



Cutaneous Lymphoma Foundation and Research: Building the Roadmap

The Cutaneous Lymphoma Foundation, along with you, are making a difference in cutaneous lymphoma research!

CLARIONS Research Award = 4 years, \$350,000, 59 applications, 14 - \$25,000 grants awarded!

The CLARIONS research award program (Curing Cutaneous Lymphoma by Advancing Research, Innovation, and Offering New Solutions) was the first award designed to fund research specifically focused on cutaneous lymphomas. The CLARIONS was successful beyond expectations, revealing a high level of interest in both the scientific and patient advocacy communities for research projects aimed at characterizing the biological foundations of cutaneous lymphomas and addressing the clinical care challenges faced by patients and caregivers every day.

As the CLARIONS Research Award Program comes to a close, the Cutaneous Lymphoma Foundation's Board of Directors are committed to continuing to fund cutaneous lymphoma specific research.

As part of their commitment, and in an effort to ensure our funding makes the greatest impact, we have enlisted the expertise of leading clinicians in cutaneous lymphoma to form our all new Research Advisory Council.

Co-chairing the Research Advisory Council are Drs. Pierluigi Porcu and Christine Eischens from Jefferson University in Philadelphia, PA. Together they worked to develop a balanced group of patients, advocates and researchers across all disciplines, as well as geographic locations, whose primary goal is to explore and construct a recommendation for the Board of Directors on where the Foundation should focus its research efforts next – our Research Roadmap. With the hard work and dedication of the Research Advisory Council, the Research Roadmap development is well underway.

We are grateful to all the medical professionals who volunteer their time and expertise to supporting the Foundation, our mission and the people we serve. We are equally as grateful for you, the members of our community, for your support and financial contributions allowing us to fund this important work.

Thank you to the physicians who served on this year's CLARIONS Scientific Review Council:

Stuart Lessin, MD, Committee Chair
KGL Skin Study Center

Lauren Pinter-Brown, MD
Chao Family Comprehensive Cancer Center

Steven Horwitz, MD
Memorial Sloan Kettering Cancer Center

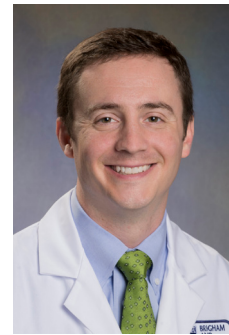
Pierluigi Porcu, MD
Thomas Jefferson University

Youn Kim, MD
Stanford Cancer Institute

Award Recipient - January, 2016 – December, 2017

Identifying Markers that Predict Cutaneous T-cell Lymphoma Disease Progression

John T. O'Malley MD, PhD
Research Fellow/Associate Physician
Brigham and Women's Hospital



Cutaneous T-cell lymphomas (CTCL) are a heterogeneous collection of non-Hodgkin's lymphomas, of which mycosis fungoides is the most common type, characterized by inflamed skin lesions and variable involvement of blood and lymph nodes. Most patients with early disease have a normal life expectancy but 20% will go on to develop life-threatening advanced skin disease. Reliable predictive models of early stage patient risk have been developed but are imperfect. We hypothesized that tissue-based prognostic biomarkers focusing on the relationships between the benign and malignant T cells in the skin will lead to more reliable predictive models in early stage patients. To address this, we assembled a group of CTCL patients that either had stable or progressive disease and examined the benign and malignant T cells using high-throughput T cell receptor sequencing (HTS) which can uniquely identify and quantify every T cell in a skin biopsy and multicolor immunostaining to identify the exact location, number and type of benign T cells.

Using HTS on 177 early stage mycosis fungoides patients (Stage IA-IIA), we have found that the frequency of the malignant T cell clone in the skin is highly predictive of eventual disease progression. Tissues that exhibit a malignant clone frequency >25% have at least 4 times the risk of disease progression compared to patients who have a malignant clone frequency <25% (Fig. 1A).

We also examined whether the types of T cells infiltrating the skin differed between stable and progressive disease patients. To examine this, a preliminary cohort of 33 CTCL patients including 13 progressive disease patients were examined for anti-inflammatory FoxP3+ T regulatory cells (Treg) and anti-tumor CD8+ cytotoxic T cells using multispectral immunofluorescence. Patients with greater CD8+ T cell skin infiltration had significantly improved

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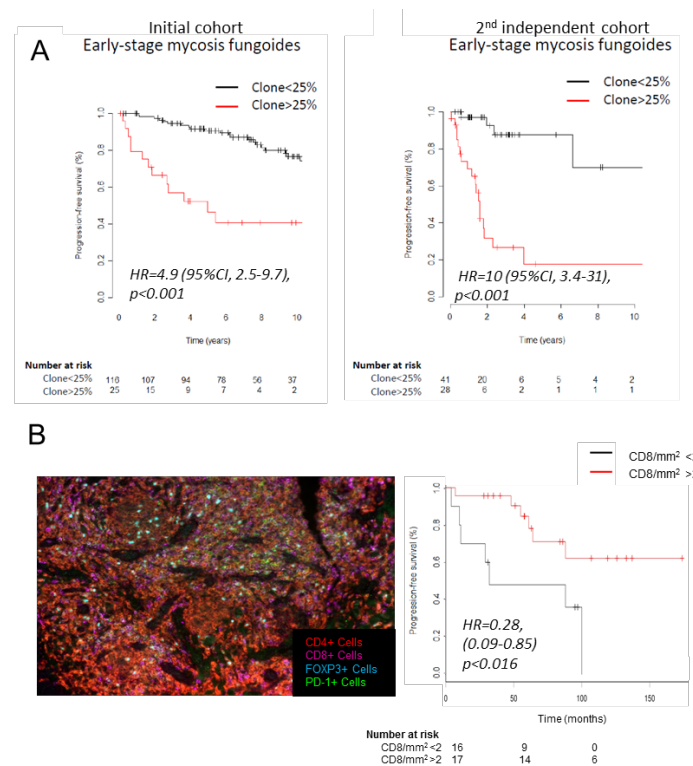


Figure 1. (A) Kaplan-Meier survival estimates of progression-free survival (PFS) of an initial (left) and 2nd independent (right) cohort of early stage (Stage IA-IIA) mycosis fungoides patients according to the malignant clone frequency. Hazard ratios (HR) are displayed at bottom which demonstrates an increased risk of disease progression in patients with MCF>25% compared to patients with MCF<25%. **(B)** Representative image of multispectral imaging of lesional skin of early stage MF patients looking at CD4+ (which contains malignant clone), CD8+ cytotoxic T cells and FoxP3+ Tregs and an immune marker of exhausted T cells (PD-1) (left). On the right are Kaplan-Meier survival estimates of PFS for a 33 CTCL patient cohort according to CD8+ cytotoxic T cell infiltration. In this case, the HR shows that patient samples containing denser infiltrates of CD8+ T cells >2/mm2 in their skin (malignant clone is CD4+) have a 72% reduced risk compared to patients who have less than 2 CD8+ T cells/mm2.

Award Recipient - January, 2016 – December, 2017

Treatment-Resistance in Cutaneous Lymphoma: the Problem Lies in Every Single Cell

Stefan M. Schieke, MD
Assistant Professor
Medical College of Wisconsin



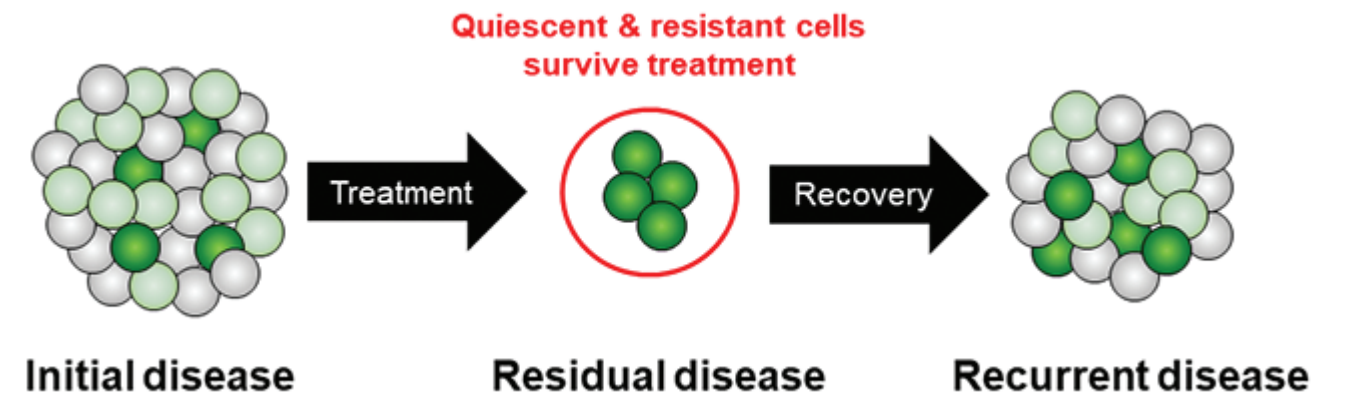
Scientists have long known about the importance of diversity. Sociologists and economists would argue that socially diverse groups are more innovative and make smarter decisions. Biologists know that diversity provides organisms with the ability to adapt to and survive harsh environmental conditions.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

Today we know that cancer is composed of numerous diverse cellular subpopulations. It is this diversity and flexibility of cancer cells that provides a huge challenge for the successful cure of the disease as some cells may behave more “aggressively” and resist treatment. Which cells in CTCL resist treatment? Is it only a few or many? Do they change over time? How could we identify them in patients to better tailor therapies and provide personalized treatments?

In our research funded by the Cutaneous Lymphoma Foundation, we have been able to provide some answers to those questions. Using cell lines from patients with CTCL, we have isolated a subpopulation of treatment-resistant

cells among the bulk population of lymphoma cells. These cells are able to survive and lead to relapse after exposure to various drugs commonly used in the clinic. We found that every cell has the potential to enter into this transient treatment-resistant state. While in this state, a single cell is enough to survive treatment and cause recurring disease demonstrating the enormous challenge and the need to look at every single cell in order to eradicate cancer.

Next, we have been looking for biomarkers allowing us to identify those drug-resistant cells even before a treatment is started. This would enable researchers and clinicians to predict and monitor the response to treatment and help define a more individualized therapeutic plan for each patient. At this point, we have defined a panel of biomarkers that makes it possible to identify rare treatment-resistant cells in cell culture and animal experiments. Most importantly, applying those biomarkers we were able to find these drug-resistant cells in biopsy samples taken from patients. Future work will focus on the mechanistic strategies lymphoma cells use to turn on their drug-resistance program and how to prevent it.



2017 Society of Investigative Dermatology (SID) Award Recipient

Tumor Clone Frequency is a Robust Predictor of Progression and Survival in Patients with Stage IB Mycosis Fungoides

Adèle de Masson, MD
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Harvard Institutes of Medicine



Most patients with mycosis fungoides present with indolent early-stage disease. A small but significant subset will develop aggressive and fatal disease. Currently we have limited ability to predict which patients are destined to progress to advanced disease. We analyzed the prognostic value of gene expression signatures, malignant T cell burden and benign T cell diversity in lesional skin of CTCL patients. We sequenced the T-cell receptor beta gene in the skin of 177 MF patients (discovery set/DS). The findings were validated on an independent cohort of 87 MF patients (validation set/VS). The frequency of the malignant clone (tumor clone frequency, TCF) in skin was significantly associated with PFS and overall survival (OS) ($p < 0.001$) and remained highly significant in a multivariate model retaining the other independent co-variables (age, stage, large-cell transformation, LDH levels; $p < 0.001$). The two cohorts also contained early stage MF patients (141 DS, 69 VS). When mycosis fungoides MF was analyzed, the TCF did not predict PFS or OS in Stage IA/TI patients; in fact, none of these patients progressed. However, TCF was highly predictive of PFS and OS in stage IB patients. For TCF > 25%, stage IB/T2 patients had decreased PFS (HR=13; 95%CI, 5-36, $p < .001$ in the discovery set, HR=11 (95% CI, 2.5-48, $p = .001$) in the validation set) and OS (HR=9.0, 95% CI, 3.0-27, $p < .001$). For plaque (T2b) vs. patch (T2a), the HR for PFS was 2.2 (95% CI, .9-5.3, $p = .08$) in the discovery set and 1.7 (95% CI, .6-4.8, $p = .3$) in the validation set, and the HR for OS was 2.0 (95%CI, .7-6.5, $p = .2$). Thus, the malignant T cell clone frequency in skin is far and away the most robust predictor of progression and survival in Stage IB MF patients, and can be used to stratify patients for more aggressive intervention.

Additional Research Credits: A DeMasson, JT O'Malley JT, CP Elco, S Garcia, SJ Divito, EL Lowry, JE Teague, NR LeBoeuf, H Robins, RA Clark, TS Kupper; Harvard/BWH/DFCI Dermatology and Adaptive Biotechnologies, Boston MA and Seattle WA, USA.

2017 American Society of Hematology (ASH) Award Recipient

Enhanced Spontaneous Anti-tumor Cytotoxicity of Natural Killer Cells in Cutaneous T-cell Lymphoma Patients

Bethany Mundy-Bosse, PhD
Research Assistant Professor
Division of Hematology
Ohio State University



Cutaneous T-cell lymphoma (CTCL) is characterized by the expansion of malignant CD4+ T cells in the skin. There are two main subtypes of CTCL, mycosis fungoides (MF) and Sézary syndrome (SS). Previous studies have demonstrated defects in cell-mediated immunity, including altered cytokine profiles and decreased neutrophil function, and patients often have recurring bacterial and viral infections. Recent studies have shown increased expression of interleukin (IL)-15 in malignant CD4+ T cells in CTCL patients (Mishra et al., Cancer Discovery, 2016). Since IL-15 can enhance NK cell differentiation and activation, we hypothesized that NK cells from CTCL patients might have phenotypic and functional alterations due to these changes in homeostatic IL-15 levels.

Enhanced Spontaneous Anti-tumor Cytotoxicity...continued

To evaluate the absolute number of NK cells in CTCL patients, CD56+Lineage- lymphocytes were quantitated by flow cytometry. SS patients had significantly fewer NK cells as compared to normal donors (mean \pm SEM of absolute cell numbers in normal donors = 0.2442 ± 0.02 , $n = 51$; SS = 0.1072 ± 0.02 , $n = 9$; MF = 0.2161 ± 0.01 , $n = 112$; unpaired t-test normal vs. SS, $p = 0.007$). NK cell counts were significantly associated with overall survival, with the short-term risk of death increasing by 87.5% for every increase in 0.1 of absolute NK cell counts on univariate analysis utilizing the Cox proportional hazards model (Figure 1A; $p = 0.0413$). We then examined the cytotoxic capacity of NK cells (CD56+Lineage-) freshly purified from both MF and SS patients by standard chromium release assay. There was a significantly higher level of specific lysis of tumor cells (K562 target cells) by NK cells from CTCL patients as compared to normal donors (Figure 1B, at 50:1 ratio mean \pm SEM of percent NK cell cytotoxicity in normal vs CTCL = 27.47 ± 4.76 , $n = 13$ vs. 52.54 ± 4.88 , $n = 12$, unpaired t-test, $p = 0.001$). Upon further transcript analysis of NK cell cytolytic mediators from CTCL patients compared to normal donors, we observed significantly increased levels of perforin (mean \pm SEM of relative RNA in normal vs CTCL = 91.39 ± 9.18 , $n = 3$ vs. 485.7 ± 43.15 , $n = 7$, unpaired t-test, $p = 0.0004$) and granzyme B (143 ± 23.79 , $n = 3$ vs. 431 ± 68.13 , $n = 7$ unpaired t-test, $p = 0.03$). In addition, we observed increased levels of interferon-gamma and Fas Ligand (5.149 ± 0.4775 , $n = 3$ vs. 13.93 ± 1.52 , $n = 7$; unpaired t-test $p = 0.007$), indicating NK cells from CTCL patients exhibited consistently elevated activation patterns as compared to normal donor NK cells. We then examined the expression pattern of the IL-15 receptor complex on NK cells in CTCL patients as compared to normal donors by RNA sequencing. There was a significant elevation in IL-15R α (mean \pm SEM of relative RNA in normal vs CTCL = 0.8677 ± 0.1018 , $n = 3$ vs. 1.772 ± 0.2055 , $n = 7$; $p = 0.03$) and IL-15R γ (57.02 ± 7.525 , $n = 3$ vs. 154.7 ± 12.14 , $n = 7$; $p = 0.001$), while IL-15R β was also elevated (128 ± 23.68 , $n = 3$ vs. 223.2 ± 25.43 , $n = 7$; $p = 0.056$). Next we evaluated protein levels of phosphorylated signal transducer and activator of transcription 5 (p-STAT5) in fresh whole blood as the surrogate marker of IL-15 signaling. Preliminary findings demonstrate an increase in p-STAT5 in CTCL patients as compared to normal donors (mean \pm SEM of fluorescent intensity in normal donors vs CTCL patients = 1506 ± 90.82 , $n = 5$ vs. 1270 ± 50.59 , $n = 4$; unpaired t-test; $p = 0.07$).

These data suggest that NK cell activation is enhanced in patients with CTCL, including MF patients with disease localized to the skin. Further investigations aimed at understanding the disconnect between NK cell numbers, activation,

Additional Research Credits:

Mundy-Bosse BL^{1,2}, Denlinger N¹, Huang S¹, Chen L², Mao HC², Kline D³, McLaughlin E³, Youssef Y⁴, Young K², Lozanski G⁴, Freud AG^{2,4}, Porcu P⁵, William BM¹, Caligiuri MA^{1,2}, Mishra A^{2,6}

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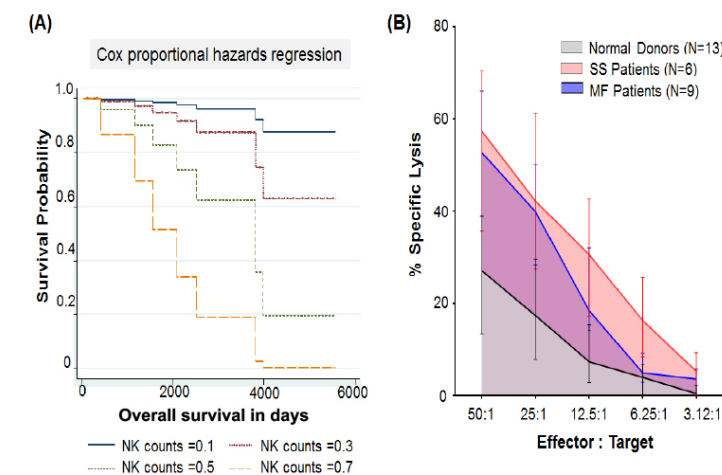


Figure 1. (A) Cox proportional hazard regression of absolute NK cell numbers in CTCL patients. **(B)** NK cells (CD56+/Lineage-) were isolated from fresh peripheral blood samples from normal donors, SS patients, and MF patients to a purity greater than 99.0% by FACS. K562 leukemic cells were used as targets in a standard chromium release assay.



Research Advisory Council

The Research Advisory Council is a committed group of distinguished scientists, accomplished clinical experts, leaders of cutaneous lymphoma research, and patient advocates who volunteer their time and talent to develop and expand the research programs offered and supported by the Cutaneous Lymphoma Foundation.

The Cutaneous Lymphoma Foundation is now positioned to lead the transition from the CLARIONS Research Award program to a long-term, sustained effort, with the goal of fostering transformative advances in the landscape of care for patients with cutaneous lymphoma. The Research Advisory Council will provide guidance and advice to the Cutaneous Lymphoma Foundation's Board of Directors on the development of a long-range research funding strategy, called the **CLF Research Roadmap**, through the creation of an innovative conceptual framework for Cutaneous Lymphoma Foundation sponsored research and a portfolio of funding mechanisms to identify, resource, and pursue high impact basic science and clinical research projects in cutaneous lymphoma.

Pierluigi Porcu, MD (Co Chair)	Thomas Jefferson University
Christine Eischen, PhD (Co Chair)	Thomas Jefferson University
Jaehyuk Choi, MD, PhD	Northwestern University
Alejandro Gru, MD	University of Virginia
Bradley Haverkos, MD, MPH	University of Colorado
Steven Jones, MD	Patient
Michael Khodadoust, MD, PhD	Stanford University
Stuart Lessin, MD	KGL Skin Study Center
Laura McGirt, MD	University of North Carolina
Anjali Mishra, PhD	Ohio State University
Alison Moskowitz, MD	Memorial Sloan Kettering Cancer Center
Christopher Shipp	Patient

Medical Advisory Council

The Medical Advisory Council is a committed group accomplished clinical experts and leaders in cutaneous lymphoma who volunteer their time to support the Cutaneous Lymphoma Foundation's mission and strategic patient program and services initiatives. The Council is divided into workgroups, each focused on specific projects of interest, utilizing each person's area of expertise. This Council allows the Cutaneous Lymphoma Foundation to serve its community through providing knowledgeable, up-to-date and vetted clinical information across all its efforts.

Ellen Kim, MD (Chair)	University of Pennsylvania
Wei Ai, MD	University of California San Francisco
Kim Bohjanen, MD	University of Minnesota
Francine Foss, MD	Yale University
Richard Hoppe, MD	Stanford Cancer Center
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Debjani Sahni, MD	Boston University
Michi Shinohara, MD	University of Washington
Jasmine Zain, MD	City of Hope

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Lars French, MD	University Hospital Zurich
Robert Knobler, MD	Medical University of Vienna
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Alain Rook, MD	University of Pennsylvania
Martin Weinstock, MD, PhD	Brown University
Sean Whittaker, MRCP	Guy's and St. Thomas' Hospital

Using Personalized Medicine...continued from pg 2

progression-free survival. The number of skin Tregs and total CD4+ T cells were not associated with improved survival. We are in the process of performing this analysis in a larger 120 patient cohort to confirm this preliminary finding.

In summary, both the malignant clone frequency and CD8+ T cell density in the lesional skin of CTCL patients is predictive of disease progression and will likely be a useful adjunct in risk-stratifying our early stage patients.

To learn more about Dr. O'Malley's research on predicting risk of disease progression, watch the **Facebook Live: John O'Malley, MD** video available on the CLF's YouTube channel: www.youtube.com/user/CutaneousLymphomaFnd

**Thank you to all of our generous donors,
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**DRS Martin & Dorothy Spatz
Foundation**

**for making our involvement in
cutaneous lymphoma research possible.**



CUTANEOUS LYMPHOMA FOUNDATION

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TIME SENSITIVE MATERIALS ENCLOSED



We Need Your Help...

- \$350,000 of research funding awarded over the last 4 years
- 59 applications received from top researchers in cutaneous lymphoma
- 14 grants of \$25,000 were awarded to innovative and novel research projects specifically in cutaneous lymphoma

Help continue our dedication to research, which is working to solve the clinical care challenges faced by all people affected by cutaneous lymphoma. Donate to the Cutaneous Lymphoma Foundation today. Remember, every donation helps...

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Mail: P.O. Box 374 Birmingham, MI 48012

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