Investigating the Role of Skin-Resident Memory T Cells in Cutaneous Delayed-Type Drug Hypersensitivity Reactions

Investigating the role of skin-resident memory T cells in cutaneous delayed-type drug hypersensitivity reactions

by

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ABSTRACT

Cutaneous delayed-type drug hypersensitivity reactions (dtDHRs) span the range of clinical severity from mild rash that is self-limited to severe sloughing of skin and mucosal surfaces with high mortality and no proven treatment. T cells are generally thought to be the principal actors driving these reactions. However, basic mechanisms underlying them, such as the phenotype and function of the T cells involved, remain poorly understood. Research into this topic has long been limited by lack of access to specimens sufficient for laboratory analysis. This project investigates whether skin-resident memory T cells mediate dtDHRs, using innovative techniques to overcome previous barriers to research. To begin addressing this hypothesis, we first developed a robust database of clinically- and pathologically-confirmed cases of three types of skin dtDHR: Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN), drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, and morbilliform drug eruption (MDE). The database included extensive clinical data, which allowed us to conduct a nested clinical study on clinicians' ability to identify culprit drugs correctly and the consequences of incorrect culprit drug identification. It revealed that culprit identification is complicated by patients taking multiple drugs (69% on 4+) before disease onset, clinicians used no testing or validated approaches to determine culprits, 40% of patients had concurrent infections that may have confounded a drug-induced etiology, and patients' allergy lists subsequently contained possible inaccuracies that could have adverse consequences for the care provided to them and even for public health in general. We performed transcript analysis of 187 target genes using Nanostring on formalin-fixed, paraffin-embedded (FFPE) skin samples from adult and pediatric patients with MDE (n=6), DRESS (n=6), and SJS/TEN (n=13). Only SJS/TEN > 10% TBSA blistered skin were included

to reduce diagnostic error. Healthy skin served as controls (n=10). Preliminary results revealed significantly increased CD3 and CD8, non-specific memory marker CD45R0, and central memory T cell marker CD62L in SJS/TEN and DRESS compared to healthy controls, but not in MDE samples. Skin-resident memory T cell markers CD69 and CD103 were not elevated in any DHR group. The dtDHRs largely demonstrated a Th1/Tc1 skewing. Microscopy confirmed that the majority of T cells (CD3+) were CD45RO+ in all three dtDHR types yet a minority of T cells were CD103+. T cells consisted of both CD4+ and CD8+ subsets and were largely CLA+. A group of patients with MDE were identified that were profoundly lymphopenic, indicating that they were nearly depleted of circulating T cells compared to healthy controls. These lymphopenic skin samples contained CD4+ and CD8+ T cell subsets that were predominantly CD45RO+ and CLA+, and were of equivalent numbers as healthy controls. These data suggest that skin-resident memory T cells can mediate MDE, but that central memory T cells are recruited to skin in SJS/TEN and DRESS. These findings may explain why MDE is largely skin-limited while SJS/TEN and DRESS involve multiple tissues/organs. This work provides a strong platform on which future basic and clinical investigations into dtDHRs can be conducted, and it offers an appealing framework for other skin researchers interested in extracting valuable information from rare cutaneous diseases.

GLOSSARY OF ABBREVIATIONS

ALAS1	Delta-aminolevulinate synthase 1
ALDEN	Algorithm for Drug causality in Epidermal Necrolysis
BCH	Boston Children's Hospital (Boston, MA)
BMT	bone marrow transplant
BUN	blood urea nitrogen
BWH	Brigham and Women's Hospital (Boston, MA)
C.P.I.	checkpoint inhibitors
CCR[x]	C-C chemokine receptor type [x]
CD[x]	cluster of differentiation [x]
CLA	cutaneous lymphocyte-associated antigen
CTLs	cytotoxic T lymphocytes
CXCL[x]	C-X-C Motif Chemokine Ligand [x]
DAB	diaminobenzidine tetrahydrochloride
DAPI	4′,6-diamidino-2-phenylindole
DM	mean difference
DRESS	drug reaction with eosinophilia and systemic symptoms
dtDHR(s)	delayed-type drug hypersensitivity reaction(s)
EBV	Epstein–Barr virus
EM	erythema multiforme
F	female
FASLG	Fas ligand
FC	fold change
FDR	false discovery rate
FFPE	formalin-fixed, paraffin-embedded
FOV	field(s) of view
GATA3	GATA Binding Protein 3
GI	gastrointestinal tract
GNLY	granulysin
GZM[x]	granzyme [x]
H&E	hematoxylin and eosin
HHV[x]	human herpesvirus [x]
Hisp./Lat.	Hispanic or Latino
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplant
HSV	herpes simplex virus
ICU	intensive care unit
IF	immunofluorescence
IFNγ / IFNG	interferon γ
IL[x]	interleukin [x]
IS	systemic immunosuppression
ITGAE	integrin, alpha E
IVIG	intravenous immunoglobulin
JAK[x]	Janus kinase [x]
LOD	limit of detection
M	male
M. pneumo.	Mycoplasma pneumoniae
max	maximum
MDE	morbilliform drug eruption
MGH	Massachusetts General Hospital (Boston, MA)
MTX	methotrexate
N	no
NK	natural killer
nl hu	normal human
No.	number
nt	nucleotide(s)
p	p-value
p_a	false discovery rate-adjusted p value
PBMCs	peripheral blood mononuclear cells
PC	principal component
PCA	principal component analysis
POLR1B	RNA Polymerase I Subunit B
Pre-bx	pre-biopsy
PRF1	perforin
RNA	ribonucleic acid
S.S.	systemic steroids
SCAR	severe cutaneous adverse reaction
SCORTEN	Score of Toxic Epidermal Necrolysis
SELL	selectin L
SJS	Stevens-Johnson Syndrome
SJS/TEN	Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (>10% TBSA)
STAT[x]	signal transducer and activator of transcription [x]
TBP	TATA-Box Binding Protein
TBSA	total body surface area
TEN	toxic epidermal necrolysis
TNF-α	tumor necrosis factor-α
Trm, Treg	resident memory T cells, regulatory T cells
um / μm	micrometer
URI	upper respiratory infection
Y	yes

1 INTRODUCTION

1.1 **Overview of delayed-type drug hypersensitivity reactions (DHRs)**

At least two to three percent of patients taking commonly prescribed medications develop disorders known as delayed-type drug hypersensitivity reactions (DHRs) (Bigby 1986). According to the classical Gell and Coombs system, DHRs are categorized as type IV reactions, which means they are considered delayed in onset and mediated by T cells (Pichler 2003). A different system proposed by the World Allergy Organization uses the timing of symptom onset to classify DHRs as either immediate or delayed (Montañez 2017). Among the fraction of patients who suffer DHRs, the skin is the most commonly affected organ. Cutaneous manifestations range from the mild and self-limited to the severe and life-threatening. The latter are often referred to as severe cutaneous adverse reactions (SCARs) and are an important cause of morbidity and mortality in routine patient care. For this work, we chose to focus on the two SCARs with the highest mortality rates: Stevens–Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) and drug reaction with eosinophilia and systemic symptoms (DRESS). We decided to study these in comparison to a mild DHR, morbilliform drug eruptions.

- ***Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN)***

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) represent two ends of a spectrum of severe mucocutaneous blistering disease with SJS-TEN overlap falling between them. Patients are febrile and experience painful blistering and sloughing of their skin. Frequently, mucosal areas such as the eyes, mouth, and genitals are also involved (Figure 1). Patients with SJS have detachment of less than 10% of their body surface area, those with SJS-TEN overlap have 10-29%, and those with TEN have more than 30% (Bastuji-

Garin 1993). Histologically, SJS/TEN is characterized by sub-epidermal blistering with full-thickness necrosis of the epidermis and a sparse, perivascular lymphocytic infiltrate. Because there can be relatively higher diagnostic uncertainty for cases of SJS (which may be confused with erythema multiforme major, for example), we chose to focus exclusively on cases of SJS-TEN overlap and TEN. For simplicity, we will jointly refer to these as SJS/TEN.

The annual number of SJS/TEN cases per million people has been reported as 0.93-1.89 in Germany, 5.76 in the United Kingdom, and 12.7 in the United States (Mockenhaupt 2012, Schopf 1991, Hsu 2016, Frey 2017, Chung 2004). In the United States, the incidence is lower in children than among adults (Hsu 2017). Incidence also appears to differ between ethnic groups, with Asians and blacks having a stronger association with the disease (Hsu 2016).

Despite its relative rarity, SJS/TEN is associated with a high mortality rate: ~10% in SJS, 30% in SJS-TEN overlap, as high as 50% in TEN, with an overall rate of 25% (Roujeau 1994, 1995). Despite improvements in quality of healthcare, mortality in TEN has not declined in recent decades (Hsu 2016, Diphoorn 2016, Sekula 2013). Mortality in individual cases of SJS/TEN can be predicted using the SCORe of Toxic Epidermal Necrolysis (SCORTEN), a system that uses 7 clinical variables (i.e. age, associated malignancy, heart rate, serum BUN, body surface area involvement, serum bicarbonate, and serum glucose) to score disease severity (Bastuji-Garin 2000, Guegan 2006).

Unfortunately, there are no proven treatments for patients with SJS/TEN. Standard of care includes stopping the culprit drug and likely transferring to a burn unit. Systemic corticosteroids have often been used in the past, but they may be associated with increased progression of disease and have demonstrated no clear survival benefit (Sekula 2013,

Dodiuk-Gad 2014, Lee 2012, Law 2015). IVIg is regularly used adjunctively in children or in adults with severe cases, but even at high doses it has not demonstrated any statistically significant benefit (Huang 2012, 2016). Several case reports suggest anti-TNF-α medications may be helpful in patients with TEN (Wojtkiewicz 2008, Paradisi 2014, Fischer 2002, Hunger 2005, Kreft 2010, Zarate-Correa 2013). Novel therapeutics have been developed that block soluble mediators, and these may hold promise if they inhibit factors that mediate disease. In this project, we aimed to shed light on the factors involved in SJS/TEN in hopes of identifying potential drug targets.

- ***Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome***

In DRESS syndrome, symptoms manifest 2 to 6 weeks after drug initiation, which is later than most immune-mediated skin reactions (Bocquet 1996). Patients develop widespread erythema and edema of skin, particularly in the face and ears, and the skin may be itchy or tender (Figure 2). Patients are febrile and may have lymphadenopathy, eosinophilia and atypical lymphocytosis (Walsh 2011). Internal organs may be affected with the liver being involved in 51-84% of patients (Wei 2011, Roujeau 1994). Renal involvement is also frequent, as it has been reported in 10-57% of patients (Wei 2011, Roujeau 1994). A variety of inflammatory patterns may be seen on histology, including eczematous, interface dermatitis, and erythema multiforme-like morphologies. The third most commonly involved organ is the lung, where symptoms may be nonspecific or may include pleuritis, acute respiratory distress syndrome, and interstitial pneumonitis (Kano 2010, Matsuno 2012). In 4-27% of patients, involvement of the heart has been reported (Thongsri 2017). Other organs may be involved as well.

DRESS is more common in adults but can occur in children as well (Cacoub 2011). Mortality in DRESS is about 10% (Kardaun 2013). Degree of systemic involvement is the major determinant of prognosis (Kano 2010). The mainstay of treatment in DRESS has been systemic corticosteroids, though an elevated risk of opportunistic infections and other complications may be associated (Cho 2017). A rapid response to treatment was reported in several patients with DRESS on cyclosporine, which thus could be considered as an alternative when systemic corticosteroids are contraindicated or ineffective (Kirchhof 2016, Kuschel 2018, Ton 2020). Other potential treatments include plasmapheresis and other immunosuppressive medications (Husain 2013).

- ***Morbilliform drug eruptions (MDE)***

In MDE, patients develop a self-limited maculopapular exanthema (Bolognia 2012, Figure 2). Classically, it appears 7 to 14 days after initial drug exposure, but it returns faster upon re-challenge. The skin may be itchy, and patients may or may not have a fever and/or eosinophilia. Findings on histology are non-specific and may include interface changes and a mild superficial perivascular and interstitial lymphocytic infiltrate with eosinophils seen in up to 70% of cases (Gerson 2008). Mortality is ~0%. Depending on severity, the culprit drug can either be continued or stopped and topical steroids initiated.

1.2 Drugs and genes in selected dtDHR

Virtually any drug has the potential to cause a dtDHR, but some are more commonly implicated than others. Per EuroSCAR, drugs with high risk of causing a SCAR include sulfonamides, aromatic anticonvulsants, allopurinol, oxicam nonsteroidal anti-inflammatory drugs, and nevirapine (Mockenhaupt 2008). The most commonly reported culprits in DRESS

specifically are anticonvulsants and allopurinol (Kardaun 2013). Most classes of drugs can induce an exanthematous eruption in ~1% of patients exposed. A greater proportion (>3%) of patients exposed to aminopenicillins, allopurinol, sulfonamides, cephalosporins, and aromatic anticonvulsants develop MDE.

Certain HLA alleles are known to be associated with increased risk of developing SJS/TEN or DRESS syndrome when exposed to particular drugs. The strength of genetic associations with elevated risk of severe hypersensitivity corresponds to the prevalence of risk alleles in different ethnic populations (e.g. HLA-B∗15:02 and HLA-B∗58:01 in Asians) (Ferrell 2008, Phillips 2011). Genetic associations are also usually drug-specific. For example, HLA-B*15:02 is associated with carbamazepine-induced SJS/TEN. Because of its high negative predictive value, it has been incorporated as a screening test before carbamazepine prescription in routine clinical practice throughout Southeast Asia, where reductions in carbamazepine-induced SJS/TEN have been significant (Chen 2011).

1.3 Microbial factors in selected dtDHRs

Microbes may be implicated in the development or exacerbation of dtDHRs as well. Although SJS/TEN is largely a drug-induced phenomenon, for example, a fraction of cases may be attributed to Mycoplasma pneumonia infection, viral infection, and even collagen vascular diseases (Auquier-Dunant 2002, Olson 2015, Chung 2013). This means infection should always be considered when a patient presents with SJS/TEN, even if they are taking medications implicated in dtDHRs concurrently; otherwise, the true etiology could be missed and the patient could be incorrectly labelled allergic to a medication they need. DRESS patients who experience reactivation of HHV-6 may have increased T cell activity during the

drug eruption and an induction of the synthesis of proinflammatory cytokines (e.g. TNF-α and IL-6) (Yoshikawa 2006). Additionally, sequential reactivation of multiple herpes viruses (HHV-6, HHV-7, Epstein-Barr virus, and cytomegalovirus) within a single host and in a particular order were found to be coincident with development of clinical symptoms in DRESS syndrome (Shiohara 2007).

1.4 Biomarkers and culprit drug identification for selected dtDHRs

Biomarkers of disease can be helpful for establishing a diagnosis (as well as informing treatment and surveillance), especially in severe forms of disease. At present, availability of biomarkers for dtDHRs is limited. The most prominently discussed biomarker for severe dtDHRs is granulysin, a key cytotoxic molecule responsible for disseminated keratinocyte necrosis. In SJS/TEN, serum granulysin levels reportedly correlate with acute disease severity as well as mortality (Chung 2008, 2015). Levels are markedly increased prior to any skin detachment or appearance of mucosal lesions, then drop rapidly in less than 5 days after disease onset (Abe 2009). Similarly, in DRESS syndrome prolonged elevation of serum granulysin levels has also been observed and might be used for early diagnosis and predicting disease prognosis (Saito 2012). For patients with DRESS, CD4+ T cells may be elevated in the acute stage, and this reportedly correlates with severity of clinical symptoms (Shiohara 2010). Levels of IL-15 have been reported to correlate with disease progression and mortality of SJS/TEN at early stages (SC 2017). Further research into the pathobiology of dtDHRs could add value in this area if unique bio-molecular signatures are discovered, especially for severe forms of disease.

It is critical that offending medications be correctly identified in patients with dtDHRs in order to remove the culprit (which is the mainstay of treatment for SJS/TEN), prevent inadvertent re-exposure and recurrence of disease, as well as to avoid unnecessary withdrawal of non-culprit medications patients may need. Unfortunately, there is no standard method for confirming offending drugs. The gold standard for identifying a culprit drug is re-exposure to the drug, but given potentially life-threatening risk this approach is not used routinely. One alternative could be the use of HLA genotyping. It has already proven useful in screening at-risk populations for susceptibility to SCAR, so the genotyping could similarly be used to reveal drug-specific susceptibilities (Phillips 2011, SC 2016). In SJS/TEN patients, one validated, structured approach to assessing culprit drugs is the Algorithm for Drug causality in Epidermal Necrolysis (ALDEN) (Sassolas 2010). Its clinical impact may be limited, however, as it is unclear whether clinicians are using it.

1.5 T cells and the pathobiology of dtDHR

T cells are thought to be major protagonists in dtDHRs, as the various types all include involvement of the T cell receptor (Lerch 2004, Pan 2019). In response to certain environmental conditions, particular T-cell subpopulations develop and produce cytokines that direct the immune response. The subpopulations include T-helper type 1 (Th1), Th2, Th17, cytotoxic T lymphocytes (CTLs), and regulatory T cells, among others. Despite evidence that T cells are involved, the phenotype, function, and mechanisms by which T cells become activated in dtDHRs are unknown.

- ***A Spotlight on SJS/TEN***

Of the various dtDHR types, SJS/TEN has been the most studied by far so we have the most information about its pathogenesis. Nevertheless, it remains unclear what exactly causes the characteristic full-thickness epidermal necrosis seen in this disease. One theory focuses on the apoptosis-inducing receptor-ligand pair Fas and FasL that are expressed on T cells as well as keratinocytes. Some researchers argue that a death-assuring interaction between the two proteins on the membrane of adjacent cells leads to widespread necrosis of epidermal cells in patients with SJS/TEN (Viard 1998). This is mediated by their induction of a pro-death signaling pathway that triggers the caspase cascade and results in intracellular DNA degradation (Posadas 2002).

A different hypothesis argues that the necrosis is caused by cytotoxic CD8+ T cells, whose soluble mediators (e.g. perforin and granzymes) are more important for keratinocyte death in SJS/TEN than the Fas-FasL interaction (Nassif 2002). Granzymes are serine proteases stored in cytoplasmic granules and, when released, can induce apoptosis in target cells (Chen 2018). Activated cytotoxic T cells (and natural killer cells) produce perforin, which binds to and creates a channel through the target cell's membrane; this permits granzyme B to enter the target cell and activate programmed cell death (Voskoboinik 2015). Elevated levels of perforin and granzyme B appear to correlate with disease severity in DHRs (Posadas 2002).

Secretory granulysin is also reportedly a key mediator for the widespread keratinocyte death observed in SJS/TEN (Abe 2009). The blister fluid of patients with SJS/TEN was found to be enriched with granulysin more than other cytotoxic proteins (e.g. perforin, granzyme B, FasL), and the granulysin correlated with level of cytotoxicity (Chung 2008). Additional

work has shown that granulysin is also upregulated in patients with DRESS but not those with MDE (Abe 2009, Weinborn 2016, Su 2017).

- ***Other cytokines/chemokines***

Beyond the aforementioned perforin, granzymes, granulysin, and Fas-FasL, a number of soluble mediators are likely involved in DHR. TNF-α plays a major regulatory role in regulating immune responses by inducing cell apoptosis, activation, and differentiation (Liu 2005). It has been found to be highly expressed in patients with SCAR and may also be responsible for the extensive necrosis seen in their skin lesions (Paquet 1994, Paul 1996). IFN-γ was similarly found to be increased in the skin tissue, blister fluid, and plasma of patients with SJS/TEN and DRESS (Posadas 2002, Nassif 2004, Caproni 2006). The cytokine IL-15 can induce proliferation of various leukocytes and has been associated with the disease severity and mortality in SJS/TEN (Mockenhaupt 2017). A number of other cytokines and chemokine receptors, including IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-18, CCR3, CXCR3, CXCR4, and CCR10, are reportedly upregulated in the skin lesions, blister fluids, PBMCs, or plasma of patients with DHRs and play a role in their immune regulation (Posadas 2002, Mockenhaupt 2017, Nassif 2004, Caproni 2006, Tapia 2004, Correia 2002, Paquet 2000).

1.6 The case for tissue-resident memory T cells (Trm)

Skin Trm are T cells (CD3+) with a memory phenotype (CD45R0+, CD69+, and/or CD103+) that take up long-term residence in the skin. Memory T cells that are not tissue-resident but circulate instead are known as central memory T cells (Tcm). Previous work has demonstrated that Trm play a key role in multiple human tissue-specific immune and

inflammatory diseases like psoriasis and allergic contact dermatitis (Park 2015). It is known that skin hypersensitivity responses can be locally induced with patch-testing for some drugs (e.g. abacavir). The fact that fixed drug eruptions (mild rash, self-limited, no mortality) recur at the same site with each drug exposure suggests there is something unique going on with the cells in that particular skin area. Further, patients who have very few circulating lymphocytes, and thus are effectively deprived of their central memory T cell population, still develop cutaneous drug eruptions. Given all this, it is reasonable to suspect that dtDHRs may be mediated by skin Trm.

The existing literature on Trm in dtDHR is limited to a small prospective study which found that intradermal culprit-drug challenge in patients with history of DRESS leads to development of a Trm phenotype over time (Trubiano 2019). To date, there are no published studies investigating whether Trm mediate these diseases.

- ***Honorable mention: Tregs***

Skin-resident regulatory T cells (Tregs, CD3+CD4+FoxP3+ phenotype) reportedly limit severity of acute disease by regulating the cytotoxic effector T cell responses (Chen 2018). Acute DRESS syndrome is characterized by dramatic expansions of functional Tregs, while in SJS/TEN their frequency is normal and function is inadequate (Takahashi 2009).

- ***Innate immune cell populations***

Innate immune cell populations include NK cells, innate lymphoid cells, monocytes, macrophages, dendritic cells, and eosinophils. In early DRESS syndrome, eosinophilia can be found in 60-95% of patients (Kardaun 2013, Shiohara 2006). The involvement of these cell

populations in dtDHRs is largely unknown. In this project, we included markers for some of these cell types as well to better understand their involvement.

1.7 Previous challenges to research

Research into dtDHR has been limited historically for several reasons. First, due to the relative rarity of these diseases, samples sizes were often too small for well-powered prospective studies. Researchers often had to pool DHR types together to increase sample size, but this mixed-disease approach sacrificed the ability to draw conclusions about individual DHR types.

Another major obstacle to researching dtDHRs has been the fact that access to skin samples appropriate for research has been limited. Available skin specimens are typically formalin-fixed paraffin-embedded (FFPE). This method of tissue preservation is useful for pathologic analysis but precludes most research applications, thus rendering large banks of potentially informative skin samples unusable. Furthermore, no adequate mouse models for these diseases have thus far been developed.

1.9 **Specific Aims**

Delayed-type DHR warrant further investigation as they are a significant public health concern and our lack of knowledge regarding their pathobiology negatively impacts patient care in a number of ways. First, clinicians are ill-equipped to treat severe forms of disease like SJS/TEN (Lissia 2010). Second, there are no effective assays to identify culprit drugs and clinicians' efficacy at assessing them at present is unknown. Because of this, patients may be incorrectly labeled as having an allergy to a drug and consequently forced to use a less

effective, more expensive alternative. Third, while physician-based assessment remains the gold standard for diagnosing these reactions, it is not always possible for clinicians to distinguish diagnoses and this leads to inaccurate characterization of drug reactions (Wheatley 2015). Given this, it is surprising and alarming that we know so little about the pathobiology of dtDHR. In this thesis work, we sought to begin filling in this important knowledge gap.

Given what is known so far about T cells in dtDHR as mentioned above, our overarching hypothesis is as follows:

- *Tissue-resident memory T cells mediate cutaneous delayed-type drug hypersensitivity reactions.*

To begin to address this hypothesis, our work aimed to fulfill two specific aims and their respective subaims:

1. **To identify validated cases of dtDHRs for laboratory analysis.**
    - Sub-Aim 1. To create a database of validated cases of dtDHRs. Note: Due to the extensive data we generated in this sub-aim, we were able to conduct a nested retrospective clinical study related to the identification of culprit drugs (leading to a second sub-aim).

    - Sub-Aim 2. To assess clinicians' ability to identify culprit drugs correctly and consequences of incorrect identification.

2. **To investigate the role of T cells in dtDHR patient skin samples using novel technologies.**
    - Sub-Aim 1. Transcriptional profiling to assess T cell phenotype, function, and concurrent inflammatory milieu in dtDHRs.
    - Sub-Aim 2. Immunofluorescence staining/microscopy to interrogate T cell phenotype in dtDHRs.

- Sub-aim 3: Immunofluorescence staining/microscopy in lymphopenic patients to assess the contribution of Trm to dtDHRs.

In this project, we overcame previous research obstacles by taking advantage of an approach that allowed us to maximize the number of samples we studied across all dtDHR types. We also made use of a number of new technologies that have been developed specifically for working with FFPE tissue.

2 METHODS

2.1 Patient samples

A retrospective study was conducted on FFPE skin samples obtained from adult and pediatric patients from January 1, 2000 to present with clinically-diagnosed and dermatopathology-confirmed cases of SJS/TEN, DRESS, and MDE. Pathology department databases at Massachusetts General Hospital, Brigham and Women's Hospital, and Boston Children's Hospital were first searched using the following terms: "toxic epidermal necrolysis," "toxic," "epidermal," "necrolysis," "Stevens-Johnson syndrome," "Stevens-Johnson," "full-thickness necrosis," "drug reaction with eosinophilia and systemic symptoms," "drug rash with eosinophilia and systemic symptoms," "DRESS," "drug hypersensitivity reaction," "hypersensitivity reaction," and "drug reaction." All cases were read by board-certified dermatopathologists. Each result from the initial search was cross-referenced and screened using the electronic medical record at its respective institution to identify likely candidate cases and to collect extensive clinical data. A board-certified dermatologist with expertise in dtDHRs verified each candidate case to confirm or exclude the diagnosis. Alternative pathology or clinical diagnoses, cases lacking sufficient clinical data to confirm diagnosis, and cases of pure SJS (<10% body surface area involvement) or erythema multiforme (EM) were all excluded. Pure SJS was excluded due to variability of diagnosis amongst dermatologists. Scrolls were collected from a subset of confirmed cases whose stored FFPE skin blocks had up to 40 micrometers of tissue to spare. Healthy human skin discarded during plastic surgeries served as controls. Representative patient images were obtained from electronic medical records.

2.2 RNA extraction and transcript analysis

Total RNA was extracted from FFPE samples using the RNeasy Micro Kit (Qiagen, Germantown, MD, USA). Total RNA quantity and quality was measured using the BioDrop™ DUO spectrophotometer. We further evaluated the quality of our isolated RNA in several samples using a fragment analysis system (Agilent Bioanalyzer, RNA NanoChip). RNA was concentrated as needed using the RNA Clean & Concentrator Kit (Zymo Research, Irvine, CA, USA). For gene expression profiling, NanoString Technologies (Seattle, WA, USA)provided a custom-designed codeset of 200 genes (Table 7) and used its nCounter platform to perform transcription profiling on all RNA samples in this project. After processing, there were multiple checks for quality control, including the proportion of fields of view (FOV) successfully counted by the nCounter Digital Analyzer, binding density, noise threshold, expression of positive and negative control genes, and expression of endogenous and housekeeping (HK) genes. Samples that were found to be suboptimal were flagged and removed prior to differential expression analysis. Patient samples with values that did not meet optimal thresholds (e.g. in FOV, binding density, etc.) and housekeeping genes with expression levels that fell below the established noise threshold were excluded from the differential expression analysis.

2.3 Immunohistochemistry

FFPE skin sections 5-6 mm thick were stained via immunohistochemistry for CD3, CD4, and CD8 (Table i) following heat-induced antigen retrieval. Slides were developed with 3,3'-diaminobenzidine tetrahydrochloride (DAB). H&E stains were carried out on FFPE tissue sections (4 μm) by standard immunohistochemical techniques.

2.4 Immunofluorescence microscopy, imaging, and analysis

FFPE skin sections 5-6 mm thick were baked, deparaffinized, and rehydrated. Skin sections underwent acidic or basic (depending upon the epitope) antigen retrieval at 96oC, were blocked for non-specific protein binding, then stained for the primary antibodies listed in Table i, followed by secondary staining with different combinations of antibodies listed in Table ii and counterstained with DAPI. Tissue was imaged using the Mantra™ Quantitative Pathology Workstation and analyzed using the InFORM® analysis software (Akoya Biosciences, Menlo Park, CA, USA).

2.5 Statistical analysis

Data were first normalized using a positive control normalization to correct for technical noise across the samples, followed by an HK gene normalization selecting for HK genes that had expression values greater than the noise threshold and a mean value of expression of at least 200 counts. The geometric mean was used for normalization. Genes expressed below the lower limit of detection (LOD) were identified using negative control subtraction, defined as the mean expression of the negative control genes plus two times the standard deviation. Genes below the LOD in all samples were dropped. Correlation analysis between metadata was performed to identify potential confounders; findings were confirmed using principal component analysis (PCA) of metadata. Continuous demographic information of patients was summarized with median and range while the categorical demographic information was summarized with frequency and percentage. PCA was conducted using the R function prcomp. PCA plots were generated using the ggplot package. Scatter plots of the first and second principle component (PC) were color-coded by risk factor categories in the individual plots.

Normalized gene expression data underwent log2 transformation. Assessments of differences in gene expression between normal patients and patients with disease, or between patients with different types of disease, were based on unpaired Student's t-tests with nominal 2-tailed p values of 0.05 as the significance cut off. The mean difference (DM) for each gene between two compared categories was calculated as the difference between the mean of the Log2 gene expression values (DM= mean log2 category1- mean log2 category2). Fold changes (FC) of gene expression were calculated as the Anti Log2 of the mean difference (FC=2^ (mean log2 category1- mean log2 category2)). Adjusted p-values (p_a-value) were estimated using the false discovery rate (FDR) to correct for multiple comparisons.

Heatmaps (Figures 9 & 10) were constructed using the gplots package in R to visualize the relationships between Log2 gene expression levels and the compared categories. Genes shown in the heatmaps were those with: (a) significant differences (nominal p-value<0.05) and, (b) a mean difference larger than 1. All the analyses were conducted using R i386 3.5.1.

2.6 Study approval

Approval for this research was granted by the Partners Human Research Committee, which is the Institutional Review Board of Partners HealthCare (Protocol #2016P001357).

3 RESULTS

3.1 Rationale

Cutaneous delayed-type drug hypersensitivity reactions (dtDHRs) range in severity from mild rash to severe sloughing of skin and mucosal surfaces with possible internal organ involvement. T cells are thought to be major protagonists in dtDHRs yet the phenotype, function, and mechanisms by which T cells become activated in dtDHRs are unknown. Tissue-resident memory T cells (Trm) persist long-term in skin in robust numbers and play a key role in many inflammatory skin diseases (Park 2015). Our overarching hypothesis is that skin Trm mediate dtDHRs. This thesis work begins addressing this hypothesis through a first-of-its-kind retrospective study of selected dtDHRs, namely Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) with > 10% body surface involvement, drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, and morbilliform drug eruption (MDE). It surmounts previous barriers to research in this area in several innovative ways that will be highlighted in this section.

3.2 Aim 1: To identify validated cases of dtDHRs for laboratory analysis

In order to gain insights into the pathobiology of dtDHRs, access to samples of diseased tissue is required – and the more samples available the better. Relative rarity of severe forms of dtDHR and a low biopsy rate for MDE and DRESS have contributed to a lack of diseased specimens, severely limiting investigation into this important area. The retrospective nature and multi-institutional design of this study enlarged the pool of available specimens of

disease. This allowed us to proceed with our first aim, which was to identify validated cases for use in subsequent laboratory studies on selected dtDHRs.

3.2.1 Sub-aim 1: To create a database of validated cases of dtDHRs

Pathology department databases at Massachusetts General Hospital (MGH), Brigham and Women's Hospital (BWH), and Boston Children's Hospital (BCH) were first searched for pathology reads possibly consistent with one of the three dtDHRs from January 1, 2000 to present. Search terms used were as listed in the Methods section. This yielded an initial caseload of 1,598 reads (Figure 3). Each case from the initial search was then cross-referenced and screened using the electronic medical record at its respective institution to identify likely candidate cases and to collect extensive clinical data. This yielded a total of 246 cases.

The data collected for each likely candidate included documentation of sex, gender, race or ethnicity, age at presentation, presence of malignancy, history of drug allergy, HIV status, history of bone marrow transplant, treatment with donor leukocyte infusion, history of acute skin graft-versus-host disease, history of autoimmune disorder, whether on immunosuppressive medications, other co-morbidities, recent history of infection prior to start of rash, date rash began, date disease began if different than rash, administration of immunosuppressive medication prior to biopsy, date of biopsy, biopsy results, whether dermatology service was consulted, diagnosis of dermatology consulting service, whether allergy service was consulted, maximum total body surface area involved, fever >38degC , eosinophilia, positive testing for HHV6/EBV/HSV/M. pneumo., obvious or suspected culprit drug(s), other medications taken, treatment received, whether patient was listed as allergic

to obvious or suspected culprits, whether listing(s) persist today, survival of incident, current living status, and availability of photos. For SJS/TEN cases, also documented were the SCORTEN (if calculated) and involvement of eyes, oropharynx, vagina, penis/scrotum, urethra, respiratory tract, and gastrointestinal tract. Eye involvement was confirmed by consultation with ophthalmology service for nearly all cases; vaginal involvement was determined by consultation with gynecology service for most cases. For DRESS cases, also documented were presence of facial or ear erythema and edema, lymphadenopathy, eosinophilia, atypical lymphocytes, and involvement of kidneys and other organs.

A board-certified dermatologist with expertise in dtDHRs then vetted each candidate case to confirm or exclude the diagnosis, yielding 55 cases of dtDHR that were clinically-diagnosed and biopsy-confirmed cases of disease. Alternative pathology/clinical diagnoses, cases lacking sufficient clinical data to confirm diagnosis, and cases of pure SJS or erythema multiforme (EM) were all excluded. Each confirmed case of disease corresponded to a single patient (Figure 4).

Of the 55 cases, there were 42 cases of SJS/TEN, 7 of DRESS, and 6 of MDE (Table 1). A dozen cases of SJS/TEN would have been quite a good outcome given the disease's rarity, so having 42 cases is exceptional. It is even more impressive a feat considering our exclusion of pure SJS cases (i.e. those with < 10% BSA involvement). There were fewer cases of DRESS and MDE, likely because clinicians are more confident in these diagnoses and biopsy them at lower rates.

Across all dtDHR, there were 26 males (47%) and 29 females (53%). Although cases of dtDHR are known to skew female, a two-tailed binomial test showed the slight female

predominance observed was not statistically significant (p = 0.099). SJS/TEN and MDE cases also appeared to skew female with 57% and 67%, respectively. Likewise, however, neither observation proved statistically significant: p = 0.080 for SJS/TEN and p = 0.234 for MDE. Interestingly, DRESS cases appeared to skew strongly male (86%), but this deviation still failed to reach statistical significance (p = 0.055). A plurality of patient cases overall was White or Caucasian (44%), followed by 18% Asian, 13% Black or African American, and 7% Hispanic or Latino. "Other"-identified and patients with undocumented race/ethnicity together comprised 19% of all cases. Within each type of dtDHR, whites accounted for a plurality and Asians were consistently represented. Blacks comprised 17% of SJS/TEN cases but were not found among DRESS and MDE cases.

3.2.2 Sub-aim 2: To assess clinicians' ability to identify culprit drugs correctly and consequences of incorrect identification

A lack of sufficient clinical data in dtDHR, particularly for SJS/TEN (due to low sample sizes, incorrect diagnoses), has been an obstacle to performing rigorous clinical research. Because of the high number of well-vetted cases of SJS/TEN and the extensive clinical data collected in Specific Aim 1 Sub-aim 1, we were able to address a major question in the field: how successful are clinicians at correctly identifying culprit drugs? We hypothesized that clinicians have difficulty identifying culprit drugs, leading to errors in patients' allergy lists.

Forty-two cases of SJS/TEN were pulled from our validated dtDHR database (Table 2). A summary of notable comorbidities, treatment, and outcome are shown in Table 3. Twenty-

six percent of patients did not survive their disease incidents consistent with mortality estimates of SJS/TEN.

Dermatologists were the primary consultants contributing to culprit drug identification as all cases underwent dermatology consultation while only a minority (12%) of cases included allergy consults. Most patients were taking two or more drugs at/preceding the time of disease onset, complicating culprit drug identification. In fact, only 7% of patients were taking one drug at the time of or immediately preceding onset of disease, while the vast majority of patients (69%) were taking 4 or more drugs at/before disease onset (Table 4). All cases underwent dermatology consultation, though only a minority included allergy consults (Table 5). In all 42 cases, disease was attributed to drug (Table 5). In no cases did consultant documentation refer to a scoring system (e.g. ALDEN), to drug half-life (despite the fact that some drugs had been discontinued prior to onset of disease), or to HLA testing to determine drug etiology. Some documents referred to epidemiologic data in determining culprit drug, though none included ethnicity/race data.

When looking at patients' subsequent allergy lists, despite the fact that most patients were taking several drugs (including several likely culprit drugs) at the time of disease, many cases listed only 1 drug as culprit and added it to the allergy list (resulting in possible false negatives in the allergy list) (Table 5). In 2 cases, though culprit drugs were identified, no drug was added to the allergy list (resulting in probable false negative). In 6 cases, consultants specifically asked primary providers to list more than one drug on the allergy list yet only 1 drug was added (resulting in potential false negatives). In 40% of cases, more than one drug was added to the list and in some cases entire classes of drugs were added

(e.g. all beta lactams to a case of amoxicillin-induced disease) (probable false positives). In many cases, listing of 2 or more drugs included different classes of antibiotics (e.g. sulfas + cephalosporins) (probable false positives). Inclusion of antibiotic classes on allergy lists forces patients to use alternative classes in the future; if they do so needlessly, this can contribute to the public health concern of antibiotic resistance.

Notably, 40% of patients had confirmed infections at the time of disease onset (ex. pneumonia, sepsis, urinary tract infection) (Table 6). Several additional patients had upper respiratory infection (URI) symptoms preceding rash though it is very difficult to delineate true viral URI from mucosal involvement of SJS/TEN preceding rash. It is possible that infection alone could trigger SJS/TEN in which case patients were incorrectly labeled as allergic to one or more drugs.

In summary, our findings suggest that dermatologists do indeed have a challenging time identifying culprit drugs. This is likely due to the large number of drugs many patients are taking and dermatologists' lack of usage of tools designed for identifying culprit drugs. The consequence of incorrect identification is that patients' allergy lists may not be accurate (potentially at their peril), a microbial etiology may have been missed, and anti-biotic stewardship may be compromised.

3.3 Aim 2: To investigate the role of T cells in dtDHR patient skin samples using novel technologies

Another major obstacle to researching dtDHRs has been the fact that available skin samples are typically FFPE specimens. This method of tissue preservation was largely incompatible

with research applications in the past, and it consequently rendered large banks of potentially informative skin samples unusable. The second aim of this project was to investigate the role of T cells in selected dtDHRs using novel technologies. Techniques included 1) gene expression profiling on skin T cells with NanoString's nCounter platform, and 2) performing multi-spectral immunofluorescence (IF) staining and imaging with the Opal™ tyramide-signal amplification staining system concurrently with Mantra™ Quantitative Pathology Workstation and inForm analysis software. All of these techniques are compatible with FFPE samples and thus offered an exciting opportunity for a robust retrospective examination of dtDHRs. For this study, we pulled dtDHR samples identified through Aim 1 Sub-aim 1 above, and in some cases compared them to samples of healthy human skin discarded during plastic surgeries as controls.

3.3.1 Sub-aim 1: Transcriptional profiling to assess T cell phenotype, function, and concurrent inflammatory milieu in dtDHRs

Gene expression profiling was used to test the hypothesis that *genes associated with the Trm phenotype are upregulated in skin dtDHR.* To address this, we first collected 42 total specimens (17 cases of SJS/TEN, 6 of DRESS, 7 of MDE, and 12 healthy controls) in the form of FFPE scrolls. Amount of tissue available in the scrolls ranged from two to four 10-micrometer-thick sections. We extracted RNA and quantified it as per the Methods section. Samples with RNA concentration too low for downstream analysis underwent concentration as per the Methods section. Fragment analysis of a subset of our RNA samples showed they were highly degraded, struggling to meet even our generous goal of having at least 50+% of the RNA > 300 nucleotides in length (Figure 5). Despite this, we found that the samples were still utilizable with Nanostring's innovative nCounter Technology. RNA was isolated from a

total of 37 samples: 14 cases of SJS/TEN, 6 of DRESS, 7 of MDE, and 10 healthy controls. These samples were then processed by the nCounter Digital Analyzer using a custom-designed probe set consisting of 188 genes of interest and 12 housekeeping genes (Tables 7 & 8). After processing was completed, the samples underwent rigorous quality control checks as per the Methods section. Thirty-five (95%) of the 37 patient samples met all quality control standards (Figures 6 & 7). Two patient samples (BWH-SJS-2 and BWH-MDE-2) had sub-optimal field of view counts and were excluded from subsequent differential expression analysis. Three housekeeping genes (TBP, POLR1B, and ALAS1) had expression levels that fell below the established noise threshold and were therefore excluded. Of the 188 target genes, 187 (99.5%) had expression levels that met inclusion criteria, but one (IL17A) fell below the limit of detection in all samples and was dropped as a result. Overall, the assay was very successful and proved robust enough to extract biology from FFPE skin samples as old as 7 years of age with small amounts (total ≤40um-thick/sample) of starting tissue, and as little as 103 ng of considerably degraded RNA.

Samples' metadata was collected to assess for possible confounders and included factors such as gender, age at presentation, race/ethnicity, whether patient received immunosuppression for rash prior to biopsy, immunosuppressed state, biopsy year, biopsy site, RNA extraction date, whether RNA was concentrated, and Nanostring batch number (Table 9). Systemic treatment was counted even if patient received a single dose to treat disease in period leading up to biopsy. Patients qualified as being in an immunosuppressed state if there was underlying active disease and/or they were receiving treatment for underlying disease. One patient was on immunomodulator medication (checkpoint

inhibitors). Correlation analysis between metadata elements was performed, and no concerning correlations were observed (Figure 8).

Using a false discovery rate-adjusted p value (p_a) to correct for multiple comparisons, statistical analyses showed that 91 genes were significantly ($p_a < 0.05$) differentially expressed in SJS/TEN samples compared to healthy controls, along with 61 genes in DRESS, and 38 genes in MDE. Hierarchical analysis of all three dtDHR types demonstrated highly segregated clustering away from healthy controls (Figures 9 & 10). Interestingly, a single SJS/TEN case was noted to cluster with the healthy controls away from the other SJS/TEN samples. Review of Table 9 shows that this case, MGH-SJS-8, was a sample from a 12-year-old black male with history of congenital toxoplasmosis (complicated by reactivation with macular scarring and vision loss) who had received systemic steroids for his SJS/TEN (up to 40% TBSA) for more than a week prior to biopsy, which still found full thickness epidermal necrosis consistent with SJS/TEN. This suggests that the skin's transcription profile can return to "normal" while disease is still evident on histopathology.

Transcriptional profiling to assess the role of Trm in dtDHRs

Indiviudal dtDHR types were compared to healthy controls. Dissimilarities in differential expression of genes related to T cell phenotype were readily apparent among them. In SJS/TEN and DRESS samples compared to healthy controls, results revealed significant ($p_a < 0.05$) upregulation (fold change ≥ 1.5) in the following: pan-T cell marker CD3; T cell subtype markers CD8 and CD4 (with CD8 > CD4); non-specific memory T cell marker CD45R0; and central memory T cell (Tcm) marker CD62L/SELL (Table 10). Tcm marker CCR7 was also upregulated in SJS/TEN. These findings suggest that T cells may be present above normal

levels in SJS/TEN & DRESS and a Tcm phenotype predominates. In MDE samples compared to healthy controls, CD45R0 was upregulated and Trm marker CD69 was downregulated (fold change < 0.67) while Tcm markers were not significant. This suggests that memory T cells may be present in MDE, but whether they are skin-resident or centrally derived is unclear. Skin-resident memory T cell marker CD103 was not elevated in any dtDHR group. Taken together, these findings suggest that Tcm may play more of a role in mediating SJS/TEN and DRESS, and that memory T cells of unclear origin are involved in MDE.

Transcriptional profiling to identify role of T cell function

T cells participating in immune response may exhibit a skew toward either a Th1 or Th2 phenotype. On examination of genes that reached statistical significance, all three dtDHRs seem to demonstrate more of a Th1 skewing, with upregulated CXCL9, CXCL10, CXCL11, and (except in MDE) IL12Rβ1. At the same time, they appear to have appropriately downregulated Th2 markers, especially in DRESS with its reduced GATA3, IL4, and IL17(B, F) (Table 11). The IL4 receptor is uniformly upregulated across dtDHR types, but this may indicate increased presence of the soluble form, which is known to inhibit IL4-mediated cell proliferation and IL5 upregulation by T cells (Silvestri 2006). All three dtDHRs also demonstrate skewing toward the Tc1 functional subset of CD8+ T cells with upregulated granzyme A and granzyme B in all 3, plus upregulated granulysin and perforin in SJS/TEN and DRESS. Evidence of possible Fas-Fas ligand interactions was limited to DRESS samples,

suggesting this mechanism of Tc1 effector cell cytotoxicity may not be strongly favored in dtDHR.

Transcriptional profiling to identify role of other potential players

In this project, we were chiefly interested in using transcriptional profiling to investigate T cell phenotype and function, but given the dearth of information on dtDHR pathobiology we also looked at other factors such as IDO1, caspases, JAKs and STATs, as well as NK cell markers. Some of these were detected and will be further studied as they may hold additional insights into our understanding of the immunopathogenesis of dtDHRs. For example, a preliminary review of the JAK/STAT pathway shows JAK3 is upregulated across the board in these dtDHR (Table 12). This includes a 3.6-fold increase in SJS/TEN, which is the most severe dtDHR and unfortunately lacks any proven treatments. These data suggest a JAK3 inhibitor might be a useful therapy for patients with SJS/TEN. The non-selective JAK1/JAK3 inhibitor Tofacitinib is already FDA-approved for use in rheumatoid arthritis. However, a selective medication may be preferred given JAK3's expression is relatively specific to lymphocytes. Several JAK3-selective medications are currently in clinical trials (Farmer 2015, Pfizer 2018). JAK2 is upregulated in SJS/TEN to a slightly lesser degree (2-fold change), but the non-selective JAK1/JAK2 inhibitor ruxolitinib (already FDA-approved for myelofibrosis and polycythemia vera) could be an alternative as well (Stern & Divito 2017).

3.3.2 Sub-aim 2: Immunofluorescence staining/microscopy to interrogate T cell phenotype in dtDHR

Immunoflurescence (IF) staining and microscopy afforded us a different but complementary and similarly powerful tool with which to adress our hypothesis that Trm mediate dtDHRs.

Samples of confirmed cases of dtDHR identified in Aim 1 Sub-aim 1 above were pulled from the pathology departments for staining and microscopy. Each sample was first stained by H&E to confirm diagnosis and assess overall histologic pattern and inflammatory infiltrate (Figure 11).

Samples were then stained using the FFPE-friendly Opal™ tyramide-signal amplification technique for CD3, CD45RO, CD45RA and CD103. This system was particularly appealing due to its promise of multiplexed staining, which theoretically allows for multiple stains on a single slide in series using antibodies from the same host species while avoiding the cross-reactivity problems that would plague other techniques. Stained slides were imaged and anlayzed per the methods section. Results demonstrated that T cells in dtDHRs were more commonly CD45R0+ than CD45RA+, and few were CD103+ (Figure 12). Also, there were many CD45R0+ cells that were negative for CD3.

Notably, optimization of this single four-antibody/antigen staining protocol took approximately 5 months to complete. In addition, we attempted to create 3 additional multiplex panels that were even more challenging, spending over 12 months in optimization. Given the time, cost, and resources required for further development, we decided to instead employ our alternative, which is standard IF, for subsequent analysis. We therefore stained for CD3, CLA, CD8 plus DAPI using standard IF techniques, but continued to image using the Mantra™ Quantitative Pathology Workstation and inForm analysis software. With this method, results showed that CD3+ cells consisted of both CD8+ and CD4+ subsets, and were largely CLA+ (Figure 13).

In summary, these results showed that T cells in dtDHRs are skin-homing (with CLA positivity), largely of a memory phenotype (with CD45R0 > CD45RA), and include both CD8+ and (by inference) CD4+ T cell subsets. Regarding the composition of the T cell infiltrate in dtDHRs, these protein expression profiling results are therefore in agreement with the gene expression profiling results we shared in Aim 2 Sub-aim 2. However, one important limitation was that these stains did not permit us to distinguish between central and tissue-resident memory T cells. We attempted staining for CCR7 and CD62L without success before ultimately adopting a ready-made alternative.

3.3.3 Sub-aim 3: Immunofluorescence staining/microscopy in lymphopenic patients to assess the contribution of Trm to dtDHRs

A group of patients with MDE were identified that were profoundly lymphopenic (<500 lymphocytes/mL blood) following chemotherapy for underlying malignancies. This indicated that they had severely reduced circulating T cells. As such, these patients had very low numbers of naïve, effector, and central memory T cells at the time of their MDE. This unique clinical scenario provided a serendipitous and elegant model for studying a possible role of Trm in patients with dtDHRs.

Five patients who were diagnosed clinically and histopathologically with MDE and who were profoundly lymphopenic had skin tissue available for analysis. All patients had underlying AML. FFPE skin samples were first analyzed to quantify and characterize the T cell infiltrate via immunohistochemistry (IHC) compared to normal healthy control skin. The samples were found to contain CD4+ and CD8+ T cell subsets equivalent to healthy control skin,

suggesting that despite the absence of circulating T cells, T cells in skin were unaffected by chemotherapy and the development of lymphopenia (Figure 14).

Each lymphopenic sample was then stained using standard IF for CD3, CLA, and CD8 (Figure 15). Each was also stained for CD45R0, CD45RA, and CD3 (Figure 16). T cells in lymphopenic MDE samples were found to express the skin-homing molecule CLA+ and to be predominantly CD45RO+. In the Tcm-depleted setting of lymphopenia, this is consistent with the skin Trm phenotype. These findings complement the preceding microscopy and transcriptional profiling data very well and indicate that Trm may be more important in mediating MDE while central memory T cells may play a larger role in mediating SJS/TEN and DRESS.

4 DISCUSSION, CONCLUSIONS, AND FUTURE DIRECTIONS

Cutaneous delayed-type drug hypersensitivity reactions (dtDHRs) are an important source of morbidity with high mortality in severe forms of disease. The most severe form, SJS/TEN, has no adequate treatment options beyond stopping the culprit drug and giving supportive care. Despite the clear need for understanding these potentially fatal diseases, the pathobiology remains poorly understood. The most significant barrier to research in the past was lack of access to specimens sufficient for laboratory analysis, largely due to the relatively low incidence of severe forms of disease, low biopsy rate of others, and formalin fixation of stored disease specimens. In this project, we sought to overcome some of these obstacles to improve our collective understanding of dtDHRs and to advance the field of skin research in general.

- ***A robust database of SJS/TEN cases created***

In our first specific aim, we sought to identify validated cases of dtDHRs for laboratory analysis. With our retrospective and multi-institutional (MGH, BWH, BCH) study design, we were able to successfully generate a database with an impressive number of SJS/TEN cases (N=42). Our vetting process was extremely rigorous, as we limited cases only to those that were clinically diagnosed, dermatopathology-confirmed, and involved > 10% TBSA. Restricting to 10+% TBSA was necessary to limit concerns about incorrect diagnoses. One limitation is that although the SJS/TEN numbers were large, the number of DRESS and MDE cases was comparatively low. This is likely due to a lower biopsy rate of these diseases. Going forward, we will overcome this limitation by continuing to collect cases as well as expanding the source group of hospitals (MGH, BWH, BCH) to include a fourth institution, Beth Israel Deaconess Medical Center.

- ***Database supports studies into important clinical questions***

Due to the large number of well-vetted cases of SJS/TEN and the extensive clinical data collected from our database, we were able to address an important clinical question pertaining to how successful clinicians are at correctly identifying culprit drugs. Correct identification of offending medications is not only important for accurate research but also critical for patient care. Patients (especially with severe forms of disease) should not be re-exposed to culprits, and they should not be needlessly forced to use second- or third-line treatments which may have less efficacy or more side effects. Costs to society of incorrect culprit drug identification must also be factored in given concerns about unnecessary contribution to antibiotic resistance. We conducted a nested retrospective clinical study to assess this and found that clinicians' ability to correctly identify culprits is lacking, with several likely false positives and false negatives. We found that many patients diagnosed with SJS/TEN were confirmed to have concurrent infection. This calls the determination of etiology in those cases into question, as a medication may not have been the true culprit at all. We are currently following up on this work to see what information doctors are using to make their determinations and assessing whether there is an intervention that could improve diagnostic accuracy. Specifically, we are applying the validated ALDEN algorithm retrospectively to assess how well clinicians performed at identifying culprit drugs.

- ***Innovative techniques used to study T cells in dtDHR***

In our second specific aim, we investigated the role of T cells in dtDHR patient skin samples using novel technologies to overcome the difficulties of conducting laboratory analysis on FFPE tissues. For transcription profiling with NanoString's innovative nCounter Technology,

we succeeded at obtaining RNA of sufficient quality and quantity from tiny (≤40um-thick starting tissue), degraded, and aged FFPE samples that would be prohibitive in more traditional assays. The nCounter assay proved itself to be a robust technique that can make an enormous number of FFPE specimens sitting in long-term storage available for retrospective investigations into many skin diseases, especially rarer ones like SJS/TEN. For protein expression profiling, we performed multiplexed, multi-spectral IF staining and imaging with the Opal™ tyramide-signal amplification staining system concurrently with Mantra™ Quantitative Pathology Workstation and inForm analysis software. We transitioned to standard IF staining for practicality but have continued using the Mantra Workstation and the inForm software with strong results.

- ***Tcm appear to mediate SJS/TEN & DRESS***

Overall, our transcription profiling results suggested that memory T cells are present above normal levels across all 3 of our selected dtDHR types. Counter to our overarching hypothesis that skin Trm mediate dtDHRs, however, our phenotypic findings indicated that the protagonists mediating SJS/TEN and DRESS are Tcm instead. MDE shared the memory T cell phenotype, though the verdict on Trm vs Tcm was unclear. Functionally, a skewing toward Th1 and away from Th2 among all 3 selected dtDHRs was observed. Although IFNγ itself was not differentially expressed, CXCL9 (a.k.a. "monokine induced by γ interferon"), CXCL10 (a.k.a. "interferon gamma-induced protein 10"), and CXCL11 (a.k.a. "interferon-gamma-inducible protein 9") are IFNγ-induced cytokines and were strongly upregulated in all 3 diseases (Tokunaga 2018, Luster 1987, Cole 1998). The CD8+ Tc1 effector subtype was clearly favored as well. Considering that both SJS/TEN and DRESS involve systemic reactions

(fever + possible internal organ involvement in both), a prominent role for memory T cells that are recruited from the circulation to the skin is highly plausible.

In terms of limitations, the aforementioned low sample size for DRESS and MDE cases has consequences for our transcript findings as well. There were several markers that did not reach statistical significance in these diseases that may become significant with an expanded sample size. For example, the Tcm marker CCR7 in DRESS had a fold change of 1.9 (identical to that of SJS/TEN) but has a $p_a = 0.08$ so it was determined to be not significant in this study. As we know that fellow Tcm marker CD62L is elevated with $p_a = 0.006$, and both were upregulated in SJS/TEN, it would seem there is a good chance that expanding the power of the study will allow us to pick up CCR7 and other "borderline" genes. Also, although our custom-designed NanoString panel of 200 genes largely served us well beyond expectations, one limitation is that it does not include a number of genes that would have been useful for performing in-depth pathway analysis. Expanding our codeset to include more target genes will therefore be helpful in the future.

- ***JAK3 identified as potential therapeutic target for severe forms of disease***

As there are no proven treatments for patients with SJS/TEN, finding potential therapies is an ongoing pressing need. Given the importance of IL-15 to the development, survival, and function of CD8+ T cells, as well as its signaling through the JAK/STAT pathway, Stern & Divito (2017) theorized that JAK inhibitors could be of use in treating SJS/TEN. The data we have presented offers some experimental support for this theory. Further, our work suggests

selective JAK3 inhibition may have the potential to treat patients with DRESS as well. Prospective experimental work providing additional evidence is needed.

- ***Trm may be protagonists in MDE***

Since RNA transcript does not necessarily correspond to translated protein, we confirmed our transcript findings with protein expression profiling. Overall, our gene expression and protein expression findings complemented each other well. Microscopy findings agreed that memory T cells are present in all 3 dtDHR types, with CD45R0 > CD45RA, and that both CD8 and CD4 subsets are present. T cells were largely of a skin-homing phenotype (CLA+). Our IF microscopy work from a group of post-chemotherapy patients with lymphopenia (& thus were depleted of Tcm) who developed MDE showed T cells in skin survived chemotherapy, are skin-homing (CLA+), and have a memory phenotype (CD45R0+). These results helped clarify the gene expression data and suggest that MDE may be mediated by Trm. Unlike the systemic reactions seen in SJS/TEN & DRESS, MDE is largely limited to the skin. Thus, involvement of Trm is plausible from a clinical perspective as well. Further microscopy work showing lack of Tcm markers and/or presence of Trm markers like CD69 would be valuable.

- ***Closing thoughts***

Despite its limitations, we are delighted that this project was overall a success. With this work, we not only have started to address the Trm hypothesis but also have built a strong platform on which we can conduct further basic and clinical research in this area. We have also demonstrated a framework that other skin researchers can use to begin extracting valuable information from rare cutaneous diseases. We are hopeful this will have broad impact at both the benchtop and the bedside.

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7 TABLES AND FIGURES

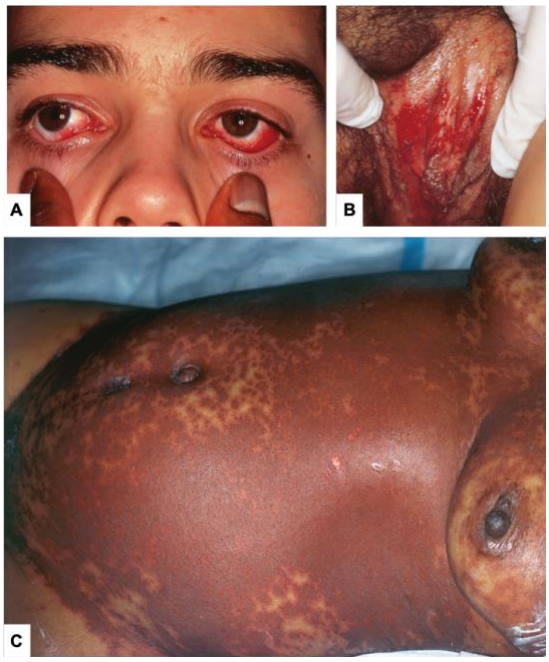


Figure 1. Mucocutaneous involvement in SJS/TEN; adapted from (Bolognia 2012).
A: Erythema and conjunctival erosions. **B**: Erosions of the genital mucosa. **C**: Characteristic dusky red color of the early macular eruption in TEN. Lesions with this color often progress to full-blown necrolytic lesions with dermal– epidermal detachment.

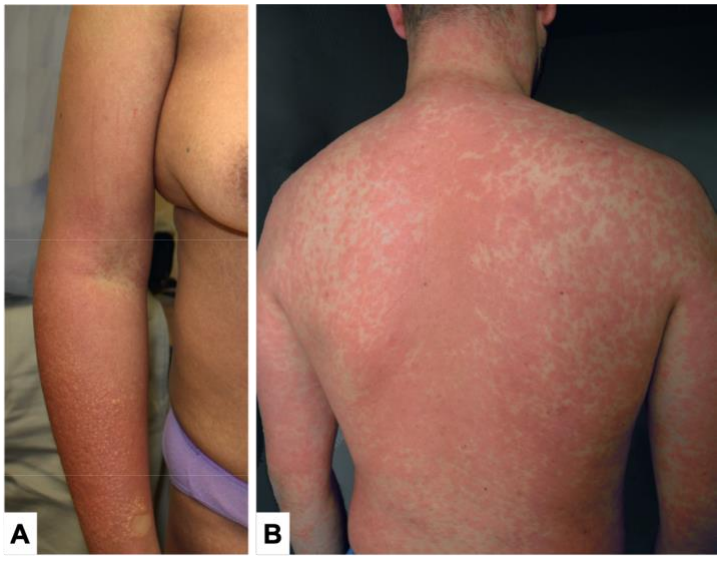


Figure 2. Clinical images of DRESS & MDE; adapted from (Bolognia 2012).
A: Edema and vesiculation on the forearm in DRESS. **B:** Erythematous papules and urticarial lesions with confluence on the midback induced by amoxicillin.

Table i. List of primary antibodies.

Label (anti-human)	Conjugate	Host	Isotype	Clone	Lot	Catalog #	Vendor
CD103 (ITGAE)	None	Mouse	IgG1,k	Ber-ACT8	B166277	350202	Biolegend
CD3	None	Rabbit	IgG	Polyclonal	20061853	A0452	Dako
CD4	None	Rabbit	IgG	EP204	0000081016	104R-24	Cell Marq.
CD45R0	Biotinylated	Mouse	IgG2a,k	UCHL1	B177325	304202	Biolegend
CD45RA	None	Mouse	IgG2a,k	158-4D3	5788-2P150622	NBP2-15193	Novus
CD8	None	Mouse	IgG1,k	C8/144B	20024879	M7103	Dako
CLA	None	Rat	IgM,k	HECA-452	B193396	321302	Biolegend

Table ii. List of secondary antibodies.

Label	Conjugate	Host	Isotype	Clone	Lot	Catalog #	Vendor
Anti-mouse IgG	AF555	Goat	Polyclonal IgG	Poly4053	B236509	405324	Biolegend
Anti-mouse IgG	AF647	Goat	IgG	Polyclonal	2069817	A-21236	Invitrogen
Anti-rabbit IgG	AF555	Donkey	Polyclonal Ig	Poly4064	B263040	406412	Biolegend
Anti-rabbit IgG	AF647	Goat	IgG	Polyclonal	2098544	A21245	Invitrogen
Anti-rat IgM	AF488	Goat	IgG	Polyclonal	2047153	A21212	Invitrogen
Streptavidin	AF488	N/A	N/A	N/A	B187261	405235	Biolegend

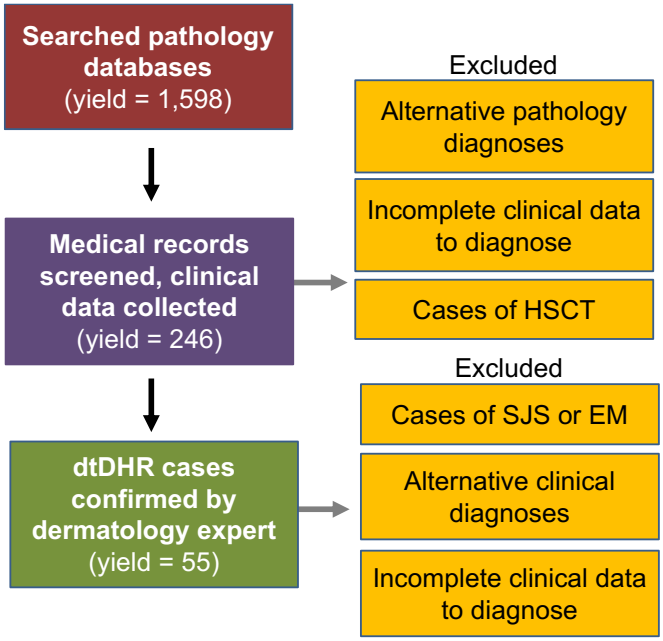


Figure 3. dtDHR database creation schematic. This illustration shows the stepwise process used to generate the database of validated dtDHR cases. HSCT = hematopoietic stem cell transplant.

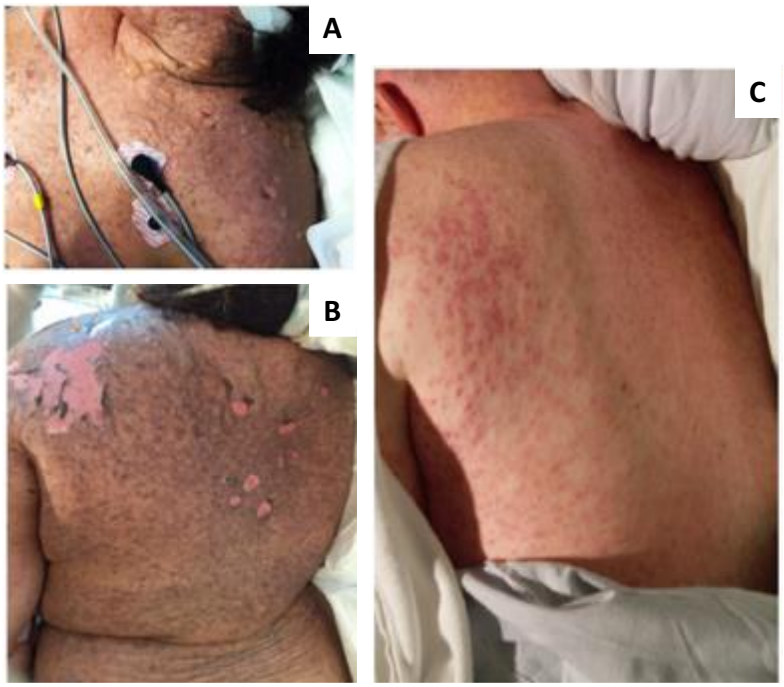


Figure 4. Representative images of patients with SJS/TEN and DRESS. A and **B**: left anterior shoulder (A) and back (B) of 23-year-old white female with SJS/TEN involving 100% body surface area. **C**: left back of 70-year-old white male with DRESS. Note: No photos of MDE cases were available.

Table 1. Cohort characteristics of patients with confirmed cases of selected dtDHRs.

	All patients	SJS/TEN	DRESS	MDE
Cohort N value	55	42	7	6
Age in years, median (range)	40 (1-86)	38 (1-82)	64 (20-84)	60 (39-86)
Sex, N value (%)				
Male	26 (47)	18 (43)	6 (86)	2 (33)
Female	29 (53)	24 (57)	1 (14)	4 (67)
Race/ethnicity, N value (%)				
White or Caucasian	24 (44)	17 (40)	4 (57)	3 (50)
Black or African American	7 (13)	7 (17)	0 (0)	0 (0)
Hispanic or Latino	4 (7)	3 (7)	1 (14)	0 (0)
Asian	10 (18)	6 (14)	2 (29)	2 (33)
Other	2 (4)	2 (5)	0 (0)	0 (0)
Unknown	8 (15)	7 (17)	0 (0)	1 (17)

Table 2. Detailed characteristics and outcomes of 42 patients with SJS/TEN.

Age/ Gender	Race/ethnicity	TBSA (max)	Mucosae +/- respiratory tract involved	Died?
1/F	White	>10%	eyes, oropharynx, vagina	N
1/F	Black	>10%	eyes, oropharynx, vagina	N
1/F	Unknown	60%	eyes, oropharynx, vagina, respiratory tract, GI tract	N
3/M	Unknown	>10%	oropharynx, penis/scrotum, respiratory tract	N
11/F	Asian	>10%	eyes, oropharynx, vagina	N
12/M	Black	40%	eyes, oropharynx	N
14/M	White	90%	eyes, oropharynx, penis/scrotum, respiratory tract, GI tract	N
16/F	Black	80%	eyes, oropharynx, vagina, respiratory tract	Y
17/F	Unknown	>10%	oropharynx, vagina, urethra	N
18/F	Unknown	≥30%	eyes, oropharynx, vagina	N
19/M	Black	54%	eyes, oropharynx	N
20/F	White	70%	eyes, oropharynx, vagina, respiratory tract	N
22/F	Hispanic/Latino	90%	oropharynx, vagina, respiratory tract	N
22/M	Unknown	95%	eyes, oropharynx, penis/scrotum, respiratory tract	N
23/F	White	100%	eyes, oropharynx, vagina, urethra, respiratory tract	N
24/M	Other	60%	eyes, oropharynx, penis/scrotum, urethra	N
26/F	Hispanic/Latino	30%	eyes, oropharynx, vagina, urethra, respiratory tract	N
30/M	Other	50%	eyes, oropharynx, penis/scrotum, urethra, respiratory tract	N
32/F	Hispanic/Latino	40%	eyes, oropharynx, respiratory tract	N
34/M	Asian	80%	eyes, oropharynx, penis/scrotum	N
37/F	White	>35%	respiratory tract, GI tract	N
39/F	Black	60%	eyes, oropharynx, respiratory tract	N
39/F	White	40%	oropharynx	N
40/F	Black	60%	eyes, oropharynx, respiratory tract	N
40/M	White	45%	eyes, oropharynx, penis/scrotum, respiratory tract	Y
41/M	White	30%	eyes, oropharynx, penis/scrotum, urethra	N
42/F	Asian	20%	eyes, oropharynx, vagina, urethra, respiratory tract	N
48/F	White	20%	eyes, oropharynx, vagina, urethra	N
49/F	White	30%	eyes, oropharynx, respiratory tract	Y
53/M	Asian	30%	oropharynx, penis/scrotum, respiratory tract, GI tract	Y
54/M	Unknown	30%	eyes, oropharynx	N
55/M	White	100%	eyes, oropharynx, respiratory tract	Y
57/M	White	50%	eyes, oropharynx, penis/scrotum, respiratory tract	N
58/M	White	18%	GI tract	Y
60/M	Unknown	70%	oropharynx, penis/scrotum	N
61/F	Asian	70%	eyes, GI tract	N
68/M	Asian	>30%	Urethra	Y
75/F	White	25%	eyes, oropharynx, vagina	Y
78/M	White	50%	oropharynx	Y
81/F	Black	>10%	eyes	N
82/F	White	40%	oropharynx	Y
82/F	White	50%	eyes, oropharynx, respiratory tract	Y

Age = years. TBSA (max) = maximum documented percentage of total body surface area involved. GI tract = gastrointestinal tract. "Died?" = whether or not patient died during disease episode.

Table 3. Summary of clinical course of 42 patients with SJS/TEN.

Clinical overview	
Notable comorbidities	**N value (%)**
HIV+	1 (2)
Active cancer	3 (7)
Autoimmune disease	7 (17)
Treatment	
IVIG	21 (50)
Systemic steroids*	22 (52)
Burn/medical ICU	37 (88)
Died during episode	11 (26)

*Systemic steroids includes treatment for other etiologies (e.g. concurrent autoimmune hepatitis)

Table 4. Number of medications taken at time of or preceding onset of SJS/TEN.

No. Drugs Taken at Time of Disease	
1 Drug	N = 3 (7%)
≥ 4 Drugs	N = 29 (69%)

Table 5. Attribution to drug by consultants and primary providers.

	N value (%)	Potential risk
Dermatology consult	42 (100)	
Allergy consult	5 (12)	
No. cases attributed to drug(s)	42 (100)	
Labelled allergic to 1 drug	20 (48)	Potential False Negative
Labelled allergic to ≥ 2 drugs	17 (40)	Potential False Negative
None added to allergy list	2 (5)	Probable False Negative
Incomplete addition to allergy list	6 (14)	Potential False Negative OR False Positive

Table 6. Confirmed infections & infectious symptoms preceding/concurrent with disease in patients with SJS/TEN.

Preceding or concurrent infection	N value (%)
Confirmed infection, excluding URI	17 (40%)
URI symptoms	12 (29%)

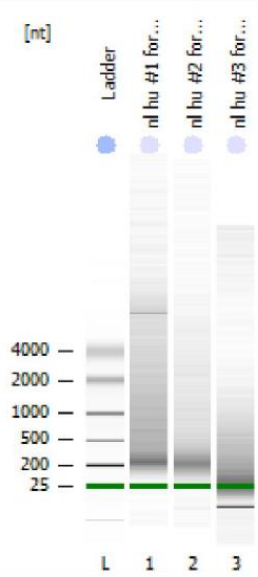


Figure 5. Fragment analysis of RNA isolated from 3 FFPE skin samples. Fragment analysis on 3 healthy control samples (nl hu #1, nl hu #2, nl hu #3) showed highly degraded RNA (50+% < 300 nt).

Table 7. List of genes of interest in Nanostring codeset, N = 188.

AHR	CCL5	CD3D	CX3CL1	HLA-DRB1	IL15	IL22RA2	ITGAL	KLRG2	STAT4
ARG1	CCL7	CD3E	CX3CR1	HLA-DRB3	IL16	IL23A	ITGAM	LAMP1	STAT5A
B2M	CCL8	CD4	CXCL1	HLA-E	IL17A	IL23R	ITGAX	MICA	STAT5B
CASP1	CCR1	CD40	CXCL10	Hobit	IL17B	IL27	ITGB2	MICB	STAT6
CASP10	CCR10	CD40LG	CXCL11	ICAM1	IL17F	IL2RA	JAK1	NLRP3	TBX21
CASP2	CCR2	CD44	CXCL2	ICAM3	IL18	IL2RB	JAK2	NOS2	TGFB1
CASP3	CCR4	CD45R0	CXCL9	ICOS	IL18R1	IL2RG	JAK3	PDCD1	TGFBR1
CASP8	CCR5	CD45RA	CXCR3	ICOSLG	IL18RAP	IL32	KIR_AS1	PDCD1LG2	TGFBR2
CCL11	CCR6	CD69	CXCR4	IDO1	IL1A	IL4	KIR_AS2	PDCD2	TNF
CCL13	CCR7	CD7	FADD	IFNA2	IL1B	IL4R	KIR_IS1	PECAM1	TNFRSF1B
CCL16	CD14	CD80	FAS	IFNG	IL1R1	IL5	KIR_IS2	PRF1	TNFRSF4
CCL18	CD1A	CD86	FASLG	IFNGR1	IL1R2	IL6	KLRAP1	S1PR1	TNFRSF9
CCL2	CD1D	CD8A	FOXP3	IL10	IL1RAP	IL6R	KLRB1	sCTLA4 (sol)	TNFSF10
CCL20	CD209	CD8B	GATA3	IL10RA	IL1RN	IL6ST	KLRC1	SELE	TNFSF11
CCL22	CD244	CSF1	GNLY	IL12A	IL2	IL7	KLRC4	SELL	TNFSF4
CCL23	CD27	CSF1R	GZMA	IL12B	IL20	IL7R	KLRD1	SELPLG	TYROBP
CCL24	CD274	CSF2	GZMB	IL12RB1	IL21	IL8	KLRF1	STAT1	VCAM1
CCL26	CD276	CTLA4_all	GZMK	IL13	IL21R	IL9	KLRF2	STAT2	
CCL27	CD28	CTLA4-TM	HLA-DRA	IL13RA1	IL22	ITGAE	KLRG1	STAT3	

Table 8. List of housekeeping genes in Nanostring codeset, N = 12.

ABCF1	EEF1G	GAPDH	HPRT1	POLR1B	TBP
ALAS1	G6PD	GUSB	OAZ1	POLR2A	TUBB

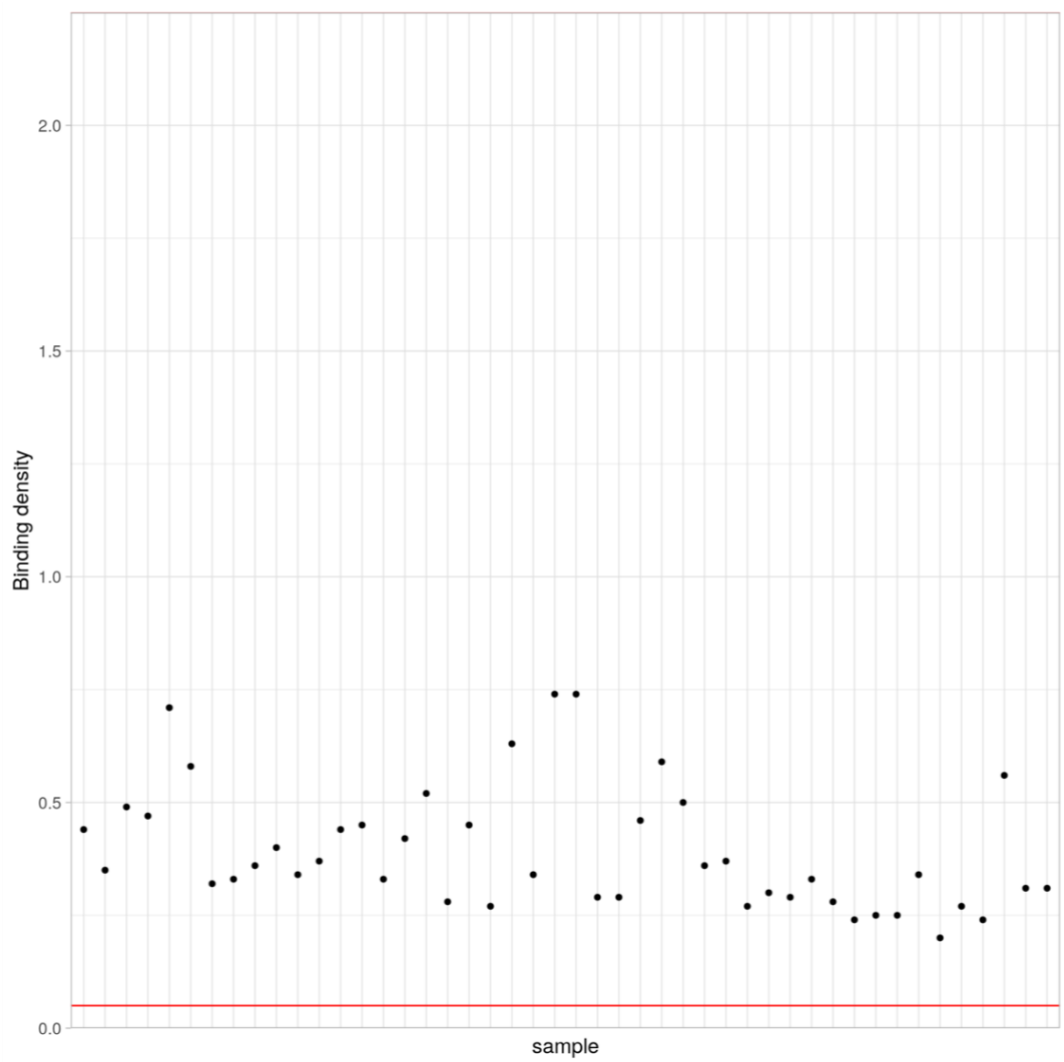


Figure 6. Quality control: binding density of each sample. The binding density is a measure of the number of optical features per square micron. It is useful for determining whether or not data collection has been compromised due to image saturation. Typically, the range for binding density will be between 0.05 and 2.25. All samples were within the correct limits for the binding density.

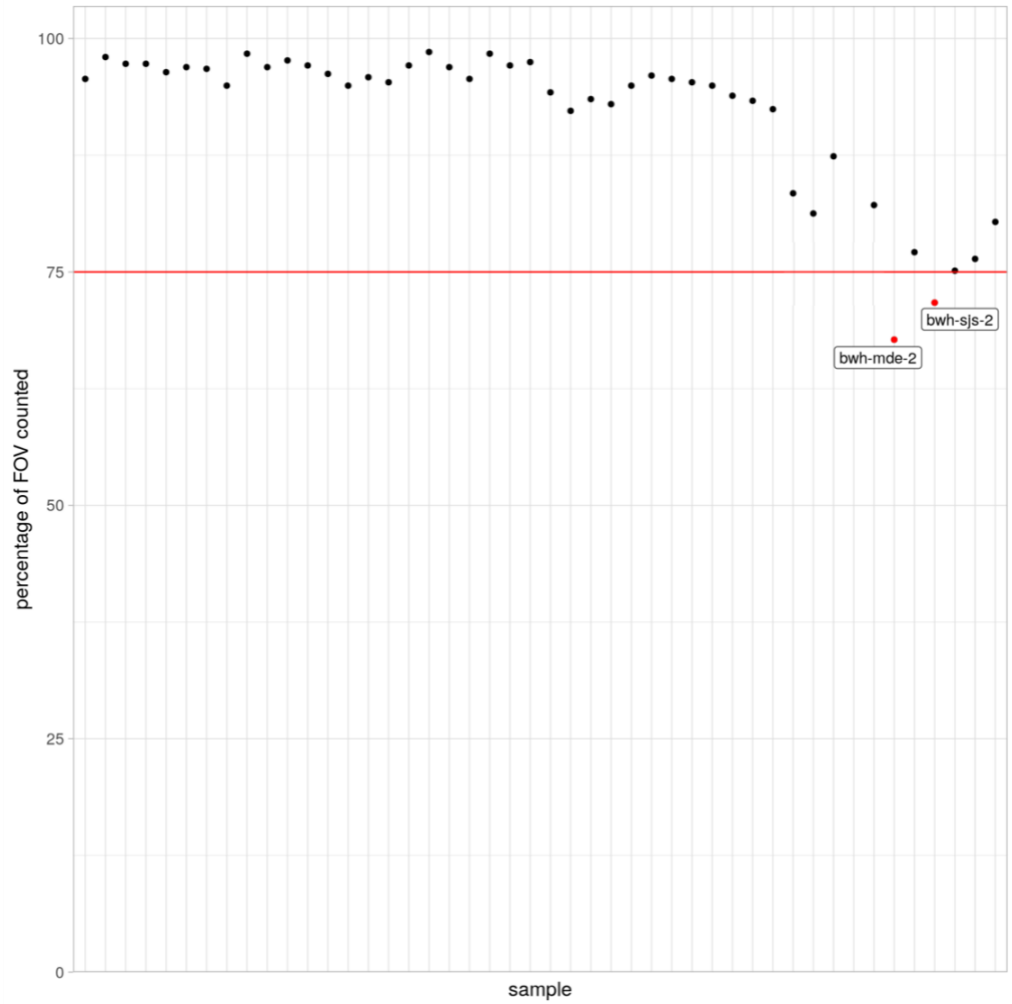


Figure 7. Quality control: proportion of FOV counted in each sample. The nCounter Digital Analyzer images each lane in discrete units, called fields of view (FOV). Optical issues, such as an inability to focus due to bubbles or insufficient oiling of the cartridge, can prevent successful imaging of a FOV. The Digital Analyzer reports the number of FOVs successfully imaged as FOV Counted. Significant discrepancy between the number of FOV for which imaging was attempted (FOV Count) and for which imaging was successful (FOV Counted) may be indicative of an issue with imaging performance. At least 75% of FOVs should be successfully counted to obtain robust data. Samples below that threshold (BWH-SJS-2, BWH-MDE-2) are marked as red.

Table 9. Excerpt of sample metadata collected.

Group	Sample ID	Gender	Age	Race/ ethnicity	Pre-bx sys. IS?	IC/IS?	Biopsy year
SJS/TEN, N = 14	BCH-SJS-1	F	1	Black	S.S.	N	2016
	BCH-SJS-2	F	1	White	S.S.	N	2011
	MGH-SJS-1	F	23	White	IVIG	N	2016
	MGH-SJS-2	M	22	Unknown	N	N	2012
	MGH-SJS-3	F	11	Asian	IVIG	N	2013
	MGH-SJS-4	F	20	White	S.S.	N	2013
	MGH-SJS-5	M	30	Hisp./Lat.	N	N	2011
	MGH-SJS-7	M	1	White	N	Y (S.S.)	2014
	MGH-SJS-8	M	12	Black	S.S.	N	2011
	MGH-SJS-10	M	55	White	N	N	2013
	MGH-SJS-11	F	39	White	N	N	2010
	MGH-SJS-12	M	58	White	N	Y (HIV)	2016
	*BWH-SJS-2	F	54	Hisp./Lat.	S.S.	N	2018
	BWH-SJS-3	F	59	White	S.S.	N	2017
DRESS, N = 6	MGH-DRESS-1	M	84	White	N/A	N	2013
	MGH-DRESS-2	M	70	White	N	N	2014
	MGH-DRESS-3	M	42	Asian	S.S.	N	2011
	BCH-DRESS-1	F	17	Hisp./Lat.	N	N	2014
	BCH-DRESS-2	F	4	White	S.S.	Y (s/p BMT)	2015
	BWH-DRESS-1	F	50	White	N	N	2013
MDE, N = 7	MGH-MDE-1	M	57	White	N	C.P.I.	2015
	MGH-MDE-2	F	54	White	N	N	2016
	MGH-MDE-3	M	73	White	N	N	2013
	MGH-MDE-4	M	31	White	N	N	2014
	BWH-MDE-1	F	37	White	S.S.	N	2012
	*BWH-MDE-2	M	65	White	N/A	Y (MTX)	2015
	BWH-MDE-3	F	47	White	N	Y (HIV)	2014
Healthy, N = 10	nl hu-1	F	57	N/A	N/A	N/A	2014
	nl hu-2	N/A	N/A	N/A	N/A	N/A	2014
	nl hu-3	F	63	N/A	N/A	N/A	2014
	nl hu-8	M	72	N/A	N/A	N/A	2016
	nl hu-11	N/A	N/A	N/A	N/A	N/A	2017
	nl hu-12	N/A	N/A	N/A	N/A	N/A	2017
	nl hu-4	F	N/A	N/A	N/A	N/A	2014
	nl hu-9	N/A	N/A	N/A	N/A	N/A	2017
	nl hu-13	N/A	N/A	N/A	N/A	N/A	2018
	nl hu-18	F	61	N/A	N/A	N/A	2018
	nl hu-19	N/A	N/A	N/A	N/A	N/A	2018

N/A = not available. Age = age at presentation in years. Hisp./Lat. = Hispanic or Latino. Pre-bx sys. IS? = systemic immunosuppression for rash before biopsy? IC/IS? = immunocompromised/immunosuppressed? BMT = bone marrow transplant. MTX = methotrexate. S.S. = systemic steroids. C.P.I. = checkpoint inhibitors.
*Excluded from differential expression analysis due to sub-optimal values.

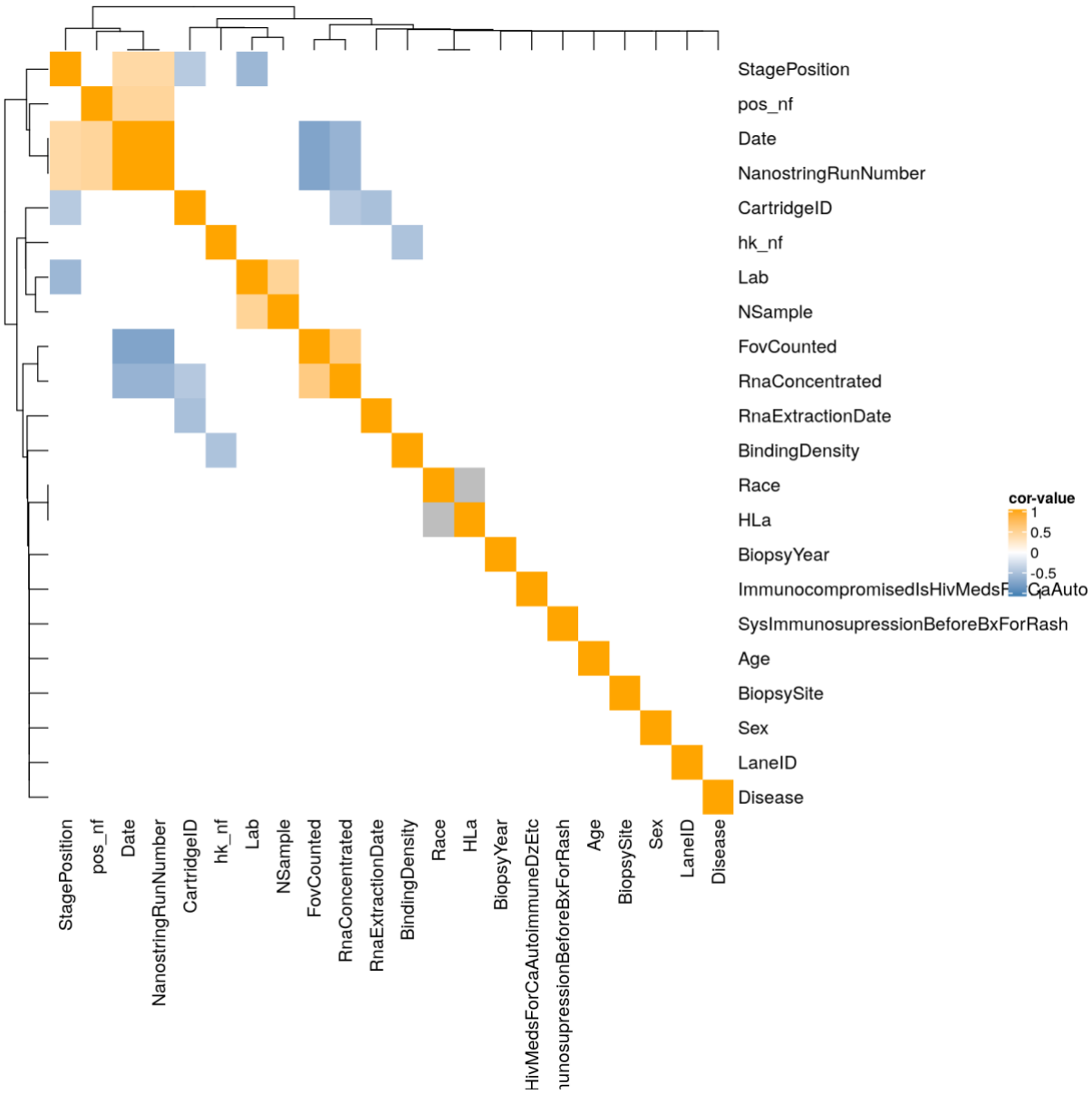


Figure 8. Correlation analysis between metadata elements. This analysis was performed to assess for confounding factors. No concerning correlations were observed.

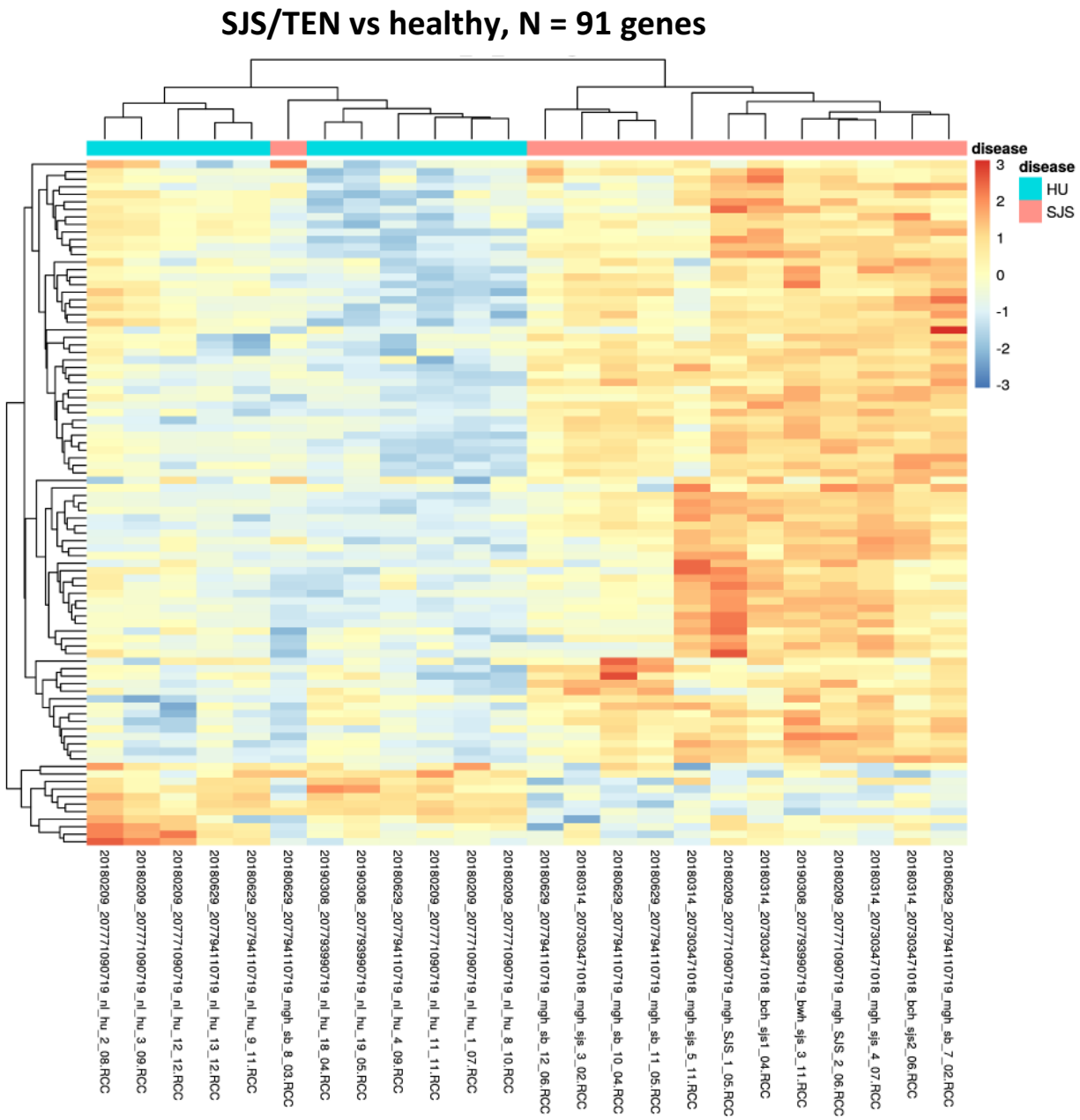


Figure 9. Heatmap of SJS/TEN samples vs healthy controls. There are 91 statistically significant ($p_a < 0.05$) genes that are differentially expressed in SJS/TEN samples relative to healthy controls. Diseased samples cluster together away from healthy controls, with the notable exception of MGH-SJS-8 (labelled in the heatmap above as "20180629_207794110719_mgh_sb_8_03.RCC").

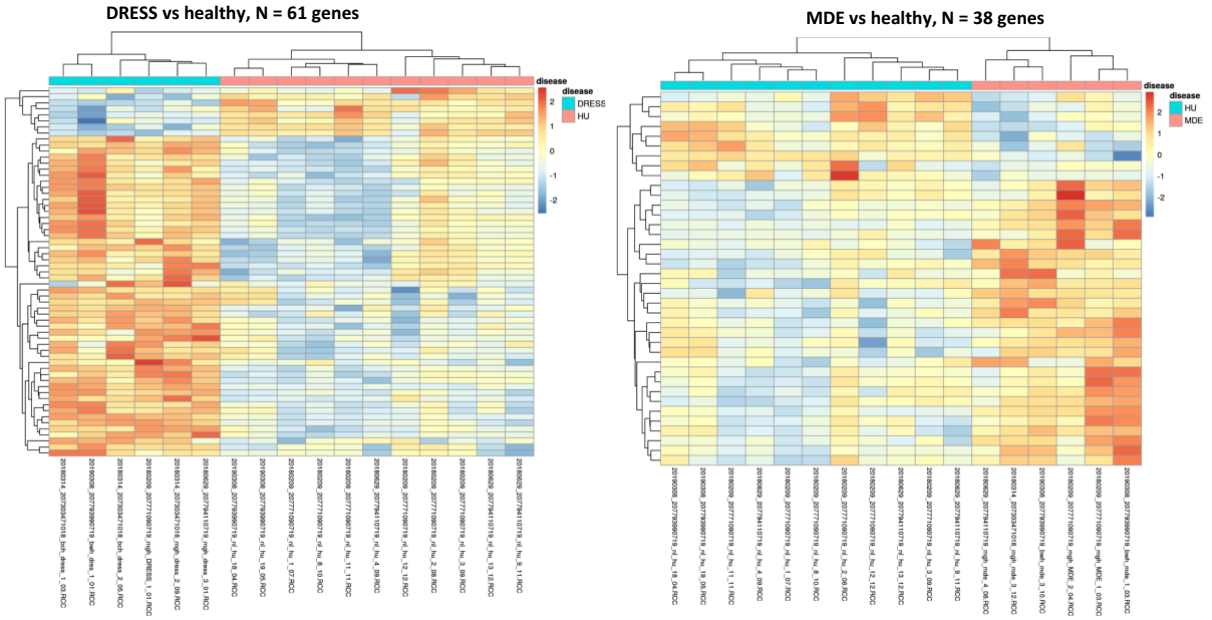


Figure 10. Heatmaps of DRESS and MDE samples vs healthy controls. There are 61 statistically significant ($p_a < 0.05$) genes that are differentially expressed in DRESS samples (left) relative to healthy controls. There are 38 such genes in MDE samples (right). All diseased samples cluster together away from healthy controls.

Table 10. Fold change in expression of T cell phenotyping genes* across dtDHRs.

Marker	SJS/TEN, N=13	DRESS, N=6	MDE, N=6
CD3E	1.9	3.4	--
CD8A	2.6	4.1	--
CD4	1.7	1.9	--
CD45R0	3.3	2.4	2.0
CD45RA	2.3	--	--
CD62L/SELL	3.5	2.9	--
CCR7	1.9	-- (1.9, p_a=0.08)	--
CD69	--	--	0.43
CD103/ITGAE	--	--	--

*Only statistically significant results are shown. "--" = non-significant results.
>1.5 = upregulated; <0.67 = downregulated

Table 11. Fold change in expression of Th1-, Th2-, and Tc1-related genes*.

	Marker	SJS/TEN, N=13	DRESS, N=6	MDE, N=6
Th1	CXCL9	29.7	17.9	15.2
	CXCL10	45.1	9.9	15.3
	CXCL11	23.0	4.4	5.9
	IFNG	--	0.94	--
	IFNGR1	--	0.91	--
	IL12A	--	1	--
	IL12B	--	0.69	--
	IL12RB1	1.9	1.7	--
	TBET/TBX21	--	1.3	--
	STAT1	8.1	4.6	4.5
	STAT4	--	--	--
Th2	IL4	--	0.7	--
	IL4R	2.7	2.6	2.1
	IL5	--	--	--
	IL13	--	--	--
	GATA3	0.23	0.43	--
	STAT6	1.3	--	--
Th17	IL17B	--	0.55	--
	IL17F	--	0.44	--
	IL23A	--	--	--
	IL23R	--	--	--
	IL6	--	--	--
	STAT3	1.7	1.4	1.6
Tc1	GNLY	4.0	4.9	--
	GZMA	5.4	9.6	3.5
	GZMB	10.5	8	3.9
	PRF1	4.7	4.7	--
	FAS	--	0.73	--
	FASLG	--	1.8	--

*Only statistically significant results are shown. "--" = non-significant results.
>1.5 = upregulated; <0.67 = downregulated

Table 12. Fold change in expression of JAK/STAT genes*.

Marker	SJS/TEN, N=13	DRESS, N=6	MDE, N=6
JAK1	--	--	--
JAK2	2.0	--	--
JAK3	3.6	4.9	2.7
STAT1	8.1	4.6	4.5
STAT2	3.8	2.2	2.4
STAT3	1.7	1.4	1.6
STAT4	--	--	--
STAT5A	1.5	--	--
STAT5B	--	--	--
STAT6	1.3	--	--

*Only statistically significant results are shown. "--" = non-significant results.
>1.5 = upregulated; <0.67 = downregulated

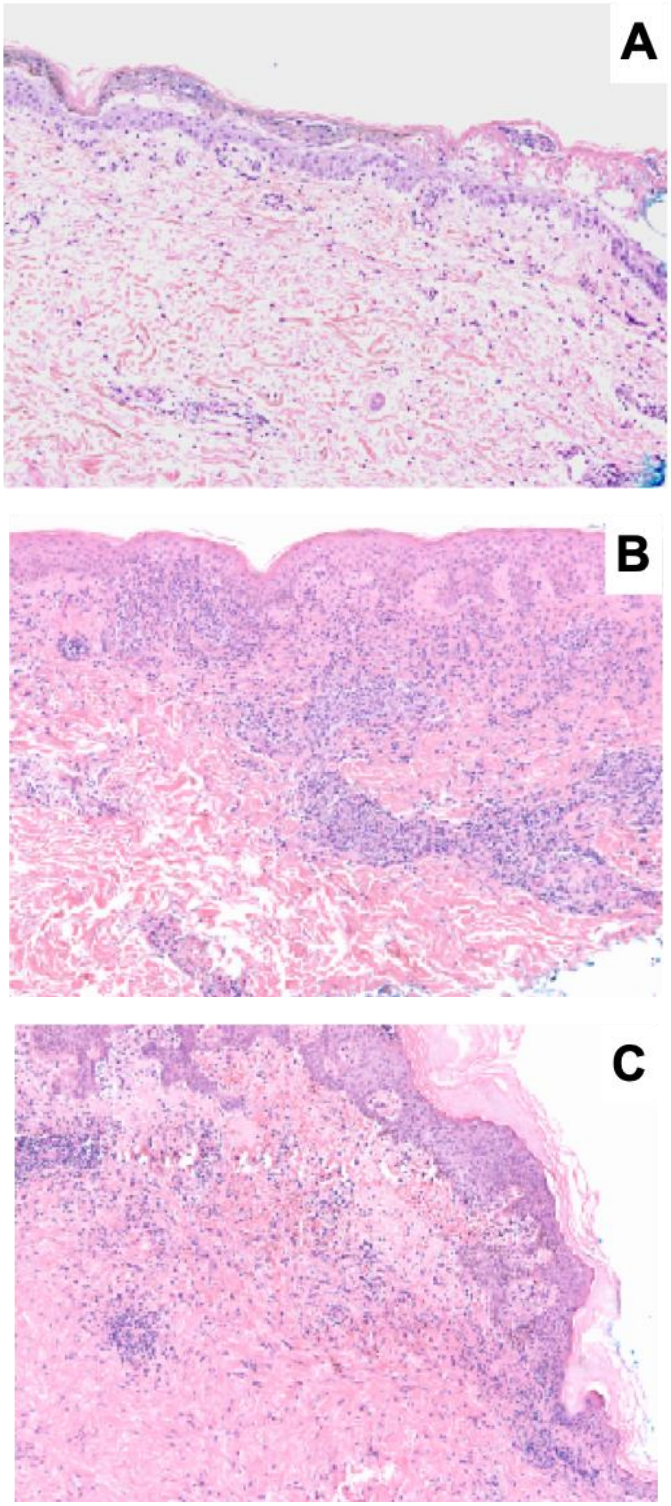
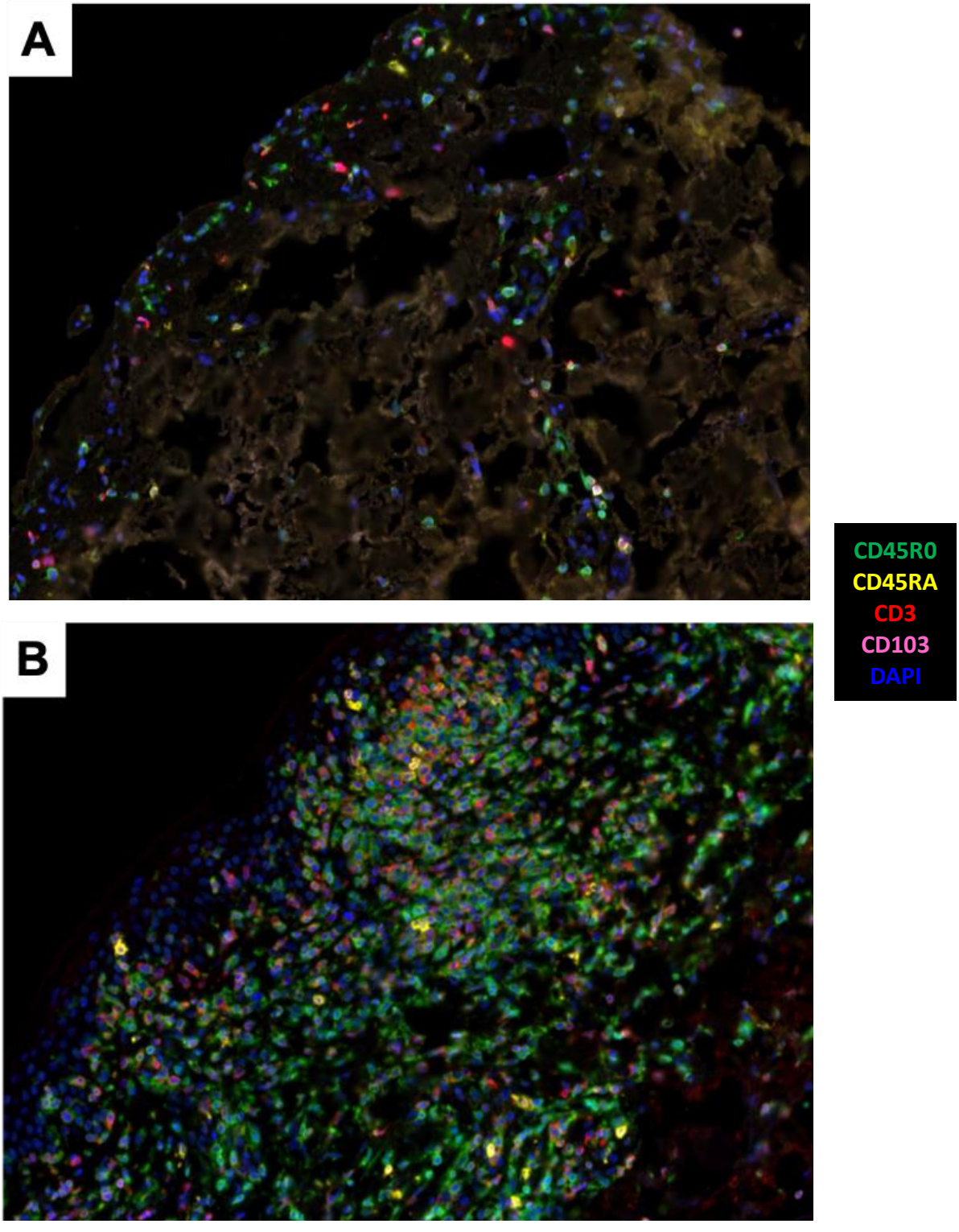


Figure 11. Representative histology of selected dtDHRs. H&E staining was performed on every case. TEN (**A**) notably has a pauci-inflammatory infiltrate compared to the other two dtDHR types. DRESS (**B**) and MDE (**C**) exhibit a marked superficial perivascular and interstitial lymphocytic infiltrate.



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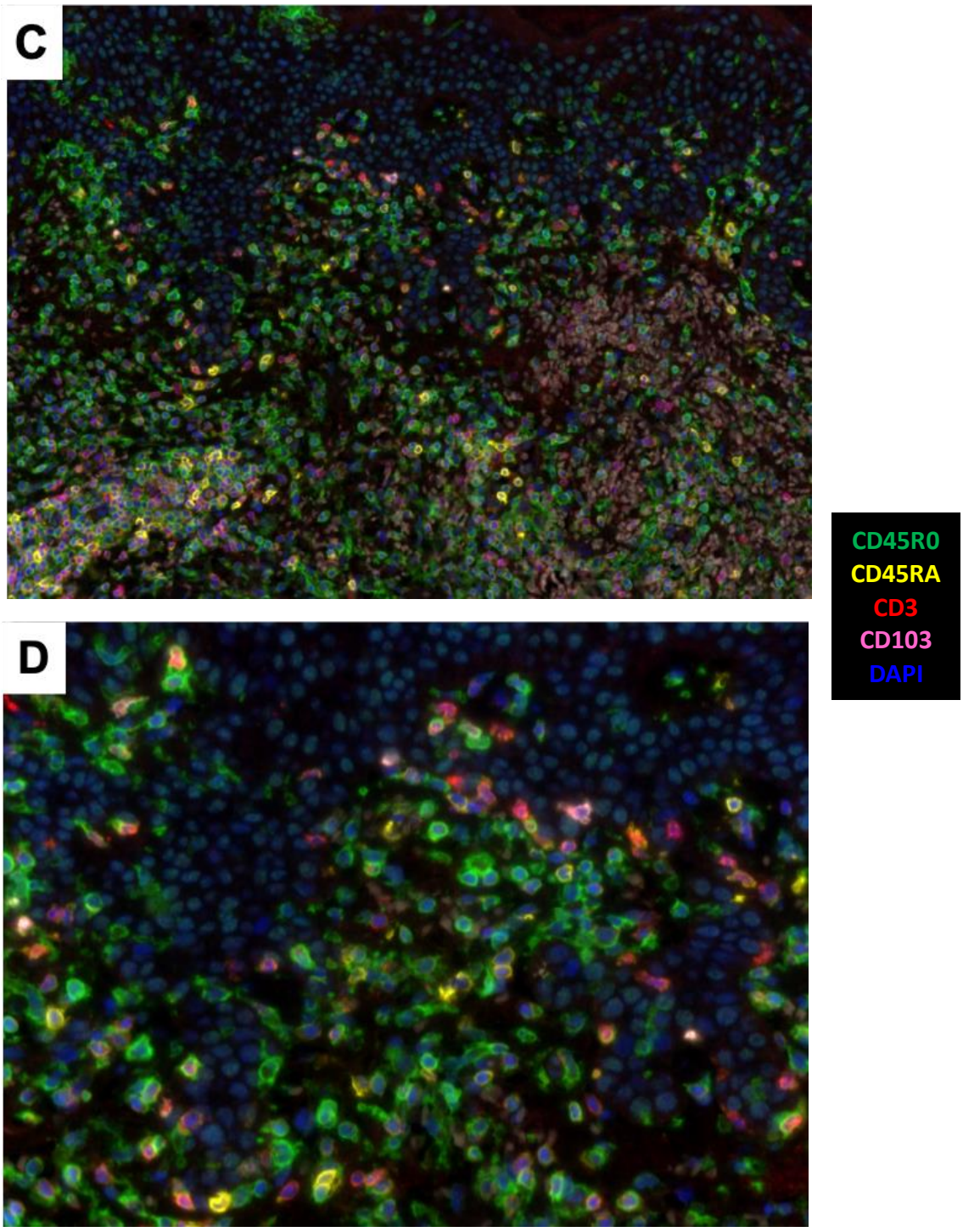
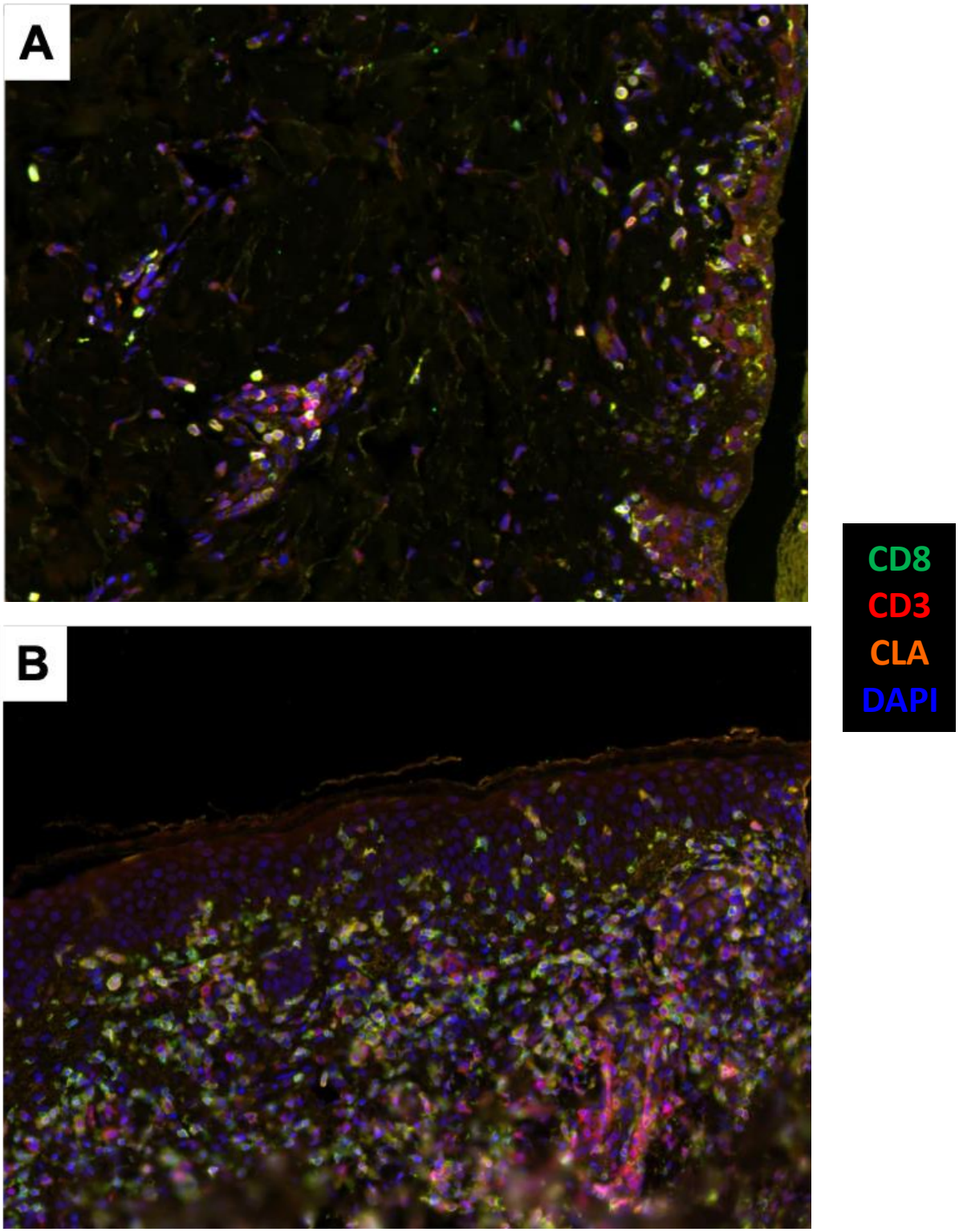


Figure 12. Multiplexed IF profiling of memory and naïve/effector T cells in selected dtDHRs. All dtDHR types were stained for CD45R0, CD45RA, CD3, CD103, and counterstained with DAPI. CD45RO+ T cells appear yellow in these images because of red's overlap with green. CD45RA+ T cells appear orange because of red's overlap with yellow. **A**: SJS/TEN at 200x magnification. **B**: DRESS at 200x magnification. **C**: MDE at 200x magnification. **D**: MDE at 400x magnification.



(Figure continued below….)

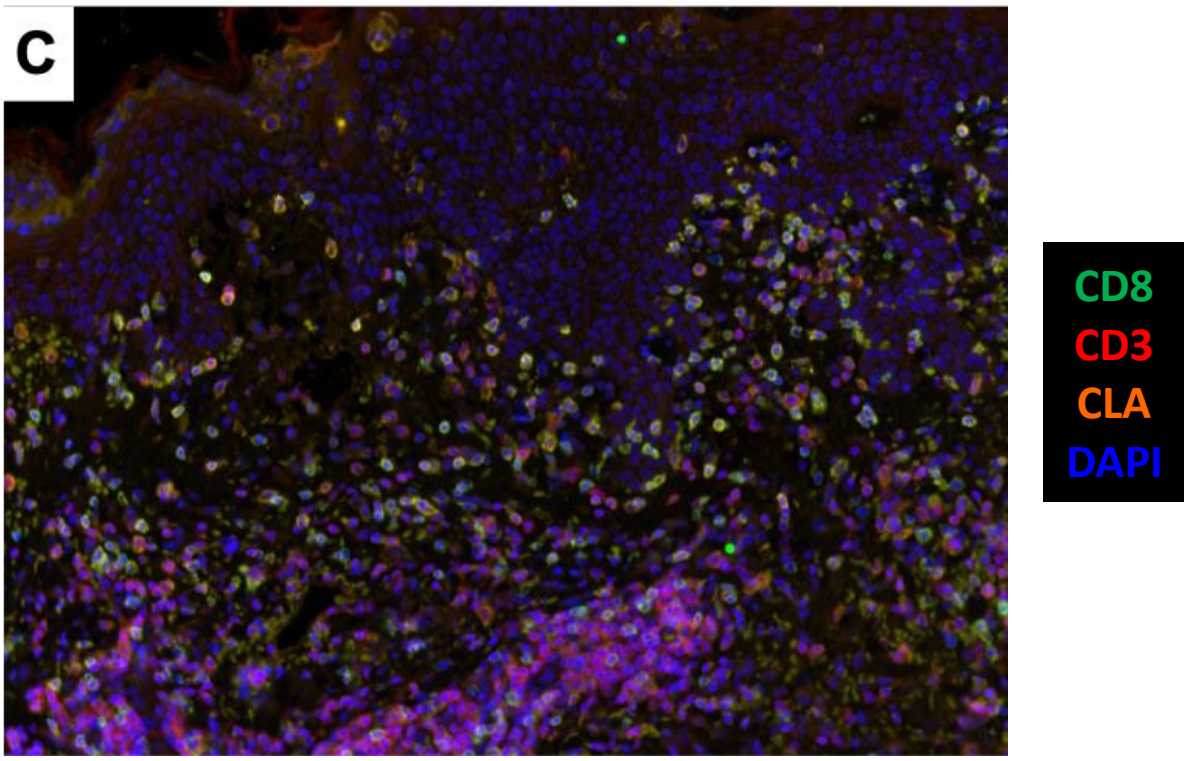


Figure 13. Standard IF profiling of T cell infiltrate in selected dtDHRs. All dtDHR types were stained for CD8, CD3, CLA, and counterstained with DAPI using standard IF. CD8+ T cells appear yellow in these images because of red's overlap with green. CLA+ T cells appear orange-red because of red's overlap with orange. **A**: SJS/TEN at 200x magnification. **B**: DRESS at 200x magnification. **C**: MDE at 200x magnification.

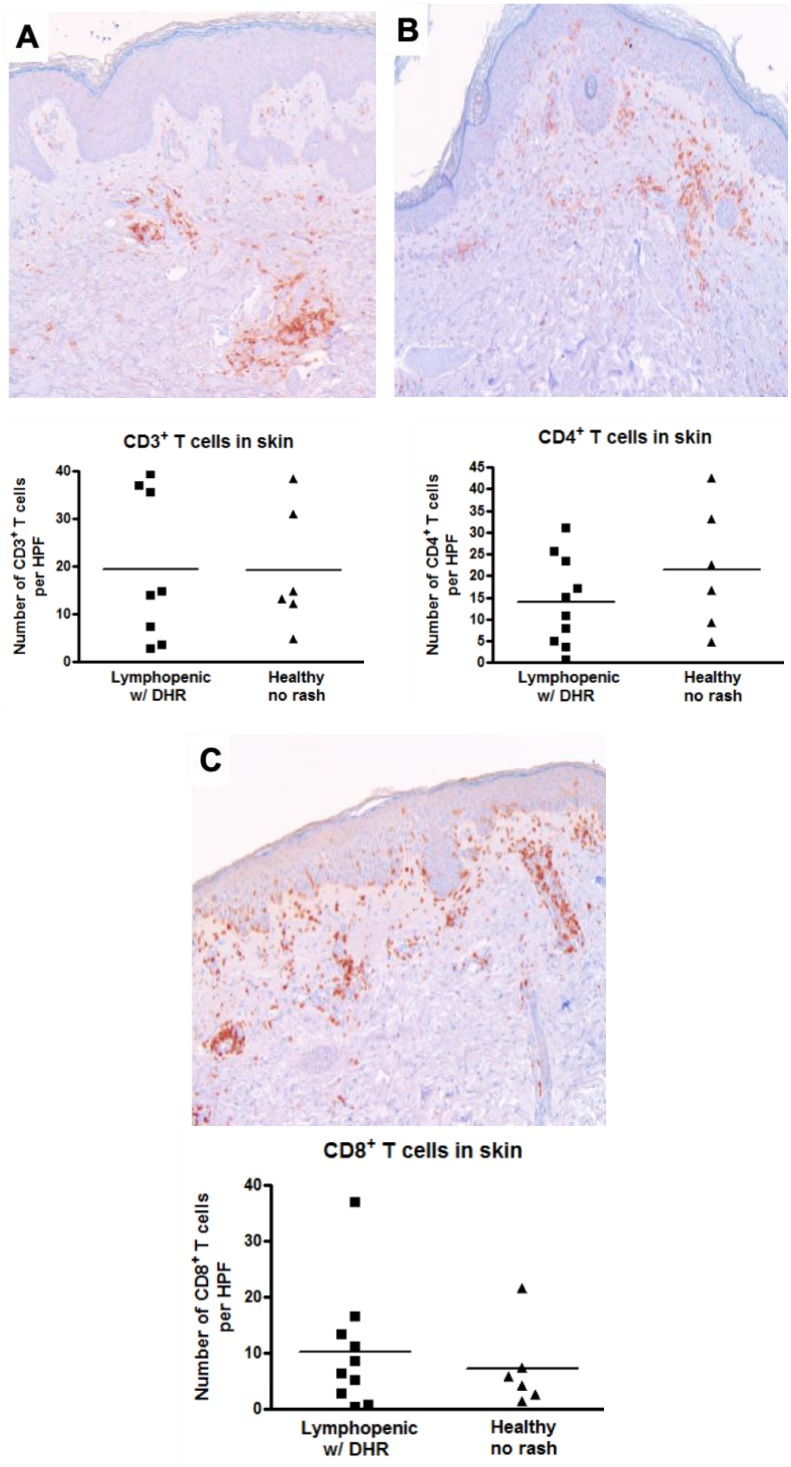


Figure 14. Analysis of T cell infiltrate in lymphopenics with MDE vs healthy controls. All 5 lymphopenic cases were stained with IHC for CD3, CD4, and CD8. They were then quantified and compared to numbers obtained for healthy controls. CD3+ (**A**), CD4+ (**B**), and CD8+ (**C**) IHC images and scatter dot plots were generated for comparison. CD4+ and CD8+ subsets were found to be equivalent to healthy controls.

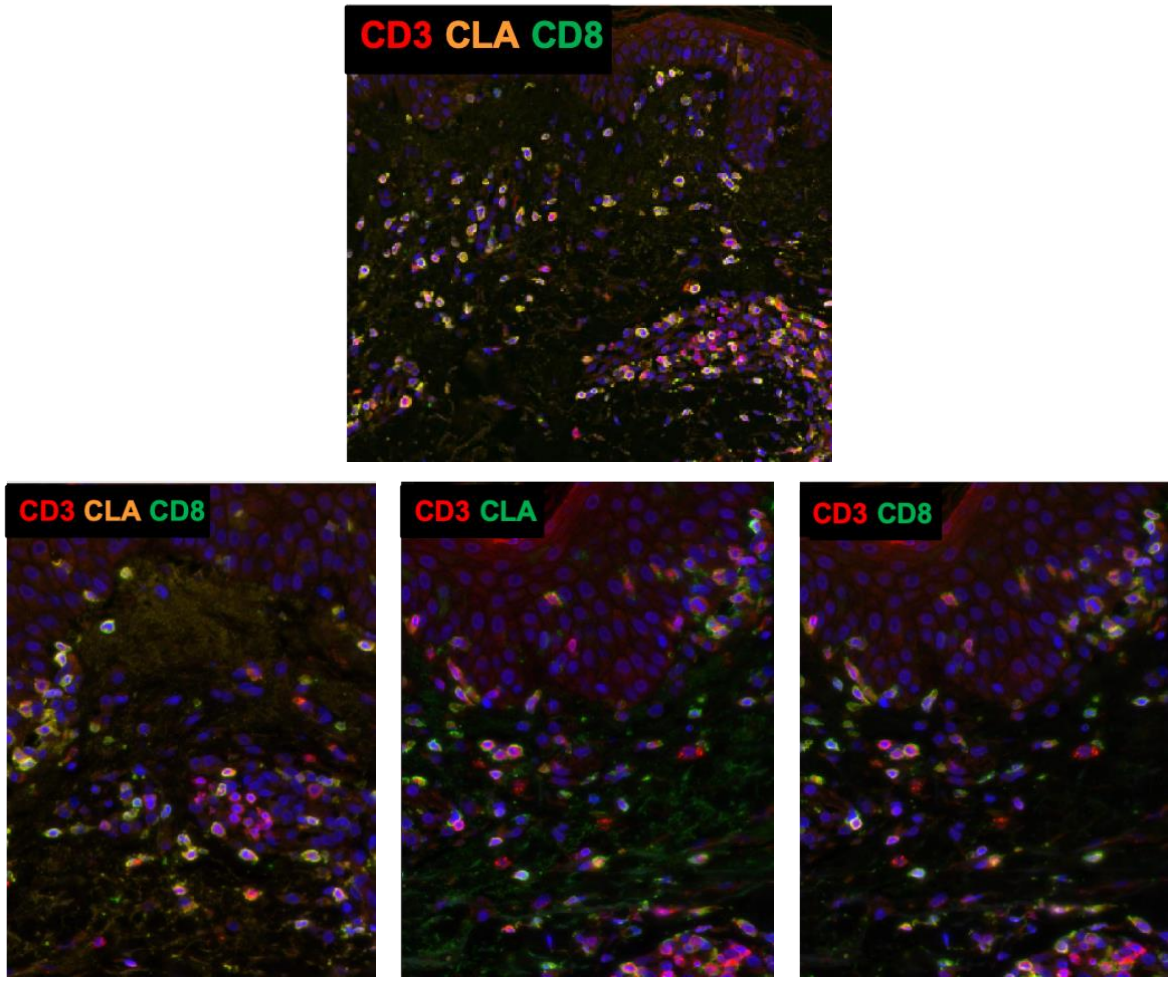


Figure 15. IF profiling of CD3, CLA, & CD8 in lymphopenics with MDE. All lymphopenic samples with MDE were stained for CD3, CLA, and CD8. **Upper**: shows all 3 markers at 20x magnification. **Lower left**: shows all 3 markers at 400x magnification. **Lower middle**: shows overlay of CD3 & CLA where CD3+CLA+ cells appear yellow. **Lower right**: shows overlay of CD3 & CD8 where CD3+CD8+ cells appear yellow.

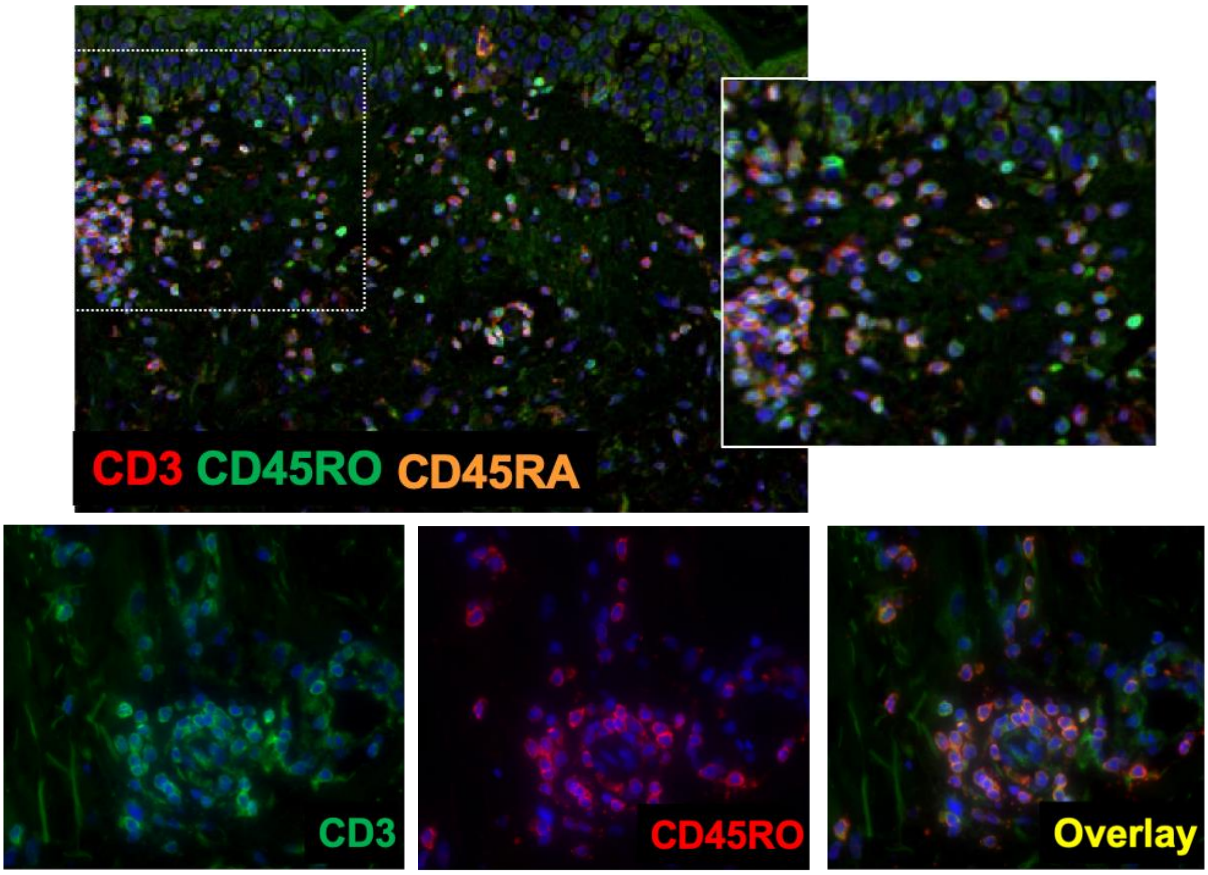


Figure 16. IF profiling of CD3, CD45R0, & CD45RA in lymphopenics with MDE. All lymphopenic samples with MDE were stained for CD3, CD45R0, and CD45RA. **Upper**: shows all 3 markers at 20x magnification with inset at 40x magnification. **Lower left**: shows CD3 alone at 40x magnification. **Lower middle**: shows CD45R0 alone at 40x magnification. **Lower right**: shows overlay of CD3 & CD45R0 where CD3+CD45R0+ cells appear yellow.