

# COLLAGEN CONNECTION

SUMMER 2019

## SINGLE CELL RNA SEQUENCING CORE

by Tracy Tabib

What happens when you donate a biopsy for research? There are a number of possible uses but among them is now a cutting-edge technology: single cell RNA-sequencing. This means we can obtain an unprecedented level of information from a small piece of skin.

We begin by digesting the matrix fibers that holds the cells together (such as collagen). Then, once the cells are separated, they can be partitioned into individual oil reaction bubbles using new instruments such as 10X Genomics' Chromium. This lets us examine each cell and its unique function at a resolution never before possible. By tagging that cell and every molecule that it produces, we can track what every cell is making. What proteins is a cell from a blood vessel making? What proteins are the immune cells making? And how do these interact with all our other cells? Single cell RNA-seq gives us the tools to pursue answers to these questions. A healthy donor's cells can be compared to the cells from a fibrotic biopsy and from this we can determine where abnormalities arise.

Previously, researchers had been limited to looking at the skin and

## Meet Our Staff



Tracy Tabib, MS

Tracy Tabib is a senior laboratory specialist working in the Scleroderma Center in the Division of Rheumatology and Clinical Immunology (DRCI) at the University of Pittsburgh. She has an MS from Duke University and 8 years of research experience in cell/cancer biology and data analysis. She has worked at the University of Pittsburgh for almost 3 years

and joined the DRCI in May of 2016 working in the laboratory of Dr. Robert Lafyatis. Tracy lives in Oakland and in her spare time enjoys hiking, gardening, and working with animals. Tracy plays a key role in our Center's single cell RNA sequencing core. Please read Tracy's feature article to understand the importance of this technology.

all its cell types in bulk, mixing all of their information together and losing their unique signatures. Oftentimes, this resulted in small but important populations (such as white blood cells) being masked or having their signals diluted out. But now, with single cell RNA sequencing, we can isolate these cells and directly compare them to cells from healthy tissues and answer important questions such as: do cells from diseased tissues produce more of certain genes? Are there increased numbers of cells from certain populations? Are there shifts in ratios? Finding a potential troublemaker cell type now becomes possible and these can subsequently be targeted with therapeutics and pharmaceuticals.

Critically, we can also see how any intervention is affecting the other cell types in the tissue, regardless of whether or not they are the target.

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This technology is also incredibly dynamic and rapidly advancing. One of the latest developments allows researchers to tag surface proteins to see what is already present on a cell's outer membrane. This allows for better profiling of cells' identities and also helps to establish good candidate markers for sorting and separating cells for future experiments.

Excitingly, we can also much more fully characterize rare cell types that are otherwise very difficult to isolate from small skin biopsies. What are the cells of the peripheral nervous system making, for example? Well, we can find answers with this technique! (The answer: lots of myelin proteins that provide protective insulation for our nerves.). With this new technology at our fingertips, a plethora of complex problems can now be teased apart to provide answers to long-standing questions and generate new inquiries that we never before would have been able to consider.

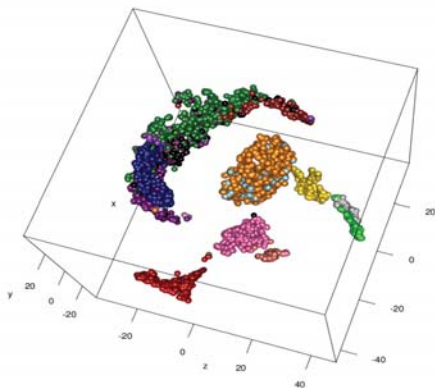


Figure 1: Three dimensional tSNE graph representing single cells as dots, with similar cells grouped together and colored by cell type. Cells were dissociated from whole tissue and analyzed by scRNA-seq. The above graph shows relationships and similarities derived from their gene expression.

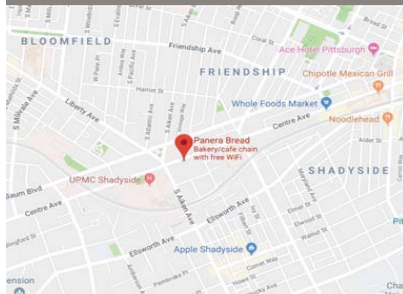
2018 UPMC EXCELLENCE IN PATIENT EXPERIENCE AWARD

**Dr. Robyn Domsic** won a **2018 Excellence in Patient Experience Award.**



Her patients rated her as a top UPMC provider by specialty at the 95% national percentile. Dr. Domsic is one of 50 recipients of this award and will receive the award at the reception to be held at the 2018 Loren Roth Quality and Patient Safety Awards Reception at the UPMC Passavant Cumberland Woods Conference Center.

## Pittsburgh Area Scleroderma Support Group

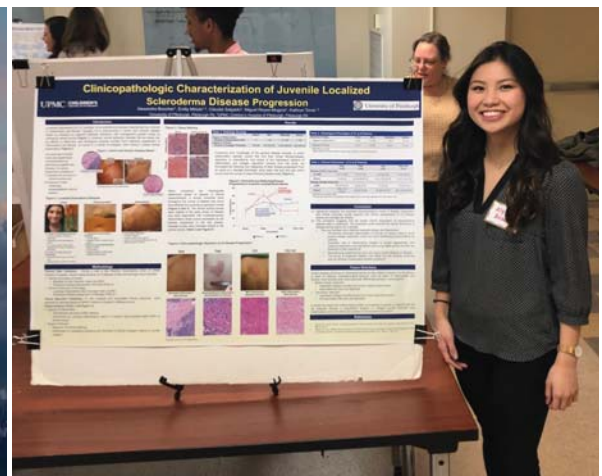
Remaining Dates for 2019

- Sep 28
- Oct 26
- Nov 30
- Dec 28

- Meet others affected by Scleroderma
- Share concerns and information
- Give and receive encouragement
- Occasional guest speakers
- Caregivers and family members welcome

Where: Panera Bread (Meeting Room)  
5430 Centre Ave  
Pittsburgh, PA 15232  
Time: 3:00 - 4:30 pm

Meetings are free of charge  
For more information contact  
sfgrouppitt@gmail.com



The Scleroderma Center's Research Assistant, Aly Boucher presents a poster on pediatric localized scleroderma.



## A FAMILY GIVES BACK -AMAZING GRACE



Grace's journey with Scleroderma started in 2017 when a dermatologist noticed the line on her forehead we now know is localized scleroderma. This doctor had never seen a case in person but was very confident in her diagnosis and urged Grace to see a pediatric rheumatologist quickly. We are forever grateful to our dermatologist for catching this early.

Grace visited a pediatric rheumatologist at the University of Minnesota the next month and was also diagnosed with juvenile polyarticular arthritis. At the same time, we learned of Dr. Torok and made an appointment to visit Children's Hospital of Pittsburgh. We knew we would need the support of Dr. Torok along with our local Rheumatologist. Grace's en coup de sabre and Parry Romberg Syndrome diagnoses were confirmed by Dr. Torok in 2018 and she began treatment.

Our Amazing Grace is incredibly strong both in body and spirit. She is a true fighter and always tries

to find the positive in what has been a life altering and sometimes painful condition. She reminds us everyday how resilient kids can be.

When Grace was diagnosed, the first thing we did was turn to the internet to research all that we could about scleroderma. After learning more about the disease, treatments, treatment side effects, we discovered that there is currently no defined cure. We realized how little research there is dedicated to scleroderma and even more so for juvenile onset scleroderma.

We knew we had to do whatever possible to help raise funds for research and hopefully discover new treatments leading to a cure. We discovered the work Dr. Torok is doing at the University of Pittsburgh Scleroderma Center and knew immediately that we needed to raise funds for her research team. The dedication of Dr. Torok and her team give us hope that they are going to find answers, better treatments, and one day a cure.

We are still at the beginning our fundraising efforts and started off by putting together a Facebook fundraiser to send funds to Dr. Torok, with confidence that 100% of these funds would directly be used for



pediatric scleroderma research at the University of Pittsburgh. Part of this fundraising includes spreading awareness. We are sharing Grace's story throughout the community via Facebook, and our local Scleroderma Foundation Chapter Walk for a Cure in Minnesota. We are committed to continue to spread awareness and raise money for scleroderma research. We are currently working on a few fundraisers including a bingo night, garage sales and teaming with our community. We know that starting small is the way we can make a difference and will keep working to raise more research dollars on a larger scale. We won't stop until a cure is found!





## CLINICAL DRUG TRIALS AND OBSERVATIONAL STUDIES

Our Scleroderma Center is committed to participating in clinical trials. We feel it is a vital step in working together to find treatments for scleroderma. Without clinical trials, our field will not advance. If you are interested in participating in a trial or would like additional information, please contact Maureen Laffoon at 412-648-7871 or [laffoonm@pitt.edu](mailto:laffoonm@pitt.edu).

### CURRENTLY ENROLLING:

#### ***Evaluation of Brentuximab Vedotin for Diffuse Cutaneous Systemic Sclerosis: A Phase I/II Multicenter, Randomized, Double-Blinded Safety Study (BRAVOS):***

Brentuximab vedotin (name brand Adcetris) is a drug that was developed and has FDA approval for the treatment of lymphoma. This research is being done to evaluate the safety and tolerability of brentuximab in the treatment of diffuse skin disease in scleroderma. Patients must be early in their disease with worsening skin to participate. Two of three patients will receive brentuximab vedotin, and the other individual, placebo. The study lasts 48 weeks and involves 14 visits. Patients will be able to remain on their current scleroderma medications.

#### ***Dimethyl Fumarate, Tecfidera, In Pulmonary Arterial Hypertension (PAH) Associated With Systemic Sclerosis (DMF):***

Tecfidera is an investigational drug that reduces the activity of chemicals in your body that are triggered by inflammation. This process is called oxidative stress. Oxidative stress is increased with pulmonary arterial hypertension associated with systemic sclerosis. The study is designed to see if Tecfidera is safe and whether it is associated with any improvement in 6-minute walk test in people with pulmonary arterial hypertension associated with systemic sclerosis. Half of the subjects will receive Tecfidera and half placebo. Other PAH medications can be continued throughout the study. This is a 36 week study with a total of 6 visits.

#### ***An Observational Study of the Effect of Mycophenolate Mofetil (Cellcept) in Early Diffuse Scleroderma (MMF STUDY):***

This is a NIH-supported single-center study (being performed only in Pittsburgh) to observe the effect of mycophenolate for the treatment of early diffuse scleroderma. Mycophenolate is one of the most commonly used medications to treat diffuse scleroderma and scleroderma-related pulmonary fibrosis, however we know little about how to predict who will respond well to the medication. Patients whose physician recommends they should be clinically treated with mycophenolate are eligible to be in this study. When in this study your doctor may change your medications at any time. We simply observe the effect of mycophenolate, collect data on its effect on skin, and collect blood and oral swab samples every 3 months

for the first year a patient is treated with mycophenolate. This study can easily be combined with regular patient visits.

#### ***A Phase II double-blind, clinical trial addressing the treatment of patients with active and symptomatic scleroderma-related interstitial lung disease (SLSIII):***

This study compares the safety and effectiveness of adding on pirfenidone to current mycophenolate (Cellcept) therapy for scleroderma-related lung disease. Pirfenidone is FDA approved for the treatment of pulmonary fibrosis. This research is designed to test whether combining pirfenidone and mycophenolate will result in a more rapid and possibly greater improvement in lung function than occurs when mycophenolate is used alone. There are 13 office visits and 11 phone calls over 18 months.

#### ***A Phase I Open-Label Study to Determine the Safety and Tolerability of AVID200 in Patients with Diffuse Cutaneous Systemic Sclerosis (AVID):***

AVID200 is a new drug that interferes with proteins that are thought to play a major role in the development of fibrosis in scleroderma patients. This trial seeks to assess safety and tolerability of AVID200 in those with diffuse cutaneous systemic sclerosis (dcSSc). The drug will be administered by intravenous (IV) infusion once every 2 weeks. Patients will receive a total of 3 infusions, and there will be additional follow-up safety visits for a total of 9 visits over the 23-week study. All participants will receive AVID200.



### ***Autologous Stem Cell Transplantation with CD34-Selected Peripheral Blood Stem Cells PBSC in Patients with Treatment Resistant Systemic Sclerosis:***

This study will utilize intermediate doses of radiation and chemotherapy, paired with intense immunoablative serotherapy, in preparation for an autologous stem cell transplant. Here, CD34 selection will remove potentially autoreactive lymphocytes. The hypothesis is that after T and B-cell depleted autologous graft infusion a normal immune system will reconstitute without regeneration of autoimmunity. In addition, we propose this conditioning regimen is a safer strategy from a cardiopulmonary, urinary, and bone marrow perspective compared to the published work of other centers, nevertheless, leads to improved SSc symptoms with possible delay of disease recurrence or progression.

### ***Validation of the preliminary Assessment of Scleroderma associated Raynaud's Phenomenon (ASRAP) questionnaire:***

A team of Scleroderma specialist developed a new questionnaire for assessing the severity and impact of Raynauds phenomenon (RP) in people affected by systemic sclerosis (SSc). We hope the Assessment of Scleroderma-associated Raynaud Phenomenon (ASRAP questionnaire in short) questionnaire will help us measure the severity of Raynauds more accurately. Before we start using this questionnaire in clinical trials, we must first undertake work to ensure it is reliable, feasible and measures Raynaud's symptoms effectively. To achieve this, we are

undertaking a study to test the new questionnaire across a large group of people with SSc. The study itself will involve the completion of the new ASRAP questionnaire and then complete a small pack of additional self-administered questionnaires, which shall collect information on RP symptoms. This study only requires one visit. We anticipate approximately 20 minutes of your time to complete the study.

### ***Optimizing Raynaud Phenomenon Outcome Measures in Systemic Sclerosis (ROSS):***

The purpose of this research study is to improve clinical trial design when studying Raynaud phenomenon (RP) in systemic sclerosis (SSc). We are validating a new patient-reported questionnaire, testing a smart phone application to assess Raynaud attack frequency/duration and comparing new imaging techniques for skin blood flow in the hands. The study is over one year, as we recognize Raynaud symptoms change across seasons, and wish to gain a better understanding of how seasons affects these new outcome measures. Ultimately, improving outcome measures could aid in having a drug show a positive change and gain approval for Raynaud treatment. The observational study will last 48 weeks, although those who start a new medication for Raynaud medication will be asked to be follow for a longer period so the effect of the new medication can be captured. There are six study visits within those 48 weeks. At each visit, all subjects will be asked to complete a one week diary the number of Raynaud attack, and on the day of the visit complete a small packet of self-administered questionnaires that collect

information on your Raynaud symptoms, function, mood, and quality of life. At all visits except week 36, subjects will undergo non-invasive blood flow imaging using a laser speckle contrast imaging (LSCI) and infrared thermography (IT) machine imaging of their hand. Visits will be 90 minutes on the first day, and thereafter 30-60 minutes.

### ***ENROLLING SOON:***

### ***A Multicenter, Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study Evaluating the Safety and Efficacy of Intravenous Iloprost in Subjects With Systemic Sclerosis Experiencing Symptomatic Digital Ischemic Episodes (AURORA Study):***

This research study is being done to find out more information about the study drug iloprost for the treatment of moderate to severe symptomatic RP in people with SSc. Iloprost has been approved in the United States and Europe as an inhaled drug to treat people with pulmonary arterial hypertension (high blood pressure that affects the heart and lungs). The drug is also approved in Europe and New Zealand as an intravenous (IV) drug for the treatment of severe disabling RP unresponsive to other therapies. The main purpose of this research study is to see if the study drug iloprost has an effect on how often symptomatic RP attacks occur compared to the frequency of symptomatic RP attacks with a placebo. We will also study the effect of iloprost on how long symptomatic RP attacks last and the severity of the symptomatic RP attacks, compared to attacks with a placebo. The IV drug is administered on 5 consecutive days and the study last 9 weeks.



**NIH Grant Awarded**



Dr. Torok and collaborator Dr. Heidi Jacobe at UT Southwestern have been awarded an R01 grant from the

National Institutes of Health entitled: “Refining outcome measures through observational cohorts to advance trials in morphea”. This grant will leverage the National Registry for Childhood Onset Scleroderma at the University of Pittsburgh/Children’s Hospital of Pittsburgh and the Morphea in Adults and Children (MAC) registry at UT Southwestern and multidisciplinary expertise of Drs. Torok and Jacobe to validate localized scleroderma (morphea) skin activity and damage measures such as lesion color, texture, pigment, as well as questionnaires for future use in clinical trials, and develop a data platform to visualize clinical data and questionnaire results over time. This study is an important step toward pediatric localized scleroderma clinical trials. We will keep you updated on our progress!

**CORT Small Grant Awarded**

Dr. Torok was awarded a Centers of Research Translation (CORT) grant from the National Institutes of Health to explore the transcriptome (which RNA and how much is being

produced) in the skin of children with localized scleroderma (LS, morphea). RNA is a copy of genetic information that instructs cells to make specific proteins within the body. LS is an autoimmune disease that disturbs macrophage and T cell production of inflammatory cytokines (special proteins) that stimulate thickening and scarring of connective tissue. Dr. Torok and her team hope to identify genetic profiles and targets that provide more information on how LS develops and progresses, which will eventually aid in development of biomarkers and therapeutic treatments.

**Scleroderma Foundation Grant Awarded**

Dr. Torok and Dr. Heidi Jacobe (UT Southwestern) have been awarded a 2-year grant from the Scleroderma Foundation entitled “Transcriptional Profiling of Inflammatory and Fibrotic Skin Signatures in Localized Scleroderma”. This grant will leverage the National Registry for Childhood Onset Scleroderma (Pittsburgh) and the MAC Cohort (UTSW) to conduct single cell RNA sequencing on skin samples from LS patients. LS can be disfiguring and disabling especially if the disease begins during childhood and affects growth, resulting in reduced joint range of motion, uneven extremity size, and distorted facial features. Intervening during active LS is essential for minimizing scarring,

skin thickening, and long-term consequences for children. This study utilizes innovative single cell RNA sequencing that will identify

genes in individual cells turned on or off during LS inflammation or fibrosis in the skin. Identifying genes involved in inflammation and fibrosis using skin specimens may help us understand why LS occurs and help develop more effective therapies, leading to improved outcomes in both children and adults.

**Genetic Research**

Dr. Torok and her research team are performing genetic research in collaboration with the National Institutes of Health (NIH). We are collecting saliva DNA samples from trios: a child with scleroderma, their mother, and father. In some cases, siblings and grandparents are also participating. The goal of this exciting new research is to determine which genes are turned on or off in children with systemic sclerosis or localized scleroderma in order to develop new therapies and diagnostic tools.

If you/your child has pediatric onset systemic sclerosis, or early onset localized scleroderma (prior to age 8) and you are interested in participating, please e-mail us at [scleroderma@chp.edu](mailto:scleroderma@chp.edu) for more information. Compensation is offered to healthy individuals.



We would like to thank the following donors for their support of scleroderma research



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Your contributions to the Scleroderma Center are greatly appreciated and help support research and patient education programs. You can remember or honor a loved one by using the envelope enclosed in this newsletter to send your donation.

For additional information on giving to the Scleroderma Center, please contact Rose Jandrasits at 412-864-1958 or krj13@pitt.edu.



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