Cutaneous Lymphoma Foundation and Research

We are delighted to share with you this year’s Research Report, where you’ll learn about the important work being completed by the recipients of both our Young Investigator and CLARIONS (Curing Cutaneous Lymphoma by Advancing Research, Innovation and Offering New Solutions) Research Awards. It is exciting and encouraging to see the progress being made in cutaneous lymphoma.

The CLARIONS Research Award program was originally developed as a response to a one-time donation. The program was intended to offer researchers seed money to begin work on new and innovative projects. As many of the recipients have expressed, this funding has gained them, and their work, the necessary credibility to secure additional funds through larger agencies.

The overwhelming response to both the CLARIONS Research and the Young Investigator Awards is very encouraging and lends to the decision to keep the Foundation’s research support efforts going. There are great strides being made in developing the next steps for how we can make the biggest impact in cutaneous lymphoma research. What we do know is, we cannot do this alone, and will need the support of our community to keep these great efforts going. We are looking forward to announcing the new research program soon.

Joseph Eischens
Board President
Cutaneous Lymphoma Foundation

Thank you to this year’s Scientific Review Committee for their time, efforts and expertise in reviewing this year’s applications:

Steven Horwitz, MD  
Memorial Sloan-Kettering Cancer Center

Lauren Pinter-Brown, MD  
Chao Family Comprehensive Cancer Center

Youn Kim, MD  
Stanford Cancer Center

Pierluigi Porcu, MD  
Sidney Kimmel Cancer Center

Stuart Lessin, MD  
Bryn Mawr Skin & Cancer Institute
Identifying Markers that Predict CTCL Disease Progression

Cutaneous T-cell lymphomas are cancers arising from T cells that travel to and protect the skin. Most patients with early disease have a normal life expectancy but 20% will go on to develop life-threatening advanced skin disease. Currently, there is no way to predict which patients will develop advanced, potentially fatal disease. The goal of this project is to identify markers that can better stratify an individual’s risk of developing progressive disease while they are still at an early stage of CTCL.

"The goal of this project is to identify markers..."

We have assembled a well-characterized group of CTCL patients, one cohort with stable disease long-term and another cohort who developed worsening disease. We are using two new technologies. One is high-throughput T cell receptor sequencing (HTS) which can uniquely identify every T cell in a skin biopsy specimen, including the malignant clone. The second technology is multicolor immunostaining which can stain up to 7 markers on one tissue sample. By studying lesional skin biopsies with these two techniques, our aim is to uncover novel markers predictive of progressive disease.

In a collaboration with Drs. Rachael Clark, Thomas Kupper and Adele de Masson D’Autume, we analyzed 210 patients and determined their progression-free and overall survival. We analyzed the number of malignant and benign T cells by HTS and found reliable prognostic indicators that identify patients at risk of progressive disease. In summary, we have evidence that HTS provides powerful prognostic information on who will develop worsening disease, even in the earliest stage patients.

For the second year of funding, we will compare the numbers of CD8+ T cells, a cell type important for antitumor responses, and regulatory T cells, a cell type that suppresses immune responses to cancer, between stable and progressive disease patients using multicolor immunostaining of skin biopsy specimens (Figure 1). If patients at high risk for disease progression could be identified earlier, more intensive therapy could be instituted at a time when the disease is more responsive.

Scientists have long known about the importance of diversity. Sociologists and economists would argue that socially diverse groups are more innovative and make smarter decisions. Biologists know that diversity provides organisms with the ability to adapt to and survive harsh environmental conditions. How does this seemingly fundamental concept of “strength through diversity” apply to cancer, in particular cutaneous T-cell lymphoma (CTCL)?

Today we know that cancer is composed of genetically and functionally diverse cellular subpopulations. It has been proposed that cancer cell diversity and heterogeneity provide an explanation for treatment failure and recurrence of disease as some cells may behave more “aggressively” and resist treatment. Withdrawal from a fast-growing state is considered to confer increased stress resistance and survival. While this quiescent tumor cell subpopulation has been identified in several cancers, its presence and function in CTCL has not been studied so far.

Using cell lines from patients with CTCL, we have isolated a subpopulation of quiescent, slow-cycling cells that exists among the bulk population of lymphoma cells. Cells from this subpopulation are better equipped to survive and recover when exposed to chemotherapy indicating their potential to be the source of relapsing disease after treatment. While this illustrates the presence of functionally diverse subpopulations among lymphoma cells, a more detailed analysis is needed to determine how to target this subpopulation. In the project funded by the Cutaneous Lymphoma Foundation, we are working on the identification of a molecular marker which would allow us to detect these cells, monitor their response to treatment and help to design novel therapeutic strategies.

To achieve this goal, we are using mouse models to grow tumors form human CTCL cell lines to identify and isolate the slow-cycling subpopulation. These cells will then be used to study their molecular features, behavior and role during progression of disease both before and after chemotherapy. The next big step will be a comprehensive study of all genes which are expressed in the quiescent cells and comparing this profile to the rest of the lymphoma cells. This will allow us to identify and validate potential molecular markers and give us first clues as to what the molecular mechanisms are that regulate the treatment-resistant, slow-cycling state.

Understanding Tumor Cell Diversity: A Key to Outsmart Cutaneous Lymphoma?

Stefan M. Schieke, MD
Assistant Professor
University of Wisconsin School of Medicine and Public Health

"This will allow us to identify and validate potential molecular markers..."
In previous studies Dr. Fuschiotti and colleagues have shown that tumor cells isolated from the blood and skin of patients with Cutaneous T Cell Lymphoma (CTCL) express high levels of the cytokine called Interleukin-13 (IL-13) and express on their cell surface two protein receptors that bind specifically to IL-13, notably the Interleukin-13 receptors alpha 1 (IL-13Rα1) and alpha 2 (IL-13Rα2). They also showed that malignant cells use IL-13 to grow and that by blocking IL-13, tumor cell proliferation is inhibited.

Based on these results, current work in Dr. Fuschiotti’s lab is focused on elucidating the cellular events that result in the expression of IL-13Rα1 and IL-13Rα2. Their hypothesis is that the binding of IL-13 to IL-13Rα1 and IL-13Rα2 will activate distinct signaling pathways characterized by the activation of specific proteins and determine the effect on tumor cell proliferation. The overall goal is to identify molecules that are specifically activated by IL-13 in tumor cells and that are responsible of tumor growth. These molecules could help in diagnosing CTCL and may be developed for innovative therapeutic approaches.

The project funded by the Cutaneous Lymphoma Foundation focuses on studying the signaling pathways occurring through the IL-13Rα2 receptor, which is highly expressed by tumor cells at all stages of CTCL tumors (see Figure). However, little is known about what happens after IL-13 binds to it, and whether this binding affects the proliferation of tumor cells. Fuschiotti proposes to use a combination of biochemistry and biophysical techniques on well-characterized tumor samples. Among these technologies, the lab will use mass spectrometry to determine the proteins that bind to IL-13Rα2 in tumor cells, and to discover the other proteins they interact with. The group proposes to block the function of these identified proteins with specific antibodies or compounds and determine the effect on tumor cell proliferation. The overall goal is to identify molecules that are specifically activated by IL-13 in tumor cells and that are responsible of tumor growth. These molecules could help in diagnosing CTCL and may be developed for innovative therapeutic approaches.
Total Electron Irradiation (TSI) therapy is a whole body technique that uses clinical linear accelerators to treat the patient’s skin. While the method is known to be effective in treating patients with cutaneous diseases such as the Cutaneous T-cell Lymphoma (CTCL), the uniformity of the treated surface is not well characterized. Traditionally, the radiation dose on a patient is characterized by attaching a number of dosimeters or radiation dose detectors throughout the patient’s skin. As a result, there is a possibility of under-treated areas (cold spots) or over-treated areas (hot spots) due to the low resolution data available to the therapist. Researchers in the Radiation Oncology Department at Stanford University are developing a flexible dosimeter device that can characterize the radiation beam profile before reaching the patient and around the patient’s body, without modifying the patient stage.

Since the shape of the dosimeter is similar to a rubber band, it can be attached to any part of the body or embedded in undergarments where radiation dose information is required. Preliminary dosimetry or radiation dose measurement was performed using a preclinical X-Ray device, and the dose information was measured using an in-house developed readout device. Results showed good accuracy when exposed to an increased amount of X-Ray dose.

In the horizon, we will apply the flexible dosimeters on CTCL patients in order to evaluate the accuracy of the TSI therapy. We will characterize the radiation beam uniformity before the beam reaches the patient stage and search for cold spots or hot spots on the patient’s skin. With successful characterization of the two parameters, we expect to aid the therapist in visualizing the invisible treatment beam, thus enabling them to aid the therapist in visualizing the invisible treatment beam, thus enabling them to increase the accuracy of the treatment procedure.

Analysis of Molecular Etiology and Bacterial Triggers of Cutaneous T Cell Lymphoma

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It has been a great honor to receive the CLARIONS and Young Investigator awards this year, and I can’t thank the Cutaneous Lymphoma Foundation enough. It has been absolutely crucial to have these funds to start the avenue of research I want to do.

~John O’Malley, MD
**Analysis of Molecular Etiology and Bacterial Triggers of Cutaneous T Cell Lymphoma** (continued from page 7)

Our group is continuing to recruit patients to gain greater insight into the heterogeneity of CTCL and to better understand the contribution of bacteria to the pathogenesis of this disease. We are using our newly established animal model to test how microbiota contributes to the pathogenesis of CTCL, as well as to probe molecular pathways that may prove to be promising targets for future therapeutic interventions.


5. Jackow, C. M., Cather, J. C., Hearne, V., Asano, A. T., Musser, J. M. & Duvic, M. Association of erythrodermic cutaneous t-cell lymphoma and other medical professionals specializing in cutaneous lymphomas coming together for three days to share and discuss their work and knowledge? Well it happened -- the World Congress of Cutaneous Lymphoma has met and here is what we learned.

6. We must continue funding the work of researchers in cutaneous lymphoma in order to continue supporting you!

7. The original plan worked -- after four years, hundreds of applications from around the world, fourteen awards issued equaling $350,000 spent to fund cutaneous lymphoma research, and now the CLARIONS Research Award is coming to a close... But, it’s not over!

8. After getting an overwhelmingly positive response from the CLARIONS program we now realize a small patient advocacy organization CAN make a difference in research, and we are forging forward. The Cutaneous Lymphoma Foundation has formed a new research committee and begun work to develop a new award.

9. We, together with you, are committed to continuing to make an impact on cutaneous lymphoma research.

Stay tuned because the announcement will be coming soon.

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**Coming Soon...**

**New Research Award Program**

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**Malignant T Cell Plasticity in Cutaneous T Cell Lymphoma**

Differing clinical presentations of CTCL reflect the fact that lymphomas can arise from discrete T-cell subsets. Mycosis fungoides (MF) and Sézary syndrome arise from resident memory T cells (T<sub>RM</sub>) and central memory T cells (T<sub>CM</sub>), respectively. However, some patients have a combination of features.

We studied patients by flow cytometry and TCR sequencing (HTS). Patients with blood disease and discrete skin lesions often had malignancies of migratory memory T cells (T<sub>M</sub>), a recently described, slowly recirculating T cell subset. These patients required both systemic and skin based therapies.

A second cohort of leukemic patients with erythroderma had complete clearance of blood disease after alemtuzumab or stem cell transplantation but subsequently recurred with fixed MF-like skin lesions. HTS confirmed that the malignant clone in fixed lesions was identical to the previous leukemic clone. This is consistent with findings that T<sub>CM</sub> can differentiate into T<sub>RM</sub>. These patients responded to skin directed therapy. Although healthy T<sub>CM</sub> can give rise to T<sub>RM</sub>, they have never been shown to differentiate into circulating T<sub>CM</sub>.

We studied 3 patients with MF who then developed peripheral blood disease. In one case, fixed lesions and leukemic disease were caused by two different clones. In 2 cases, the clone in skin and blood were identical and circulating malignant cells had T<sub>CM</sub> markers, supporting either de-differentiation of T<sub>CM</sub> to T<sub>RM</sub> or the presence of a stem cell population that could give rise to both. A subpopulation of malignant T cells in these patients did have surface receptors suggestive of T stem cell memory cells (T<sub>ScM</sub>).

In summary, the evolution of clinical presentations in CTCL is partially consistent with the normal biology of T<sub>CM</sub>/T<sub>ScM</sub> differentiation with the exception of patients who undergo T<sub>ScM</sub> to T<sub>CM</sub> conversion, either through de-differentiation or via the presence of a previously unsuspected stem cell pool.

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**Low-dose Radiation Preferentially Kills Malignant T Cells, recruits Benign T Cells and Normalizes the Immune Milieu in Mycosis Fungoides**

Topical therapies can suppress mycosis fungoides (MF) but none appear curative. We previously reported that low-dose radiation (LDR, 4 gray x 2) could lead to remission and malignant T cell depletion in skin. We now extend our findings in 11 patients. Before therapy malignant T cells were on average 29% of the total T cell population as measured by TCR sequencing (HTS). 8 weeks after

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**This publication, along with our ongoing research efforts, has been made possible, in part by, the Drs. Martin Foundation and Dorothy Spatz Foundation.**

**Pictured left to right:**

Carol Price, Susan Thornton and Richard Thompson.
Targeting the epigenome is a promising strategy in the treatment of advanced stage cutaneous T-cell lymphoma (CTCL). CTCL is a malignancy of mature CD4+ T-cells which initially involves the skin but may progress to involve blood and visceral organs. There is no curative treatment, and drug resistance is a common problem. A hallmark feature in the development and progression of CTCL is global dysregulation of the epigenome resulting in aberrant gene expression, increased expression of oncogenes, and silencing of tumor suppressors.

Bromodomain 4 (BRD4) is a master epigenetic regulator of gene expression recently identified as a survival factor in many hematologic and solid malignancies. A member of the bromodomain and extra terminal (BET) protein family, BRD4 binds chromatin in super-enhancer regions to direct downstream gene expression through interaction with cofactors such as Mediator and p-TEFb. Recently, a small molecule specific inhibitor of BRD4, JQ1, has been investigated as an antitumor agent. The role of BRD4, and therefore the efficacy and mechanism of JQ1 in CTCL is not known.

Our group recently reported the critical role of IL-15 signaling in the development and progression of CTCL (Mishra et al, Cancer Discovery, 2016). Utilizing CTCL-derived cell lines, patient samples, and the newly characterized IL-15 transgenic mouse model of CTCL, we describe the effects of IL-15 signaling on BRD4 expression, and demonstrate for the first time regulation of BRD4 binding at regulatory regions of IL-15 receptor genes, we performed ChIP-sequencing for BRD4 binding in CD4+ T-cells from a healthy donor, fresh CTCL cells, and JQ1-treated CTCL cells. At the gene locus for IL-15RA (Chromosome 10p14-p15), we observed increased BRD4 binding at the transcription start site. This occupancy is reversed upon treatment with JQ1, to a level comparable to that of healthy donor CD4+ T-cells. This pattern is recapitulated in regulatory regions for IL-15RB and IL-15Rγ loci.

To determine if decreased BRD4 occupancy following JQ1 treatment results in decreased IL-15 receptors gene expression, immunoblotting was performed for each receptor subunit. Treatment of the CTCL cell line HuT78 cells, with JQ1 results in significant reduction in the expression of all three IL-15 receptor subunits compared to vehicle. IL-15RA expression decreased 2.3-fold, IL-15RB by 17-fold, and IL-15Rγ by 122-fold (Figure 1A).

To determine the efficacy of JQ1 as an anti-tumor agent, CTCL-derived cell lines were treated with increasing doses of JQ1 for 72 hours. Cell viability and cell cycle analysis was performed and IC50 values were calculated for each cell line. At 10μM dose, there were significant decreases in % cell viability for all 5 cell lines (MyLa 66±2.56; HuT102 37±1.39; HuT78 30±1.86; HH 19±2.15; SeAx 36±0.79; p<0.0001 for all of the above). IC50 for MyLa is 21μM, HuT102 0.445μM, SeAx 4.45μM, HuT78 0.167μM, and HH 0.461μM. Treatment of these cell lines

**“Recently, a small molecule specific inhibitor of BRD4, JQ1, has been investigated as an antitumor agent.”**

with JQ1 also resulted in a dose-dependent increase of cells in subG0 phase of the cell cycle, corresponding with increased Annexin V staining.

IL-15 transgenic mice universally develop CTCL by 3-4 weeks of age. We treated these mice with 50mg/kg JQ1 (n=8) or a vehicle control (n=5) beginning at 4 weeks of age for 4 weeks. Scoring of the morphology, and severity of skin lesions histologically (Figure 1B) demonstrated a significant difference between JQ1 treated animals and controls (Figure 1C, p=0.0041), with JQ1-treated animals having milder disease.

We conclude that BRD4 binding at regulatory regions enhances IL-15 receptor expression in CTCL. Increased receptor expression may augment IL-15 signaling, a known oncogenic mechanism in this malignancy. Furthermore, JQ1 reverses the effects of BRD4 on IL-15 receptor expression, results in significant cytotoxicity in cell lines, and prevents development of severe disease in a mouse model of CTCL. BRD4 therefore represents a promising therapeutic target in CTCL.

Low-dose Radiation Preferentially Kills Malignant T Cells, Recruits Benign T Cells and Normalizes the Immune Milieu in Mycosis Fungoides (continued from page 9)

The total number of T cells in skin decreased by 4-fold after therapy but benign T cells were relatively spared. 38% of the benign T cell clones present in skin before therapy were also present after. LDR also led to recruitment of new benign T cell clones into skin; new benign clones were 51% of the total T cell population after therapy.

We carried out NanoString based immune profiling to further characterize the effects of LDR. Pretreatment MF skin expressed many pro-inflammatory genes including chemokines and cytokine receptors as well as markers of cellular exhaustion including PD-1 and Tim-3. This inflammatory/exhausted phenotype was completely reversed by LDR in patients with CR. 8 weeks after therapy post-radiation biopsies were indistinguishable from normal skin by using principal component analysis. One patient each with PR and SD had persistent inflammatory signatures and continued expression of exhaustion associated genes.

In summary, LDR was highly effective in 80% of MF patients and has led to long-term clinical cures extending up to five years. LDR led to complete or near eradication of malignant T cells from skin and complete normalization of the MF-associated inflammatory/exhausted gene signature.
Support the Cutaneous Lymphoma Foundation to Help Advance Cutaneous Lymphoma Research

Please consider making a donation to the Cutaneous Lymphoma Foundation to help fund both research specific to cutaneous lymphoma, and the education and support of those affected by this disease. Your generous donation will make a difference in the lives of those suffering from cutaneous lymphoma.

Support the Cutaneous Lymphoma Foundation
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