



Clinical Transcriptomics in Systemic Vasculitis (CUTIS)

Vasculitis Clinical Research Consortium (VCRC)

Protocol Number 5563

This protocol is for research purposes only, and should not be copied, redistributed or used for any other purpose. The procedures in this protocol are intended only for use by Consortium investigators in carefully controlled settings. The Chair of this study should be consulted before using or attempting any procedure in this protocol.

Study Chairs:

Peter C. Grayson, MD, MSc

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Robert Micheletti, MD

The University of Pennsylvania

Peter A. Merkel MD, MPH

The University of Pennsylvania

Table of Contents

1. Protocol Synopsis Table	3
1a. Definitions.....	5
1b. Brief Summary.....	5
1c. Detailed Summary	5
2. Study Endpoints	7
2a. Primary Outcome	7
2b. Secondary Outcomes	7
3. Background and Rationale.....	7
3a. Background	7
3b. Rationale	8
4. Study Design and Methods.....	9
4a. Overview:.....	9
4b. Study Diagram	9
4c. Identification of Patients.....	9
4ci. Inclusion Criteria.....	10
4cii. Exclusion Criteria	10
4d. Study Procedures	10
4di. Visit Frequency/ Visit Schedule	12
4dii. Recruitment.....	13
4e. Data Elements for Collection.....	13
5. Safety Monitoring and Adverse Event Reporting.....	13
5a. Nature of Study	13
5b. Definitions.....	13
5c. Standard Elements.....	14
5d. Reporting Timelines.....	14
5e. RDCRN Adverse Event Data Management System (AEDAMS).....	14
5f. Toxicity Grading of Adverse Events	15
5g. Relation to Study Therapy	16
6. Data Analysis and Statistical Considerations	16
6a. Sample Size.....	16
6b. Analysis Plan	16
6c. Secondary Outcomes.....	17
7. Data Management	17
7a. Registration	17
7b. Data Entry	18
7c. Data Quality Control.....	18
8. Protection of Human Subjects	18

1. Protocol Synopsis Table

Protocol Number:	5563
Protocol Title:	Clinical Transcriptomics in Systemic Vasculitis (CUTIS)
Study Chairs:	Peter Grayson, Robert Micheletti, Peter Merkel
Statistician:	NIAMS Bioinformatics Core / Grayson
Consortium	Vasculitis Clinical Research Consortium
Participating Sites:	VCRC sites + NIAMS VTRP
Activation Date:	Pending
Sample Size:	40-56 subjects with vasculitis
Target Enrollment Period:	Jan 2015 – Sept 2016
Study Design:	<ul style="list-style-type: none"> • Multi-center observational study to evaluate the histopathology and transcriptome of cutaneous lesions in patients with several different types of vasculitis
Primary Study Objectives:	<ul style="list-style-type: none"> • To characterize the histopathology and transcriptome of vasculitic skin lesions in patients with different types of cutaneous vasculitis
Primary Outcome Measure:	<ul style="list-style-type: none"> • Detailed description and comparison of histopathologic patterns across different forms of cutaneous vasculitis • Whole-genome differential gene expression comparison of cutaneous lesion mRNA across several forms of cutaneous vasculitis
Secondary Study Objectives:	<ul style="list-style-type: none"> • Comparison of transcriptomic signatures in idiopathic vasculitides to known signatures in pediatric monogenic diseases with cutaneous vasculitic features with focus on specific set of pathways including type 1 interferon and interleukin-1 • Whole-blood RNA and DNA samples will be collected for potential ancillary analyses
Secondary Outcome Measures	<ul style="list-style-type: none"> • Pathway and enrichment analyses for pre-specified gene set signatures between pediatric autoinflammatory diseases and idiopathic vasculitides • Within-subject comparison of blood and skin gene expression patterns • Use of RNA sequencing data for microbiome studies.
Main Eligibility/ Exclusion Criteria:	<p>Inclusion Criteria</p> <ol style="list-style-type: none"> 1. Cutaneous lesion (purpuric macules, palpable purpura, retiform purpura, nodules, ulcers, or urticaria) believed to be related to active vasculitis 2. A suspected or confirmed diagnosis of cryoglobulinemic vasculitis (CV), drug-induced vasculitis, eosinophilic granulomatosis with polyangiitis (EGPA), IgA vasculitis, isolated cutaneous vasculitis, either granulomatosis with polyangiitis (GPA) / microscopic polyangiitis (MPA),

- polyarteritis nodosa (PAN), or urticarial vasculitis.
3. Willing and able to provide written informed consent.

Exclusion Criteria

1. Age < 18 years
2. Patients who, in the physician's judgment, are poor candidates for biopsy or who are at elevated risk of complications due to infection, bleeding, etc., or in whom risk is felt to outweigh the potential benefit of knowledge gained
3. Patient with ANC <1500, PLT <50, or Hgb <7.0
4. Other uncontrolled disease (co-morbidity) that could prevent a patient from fulfilling the study requirements or that would substantially increase the risk of study problems
5. Active infection at or near the potential biopsy site, poor circulation, or site such as bony prominence or other structure that is felt to increase the risk of complications
6. Pregnant or nursing women
7. Persons unable to provide informed consent

Statistical Considerations (analysis plan): Histopathology will be described and quantified by a Dermatopathologist within the NIH using standardized data collection form. Intra-rater reliability will be assessed. Abundance of cellular populations and patterns of structural involvement will be compared using non-parametric statistical methods. Gene expression data will be analyzed by the NIAMS Bioinformatics core under direction of Dr. Grayson. Analysis of variance (ANOVA) will be used to identify differentially expressed genes between different types of vasculitis. Unsupervised clustering methods and principle component analysis will be used to classify samples in a disease agnostic fashion. Enrichment analyses will be performed to study specific tissue pathways of interest including gene signatures related to type 1 interferon, interleukin-1, low-density granulocytes, and various other cytokine/chemokine pathways. Signatures will be compared to known signatures in pediatric monogenic diseases with vasculitic features.

Sponsors: Office of Rare Diseases Research, National Center for Advancing Translational Sciences (ORDR, NCATS), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

1a. Definitions

- Macule: flat lesion that is less than 1cm in diameter with a change in color of the skin
- Papule: solid raised lesion that has distinct borders and less than 1cm diameter
- Purpura: non-blanching red or purple discolorations measuring 0.3 – 1cm in diameter
- Retiform: composed of crossing lines and interstices
- Nodule: raised solid lesion more than 1cm that may be in the epidermis, dermis, or subcutaneous tissue
- Ulcer: discontinuity or break in skin

1b. Brief Summary

This study employs a multi-center approach to evaluate cutaneous vasculitis across several forms of idiopathic vasculitis. Patients with cutaneous manifestations of vasculitis will be evaluated by teams of primary vasculitis care providers and Dermatologists in order to facilitate optimal selection of patients and sampling of lesions. A punch skin biopsy at a site of active vasculitis will be the source of material for histopathologic and transcriptomic evaluation. The histopathology of cutaneous vasculitis will be characterized using a standardized, systematic approach. This approach includes a detailed description of abnormalities and extensive immunophenotyping of cell populations in the epidermal, dermal, and subcutaneous layers of skin. In addition to histologic description, skin biopsy material will be used a source of RNA for whole-genome gene expression profiling studies. Differential gene expression comparisons will be made across types of vasculitis. Disease-agnostic computational approaches will be used to discover novel ways to classify subgroups based upon global and specific patterns of gene expression. Additional samples will be collected (whole blood RNA, DNA) for potential secondary studies.

1c. Detailed Summary

Vasculitis is defined as inflammation directed towards blood vessels. There are many forms of vasculitis, and the underlying cause is unknown for most types of vasculitis. Vasculitis can be isolated to a single organ, or it can be systemic. Patterns of organ involvement help to differentiate different types of vasculitis. The skin is a frequent site of involvement in many types of vasculitis. Cutaneous manifestations of vasculitis include petechia, purpura, urticaria, infiltrative erythema, hemorrhagic vesicles and bullae, nodules, ulcers, digital gangrene, and livedo racemosa. Although a broad range of skin manifestations overlap across different types of vasculitis, the pattern of affected cutaneous blood vessels and the cell populations involved at sites of active disease can help differentiate between different forms of vasculitis.

Because there are few diagnostic tests specific to vasculitis, classification schemes are often necessary to distinguish between the many different forms of vasculitis. Traditionally, size of affected vessels serves to differentiate between small, medium, and large vessel vasculitis. However, this classification scheme is heuristic and does not account for potential biologic differences. What is needed is a better understanding of the molecular pathways involved in the different types of vasculitis. Whole genome gene expression profiling can help to identify pathways of disease and suggest novel methods for classification on the basis of disease biology rather than clinical phenotype. The skin serves as an excellent source of tissue for transcriptomic studies in vasculitis. Skin is easily accessible for detailed clinical evaluation and biopsy and may provide more disease-specific information than circulating blood. To date, no studies have evaluated

the transcriptome of vasculitic cutaneous lesions. These types of studies could serve to identify novel biomarkers, treatment paradigms, and classification schemes in vasculitis.

This study will examine histopathology and the transcriptome of cutaneous vasculitis across eight different types of vasculitis. A single punch biopsy will be the tissue source for both the histopathologic and transcriptomic material to facilitate comparison across these assessment modalities. A multi-center and cross-disciplinary approach will be used. The University of Pennsylvania will serve as the central coordinating site and all samples will be sent to the National Institute of Health for processing and analysis. Teams of primary vasculitis care providers and Dermatologists will be engaged at each participating site. The vasculitis providers will clinically assess and categorize any systemic features of disease, quantify disease activity using validated assessment tools in vasculitis, and help determine the underlying diagnosis. The Dermatologist will assess and classify the cutaneous aspects of disease, perform skin biopsy, and assist with interpretation of biopsy results.

A purposeful sampling strategy will be employed for patient recruitment/selection. The study will be restricted to the following forms of vasculitis:

- Cryoglobulinemic vasculitis (CV)
- Drug-induced vasculitis
- Eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss)
- IgA vasculitis
- Isolated cutaneous vasculitis
- Granulomatosis with polyangiitis (GPA, Wegener's) or microscopic polyangiitis (MPA)
- Polyarteritis nodosa (PAN)
- Urticarial vasculitis

Five subjects from each of these groups will be recruited into the study. Other types of vasculitis with cutaneous disease will not be included. Previously published transcriptomic data from biopsies of unaffected skin in healthy controls will be used as in silico controls. Skin lesions from other diseases, e.g. systemic lupus erythematosus (SLE), may be used as an inflammatory disease controls. Disease control patients will be recruited through existing protocols with the NIAMS, independent of this protocol.

Although the skin manifestations of vasculitis are broad, this study will restrict lesions sampled to a short list of potential manifestations. Restriction of the type of lesions included in this study will insure homogeneity of samples. Lesions will be restricted to the following:

- Purpuric macule
- Palpable purpura
- Retiform purpura
- Nodule
- Ulcer

Skin biopsies will be performed either in the context of ongoing clinical care or purely for research purposes. In cases of diagnostic uncertainty where it may be unclear how to classify a particular patient, diagnosis will be re-assessed 6 months after biopsy prior to final determination of the diagnosis. All participating Dermatologists will receive study-

specific training regarding the skin biopsy procedure and study documentation, per standardized operating procedure.

All skin biopsies will be processed according to protocol. A portion of the sample will be submitted for histologic review and the remainder of the sample will be used for transcriptomic analysis. If a biopsy is performed for clinical indications, the portion of tissue used for histology will be processed and initially evaluated at the local center per standard practice. Processed slides and unprocessed tissue blocks will be sent to the NIH for further interpretation and processing as needed. If a biopsy is performed for research, the portion of tissue used for histology will be cryopreserved and sent to the NIH for processing and review. All samples submitted for transcriptomic study will be immediately preserved in RNA later tubes and sent to the NIH for processing and analysis.

A standardized histology assessment will be performed on all samples. This assessment will include detailed immunophenotyping, direct immunofluorescence, and assessment of defined structures within different layers of skin. A team of vasculitis care providers, Dermatologists, and dermatopathologists will participate in histologic review. A similar process has been developed and implemented by our group to study skin biopsies from patients with pediatric monogenetic diseases including CANDLE, SAVI, and NOMID.

Analysis of transcriptomic data will occur at the NIH in conjunction with the NIAMS bioinformatics core. Our group has extensive experience conducting whole-genome gene expression studies in blood and tissue from patients with vasculitis.

Additional samples will be collected per study protocol for potential ancillary studies. These may include studies related to DNA, the cutaneous microbiome, and comparative transcriptomic analysis between circulating blood and affected tissue.

2. Study Endpoints

2a. Primary Outcome

1. Histopathologic description of cutaneous vasculitis across several different forms of systemic vasculitis
2. Transcriptomics of cutaneous vasculitis

2b. Secondary Outcomes

1. Pathway analysis and enrichment study comparisons between idiopathic vasculitis and pediatric monogenic diseases
2. Within-subject transcriptomic comparisons between skin and whole blood

3. Background and Rationale

3a. Background

Vasculitis is defined as inflammation directed at blood vessels. The systemic idiopathic vasculitides are a group of rare diseases involving inflammation of arteries and other tissue with resulting organ- and life-threatening disease courses. Cutaneous involvement is a common feature of disease across several different forms of vasculitis. Petechia, purpura, urticaria, infiltrative erythema, hemorrhagic vesicles and bullae, nodules, ulcers, digital gangrene, and livedo racemosa are all potential manifestations of cutaneous

vasculitis. These varied features of disease reflect differences in the size of affected vessels, ranging from petechial lesions seen in a vasculitis affecting a few superficial, small vessels to nodular and ulcerative lesions seen in a vasculitis affected deeper muscular arteries. Skin biopsy, extending to the subcutis of early and symptomatic lesions, is crucial for obtaining a high-yielding diagnostic sample. Based on histology, vasculitis can be classified by the size of vessels affected and the dominant immune cell type mediating the inflammation (e.g. neutrophilic, granulomatous, lymphocytic, or eosinophilic).

Given the wide range of presentations for cutaneous vasculitis, it can be challenging to recognize and correctly classify patients presenting with cutaneous features of vasculitis. Additionally, it can be challenging to find effective glucocorticoid-sparing therapies to treat cases of recurrent vasculitis isolated to the skin. A better understanding of the molecular basis of cutaneous vasculitis could therefore lead to novel ways to classify and treat vasculitis.

3b. Rationale

Whole genome gene expression profiling enables molecular characterization of disease that can be used to identify novel disease-targeted therapeutics and facilitate biomarker discovery. To date, most transcriptomic studies in rheumatologic diseases have focused upon circulating blood as the tissue source. Expression profiling of peripheral blood may not accurately reflect pathology of affected tissue and is often confounded by treatment effects.

Establishing a diagnosis in the different systemic vasculitides usually requires histologic confirmation of vasculitis. Thus, tissue biopsy is often obtained early in the course of systemic vasculitis, potentially before any medications are administered to treat the disease. We have previously characterized gene expression signatures in affected nasal tissue from patients with ANCA vasculitis, demonstrating our ability to study tissue sources other than peripheral blood. Skin is easily accessible and frequently affected in many of the systemic vasculitides. Biopsy material of cutaneous vasculitic lesions is therefore an excellent source of tissue for transcriptomic analysis in vasculitis. Simultaneous characterization of histopathology and gene expression from a single punch biopsy skin sample will facilitate direct comparisons across these complimentary assessment modalities.

At the National Institute of Arthritis and Musculoskeletal and Skin Diseases (the NIAMS), there is strong clinical research interest in high throughput characterization of affected tissue in rheumatologic diseases coupled with bioinformatics core laboratory support to process tissue and perform data analysis. The Translational Autoinflammatory Disease Section led by Dr. Raphaela Goldbach-Mansky has collected RNA from cutaneous lesions across a spectrum of pediatric monogenic autoinflammatory diseases, and cutaneous vasculitis can be seen in several of these diseases. Interestingly, the clinical phenotype of certain pediatric monogenic diseases parallels phenotypes seen in specific idiopathic systemic vasculitides. Information learned from profiling tissue in pediatric monogenic diseases with vasculitic features may inform our understanding of certain idiopathic systemic vasculitides. Comparing gene expression profiles derived from cutaneous vasculitic lesions between pediatric monogenic diseases and idiopathic vasculitides is a secondary objective of this project.

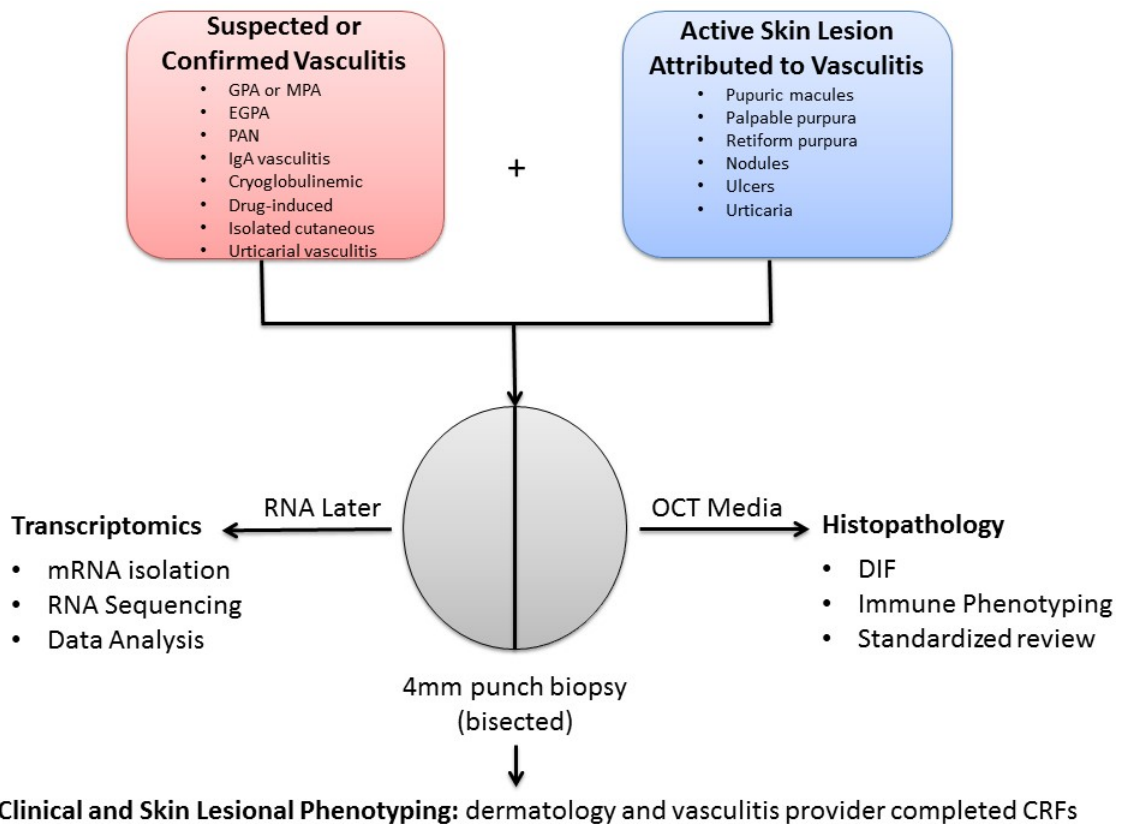
4. Study Design and Methods

4a. Overview:

This is an observational, multi-center study. Patients with known or suspected vasculitis will undergo skin biopsy and samples will constitute the tissue source for this study. Skin biopsies can be performed for either clinical or research indications. Biopsy samples will be bisected. Half of the sample will be used for histopathological analysis, and the other half will be used for transcriptomic analysis. A purposeful sampling strategy will be employed to insure that subjects representing eight different forms of vasculitis (n=5 for each type) are recruited. Publically available transcriptomic datasets from healthy subject skin biopsies will be used as an in silico control. Biopsy samples of affected skin from patients with other diseases, such as systemic lupus erythematosus, that are being collected at the National Institutes of Health on separate protocols may be used as a disease comparator group. Clinical phenotyping of all subjects will be done using standardized data collection forms. Histology will be systematically assessed and quantified using a data collection form specifically developed for this project.

4b. Study Diagram

FIGURE: Flow Diagram for Sample Collection and Processing for CUTIS Study



4c. Identification of Patients: Patients will be recruited from participating Vasculitis Clinical Research Consortium (VCRC) centers and from the Vasculitis Translational Research Program (VTRP) at the National Institutes of Health.

4ci. Inclusion Criteria

1. Cutaneous lesion (purpuric macules, palpable purpura, retiform purpura, nodules, ulcers, or urticaria) believed to be related to active vasculitis
2. A suspected or confirmed diagnosis of:
 - Cryoglobulinemic vasculitis (CV),
 - Drug-induced vasculitis
 - Eosinophilic granulomatosis with polyangiitis (EGPA)
 - IgA vasculitis
 - Isolated cutaneous vasculitis
 - Granulomatosis with polyangiitis (GPA) or Microscopic polyangiitis (MPA)
 - Polyarteritis nodosa (PAN)
 - Urticarial vasculitis
3. Willing and able to provide written informed consent

4cii. Exclusion Criteria

1. Age < 18 years
2. Patients who, in the physician's judgment, are poor candidates for biopsy or who are at elevated risk of complications due to infection, bleeding, etc., or in whom risk is felt to outweigh the potential benefit of knowledge gained.
3. Patient with absolute neutrophil count <1500, platelet count <50, or hemoglobin <7.0
4. Other uncontrolled disease (co-morbidity) that could prevent a patient from fulfilling the study requirements or that would substantially increase the risk of study problems
5. Active infection at or near the potential biopsy site, poor circulation, or site such as bony prominence or other structure that is felt to increase the risk of complications
6. Pregnant or nursing women
7. Persons unable to provide informed consent

4d. Study Procedures*Lesion selection:*

It is important to ensure sample quality and reduce variability in lesion selection and acquisition. Medical dermatologists and practitioners with experience diagnosing and treating vasculitis are best equipped to select appropriate samples. These will include typical cutaneous manifestations of vasculitis, including, in the appropriate clinical context: 1) purpuric macules, 2) palpable purpura, 3) retiform purpura, 4) nodules, 5) ulcers, or 6) urticaria.

Lesions must be well-established to increase the likelihood that true vasculitis is present but not so old that characteristic histology is replaced by non-specific inflammation and necrosis. Target lesions will preferably be 24-36 hours old or else not older than one week. Physicians will be asked to approximate and record the age of the sampled lesion based on patient history and exam.

Each sample will be biopsied via 4mm punch biopsy using appropriate technique, including sampling of the subcutis if the target lesion is a subcutaneous nodule. The biopsy will be split in half longitudinally, with half placed in frozen media and sent for standard histologic

processing and review, and half placed in RNAlater tissue tubes and sent for gene expression analysis. By reviewing the histology of the sample submitted for gene expression analysis (rather than submitting two separate samples for histology and gene expression analysis), we will ensure that we are performing such analyses on true vasculitis.

Lesion characterization:

The participating Dermatologist will describe / characterize the biopsied lesion according to lesion age (<24 hours, 24-36 hours, 2-7 days, and older than 7 days), morphology (purpuric macule, palpable purpura, retiform purpura, nodule, ulcer, or eschar), and site.

Number of skin biopsies:

Skin biopsies can be obtained for clinical indications or for research purposes. For those subjects, who undergo skin biopsy for clinical indications (i.e. diagnostic procedure), the participating Dermatologist may choose to perform either 1 or 2 skin biopsies if there are multiple lesions amenable to biopsy. If 1 biopsy is performed, the sample will be bisected. Half of the sample will be used for histologic review to be performed initially at the local pathology department. Unused tissue and processed slides will be sent to the NIH for further histologic review. The other half will be placed in RNA preserving solution for transcriptomic analysis. If 2 biopsies are performed, one sample will go the local pathology department for clinical, histologic review. The other sample will be bisected and used for research (half will be placed in frozen media and half will be placed in RNA preserving solution for transcriptomic analysis). If 2 biopsies are performed, lesion characterization will pertain to the biopsy sample used for research purposes rather than the biopsy sample used for clinical purposes.

Clinical assessment of skin disease:

The participating Dermatologist will also describe / characterize the patient's other cutaneous lesions with respect to morphology (purpuric macule, palpable purpura, retiform purpura, nodule, ulcer, eschar, gangrene, livedo reticularis, livedo racemosa, urticarial lesions, oral and nasal ulcers, splinter hemorrhages) and distribution (trunk, arms, legs, head/neck, preference for dependent areas, distal areas), checking all that apply.

Clinical assessment of systemic disease:

The participating primary vasculitis provider will describe / characterize the extent of the patient's systemic disease, noting whether signs or symptoms of constitutional, joint, kidney, lung, ear/nose/throat, eye, peripheral nerve, or central nervous system disease are present versus skin-only disease. The participating vasculitis care provider will note the specific suspected or confirmed diagnosis (cryoglobulinemia vasculitis, drug-induced vasculitis, eosinophilic granulomatosis with polyangiitis, IgA vasculitis, isolated cutaneous vasculitis granulomatosis with polyangiitis or microscopic polyangiitis, polyarteritis nodosa, or urticarial vasculitis).

A case report form will be used to record clinical phenotypic data. The participating vasculitis provider will record any therapies, including dose and duration, the patient is receiving for treatment of his or her vasculitis.

Skin biopsy procedure:

Study participants are asked to consent to one skin biopsy for research. Skin biopsies can be obtained for clinical indications or for research purposes. For those subjects, who undergo skin biopsy for clinical indications (i.e. diagnostic procedure), the participating Dermatologist may choose to perform either 1 or 2 skin biopsies if there are multiple lesions amenable to

biopsy. Skin biopsies involve the cleaning of the skin with alcohol, numbing of the skin with lidocaine, sampling of a 4mm-diameter core of skin, and placement of a single suture to close the skin defect. Skin biopsies are considered a low-risk and relatively noninvasive medical procedure that takes approximately 3-5 minutes to perform.

The principal risks associated with skin biopsy are as follows:

- A. Pain associated with infiltration of the skin with a 31-gauge or other very small needle for infusion of lidocaine for local anesthesia. The pain associated with skin biopsy is limited to the initial infiltration of the needle and is expected to be less than that associated with venipuncture or vaccination, which use much larger needles.
- B. A permanent linear scar roughly 4mm in length will result from skin biopsy. The standard skin biopsy size is 4mm in diameter, and we believe this is the smallest biopsy size suitable to obtain useful information for this study.
- C. A small amount of blood is typically lost during skin biopsy. This amount is generally too small to quantify.
- D. There is a remote risk of infection any time the skin is broken. With proper technique and care, the risk of infection of the biopsy site is exceedingly low, even among patients taking immunosuppressive medications or otherwise immunocompromised. In the rare event infection occurs, it would most likely be a superficial bacterial wound infection which would be readily treatable.

Other samples to be collected:

At the baseline study visit, the following additional samples will be collected from peripheral venipuncture: DNA (1x3mL), whole blood RNA (1x3mL), serum (2x4mL), and plasma (2x4.5mL). Plasma samples will be collected in sodium citrate tubes. Specimens will be shipped to the VCRC Biospecimen Repository at the University of Pennsylvania, and will be processed by the VCRC Laboratory at the University of Pennsylvania or the NIAMS Bioinformatics Core. The laboratory-based research studies will evaluate whether abnormalities detected in a skin biopsy can also be detected in blood.

Other potential risks of participation:

- A. Monetary: If being performed for diagnostic purposes, biopsies will occur as part of routine care. Half the sample will be sent for clinical pathology at the local site, and half will be sent for gene expression analysis by the study team at the NIH. Any fees associated with a procedure or interpretation of a biopsy performed for diagnostic purposes will be paid for through traditional means (insurance, Medicare, etc.) because diagnostic biopsy is necessary and standard practice for vasculitis. Any biopsy performed purely for research purposes will not be billed to the patient. Any processing or analysis of the sample purely for research purposes will not be billed to the patient.
- B. Time: Patients are not generally expected to travel to study visits solely for the purpose of skin biopsy outside the context of their clinical care.
- C. Confidentiality: The study team will take measures to protect the identity of participants. All data gathered will be de-identified and password-protected. Patient tissue samples will be used only as described in this protocol and will not be individually identifiable.

4di. Visit Frequency/ Visit Schedule: This is an observational cross-sectional study. Confirmation of diagnosis at 6 months from the time of biopsy, when necessary, will be coordinated by the enrolling physician. Diagnosis confirmation can take place during a routine clinic visit approximately six months later, or through a follow-up call by the physician.

4dii. Recruitment: Patients will be recruited from the VCRC centers and the NIH. The University of Pennsylvania will be the study-coordinating center. A purposeful sampling strategy will be employed. Participating centers will receive regular updates about recruitment to insure that five subjects from each of eight different types of vasculitis are recruited.

4e. Data Elements for Collection

Form	Baseline	6 Months
Eligibility Form	X	
Demographics Form	X	
Skin Biopsy Case Report Form	X	
Medical History Form	X	
BVAS	X	
Physician Global Assessment	X	
Vasculitis Medication Form	X	
Research Specimen Collection Form	X	
Histopathology Case Report Form (<i>Will be completed by study appointed scorer</i>)	X	
6 Month Confirmation of Diagnosis		X

5. Safety Monitoring and Adverse Event Reporting

5a. Nature of Study

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRBs/REBs for approval. Participant enrollment may only begin with IRB/REB approved consent forms.

The Study Chair has primary oversight responsibility of this clinical trial. This study meets the federal definition of minimal risk.

5b. Definitions

An adverse event is defined as: "...an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a study."

A serious adverse event includes those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience...the specificity or severity of which is not consistent with the risks of information described in the protocol. Therefore, expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study.

Only SAEs are reportable.

Only those events associated with the conduct of the study and as defined above are reportable.

All reported adverse events will be classified using version 4 of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

5c. Standard Elements

A set of standard elements for adverse event data will be collected in this study. These elements include: participant ID, reporter name & location, dates for event/event reported/date resolved, the event itself, event severity, whether it was expected and/or serious (as defined above), patient status, place of AE treatment (to further determine serious events), causality, and subsequent changes to protocol or consent form. Additionally, there is ample room for the reporter to write a description of the event and any other pertinent information.

5d. Reporting Timelines

- Within **24 hours** (of learning of the event), investigators must report any Serious Adverse Event (SAE) that:
 - Is considered life-threatening/disabling or results in death of subject
 - OR-
 - Is Unexpected/Unanticipated
- Investigators must report all other SAEs within **5 working days** (of learning of the event). Infections requiring treatment with intravenous (IV) antibiotics or infections commonly understood as opportunistic will fall within this **5-day** reporting period.
- Expected or unexpected AEs that are grade 1 will not be collected or reported.
- All other (suspected) AEs must be reported to the RDCRN within **20 working days** of the notification of the event or of the site becoming aware of the event.

5e. RDCRN Adverse Event Data Management System (AEDAMS)

Upon entry of a serious adverse event, the DMCC will triage the event to the Medical Review Officer, and any additional agencies of any reported adverse events via email.

Serious adverse events: The Medical Review Officer determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The Medical Review Officer may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the Medical Review Officer beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DMCC in a timely

fashion (within 20 working days). The events will be presented in tabular form and given to the Protocol Oversight-Management Team on a monthly basis.

The DMCC will prepare aggregate reports of all adverse events (serious/not serious and expected, unexpected) for IRBs/REBs and the Protocol Oversight-Management Team.

5f. Toxicity Grading of Adverse Events

The values to describe adverse events will come from the Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, developed and maintained by CTEP at National Cancer Institute. The CTCAE v.4.0 was chosen because of its widespread use as a standard for adverse event reporting in clinical trials (in oncology), its specific criteria for grading severity, its ongoing maintenance from the National Cancer Institute (NCI) and its harmonization with MedDRA (Medical Dictionary for Regulatory Activities Terminology) at the AE term level. Investigators from each consortium of the RDCRN reviewed the CTCAE from Dec. 2004 – Jan. 2005 and thought that it would meet their adverse event reporting requirements.

The CTCAE is organized broadly by System Organ Classes (SOCs) (26), shown below:

Blood and lymphatic system disorders	Metabolism and nutrition disorders
Cardiac disorders	Musculoskeletal and connective tissue disorders
Congenital, familial and genetic disorders	Neoplasms benign, malignant and unspecified (incl cysts and polyps)
Ear and labyrinth disorders	Nervous system disorders
Endocrine disorders	Pregnancy, puerperium and perinatal conditions
Eye disorders	Psychiatric disorders
Gastrointestinal disorders	Renal and urinary disorders
General disorders and administration site conditions	Reproductive system and breast disorders
Hepatobiliary disorders	Respiratory, thoracic and mediastinal disorders
Immune system disorders	Skin and subcutaneous tissue disorders
Infections and infestations	Social circumstances
Injury, poisoning and procedural complications	Surgical and medical procedures
Investigations	Vascular disorders

Each System Organ Class is identified by anatomical or physiological system, etiology, or purpose. Within each SOC, AEs are listed (alphabetically) and accompanied by descriptions of severity (grade). An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each AE must be associated with a grade. Grade refers to the severity of the AE. The CTCAE v4.0 displays Grades 1 through 5 with unique clinical descriptions of severity of each AE based on this general guideline:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling;

- limiting self care ADL***.
 - Grade 4 Life-threatening consequences; urgent intervention indicated.
 - Grade 5 Death related to AE.
- Activities of Daily Living (ADL)
 *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
 **Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Not all grades are appropriate for all AEs. Therefore, some AE’s are listed with fewer than 5 options for Grade selection. (e.g., the adverse event “chest pain - cardiac”, listed in the Cardiac disorder SOC, only has options for Grades 1-2.) Grade 5 (Death) is not appropriate for some AEs and therefore is not an option.

5g. Relation to Study Therapy

The relation or attribution of an adverse event to an investigational product is determined by the site investigator and then recorded on the appropriate case report form and/or SAE reporting form. The CTCAE provides the following descriptors and definitions for assigning an attribution to each adverse event.

Code Descriptor Definition

“Unrelated” Category Code

1 Unrelated The adverse event is clearly not related to the investigational product

“Related” Category Codes

- 2 Unlikely The adverse event is doubtfully related to the investigational product
- 3 Possible The adverse event may be related to the investigational product
- 4 Probable The adverse event is likely related to the investigational product
- 5 Definite The adverse event is clearly related to the investigational product

6. Data Analysis and Statistical Considerations

6a. Sample Size: Forty to fifty-six patients with cutaneous vasculitis will be recruited representing 8 types of vasculitis (n=5-7 for each type). For the histopathology aim, 5 patients should be adequate to characterize the representative histology of each disease. For the transcriptomic aim, our sample size is largely driven by feasibility and cost. It has been suggested that a minimum of 3 subjects are needed per group in whole-genome gene expression studies. Computational methods can be employed to evaluate small sample size data. For example, although smaller sample sizes often preclude the ability to detect statistically significant small changes in differential gene expression, analytic techniques such as pathway analysis, enrichment analysis, and principal component analysis can be used to infer biologic differences based on more global patterns of differences in gene expression. Exciting findings could be validated in future studies conducted within the VCRC network.

6b. Analysis Plan:

Histopathology Aim: All samples will be read by a team of investigators at the NIH led by a Dermatopathologist at the NIH. Pathology will be assessed in each layer of skin.

Immune cell subsets will be quantified in each layer of skin and relative to vasculature. A subset of slides will be read twice by the same investigator to determine intra-rater reliability. Comparisons across disease subsets will be largely descriptive. Nonparametric tests will be used to compare distributions of findings across diseases. There will be no adjustment for multiple comparisons.

Transcriptomic Aim: RNA isolated from skin biopsy samples will be used for RNA sequencing (RNASeq) experiments. After RNA is isolated, processed, sequenced, and aligned to the human genome, sequencing reads will be converted to a quantitative value representing transcript abundance using the Genominator software package, available through Bioconductor. The number of reads obtained for each gene will be normalized for the length of the transcript and for the depth of sequencing obtained per sample, yielding reads per kb per million reads (RPKM). Quantification of each element will then be derived from RPKM.

Differential expression analyses will be conducted to compare gene expression across disease groups using one-way ANOVA. Analyses will be performed using publically available software (R) and commercial software platforms (Partek). Different thresholds to define significance will be explored, but given the small sample size of each group, adjustment for false discovery will likely not be possible. Validation of gene set biomarkers will be performed using leave-one-out-cross validation. Pathway analysis will be conducted to delineate relevant biology using tools such as Gene Set Enrichment analysis (GSEA) and the Ingenuity Pathway Analysis. Enrichment of blood and tissue signatures from published datasets in vasculitis and will be assessed using GSEA. Clustering algorithms (k-means, agglomerative hierarchical clustering, consensus clustering) will be explored to identify *de novo* subgroups based on global patterns of gene expression and subsequent putative associations with clinical characteristics including age of lesion, site of lesion, type of lesion, disease status, and treatment status.

6c. Secondary Outcomes: The same methods will be used to study skin samples from approximately 30 subjects with different types of pediatric monogenic diseases. Histopathologic descriptions of skin will be compared between idiopathic vasculitis and the monogenic diseases. GSEA will be used to test for enrichment of gene set signatures between pediatric diseases and the idiopathic vasculitides. Whole blood RNA and DNA samples will be taken from each subject at time of biopsy. These samples may form the basis for additional studies. Gene expression in blood could be compared to tissue expression within the same subject to determine whether skin expression signatures are reflecting in the circulation. Candidate genes identified via differential expression studies could be sequenced and evaluated in a genotype/expression phenotype framework. Algorithms to mine RNA sequencing data for microbial transcripts will be explored.

7. Data Management

All study data will be collected via systems created in collaboration with the Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

7a. Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and

obtain appropriate informed consent. IRB/REB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes; the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

7b. Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

7c. Data Quality Control

As much as possible data quality is assessed at the data entry point. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. Data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Among the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, these checks will be built into the initial tables and cross tabulations that should reveal any remaining data quality issues.

8. Protection of Human Subjects

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

The proposed research project is a study of up to 56 participants with one of eight specific types of skin vasculitis. No therapeutic intervention of any type is included in the research plan.

The biological specimens to be obtained from the study participants for study purposes consist of skin and blood samples. Other relevant information obtained as part of clinical practice including physical examination findings and other pertinent medical information obtained as through the patient medical records will be reviewed for all participants.

The risks of participating in this study are expected to be minimal and are mainly due to the minimal pain and discomfort from the lidocaine injection, skin biopsy, and venipuncture participants will undergo. Future studies include genetic testing. All participants may experience the potential inconvenience of the study appointment. Lost school or work time for both patients and their family members is unavoidable in performing this study. However, every effort will be made to perform testing in an efficient manner for the study visit, possibly in conjunction with regularly scheduled clinical appointments. The medical interview is not anticipated to be psychologically harmful or stressful.

Strict patient confidentiality will be observed throughout the study. While medical records will be reviewed by members of the research team, no individually identifiable patient data will be distributed to non-research or non-care-giving team members. Your treating physician will have access to the clinical information.

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

In the very unlikely event of adverse effects from the study, the full resources of the hospital will be available to intervene as medically necessary. Licensed physicians expert in the care of patients with vasculitis are available at all times at each study site. Serious adverse events which possible, probably, or definitely occur directly as a result of study activities (venipuncture and skin biopsy) will be reported to the IRB/REB per local site IRB/REB reporting guidelines.

There are no benefits to study participants for their involvement in this research. Participants in this study are unlikely to gain direct benefit from participation. If the study leads to higher quality care or therapeutic trials for vasculitis then study patients could theoretically benefit in the future. All participants will potentially have the satisfaction of helping to contribute to medical knowledge of vasculitis. Further understanding of both the clinical and molecular aspects of skin vasculitis is of great medical importance and because progress in understand these disease is expected from this study it is felt that the potential benefits of this research outweigh the risks of participation.

Knowledge to be gained from the study could be potentially highly important. Discovery of effective biomarkers of vasculitis could lead to better care.

