Cutaneous Lymphoma Foundation
RESEARCH REPORT 2022

Continuing the Vision: Supporting Cutaneous Lymphoma Research

We are thrilled to again be accepting applications for the 2022/2023 Cutaneous Lymphoma Catalyst Research Grant. Two $50,000 grants will be presented at the 2-Day Patient Conference being held virtually, July 8-9, 2022. This year, the Foundation's Board of Directors, Research Advisory Council, and Scientific Review Committee have made provisions to ensure that at least one of this year's recipient's projects has a focus on patient quality of life.

Like everything else, scientific research looked different over the past two years with researchers shifting efforts to help in the fight against COVID-19. With labs being closed, significant delays in availability of tissue and other samples, an inability to see patients, loss of funding, and the shift in needs at many of the primary research institutions, it is no small feat that invaluable research in cutaneous lymphomas has still been completed.

As you may recall, the Cutaneous Lymphoma Catalyst Research Grant funds innovative projects from all areas of cutaneous lymphoma research, with a particular interest in: clinical impact, multi-institution collaborations, and innovative science. The Catalyst Grant was originally designed to address a near term need to accelerate or extend ongoing research that was previously approved by another funding source.

As introduced in our Research Roadmap, the Foundation's research vision is focused on answering three critical questions posed by patients, or what we refer to as our 3 Pillars of Research:

- Why did I get cutaneous lymphoma?
- What cutaneous lymphoma do I have?
- How do I treat my cutaneous lymphoma?

In the following pages you'll find highlights of the incredible research being completed from the two current 2021/2022 Cutaneous Lymphoma Catalyst grant recipients, as well as two of the four 2020 awardees. Below is how these four research projects align with the 3 Pillars of Research, including the questions these researchers are working to answer for you.

What cutaneous lymphoma do I have?
Patrizia Fuschiotti, PhD - *Single-cell transcriptome analysis of IL-4Ra-Positive cells in mycosis fungoides skin tumors*

How do I treat my cutaneous lymphoma?
Assia Angelova, PhD - *H-1 parvovirus-induced oncolysis and tumor microenvironment immune stimulation in a novel heterotypic spheroid model of cutaneous T cell lymphoma*

Pietro Quaglino, MD - *Mechanistic insights into the CD39/CD73 adenosinergic immunosuppressive axis in patients with Sézary syndrome: association with disease course and treatment response*

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Cutaneous Lymphoma Catalyst Research Grant 2020 Recipients

SINGLE-CELL TRANSCRIPTOME ANALYSIS OF IL-4RA-POSITIVE CELLS IN MYCOSIS FUNGOIDES SKIN TUMORS

Patrizia Fuschiotti, PhD
Assistant Professor of Medicine
Department of Medicine
University of Pittsburgh

Mycosis fungoides (MF) is a clonal disorder of skin-resident memory T lymphocytes and is the most common form of cutaneous T-cell lymphomas (CTCL). A striking feature of MF is the restriction of lymphocyte proliferation to the skin, which implies that the malignant cells are dependent on their specific cutaneous tumor microenvironment, including interactions with other immune and stromal cells. These non-malignant infiltrating and resident cells produce a variety of cytokines and other immunomodulator factors that affect cutaneous inflammation and are important constituents of the local microenvironment of tumors, fostering proliferation, survival, migration, and suppressing tumor-cell immunosurveillance. Thus, the pattern of cytokine production in the skin tumor microenvironment is of major importance for the pathogenesis of MF.

We have previously shown that cytokine IL13 and its receptors are highly expressed in the skin lesions of advanced-stage MF patients and that IL-13 signaling through the IL-4Rα receptor induces proliferation of malignant lymphocytes in advanced-stage MF tumors. However, IL4Rα is expressed not only by malignant cells but also by other cell types in the cutaneous microenvironment of MF tumors. Our hypothesis is that IL-4Rα expression by malignant lymphocytes, as well as by immune and stromal cells in the tumor microenvironment of advanced MF, plays an important role in MF tumorigenesis and progression.

The main goal of this project was to identify novel prospective targets for therapy as well as indicate strategies for tailoring therapy to specific patients. In this study, we employed cutting-edge, single-cell technologies that allow the simultaneous measurement of transcriptomes (RNA levels), immunophenotypes (cell surface protein levels), and T-cell clonal expansion (malignant cells) in thousands of individual cells from a large heterogeneous population such as a patient biopsy. With this novel approach we could determine the transcriptional profile of all cells that express IL4Rα on their surface as well as distinguish malignant from reactive T cells in the microenvironment of advanced-stage MF skin tumors.

Importantly, this comprehensive analysis was done on a minimal size biopsy (3mm) from patients and generated a large amount of data. Well-characterized patient samples were provided by our clinical collaborator Dr. Larisa Geskin at Columbia University. An example of the work pipeline is shown in Figure 1. Our pilot study identified patient-specific and common gene expression by IL4Rα-positive malignant and reactive lymphocytes as well as by IL4Rα-positive immune and stromal cells in the cutaneous micro-

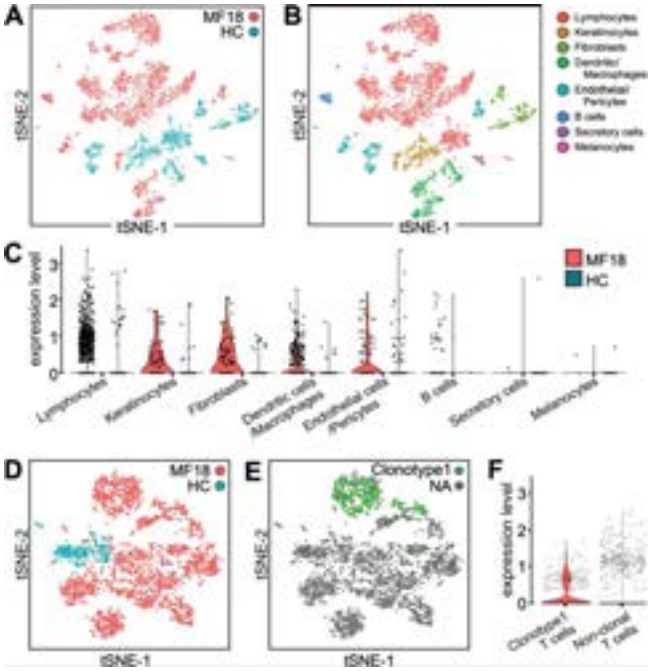


Figure 1. An example of the pipeline from skin biopsies to measuring the transcriptomes of specific cell types. Cell suspensions were prepared by enzymatic digestion from 3mm skin biopsies from five patients with advanced-stage MF and from 4 healthy controls (HC) and analyzed by single-cell RNA sequencing technology followed by extensive bioinformatic analysis. (A) The transcriptomes of all cell types from one MF patient (MF18, red) and 4 HC (blue) represented graphically so that clusters of cells with similar transcriptomes are apparent. Each dot corresponds to a single cell. (B) In the same distribution as (A), the cell types are colored according to known markers, and are also observed to cluster, in some cases overlapping with the tumor or HC signals, or both. (C) Comparison of the expression levels of IL4Ra by different cell types between the patient sample and the HCs. Each dot represents a cell. Significant differences are apparent in the lymphocytes, keratinocytes, dendritic cells/macrophages, endothelial cells and fibroblasts, consistent with (B). (D-F) Similarly to (A-C) but focusing only on the T lymphocytes from MF18 and HC control skin biopsies. The green in (E) marks cells of the malignant clone, and their expression of IL4Ra compared to benign T cells is shown in (F).

environment of MF advanced tumors. We observed up-regulation of pro-tumorigenic pathways associated with cancer cell proliferation, cell cycle regulation, and invasion as well as pathways associated with immunosuppression, chemotaxis, and tumor matrix remodeling. The proteins expressed by these particular genes may be targets for detecting the disease and its progression. In future studies, we plan to validate these candidate biomarkers in a larger pool of advanced-stage MF patients so that the most promising may be developed for clinical use.

CHARACTERIZATION OF THE SKIN MICROBIOME IN CUTANEOUS T CELL LYMPHOMA PATIENTS UNDERGOING PHOTOTHERAPY

Xiaolong (Alan) Zhou, MD, MSc
Assistant Professor of Dematology
Department of Dermatology
Northwestern University Feinberg School of Medicine

The cutaneous T-cell lymphoma (CTCL) microbiome is not well understood despite evidence that CTCL patients have an increased risk of skin infections with disease progression. Narrowband UVB phototherapy has response rates of 54-90% in early-stage disease. Here, we examined skin microbial changes of 25 phototherapy-treated CTCL patients (20 mycosis fungoides, 2 Sézary syndrome, 3 unspecified CTCL; 14 responders, 11 non-responders) using 16S gene sequencing.

For lesional skin, microbial richness was unchanged in responders pre- and post-phototherapy, but decreased after phototherapy in non-responders (Simpson, $p=0.046$). Post-phototherapy richness was greater in responders than non-responders (Shannon, $p=0.016$). For non-lesional skin, microbial richness did not differ with phototherapy for responders or non-responders (Shannon, $p=0.497$).

Bray-Curtis analysis showed that the microbial communities in non-lesional skin of responders and non-responders were distinct both before ($p<0.01$) and after ($p=0.017$) phototherapy. Moreover, microbial communities populating the lesional skin of responders and non-responders were distinct after phototherapy ($p=0.045$), but not before phototherapy ($p=0.181$). Bray-Curtis did not show intraindividual differences before and after phototherapy for responders or for non-responders in lesional skin or non-lesional skin ($p>0.35$). Non-responders were significantly enriched for *Porphyromonas* in lesional skin and *Acinetobacterium* in non-lesional skin compared to responders. Responders harbored high levels of Staphylococcus in lesional skin that decreased with phototherapy, and reduced Staphylococcus in non-lesional skin. *Corynebacterium* increased after phototherapy in lesional skin of responders but not non-responders.

CTCL skin may carry predictive signals for phototherapy response, including greater microbial α-diversity and a specific bacterial signature. Phototherapy alters the abundances of specific bacterial taxa in lesional skin and non-lesional skin in CTCL. Importantly, our data show that phototherapy reverts lesional skin microbial communities to a normal-like state in responders, while non-responders skin show signs of continued dysbiosis. Our longitudinal work is the first to demonstrate this in CTCL and lays the groundwork for follow-up trials to assess the skin microbiome as a novel biomarker for treatment response in CTCL and as an opportunity for therapeutic intervention.

H-1PV-INDUCED ONCOLYSIS AND TUMOR MICROENVIRONMENT IMMUNE STIMULATION IN A NOVEL HETEROTYPIC SPHEROID MODEL OF CUTANEOUS T-CELL LYMPHOMA

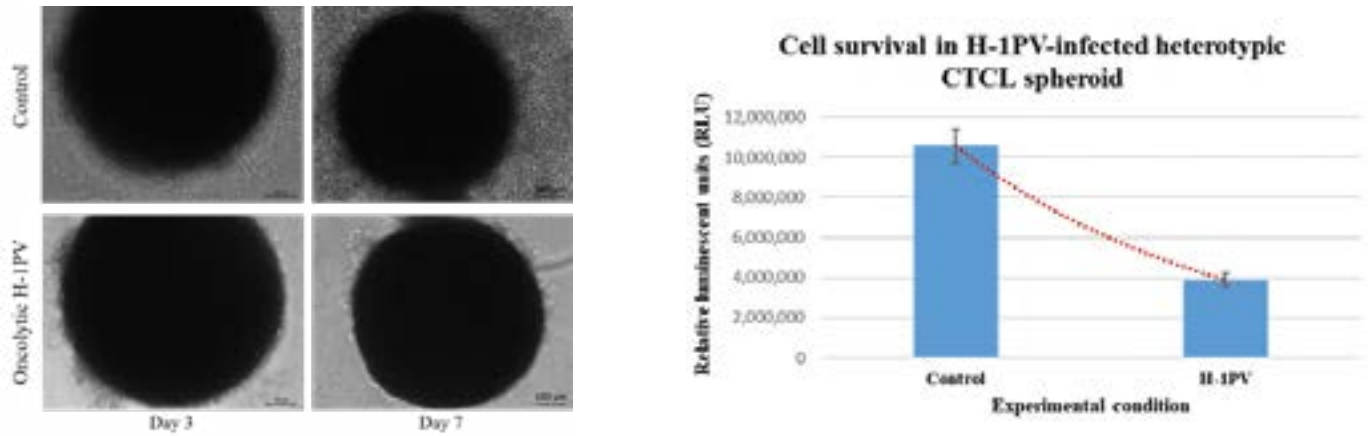
Assia Angelova, PhD
Clinical Cooperation Unit Virotherapy
German Cancer Research Center

Except for one single study from 2005 that uses an oncolytic measles virus, the oncolytic virotherapy approach remains largely unexplored in the biomedical cutaneous T-cell lymphoma (CTCL) research so far. Our project is therefore focusing on the preclinical testing of an oncolytic virus, the H-1 parvovirus (H-1PV), in in vitro CTCL models. H-1PV is considered as a promising therapeutic tool against various types of cancer, such as glioblastoma, pancreatic cancer, neuroblastoma, medulloblastoma, colorectal carcinoma, melanoma, non-Hodgkin lymphomas of B cell origin, etc. However, until very recently, no data on H-1PV oncolytic potential in CTCL were available. It is the ambition of this project to provide the first preclinical evidence demonstrating the efficiency of parvovirotherapy in CTCL. This launching step towards a future development of a novel treatment strategy against cutaneous lymphoma has been made possible by the generous support, through the Catalyst Research Grant 2021, of the Cutaneous Lymphoma Foundation.

In order to conduct in vitro experiments with higher relevance to the in vivo situation, we set the development of a three-dimensional (3D) CTCL model as one of our project goals. Heterotypic spheroids, which include various cell types in addition to CTCL, represent one such model of the tumor and its microenvironment. This model offers the possibility to analyze various cellular, viral and immunological parameters in the context of CTCL spatial growth and crosstalk with other cells.

The project encompasses three overlapping work packages, namely the establishment of the spheroid model, virological studies and immunological studies. These work packages correspond to the main research objectives, i.e., to generate CTCL spheroids, which model the presence of tumor microenvironment, to evaluate in this setting H-1PV-induced oncolysis and to measure the induction by H-1PV treatment of an immunogenic type of CTCL cell death.

The pilot experiments conducted in classical cultures demonstrated the capacity of H-1PV for infecting, replicating in and destroying CTCL cells as shown by their reduced viability in proliferation assays. As for CTCL sensitivity to H-1PV-induced tumor cell killing, the data obtained so far allow making a clear distinction between Bcl-2 (a cellular pro-survival protein)-positive (Bcl-2+) versus Bcl-2-negative cutaneous lymphomas. While the latter are readily infected, support a



Oncolytic H-1 parvovirus-induced cytopathic effects (A) and cell viability reduction (B) in a heterotypic CTCL spheroid

productive viral cycle and die within a few days after the treatment with low virus doses, the former seem to be intrinsically more resistant. Resistant CTCL cells require a longer exposure to H-1PV in order to die or – a worst-case scenario - an overgrowth of surviving cells takes place in the culture. It is these cells, which pose one of the major therapeutic challenges.

That is why we shall attempt to overcome Bcl-2+ CTCL resistance to H-1PV-induced oncolysis through a combined Bcl-2 inhibition and virus infection. To substantiate our working hypothesis, further analysis will be required, with a larger panel of CTCL cell lines and/or patients' samples. Although this will go beyond the time frame of the project, our immediate goal is to gather the evidence that is necessary to propose oncolytic H-1PV monotherapy (against Bcl-2-negative CTCL) and H-1PV combined therapy (against Bcl-2+ CTCL) for further preclinical development.

Using an improved hanging drop method, we were successful in establishing heterotypic spheroids comprising tumor, fibroblast (or keratinocyte) and endothelial cells. This methodology offers a three-dimensional reconstitution of CTCL and its microenvironment. It is therefore a better predictor of patients' therapeutic response to H-1PV than classical in vitro cultures. We could prove that H-1PV infection takes place also under 3D conditions. In a Bcl-2-negative (H-1PV-sensitive) model, the formation of spheroid invasive border was completely suppressed by parvovirus treatment.

The forthcoming project steps will shed light on the question of whether H-1PV-induced CTCL oncolysis is accompanied by the release of immunogenic signals, thus leading to immune system stimulation.

It is our hope that by establishing a novel in vitro CTCL model and proposing an original drug candidate, our research will pave the way towards the clinical translation of a novel CTCL treatment approach. ■

MECHANISTIC INSIGHTS INTO THE CD39/CD73 ADENOSINERGIC IMMUNOSUPPRESSIVE AXIS IN PATIENTS WITH SÉZARY SYNDROME: ASSOCIATION WITH DISEASE COURSE AND TREATMENT RESPONSE

Pietro Quaglino, MD
Associate Professor
Dermatologic Clinic, Dept Medical Sciences,
University of Turin

Research group participants and role in the project:

- Ada Funaro, Erika Ortolan: flow cytometry analysis and functional analysis
- Rebecca Senetta: pathology reports

The project development was strategically focused towards three different objectives which are intrinsically related; the results of these three main aims will be analyzed together to have a comprehensive view of the scenario. In detail:

- CD39/CD73 analysis
- CD38 analysis
- Clinical and disease outcome analysis of Sézary syndrome patients

Project Summary

Sézary syndrome (SS) patients show an aggressive disease that is characterized by a severe impairment in immunity which is related both to disease (the lymphocytes involved) and treatment.

(CONTINUED ON PAGE 6)

MECHANISTIC INSIGHTS...

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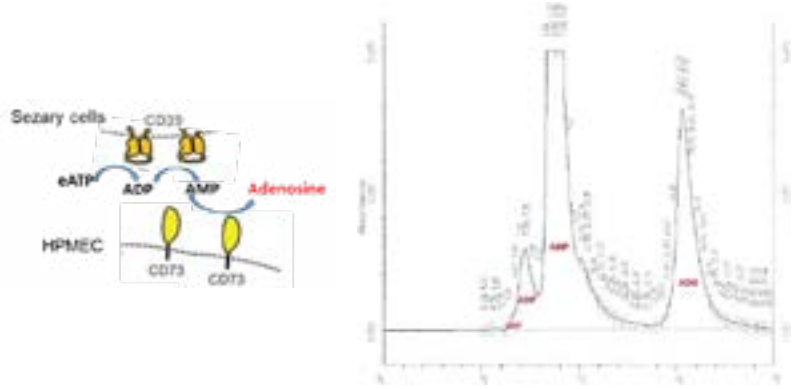
Our focus was on the mechanism that the immune suppression develops, a small molecule called adenosine, which is released in the microenvironment and induces a severe and profound immunodeficiency.

The question asked was, "Why does this patient develop this molecule?" Looking at the existing (research) literature, two different molecular pathways are identified in the blood of SS patients studied. One pathway included CD39 and CD73, which are two markers on the surface of the T cell of the disease, and the other pathway was CD38. With this project, we were trying to find which of these two pathways was involved in the production of adenosine in the patient.



CD39/CD73-mediated adenosinergic pathway.

Left panel: schematic representation of the interaction between Sézary cells and vascular endothelial cells leading to the hydrolysis of ATP into ADP and AMP by CD39. **Right panel:** extracellular ATP is hydrolyzed to ADP and AMP by CD39 expressed by CD4+CD39+ SS cells. AMP is converted into adenosine by CD73 expressed by vascular endothelial cells (HPMEC).

From this preliminary result, what became evident was that in the majority of cases, the action involved in the production of adenosine is on the pathway identified with CD39 and CD73. On the other end, a hallmark of the CD38 pathway is that it very rarely showed an expression of adenosine.

So the first pathway is more involved, some patients express CD39 much more and others express CD73, but both molecules belong to the same axis. We are working on trying to determine why some patients express one molecule over the other. We are also trying to analyze the expression of this molecule in the blood and correlate that with the skin.

Working with collaborative researchers in the genetic and pathology departments, what was found is that there is a great correlation between skin and blood in the expression of these markers. We found that if we blocked these markers on the antigen with specific antagonists, there is a dramatic decrease in the production of adenosine. So it means that these markers are definitely related to the production of adenosine.

If these results are confirmed, the consequences of this finding to clinical practice would be very important because monoclonal antibody treatments are already available that target both pathways, CD39/CD73 and CD38. This means that if you identify that a patient is characterized by an expression of one or the other pathway, the patient could be treated with the specific monoclonal antibody which would block the pathway, thus the production of adenosine and thus the immune suppression. This would be significant because we would have a treatment which would induce an improvement in the immune response, potentially leading to a response in the clinical lesions without immune suppression.

The other important point that is still being worked on, is analyzing the outcome of the patient that we know is expressing CD39 or CD73, or the minority expressing CD38. Do these patients show a difference in the disease course, and can we relate the expression of these markers to a prognostic factor of disease outcome or as predictive in the case of treatments.

This could all be important in the daily, clinical treatment of Sézary syndrome patients. It could induce higher response by selecting specific monoclonal antibodies and help identify before treatment, specific parameters related to disease course - for example, it could help determine those patients that might have a bad disease outcome and help the clinician to be more strategic in how to treat in order to get a better outcome.

In summary, the main points that we working on now are:

- To correlate the expression in the blood and the skin;
- To better identify patient clinical presentations and disease outcomes;
- To use the above to better define patient treatment to improve outcomes.

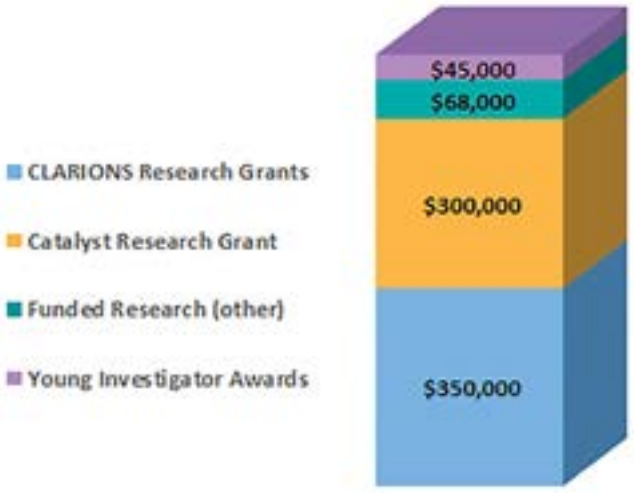
CONTINUING THE VISION...

(CONTINUED FROM PAGE 1)

Xiaolong (Alan) Zhou, MD, MSc - *Characterization of the skin microbiome in cutaneous T cell lymphoma undergoing phototherapy*

We want to take a moment to thank the incredible cutaneous lymphoma clinical and scientific community for their hard work and efforts to keep research moving forward throughout the pandemic. We are equally as grateful to the entire cutaneous lymphoma community for your continued support, allowing us to continue to fund important cutaneous lymphoma research. Don't forget, mark your calendars! We look forward to seeing you in July for our presentation of this year's Cutaneous Lymphoma Catalyst Research Grant recipients.

Awards / Grants Funded



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