proposal may reveal new therapeutic avenues for immune-mediated breast cancer control. The project will be led by a dream team consisting of a junior investigator with deep expertise in the complex immunological experiments proposed working with Dr. Crews, an extraordinarily innovative scientist who has revolutionized cancer pharmacology with development of Carfilzomib and now the PROTAC technology."

Exercise as an Adjuvant to Pembrolizumab in the Treatment of Triple Negative Breast Cancer - $50,000
Principal Investigator
Rachel J. Perry, Ph.D. Assistant Professor of Medicine/Endocrinology and Cellular & Molecular Physiology, Yale University School of Medicine

Immune checkpoint therapies have had enormous therapeutic impact in melanoma and lung cancer, but are less effective in other cancers including triple negative breast cancer. The positive impact of exercise on breast cancer outcomes and quality of life is well-documented. Dr. Perry's earlier research, funded in part by a 2019 Lion Heart award, has linked high glucose with growth of breast tumors in mouse breast cancer models. She hypothesizes that exercise will increase insulin-dependent glucose uptake in muscle and liver, depriving tumor cells of glucose. She further supposes that this rebalancing of glucose metabolism affects nutrient competition between tumor cells and T cells and may indirectly favor T cell activity promoting success of anti-tumor immune control. Dr Perry plans to evaluate the impact of exercise on the metabolism of breast tumors and immune cells in a triple negative breast cancer mouse model treated with pembrolizumab. A second series of experiments will evaluate the interplay of exercise and obesity on tumor growth in these models when treated with pembrolizumab.
Success of this proposal will clarify a major mechanism proposed to link exercise with more favorable outcomes, will reveal how exercise alters glucose metabolism and uptake by tissue depots, immune cells and breast tumor cells and will identify the functional consequences for tumor growth and T cell activation. These findings will be linked to efficacy of PD1 inhibitor Pembrolizumab, which is already approved by the US FDA, and hence may be rapidly translated to human clinical settings in follow-up studies.

Dr. Perry is a gifted young investigator who uses her expertise in metabolism to develop new cancer therapies. Her 2019 Lion Heart research is scheduled to go into a clinical trial in March 2023 to address whether normalization of hyperinsulinemia in obese, non-diabetic breast cancer patients increases chemotherapy sensitivity. This is another clear sign that our granting philosophy is working and that we are making a difference.

Functional interrogation of putative oncogenic drivers in breast cancer - $ 50,000
Principal Investigator Arnaud Augert, Ph.D.
Assistant Professor, Department of Pathology, Yale University School of Medicine

Each breast cancer is caused by a small number of genetic and epigenetic alterations. Many of the important breast cancer oncogenes and tumor suppressors have been identified because they are often changed in breast cancer. For example, the PIK3CA gene is mutated and activated in almost half of all breast cancers and it is a therapeutic target. These mutations coincide in the same tumors with a number other chromosomal changes, a subset of which may be functionally important but have not been evaluated. Dr. Augert has compiled a list of 122 candidate genes that may contribute to development or aggressiveness of breast tumors with PIK3CA mutations, as they reduplicated and overexpressed in combination with PIK3CA in human breast cancers. An innovative CRISPR-based technology will be used to systematically activate these genes in mammary cells of mice that have been engineered to express activated PIK3CA. Tumors that develop early in these mice are likely to harbor CRISPR vectors that activate oncogenic genes, which can be identified by DNA sequencing. This project is likely to reveal new breast cancer oncogenes that synergize with PIK3CA mutations. Therapeutic agents that target these new genes and the processes they regulate make excellent combination agents that synergize with PIK3CA inhibitors in patients with matching gene reduplications. This is important as clinical use of PIK3CA drugs has been constrained by toxicities and effective partner agents will reduce the dose required for efficacy.

Dr. Augert is a new investigator who recently joined the Yale faculty with a superb track record of research on non-small cell lung cancer. Success of this project will divert a major component of his research program towards breast cancer, with life-long career impact.

Grantees for 2021

Targeting DNA Replication Initiation for Cancer Therapy: Franziska Bleichert, PhD, $45,272

This research project will identify drug inhibitors of a normal cellular process, *licensing of replication origins*, as a new therapeutic approach to exploit vulnerabilities of breast cancers. Every cell must replicate its entire DNA content once and only once in preparation for each cell division. Because of the large amount of DNA to replicate, this task is divided up among many replication factories ("replication complexes") operating in parallel that each duplicate a portion of the genome. For this process to be successful, there must be a sufficient number of replication complexes, and they must have sufficient raw materials (DNA building blocks) to complete replication. Failure of these complexes can lead to inadequate DNA synthesis resulting in breaks in the DNA and cell death. The main goal of the project is to identify new drugs that will inhibit the process of replication licensing, which determines the number of DNA replication complexes. Interference with this process will reduce the number of replication complexes, straining the ability of cells to complete DNA replication. Breast cancer cells should be particularly sensitive to such inhibitors for two reasons. Hormone receptor positive and HER2 positive breast cancers have a propensity to activate DNA replication prematurely, when there may be an inadequate number of DNA precursors, and hence are already under replicational stress. Triple negative breast cancers, including those with BRCA1 or BRCA2 mutations, are often defective for the DNA repair process that fixes DNA breaks resulting from replication stress. The investigator will develop an elegant biochemical assay for high throughput screening, in collaboration with Yale Center for Molecular Discovery. ***This innovative project will target a hitherto unexplored cellular process, and have the potential to identify an entirely new class of anti-cancer drugs particularly relevant to breast cancer.*** The committee felt that this research employed an innovative and sophisticated approach. The researcher is a new investigator presenting a novel process.

Activating MKL1/SRF pathway to target breast cancer metastasis:
PI Shangqin Guo, PhD, Co-Investigator Qin Yan, PhD, $45,272

In Memoriam Nina Donohue McDonald (1954-2020)
We dedicate this grant to the memory of Nina Donohue McDonald, Lion Heart great, friend and thirteen year survivor.

Breast cancer cell populations are heterogeneous. Each tumor includes a small number of stem cells that are especially important in repopulating the tumor after therapy, and in enabling breast cancer metastasis. Dr. Guo has discovered that MKL1, a nuclear regulator that senses mechanical cues, suppresses stemness. Low MKL1 expression is associated with lower overall survival of breast cancer patients, and with lower rates of distal metastasis, especially bone metastasis. In an animal model, engineering higher MKL1 expression in tumors reduces metastatic growth. Importantly, a small molecule drug, ISX, has been identified that enhances MKL1 expression and activity. The project will test the impact of upregulating MKL1 expression in multiple breast tumor cell lines, determine whether ISX succeeds in suppressing breast cancer metastasis in these new models, and determine the molecular

mechanism by which ISX effects these outcomes. ***Overall, this work explores an entirely new approach to controlling breast cancer metastasis. Success may lead to further preclinical development of ISX, and provide a rationale for seeking additional drugs that affect the same pathway. The committee felt that this was a novel piece of research and would result in human testing soon.***

Grantees for 2020

Elucidating the role of the long coding RNA Pvt1 in Cancer: Nadya Dimitrova, PhD, $50,000
This novel proposal aims to elucidate how a novel and understudied class of genes, called long noncoding RNAs (IncRNAs) participates in cancer and may be harnessed for therapeutic applications. IncRNA Pvt1 is over expressed in breast cancer. This research using molecular biology, advanced genetic technologies and mouse models will decipher the role of Pvt1 in cancer, expand druggable space and improve patient outcomes. The committee felt that this was excellent, highly technical, ambitious research.

Exploring Complementary Insulin-Lowering Agents as Adjuvants to Chemotherapy: Rachel Perry, PhD, $50,000
The committee was enthusiastic about this proposal, citing that the hypothesis was novel and currently a major topic in the field of breast cancer research. In fact the committee stated that the next big advances may well be in metabolism and cancer. It has been known for years that rich diet, alcohol and obesity lead to breast cancer (fats produce estrogens) and that controlling glucose and insulin reduces tumors. The hypothesis that tumors need glucose is a novel one and this proposal will study this, in particular the reduction of insulin levels to inhibit tumor glucose uptake and oxidation. This study could produce better and enhanced approaches to improve the efficacy of chemotherapy, leading to new and better treatment for breast cancer.

Drug discovery with humanized bacteria identifies kinase muscle type 2 phosphorylation as potential target to treat highly aggressive metastatic breast cancer: Jesse Rinehart, PhD, $44,000
This innovative hypothesis is a novel one with application to breast cancer and other cancers. Pyruvate kinase is a key metabolic enzyme that controls the supply of energy into a cell. There are many types; one of them, PKM2 is highly expressed in many cancer types and is tied directly to cancer cell vitality. Dr Rinehart's lab discovered that their PKM2 drug induced cell death in triple negative breast cancer both in cultures and in mice. This research would complete this critical proof of concept study in mouse models. It should lead to a high impact journal publication and have immediate impact on treatment for breast cancer patients. The committee stated that this is true pilot funding and could well lead to the next major breakthrough.

Grantees for 2019

Targeting Metabolic Vulnerabilities of Triple Negative Breast Cancer: Vignesh Gunasekharan, PhD, $50,000

This research utilizes metabolic targeting to identify and inhibit cancer cells present in triple negative breast cancer (TNBC). Currently there is a dearth of targeted therapy tailored to TNBC. Chemotherapy, the only option today, has adverse side effects. TNBC expresses only one dominant isoform (instead of many) and if this isoform was inhibited, it would reduce the overall activity of the breast cancer. PCK2 is an isoenzyme that has high expression in TNBC. By suppressing PCK2 the researcher hypothesizes that the proliferation of cancer cells would be reduced. The goal of this research is to identify small molecules to selectively inhibit PCK2.

This research is the first step targeting the metabolism of enzymes at the molecular level. This piece of the research can be completed in a year and will form the basis of more research to follow which will seek to to develop an isoenzyme targeting drug. The committee was enthusiastic about this early research to be conducted by a blossoming scientist.

DEOXYMAB: A Targeted Biologic that is Synthetically Lethal to Triple Negative Breast Cancer Brain Metastases: James Hansen, MD, $50,000

This radiation oncologist/physician scientist's research is in genetics and immunology which are fertile fields for researchers. This research builds on a known lupus autoantibody which seems to cross the blood brain barrier (BBB), localizes in tumors and kills cancer cells. Dr Hansen's team has reengineered this antibody into a form called DX-1 to attack cancer cells with limited toxicity. This study aims to confirm the efficacy and safety of DX-1 by treating mice with TNBC Brain Metastases. The committee was impressed with this research because if viable it would provide a real alternative for patients with brain metastasized breast cancer. Our Lion Heart pilot funding would allow this researcher to gather sufficient data to apply for and receive large scale funding from the NIH. This research will also be attractive to drug companies. The committee felt this was a great proposal, a highly focused research team and good collaboration with a neurosurgeon.

Feasibility of Expansion and Characterization of Tumor Infiltrating Lymphocytes (TILs) from Breast Cancer: Tristen Park, MD, $50,000

This research combines breast surgical oncology and immunotherapy and will test the ability to grow tumor infiltrating lymphocytes (TILs) from the patient's own breast cancer tumors. TILs are tumor specific immune cells which eradicate tumors and would be removed from the patient, grown in the lab, resected. strengthened and then reintroduced into the patient to create an anti-cancer immune system. This research would lead to a living treatment for breast cancer patients with advanced/metastatic breast cancer and would result in durable long term tumor regression. The committee felt that this was impressive research, clinically relevant and potentially high impact.

Grantees for 2018

Exploiting HR defects in Breast Cancer with DNA-PK Inhibitor- Based Therapies: Ranjit Bindra, MD, PhD,
$50,000

The committee felt that this research submission was focused and "great science". Dr Bindra is a radiation oncologist and a physician-scientist who has an independent research lab, designs and executes clinical trials and treats patients. This proposal seeks to develop a completely novel approach to treatment of aggressive, invasive breast cancers by targeting DNA repair defects found in breast cancer tumors. By combining DNA repair inhibitors with specific chemotherapy, it is believed that this approach will selectively kill breast cancer cells and improve survival and may even be effective for breast cancer patients who have been treated and have developed resistance to potent FDA-approved inhibitors. At the successful conclusion of this year of research, Dr Bindra plans to develop his results into a major research focus, a R01 application to support a deeper, major study.

Nutritional status as assessed by skin carotenoids and chemotherapy related side effects: Leah Ferrucci, PhD, MPH, $50,000

Side effects such as nausea and nerve damage (neuropathy) are common in women receiving chemotherapy for breast cancer. These side effects can lead to delay or discontinuation of chemotherapy. These reductions in chemotherapy dose intensity significantly lessen the effectiveness of chemo. Side effects are caused in part by free radical damage induced by the chemotherapy. It is thought that antioxidants might mitigate these side effects. Carotenoids from fruit and vegetables are potent antioxidants. This proposal aims to use reflectance spectroscopy to assess skin carotenoids in breast cancer patients during chemotherapy. A trial of women in treatment divided into 2 groups (intervention vs usual care) would measure skin carotenoids levels to identify vulnerable individuals, then treat to increase carotenoid levels and thus minimize side effects to ensure that chemotherapy is effective. This research if successful would be hugely beneficial to women undergoing chemotherapy, ensuring full treatment and thus increasing positive treatment outcomes.

Imaging and Targeted Therapy of EGFR-cMET in Triple Negative Breast Cancer: Bernadette Marquez- Nostra, PhD, $50,000

Triple negative breast cancer is diagnosed by the absence of three proteins. Patients with TNBC thus are not able to benefit from therapies that target these proteins to kill cancer cells. Currently there are poor survival outcomes for these patients. The goal of this research is to evaluate the efficacy of targeting two specific proteins simultaneously: EGRF and c-MET which are more abundant in some TNBC cells than normal cells. Recently an antibody treatment was developed that blocks both of these proteins and is effective in lung cancer treatment. This research will test the antibody in animal models of TNBC. The research will tag the antibody with radioactivity to follow where it goes in the body and how much accumulates in the tumor(s) using PET scans.

This would identify patients most likely to respond to antibody therapy. The committee felt this was an exciting research with its novel incorporation of PET imaging therapies. The outcome of this research could provide a new approach to targeted therapy in a disease now considered to be non-targetable.

Grantees for 2017

Targeting KDM5s to Enhance the Response to Immune Checkpoint Inhibition in Breast Cancer: Jian Cao, Ph.D, $50,000

The goal of this research is to target KDM5s to enhance the response to immune checkpoint inhibition in breast cancer. These KDM5s release the brakes on patients' immune cells and free these immune cells to attack breast cancer tumors. The results of this research will pave the way for an application of KDM5 inhibitors for immunotherapy. Earlier work has shown a connection between the KDM5 family, innate immune response and suppression of breast cancer. This study will investigate the mechanism of anti-tumor immune response induced by KDM5 inhibition in combination with PD-1 blockade. This work will build on recent and current advances in immunotherapy as a new and viable breast cancer treatment.

New DNA Repair Alterations in Hereditary Breast Cancer Susceptibility: Rosa Munoz Xicola, Ph.D, $50,000

This research will study women with breast cancer and strong family history who do not carry the BRCA1 or BRCA 2 genes. This study hypothesizes that overall alterations in DNA repair mechanisms account for a significant amount of unknown genetic causes of breast cancer, The aim of this study is to identify these alterations in a group of 59 breast cancer patients to evaluate whether these alterations affect treatment response. From the genetic analyses and comparison work, the project will develop adequate and proper disease management, new diagnostic tools and new treatments. The 59 breast cancer patients with strong family history have already been identified for this study.

A Novel Genetic Model of Tumor Hypoxia for Breast Cancer: Zhong Yun, Ph.D, $45,000

Breast cancer tumors are often oxygen deprived (tumor hypoxia). Tumor hypoxia predicts malignant progression, metastasis, therapy resistance and poor survival in breast cancer. This research will develop genetic mice mammary tumor models which will allow labeling and monitoring of hypoxic tumors which will fill an important experimental gap and facilitate breakthrough research in hypoxia and tumor microenvironment research. This research will lay the foundation for new breast cancer therapies which will target hypoxic tumors.

Grantees for 2016

Preclinical Evaluation of Candidate Drug Combinations for Treatment of Triple Negative Breast Cancer, David F. Stern, PhD: $40,000.

Triple negative breast cancers are aggressive, appear in younger women and disproportionally affect under- represented minorities more than other breast cancers. Available therapies are not successful due to the genomic instability of this cancer. These cancers contain both basal -like breast cancers and stem-cell rich cancers (which often reconstitute after therapy and reseed metastases). Dr. Stern has already identified a small number of combinations of anti-cancer agents that suppress BOTH cancers. Currently these drugs are only used singly. Our Lion Heart grant will allow his team to further evaluate these combo "top hits" and conduct functional and mechanistic studies of these top candidates for preclinical development that would result in therapeutic trials with an overall goal of developing effective therapies. It is anticipated that our grant will allow Dr. Stern to identify two high value candidate combinations for preclinical animal testing. The committee felt strongly that without Lion Heart funding this research would not happen due to the dearth of research funding.

Targeting CaSR/GABABR1 Heterodimers to Treat Bone Metastases in Breast Cancer: John J Wysolmerski, MD, $40,000.

Breast cancer often spreads to the bone. Breast cancer in the bone resists standard chemotherapy and radiation. In order to grow in the bone, breast cancer cells must adapt to high concentrations of calcium. To do this, breast cancer cells form a heterodimer receptor which directs the cancer cells to proliferate instead of dying as calcium levels increase. Dr Wysolmerski's lab has discovered that inhibition of this receptor may kill cancer cells when calcium is elevated. This research grant is comprised of experiments which will antagonize this receptor and prove whether this inhibition will kill the breast cancer cells. This research has (as the researcher explained in his submission) hit a funding crossroads and is stalled due to a lack of research funds. Lion Heart in granting this funding is acting as a bridge funder in order to move this research to the next step, e.g. an NIH grant. This grant's success could result in a truly effective treatment for bone metastases. The committee agreed that this research was important, collaborative and cross-disciplinary.

How Does Na+/I Symporter (NIS) Expression in Metastatic Breast Cancer Correlate with Histopathological, Biological and Clinical Parameters? : Nancy Carrasco, MD, $23,000.

The most successful targeted internal radiation cancer treatment which has been used for 65 years is the administration of radioactive iodide to thyroid cancer. This therapy is effective and its toxicity is low. One key protein present in thyroid cells, (NIS), is the molecule that makes this therapy possible, by insuring that the radioiodide accumulates in thyroid cancer cells, sparing healthy cells. Dr Carrasco's team has cloned NIS and demonstrated that it is present in breast cancer cells, suggesting that the above described therapy might be effective in treating breast cancer. In conjunction with a Yale Discovery Fund award (which will identify a metastatic breast cancer population whose tumors express NIS), the Lion Heart grant will expand the study and make it more informative by investigating NIS expression by immunohistochemistry (IHC) in 100 breast cancer tissue samples housed at the Yale Breast Cancer Center. The committee felt that the Lion Heart piece of this research was the most important as it would explain and determine the percentage of NIS present in

breast cancer cells; ascertain a correlation between NIS expression and estrogen receptor, progesterone receptor and Her-2/neu status. This analysis would also reveal which patients do not express NIS in their tumors thus sparing them unnecessary radiation. The committee felt this research was truly novel and if successful would be highly beneficial.

Grantees for 2015

Donald Engelman, Ph.D,Eugene Higgins Professor of Molecular Biophysics and Biochemistry and John Deacon, Ph.D, Molecular Biophysics and Biochemistry: Targeting Chemotherapy in Breast Cancer: Weakly-acidic prodrugs bias drug uptake toward tumors.
This is a most exciting piece of research. Effective breast cancer therapy often requires chemotherapy. The most common and effective chemotherapeutics are anthracyclines. These drugs cause cardiotoxicity and oncologists only use them in advanced or super aggressive breast cancers.

The Engelman lab has developed a way to directly target tumors and and deliver these anthracyclines in a way that lessens the accumulation of toxins in healthy tissue thereby reducing side effects and improving therapy, making it more effective. Larger doses could then be more safely administered and if effective this therapy could be used for lower stage breast cancers. Lab tests have been quite successful. Now the researchers wish to test their innovations "in vivo" which will support the advancement of this technology towards clinical trials.

This lab is well known and has been highly successful. This research grant is well worth supporting. As stated in their application "this prodrug approach may have a real and near term potential to improve breast cancer therapy. This discovery has come at a time of restricted research budgets and we find ourselves unable to afford the studies necessary to adequately demonstrate its potential in order to secure long term funding to push this technology towards the clinic". This is why we, Lion Hearts, fund raise…. to support real, viable and necessary research. The committee enthusiastically awarded a grant of $*46,000.*

Vikram B Wali, Ph.D, Associate Research Scientist, Yale Cancer Center. Exploiting Metabolic Isoenzyme Shifts in Breast Cancer to Discover Novel Therapies.
This research project is a perfect example of Lion Heart's mission statement which is to fund the promising, novel research of a bright young investigator. Dr Wali's research is to develop a new therapy for triple negative breast cancer. His hypothesis is to target and exploit specific metabolic isoenzymes which may suppress cancer cell growth in triple negative breast cancer (TNBC) which is aggressive and very difficult to treat. His research is novel, interesting and promising. While it is a "long shot" the committee supported this research because of the dearth of viable therapies to treat TNBC. *Awarded grant: $20,000.*

Vikram B Wali,Ph.D and Chistos Hatzis, Ph.D, Associate Professor of Medicine, Yale School of Medicine: Evaluation of New Treatment Strategy for Triple Negative Breast Cancer Involving Combinations of Cancer Stem Cell Specific Drugs and Standard Chemotherapy.

This is research project that has the potential to change the treatment of triple negative breast

cancer. Currently TNBC does NOT respond to chemotherapy, endocrine, or currently available targeted therapies. TNBC presents the biggest challenge in the field of breast cancer. This research project will examine the function of cancer stem cells (CSC) in resisting therapies in triple negative breast cancer. By identifying distinctive CSC features, it may be possible to target them and thus find a way to develop a novel potential therapeutic strategy for treatment of TNBC. This is a multi-disciplinary project involving Cancer Cell Biology, Biostatistics and Bioinformatics.
Awarded grant: $25,000.

Grantees for 2014

Veerle Bossuyt, MD: A Pilot Study to Identify a Gene Signature of age related Involution in Healthy Breast Tissue.
This young pathologist will examine why aging increases risk of breast cancer. Physicians know that age increases risk but they do not know why. Dr Bossuyt's approach is a novel one that has been little examined. She hopes to find the biologic mechanism that causes breast cancer in aging breast tissue. If she can establish cause (or some cause) it could lead to a major grant and new approaches for treatment of breast cancer. Her co-investigators are an impressive multidisciplinary group. This research is a true pilot project. *Amount of grant: $18,000.*

Melissa Durand, MD: C-View+3D Tomosynthesis in the Clinical Environment: Can we decrease radiation dose and maintain increased cancer detection with decreased recall rates?
Melissa Durand, MD is a radiologist and professor of Diagnostic Radiology at Yale School of Medicine. Dr. Durand's research will compare Tomosynthesis and C-View with the standard mammogram protocol. If successful her study will have immediate clinical impact AND would increase breast cancer detection for women with dense breast tissue by 40%, decrease radiation dose by 50% and reduce false alarms by 15-30%. This is the first time that Dr Durand will take a leadership role in research. Her fellow investigators include a statistician which is important for this study. The panel felt this was great pilot study. *Amount of grant: $18,000.*

Christos Hatzis, PhD: Phenotypic Characterization of Mutation Heterogeneity to Enable Precision Treatment in Breast Cancer.
This PhD in Chemical Engineering with deep expertise in bioinformatics and biostatistics will examine huge data sets to develop and validate predictive tests that could lead to precise therapies for breast cancer treatments AND outcomes. Individually targeted and personalized therapies are of great interest in the breast cancer world. This is a proof of concept study that could lead to a large grant. Dr Hatzis can complete this proof of concept study within a year. The panel felt this was important, timely and necessary research. *Amount of grant: $18,000.*

Lajos Pusztai, MD: Development of a novel antibody based therapy for Triple Negative Breast Cancer that targets low density lipoprotein receptor-related protein 8 (LRP8).
This research is a small part of a huge study. The last advances in treatment of triple negative breast cancer occurred 20 years ago and currently the only treatment is chemotherapy. This type of breast cancer is aggressive and tends to occur in younger women. Gene therapy holds tremendous promise

for treatment of this deadly breast cancer. The overall goal is quite exciting and to be part of a genomic approach for treatment and better outcomes is wonderful. The panel was enthusiastic about this grant. If successful, Lion Heart would be part of this success and would have truly made a difference.

Work on these grants will commence in early January and continue through 2014 with completion in December, 2014. These 4 grants represent diverse fields and novel approaches to breast cancer treatment and outcomes. They exemplify the mission of Lion Heart which is to fund pilot research projects and to endow young, brilliant researchers. In this time of reduced research funding, Lion Heart is making a difference.

Grantees for 2013

In December, 2012 the Lion Heart Fund for Cancer Research awarded its first research grants for 2013. They demonstrate the direct impact Lion Heart funds can have.

Erin Hofstatter MD: Examination of Epigenetic Changes in Breast Tissue and Peripheral Blood of Women at Average vs Increased Risk of Breast Cancer

Using genetic sequencing technology, Erin will explore genetic and epigenetic causes of breast cancer. Currently it is not well understood why women develop breast cancer. Better methods are needed. Erin hopes to identify an epigenetic pattern that could accurately identify women at increased risk of breast cancer. Dr. Hofstatter is an academic breast cancer medical oncologist, an Assistant Professor of Medicine at the Yale School of Medicine and a member of the Yale Cancer Center Genetics and Genomics program.

Brigid Killelea MD: Atypical Ductal Hyperplasia of the Breast: Is Excision Necessary?

ADH is neither cancerous or precancerous but in 11-17% of cases it is associated with cancer. Usually in those cases a more extensive surgical excision is performed. Brigid's research will follow and compare surgery (which does not improve survival outcomes) vs hormonal therapy and close observation as a alternate choice associated with better quality of life and fewer complications. This research could be practice changing compared to how ADH is treated currently. Dr Killelea is an Assistant Professor of Surgery,Yale School of Medicine and a breast surgical oncologist.

Anees Chagpar MD: Understanding the Intersection Between Intratumoral Heterogeneity and Clinical Phenotype in Breast Cancer

It is believed that within breast cancer tumors that there may be areas in which different genetic profiles occur. If tumors are genetically diverse, this will have an enormous effect on treatment post surgically. Little research has been done in this area. Anees will study women newly diagnosed with breast cancer in order to see if diverse genetic populations occur in their tumors. Current practice is that only one core biopsy of a breast cancer tumor is done. Anees' research will entail doing multiple biopsies within various areas of tumors. Successful research in this area will not only enhance post surgical targeted treatment but may also be a predictor of how the tumor cells might behave going forward. Anees is a breast surgical oncologist, an Associate Professor of Surgery, Yale School of

Medicine, and Director of the Breast Center, Smilow Cancer Hospital.