JDRF REQUESTS PROPOSALS FOR:
BIOMARKER ANALYSIS CENTER(S) FOR MASS CYTOMETRY AND TRANSCRIPTOME ANALYSIS OF TRIALNET SAMPLES

This Request for Proposals (RFP) contains the following information:

PURPOSE OF THIS RFP

JDRF, the world's leading non-profit organization with the mission to cure type 1 diabetes (T1D), is partnering with TrialNet, an international consortium of clinical research centers with the goal to prevent or delay T1D. Both organizations support researchers worldwide who are working to better understand the natural history of T1D, develop methods to better identify persons at risk for the disease, and evaluate new therapies to potentially delay or reverse the progression of disease. Further information about JDRF and TrialNet is available online at www.jdrf.org and www.DiabetesTrialNet.org.

TrialNet has infrastructure that is world-leading for biomarker discovery, including biosamples collected to common protocols and linked to comprehensive clinical records and pathological outcomes.

JDRF requests proposals from qualified laboratories to serve as a Biomarker Analysis Centers. One Center will focus on mass cytometry analysis, and one Center on transcriptome analysis. Applicants may submit a proposal to serve as the Center for Mass Cytometry Analysis, the Center for Transcriptome Analysis, or both. The successful applicant(s) will receive access to TrialNet samples and conduct the project in close collaboration with TrialNet's Coordinating Center.

BACKGROUND

Our current understanding of T1D is that it progresses in stages: Stage 1 is characterized by the presence of 2 or more autoantibodies (AAb), Stage 2 by the presence of AAb plus dysglycemia, and Stage 3 by clinical diagnosis of T1D [1, 2]. The discovery of mechanistic biomarkers of T1D progression from stages 1 to 3 would be transformative in several key areas, most notably in: providing a better understanding of T1D natural history and disease heterogeneity; identifying disease pathways and treatment algorithms; and designing strategies to delay, prevent and ultimately reverse disease. Discovery and clinical validation of new prognostic biomarkers of disease progression therefore represents a significant unmet research need in T1D.

STATEMENT OF WORK

STUDY DESCRIPTION

The current opportunity offered by this RFP is to test the hypothesis that there are measurable cytometric/transcriptomic or combined signatures in peripheral blood mononuclear cells (PBMC) that can differentiate risk and prognosis.

To this end, TrialNet has designed a nested case-control study using available samples from the Pathway To Prevention natural history study. For the first cohort of interest, cases are defined as multiple autoantibody-positive (AAb+) subjects who have progressed to T1D (Stage 3). Eligible cases are further defined as having 2 or more samples of sufficient quantity (at least 30 million cells) any time from 1 month prior up to 3 years prior to Stage 3 T1D diagnosis. Currently n=84 subjects meet the definition of a case (approximately 250 samples). Two controls per case have been selected (where possible) using a modified risk set sampling approach. Controls have samples over the same (approximate) prior timeframe after matching on the case's age at Stage 3 (clinical T1D diagnosis). Two samples per control will be tested (approximately 336 samples). In total, approximately 586 samples will be analyzed. Additional information on the samples will be provided to applicants upon initiating a proposal with JDRF. The same information on the samples will be made available to all applicants.

JDRF is seeking applicants with the facilities and expertise required to serve as Biomarker Analysis Center(s) for Mass Cytometry and/or Transcriptome Analysis of TrialNet samples.

RESEARCH QUESTIONS TO BE ADDRESSED

Mass cytometric and transcriptomic approaches will be used to address the following Research Questions:

- Do biomarkers or signatures exist that can differentiate risk of progression in multiple AAb+ subjects to clinical diagnosis of T1D (prognostic biomarkers for Stage 1 to Stage 3)?
- Are there changes in biomarkers over time in multiple AAb+ subjects (Stage 1) that predict progression to clinical diagnosis of T1D (Stage 3)?

TrialNet further proposes to combine, where possible, phenotype with function by performing parallel studies using cell stimulation conditions, to examine functionally committed cell populations (e.g. by cytokine and chemokine receptor expression). Parallel developments in data analysis allow unbiased detection of distinct cell populations (e.g. those that differ between cases and controls, or that change over time). Multiparameter flow cytometry may also be considered.

DESCRIPTION OF FUNDING OPPORTUNITY - BIOMARKER ANALYSIS CENTERS

Applicants are invited to submit a proposal to serve as one of the Centers described below. Applicants with the facilities and expertise to conduct both types of analyses are encouraged to apply.

The awarded Center(s) will work closely with TrialNet and JDRF leadership in regards to all aspects of the performance of this study, including the handling of samples, data analysis, reporting and publication.

I. Center for Mass Cytometry Analysis

Biomarker Analysis Center for Mass Cytometry Analysis of Innate and Adaptive Immune Cells

The relatively recent, wide availability of mass cytometry offers single-cell resolution using metal conjugated monoclonal antibodies bound to cells that are then atomized, ionized, and finally detected by time-of-flight mass spectrometry. Mass cytometry avoids limitations associated with spectral overlap in conventional flow cytometry and can theoretically detect in excess of 40 parameters per cell, offering increased resolution at the single-cell level. Recent advances at the machine level offer greater speed of acquisition, and therefore higher sample throughput lending itself to the large-scale study proposed here.

JDRF and TrialNet invite laboratories with **mass cytometry** capabilities and expertise to respond to this RFP:

- Able to provide high throughput, comprehensive, multi-parameter analysis of immune cell subsets (immunomics) using a mass cytometry platform (CyTOF) and unbiased bioinformatics analyses

- Experience in handling peripheral blood cell samples in large-scale studies
- Experience in cell biology and immunology, to enable parallel studies to correlate phenotype with function
- Facility to perform multiparameter flow cytometry
- Strong data analysis capabilities
- Experience in type 1 diabetes studies is an asset, but not a requirement

Applicants should describe the tools they have to manage and the capability to integrate and analyze the diverse data types (for example -omics, phenotype, clinical); to mine the data to identify correlates of progression to autoimmunity and disease.

The successful applicant will work with TrialNet and JDRF toward the ultimate goal of this component of the proposed project: to generate a comprehensive understanding of peripheral blood immune cell phenotypes, frequency, and function in the progression of T1D through its stages, and to employ such understanding together with other data to inform translational research.

II. Center for Transcriptome Analysis

Biomarker Analysis Center for Transcriptomic Analysis in Whole Blood and Blood Subsets

The analysis of transcriptome patterns holds promise for unveiling gene pathways involved in diseases, for identifying diagnostic and early disease progression markers, and for identifying therapeutic targets.

RNA-Seq data from the TrialNet subjects will be used for detection of novel transcripts, allele-specific expression (which is not available from gene expression microarrays) and splice junctions with the aim of identifying novel biomarkers that can be linked to the onset of the disease or with the autoimmune response in T1D. Also, small RNA that are not detectable by expression arrays may be measured. The key aims will be to catalogue all species of transcript, including mRNAs, non-coding RNAs and small RNAs; to determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other posttranscriptional modifications; and to quantify the changing expression levels of each transcript during development and under different conditions.

JDRF and TrialNet invite laboratories with **transcriptomic analysis** capabilities and expertise to respond to this RFP:

- Able to provide peripheral blood cell-subset transcriptome analysis using a combination of flow sorting (e.g. derive naïve/memory, CD4/CD8, B lymphocyte subsets) and RNA-Seq (mRNA, ncRNA, miRNA) to identify cell-type specific signatures related to pathophysiological mechanisms
- Requested approaches include those that use libraries prepared from total RNA depleted of ribosomal RNA and barcoded and mixed to avoid batch effects; RNA-seq performed on Hiseq 2000 machines or equivalent to depths of ~200M reads per sample, or better
- Experience in handling peripheral blood cell samples in large-scale studies
- Experience in cell biology and immunology, to enable parallel studies to correlate phenotype with function.
- Facility to perform multiparameter flow cytometry
- Strong data analysis capabilities
- Experience in type 1 diabetes studies is an asset, but not a requirement

Applicants should describe the tools they have to manage and the capability to integrate and analyze the diverse data types (for example -omics, phenotype, clinical); to mine the data to identify correlates of progression to autoimmunity and disease.

The successful applicant will work with TrialNet and JDRF toward the ultimate goal of this component of the proposed project: to generate a comprehensive understanding of how the transcriptome relates to autoimmune status as well as progression to diabetes, and employ such knowledge to translational research activities in T1D.

Applicants with the facilities and expertise to conduct both types of analyses are encouraged to apply under a single proposal.

SAMPLES AND SAMPLE HANDLING

Laboratories should expect to receive blinded samples until laboratory work is completed and the data are delivered to the TrialNet Coordinating Center (TNCC) in the format specified by the TNCC.

Awardees will be expected to work in collaboration with the TNCC and other Biomarker Analysis Center(s), if applicable, in the handling of samples. The TNCC will establish the QC processes to be used. All laboratory procedures must be reviewed and approved by TrialNet prior to the initiation of sample testing. Samples will be analyzed in batch in the order specified by the TNCC. Data from each batch will be transmitted to the TNCC upon completion; further analysis will be contingent upon successful QC review.

Various factors, such as patient heterogeneity, collection and storage variability, and environmental exposures may influence the cellular states of blood and prepared PBMC on a transient or permanent basis. Laboratories should provide analytical strategies that account for these influences.

Additional information on the samples will be made available to all applicants who have indicated their intent to apply.

DATA ANALYSIS, DATA SHARING, REPORTING AND PUBLICATION

There is a need for improved analytical methods to process and interpret the vast amounts of data generated by high throughput platforms in the context of pathological conditions including T1D. Proposals that address this are encouraged to apply.

Applicants will need to agree to share the data in accordance with the JDRF data sharing policies and the NIH and TrialNet Data Sharing Policies.

It is expected that all information be transmitted using TrialNet data systems to the TrialNet Data Coordinating Center (TNCC). All information must be transmitted electronically to the TNCC following set guidelines that will be outlined by the TNCC. Data formats will be specified by the TNCC.

The successful applicant will be expected to summarize and interpret results of the analyses for publications in accordance with TrialNet's publication policies, and with standard policies and procedures of TrialNet and JDRF.

Applicants should state their willingness to participate in joint data analysis and publication through interactions and collaborations with other JDRF and TrialNet scientific committees, as well as other bioinformatics study groups. The samples are part of other TrialNet studies. Data generated will be used to create a platform for integration with clinical study datasets and collaborative analyses with TrialNet investigators and TNCC statisticians.

MECHANISM OF FUNDING

A detailed budget proposal and budget justification must be included in the proposal. Budgets should be determined by the applicant(s) and be relative to scope of work. Budgets should include cost per sample in addition to total cost.

Cost-effectiveness will be one consideration for choosing the awardee. Applicants are encouraged to be thorough in their assessment of costs.

Applicants should expect that costs may be negotiated prior to activation of the award.

Funding will be released based on the completion of specific milestones and deliverables determined by JDRF and TrialNet. This will include the review of sample data after completion of each batch; continued funding to complete sample analysis will be dependent upon successful QC review.

ELIGIBILITY

ELIGIBLE INSTITUTIONS

You may submit a proposal if your institution has any of the following characteristics:

- Non-profit organizations
- Public or private institutions such as universities, colleges, hospitals, and laboratories
- Eligible units affiliated with government agencies
- For-profit organizations (additional information will be requested from for-profit entities if warranted)

ELIGIBLE PRINCIPAL INVESTIGATORS

Any qualified individual with the skill, knowledge, and resources necessary to carry out the terms of the statement of work detailed above is invited to work with his/her institution to develop a proposal for support.

The Principal Investigator of the proposal must hold an M.D., D.M.D., D.V.M., Ph.D., or equivalent academic degree and a faculty position or equivalent at a college, university, medical school, or comparable institution. Investigators at for-profit agencies are also welcome to apply.

All investigators are strongly encouraged to contact JDRF program staff prior to preparing a proposal.

There are no citizenship requirements.

KEY DATES AND DEADLINES

• RFP Release Date	July 26, 2017
• **Application Deadline**	**September 14, 2017**
• Response to Applicants	December 2017
• Earliest Anticipated Start Date	January 2018

PROPOSAL REQUIREMENTS

All applicants are requested to contact JDRF program staff (Dr. Olivia Lou, olou@jdrf.org) to indicate intent to apply.

Qualified laboratories may submit a proposal to serve as the Center for Mass Cytometry Analysis, the Center for Transcriptome Analysis, or both. Applicants proposing to conduct both analyses types are encouraged to apply under a single proposal, and requested to contact JDRF staff to discuss arrangements.

The application must be completed using the templates provided on the RMS360 website (https://jdrf.smartsimple.us). Proposal section templates in MS Word should be type-written, single-spaced and in typeface no smaller than **10-point font** and have no more than **six vertical lines per vertical inch**. Margins, in all directions, must be at least ½ inch. Complete information should be included to permit review of each application without reference to previous applications.

Proposals may not exceed 15 pages total, including figures, tables, and legends, for items 1-6 in the Research Plan as outlined below. Applicants completing a single proposal to serve as two Centers may request a longer page limit if needed, by contacting JDRF staff.

RESEARCH PLAN

The Research Plan template will include the following components:

1. Introduction/background
2. Qualifications and experience of the institution, principal investigator, and key personnel
    a. Facilities and organizational structure and support for the required volume and variety of analyses and tasks
        i. State past experience in handling studies similar in size and scope.

ii. Describe existing personnel, facilities, and equipment to assure measures can be completed on total number of samples and that raw data transfer can occur within 12 months. Include description of the capacity for prompt electronic transmission of results. Specifications for data transfer will be made available from TrialNet.
iii. Describe internal and external quality control procedures and results from previous studies.

3. Approach
    a. Detailed description of analytical techniques. Ability to perform, in the quantity required, accurate and precise measurements and maintain consistent accuracy and precision for the assays listed in the Statement of Work.
    b. Applicants should describe the tools they have to manage and the capability to integrate and analyze the diverse data types; for example -omics, phenotype, clinical to mine the data to identify correlates of progression to autoimmunity and disease.
    c. Provide details of the approach including volumes and types of samples required. Proposals should request only the minimum sample volume for their assay.
    d. Describe quality control procedures to be used. Include participation in external Quality Control programs.
    e. Throughput: time to complete processing of the TrialNet samples received.
4. Relevant preliminary data
    a. Demonstrate the prior performance of the assay, including reproducibility, variability, validity, precision, etc.
    b. Demonstrate the suitability of sample type and required volumes (i.e., show data that frozen thawed PBMC can work in the assay proposed)
5. Sample Handling
    a. Plans for interaction with the TNCC and repository in handling of samples and for quality control.
    b. Include protocol for receipt, short-term storage and preservation of samples sent from TrialNet
    c. Past relevant experience in handling samples from cohorts
    d. Applicants applying for Biomarker Analysis Center for Mass Cytometry need to include the option of cell sorting on thawed samples to transfer to the RNA-Seq laboratory.
6. Describe ability to contribute to and support the scientific objectives of the study
    i. Include description of past relevant experience
    ii. Include a detailed description of protocols for data management and records maintenance
    iii. Include strategy for data analysis and for identifying markers of disease stage
    iv. Describe capacity to integrate and analyze the diverse data types; for example -omics, phenotype, clinical to mine the data to identify correlates of progression to autoimmunity and disease.
    v. State commitment to working collaboratively within TrialNet and JDRF policies for publication as part of this project.
7. Literature Cited (no page limit)
8. Completed Agreements to be included:
    a. Type 1 Diabetes TrialNet Sample Distribution Agreement
    b. NIDDK Central Repositories Sample and Data Use Agreement
9. Intellectual Property
10. Principal investigator assurance: The principal investigator agrees to accept responsibility for the scientific and technical conduct of the research project and agrees to all terms and conditions of the award. (append financial disclosure and conflict of interest assurance statements)

SUMMARY OF PROPOSAL COMPONENTS

- Applicant and Institutional Demographics (including Financial and Administrative Officer)
- Institutional Letter of Support
- Key Personnel
- Lay and Technical Abstracts
- Budget (include cost per sample at well as total cost)
- Budget Justification
- Subcontract Budget (if applicable)

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- Other Support (for the PI only)
- Organization Assurances (IRB and/or IACUC)
- Biosketches (for all Key Personnel)
- Research Plan
- Resources
- Supporting Documents (i.e. Letter(s) of Collaboration, etc.)
- Completed Type 1 Diabetes TrialNet Sample Distribution Agreement
- Completed NIDDK Central Repositories Sample and Data Use Agreement

SUBMISSION TO RMS360

Applicants must register as an applicant and submit their application in response to this RFP using JDRF's on-line application system RMS360 (https://jdrf.smartsimple.us).

REVIEW CRITERIA

Applications that are complete and responsive to the RFP will be evaluated for scientific and technical merit by an appropriate peer review group convened by JDRF in accordance with JDRF's standard confidential award policies. Applications will be shared with TrialNet, who will participate in the review process. The scientific review group will address and consider the following criteria in assigning the application's overall score, weighting them as appropriate for each application:

- Qualifications of the institution and investigators in conducting similar analyses
  - Relevant past experience
  - Facilities, equipment, and resources available
  - Organizational structure
- Technical Evaluation Criteria
  - i. Organization's ability to conduct assay and obtain consistent and precise results
  - ii. Appropriateness of the assay with respect to the study design, specific experience with the approach, and evidence of the offeror's current precision and accuracy for analysis
  - iii. Qualifications and relevant experience of the institutions in the proposed field of research and the overall methodology
  - iv. Quality of the proposed laboratory procedures and protocols in regard to sample handling and assay performance
- Ability to contribute to and support the scientific objectives of the study. Comprehensiveness and suitability of the data analysis plan in addressing the key research questions within 12 months.
- Suitable plans for collaboration, communication and reporting
- Appropriateness of budget and appropriate budget justification

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RMS360 (http://jdrf.smartsimple.us)

If you have any grant-specific questions as you work within RMS360, please contact the administrative contact listed above.

For any non grant-specific inquiries or issues, please contact SmartSimple Support Services via email support@smartsimple.com or phone (866) 239 - 0991. Support hours are Monday through Friday between 5:00am and 9:00pm US Eastern Standard Time.

REFERENCES

[1]Insel R and Dunne JL. JDRF's vision and strategy for prevention of type 1 diabetes. Pediatric Diabetes 2016 Jul; 17 Suppl 22:87-92.

[2]Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, Greenbaum CJ, Herold KC, Krischer JP, Lernmark A, Ratner RE, Rewers MJ, Schatz DA, Skyler JS, Sosenko JM, and Ziegler AG. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. Diabetes Care 2015 Oct;38(10):1964-74.