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14. ABSTRACT

Shwachman-Diamond Syndrome (SDS) is an underdiagnosed and clinically-heterogeneous disorder resulting in bone marrow (BM) failure. SDS is caused by biallelic mutations in the SBDS gene, which normally functions in ribosomal subunit joining and mitotic spindle stabilization. Despite these insights, the molecular pathways leading to BM failure are unknown because the hematopoietic stem and progenitor cells (HSPC) affected by SBDS mutations are rare and heterogeneous. To investigate the mechanisms of SDS pathogenesis, we performed single cell RNA-sequencing on primary CD34+ HSPC from normal and SDS BM. We generated a single cell map of early lineage commitment, and found that SDS hematopoiesis was left-shifted with selective loss of granulocyte-monocyte progenitors (GMPs). Differential gene expression analysis revealed dysregulation of TGFß target genes in SDS hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs), but not in lineage-committed progenitors. Proteomic analysis of primary SDS patient plasma identified increased TGFß-family ligand production. Treatment of SDS patient BM with TGFß inhibitors increased hematopoietic colony formation, supporting a causative role for TGFß-signaling in SDS pathogenesis. These data establish TGFß as a therapeutic target in SDS and translate insights from single cell biology into a novel potential therapy.

15. SUBJECT TERMS
Single cell RNA-seq; bone marrow failure; TGFß

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Introduction

Patients with Shwachman-Diamond Syndrome (SDS) suffer from hematopoietic abnormalities including neutropenia, thrombocytopenia, pan-anemia and myelodysplasia (MDS) with progression to acute myeloid leukemia (AML). The affected cell types and altered genetic networks *in vivo* remain unknown, primarily due to the rarity and heterogeneity of bone marrow progenitors. Our overarching goal was to define transcriptional signatures of bone marrow failure in SDS by performing single cell RNA sequencing (RNA-seq) on freshly isolated patient cells. Indeed, we detected dysregulation of TGFß signaling in rare hematopoietic stem cells and multipotent progenitors, and found that inhibition of this pathway improves SDS hematopoiesis. These studies may lead to clinical use of TGFß inhibitors to treat bone marrow failure in SDS.

Keywords

Single cell RNA-seq; bone marrow failure; hematopoiesis; TGFß

Accomplishments

What were the major goals of the project?

Aim 1. Define the molecular basis for the SDS myelopoiesis defect at single cell resolution.

Sub-Task 1A: Consent 6 SDS patients under protocol 10-02-0057, and 6 normal donors under protocol 09-04-0167; obtain patient bone marrow samples, purify CD34+ cells. (Start date 9/30/2014; duration 24 months; protocol renewed 11/09/2015 and 8/21/2015, respectively, by local IACUC).
Milestone: Obtain 6 patient and 6 donor samples.
Percent completion: 100%; milestone was adjusted because we obtained a sufficient number of cells from 4 normal donors and 4 SDS patients to identify key pathways.

Sub-Task 1B: Load purified cells onto C1 chips; prepare ~96 single cell RNA-seq libraries per sample; run sequencing reactions. (Start date 9/30/2014; duration 24 months)
Milestone: Generate genome-wide transcriptional profiles of CD34+ cells form normal donors and SDS patients at single cell resolution.
Percent completion: 100%

Sub-Task 1C: Process data; perform bioinformatic analyses. (Start date 3/30/2015; duration 30 months)
Milestone: Identify SDS affected progenitor cells; Define SDS gene expression networks in single cells or subpopulations of CD34+ cells; Predict microRNA-targeted mRNAs that explain pathogenesis of SDS.
Percent completion: 95%; We have identified that TGFβ signaling in signaling is selectively activated in SDS stem and multipotent progenitors. We have also identified long non coding RNAS (lncRNAs) that are upregulated and associated with TGFβ signaling that may explain the pathogenesis of SDS. We are continuing to evaluate the role of microRNAs on the targeting of mRNAs.

Aim 2. Functionally annotate SDS transcriptomes to myelopoiesis defects.

Sub-Task 2A: Obtain 2-4 frozen mononuclear cells from SDS patient repository under protocol 10-02-0057 and 2-4 normal donors under protocol 09-04-0167. (Start date 9/30/2015; duration 24 months; protocol renewed 11/09/2015 and 8/21/2015, respectively, by local IACUC).
Milestone: Obtain 2-4 SDS patient and 2-4 donor samples.
Percent completion: 100%

Sub-Task 2B: Culture CD34+ cells; over-express or knockdown key central and peripheral node genes in SDS networks; assess myeloid/neutrophil phenotypes in myelopoiesis culture ex vivo. (Start date 9/30/2015; duration 24 months

Milestone: Identify SDS myeloid phenotypes ex vivo; define genes responsible for myeloid phenotypes; functionally annotate microRNA-dependent and -independent pathways explaining SDS pathogenesis.
Percent completion: 85%; We functionally annotated TGFß signaling pathway genes to the SDS hematopoietic defect. We validated this pathway by treating SDS CD34+ cells using with pharmacological inhibitors of TGFß to rescue hematopoietic defects.

Sub-Task 2C: Quantify microRNA activity in SDS myeloid progenitor cells; perform microarray Expression profiling of microRNAs in SDS myeloid progenitor cells; re-evaluate microRNA target predictions accounting for microRNA expression changes in affected SDS progenitor cells. (Start date 9/30/2016; duration 12 months)
Milestones: Quantify microRNA activity in SDS myeloid progenitors; discern SDS pathways that are affected by reduced microRNA activity and/or altered microRNA expression
Percent completion: 70%; We have developed a dual-reporter system to evaluate microRNA activity and targeted repression and will apply this to normal versus siRNA-mediated SBDS-knockdown CD34+ cells. In addition to evaluating the role of microRNAs, we identified high priority lncRNAs which are implicated in TGFβ signaling and that are up- or down-regulated in SDS progenitors. We are currently validating these lncRNAs as possible targets for a future SDS therapy.

What was accomplished under these goals?

Patients with SDS suffer from complex and unstable hematopoietic defects. Neutropenia is most common in early stages of the disease, with some patients progressing to pan-anemia, thrombocytopenia or MDS/AML. Due to the rarity and heterogeneity of bone marrow progenitors, the affected cell types and altered genetic networks *in vivo* remain unknown. To uncover pathogenic mechanisms in SDS, we analyzed bone marrow progenitors from SDS patients by single cell RNA-seq.

Aim 1. We performed single cell RNA-seq on CD34+ hematopoietic stem and progenitor cells (HSPC) from four normal donors (n=256 cells) and four SDS patients (n=283 cells). CD34+ cells comprise several stages of hematopoietic development, which we reasoned could be distinguished by transcriptional signatures. To derive these signatures, we performed bulk RNA-seq of FACS-purified hematopoietic stem cells (HSC), multipotent progenitors (MPP), common myeloid progenitors (CMP), granulocyte-monocyte progenitors (GMP) and megakaryocyte-erythroid progenitors (MEP)[1]. We then applied tSNE based on these bulk signatures to generate single cell maps of early hematopoietic lineage commitment (**Fig 1**). Single cell mRNA expression patterns and immunophenotypes validated that our lineage map is consistent with known markers of hematopoietic development. Comparative analysis of normal and SDS maps revealed modest accumulation of HSC/MPP and a drastic reduction of GMP in SDS bone marrow, which was rescued by G-CSF therapy (**Fig 2**).

We compared gene expression between normal and SDS cells for each cell type except for GMP, which was excluded due to the low number of GMP in untreated SDS

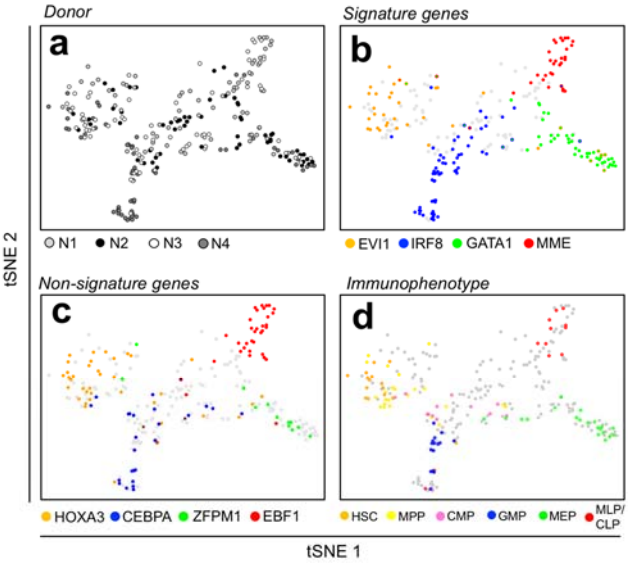

Figure 1. Supervised dimensionality reduction maps lineage commitment of CD34+ cells. tSNE plot of hematopoietic lineage commitment was derived from an empirically-defined gene expression signature. Shown here are cells from four normal donors (n^{N1}=70, n^{N2}=58, n^{N1}=69, n^{N1}=59, n^{total}=256). Cells are colored based on (a) donor identity, (b) mRNA expression of selected signature genes, (c) mRNA expression of lineage-restricted genes reported elsewhere[12], and (d) immunophenotypes. Numerical axes derived from tSNE are arbitrary, and therefore not shown.

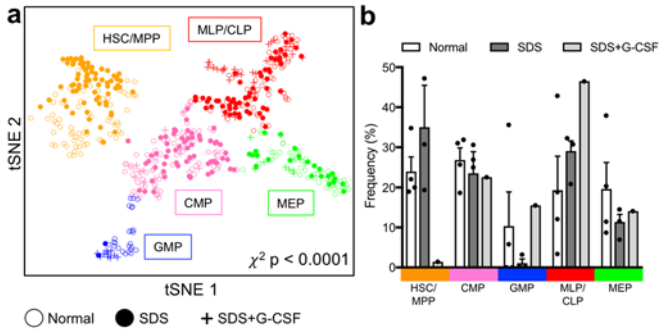

Figure 2. The cellular architecture of early hematopoiesis is altered in SDS. (a) tSNE plot of hematopoietic lineage commitment showing cells from normal donors as in Figure 1, untreated SDS patients ($n^{SDS1.1}$=72, $n^{SDS1.2}$=62, $n^{SDS2.1}$=78, n^{total}=212), and an SDS patient who was being treated with 4.2ug/kg/day G-CSF ($n^{SDS2.2}$=71). Clusters were determined using 'partitioning around medoids' version of *k*-means clustering (*k*=5), and labeled based on the enrichment of index sorted HSC, MPP, MLP, CMP, GMP and MEP as shown in Figure 1d. The sum of normal cells and SDS cells in each cluster is significantly changed using the χ^2 test. (b) Mean relative frequencies of normal, untreated SDS, and G-CSF-treated SDS cells in each cluster. No significant differences were detected from averaged data due to high interindividual variability. Error bars=SEM.

patients. Overall, 1680 genes were differentially expressed in at least one cell type. Among these genes, 94% were also significant in a comparative analysis across all cell types, but the fold changes were uninterpretable due to HSPC heterogeneity and altered cellular architecture in SDS. For example, fold reductions in GMP-enriched genes were exacerbated due to the reduced frequency of this cell type in SDS. Remarkably, 81.5% of all differentially expressed genes were specific to either HSC/MPP or CMP, indicating that these are the primarily affected HSPC in SDS (**Fig 3a**). These data demonstrate that despite the general biochemical functions of the SBDS protein in ribosomal subunit joining and mitotic spindle stabilization[2-6], *SBDS* mutations have cell type-dependent consequences.

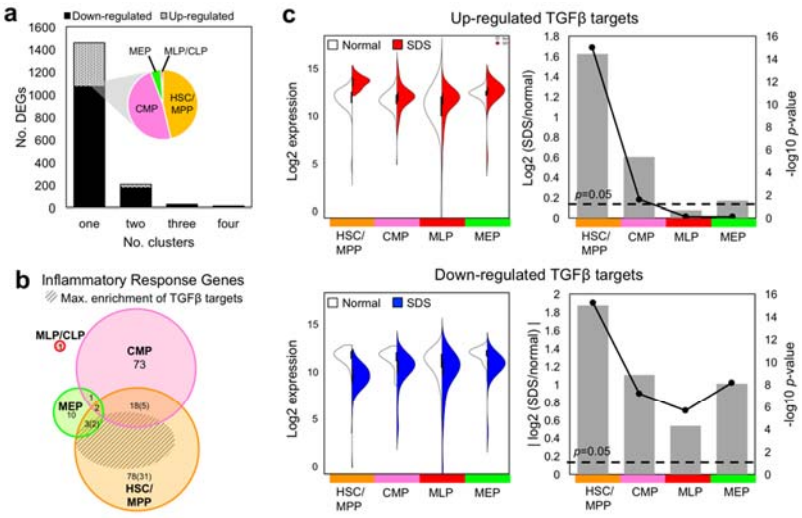

Figure 3. TGFb signaling is selectively dysregulated in SDS stem and multipotent progenitors. (a) Differentially expressed genes were identified among all SDS versus normal cells and within each cluster. To aid biological interpretation, this gene set was filtered to focus on genes with FDR adjusted p-value < .05 and log2(fold change) >|1| in at least one cluster. Plotted are the number of genes that were either up- or down-regulated in one, two, three or four clusters. GMP was excluded due to the paucity of SDS GMP. Inset pie chart shows the proportion of differentially expressed genes in each cluster. (b) Venn diagram of differentially expressed genes in each cluster that were annotated to the "Inflammatory Response" function in Ingenuity Pathway Analysis. The shaded region shows the area of maximal enrichment of TGFb targets (p=4.03x10^{-15}). (c) Left: split violin for the summed expression of 25 upregulated TGFb targets and 52 down-regulated TGFb targets in SDS HSC/MPP. Right: Log2 fold changes (primary axis, bars) and p-values (secondary axis, lines) for the gene sets plotted in 'b'. Significance was determined by Holm-Sidak's multiple comparisons test.

The Inflammatory Response was enriched among differentially expressed genes in HSC/MPP and CMP (maximum p-value 4.98x10^{-5} and 1.18x10^{-3}, respectively). However, the genes contributing to the enrichment differed between the clusters (**Fig 3b**). TGFß was the top regulator predicted for the HSC/MPP inflammatory response (p=4.03x10^{-15}), and dysregulation of this subset of TGFß targets was restricted to HSC/MPP (**Fig 3c**). Together, these data implicate dysregulated TGFß signaling in HSC/MPP as a mechanism of BM failure in SDS patients.

Aim 2. To confirm activation of TGFß signaling in SDS BM, we stained primary CD34+ cells for phospho-SMAD2 (p-SMAD2), a transcriptional modulator that translocates to the nucleus in response to TGFß. A subset of CD34+ cells from SDS BM had elevated levels of nuclear p-SMAD2 that were outside the normal range (**Fig 4a, b**). Treating SDS cells with AVID200, a decoy receptor trap designed to specifically neutralize TGFß1 and TGFß3, reduced the p-SMAD2 signal. The same trend was observed to varying degrees in two additional sample pairs (**Fig 4c**). These data are consistent with our single cell RNA-seq analysis demonstrating selective activation of the TGFß pathway in the HSC/MPP subset of SDS CD34+ cells.

TGFß induces context-dependent effects on cell growth, survival, inflammation, and extracellular matrix, among others. TGFß1 and TGFß3 have potent growth inhibitory effects on HSC[7,8], although this activity may vary among functional subsets[9]. To determine whether hyperactivation of TGFß signaling contributed to the functional impairment of HSC/MPP in SDS, we treated primary bone marrow mononuclear cells from normal donors and SDS patients with TGFß inhibitors. SD208 is an intracellular inhibitor that blocks signal transmission from TGFßR1[10]. AVID200 is a recently-developed extracellular inhibitor that functions as a TGFß1/3 receptor trap; it is a promising therapeutic agent since it avoids cardiac toxicities associated with TGFß2 inhibition. Both SD208 and AVID200 improved hematopoietic colony formation in SDS patient samples, but not in normal donor controls (**Figure 4d**). Moreover, significant effects were observed for myeloid and

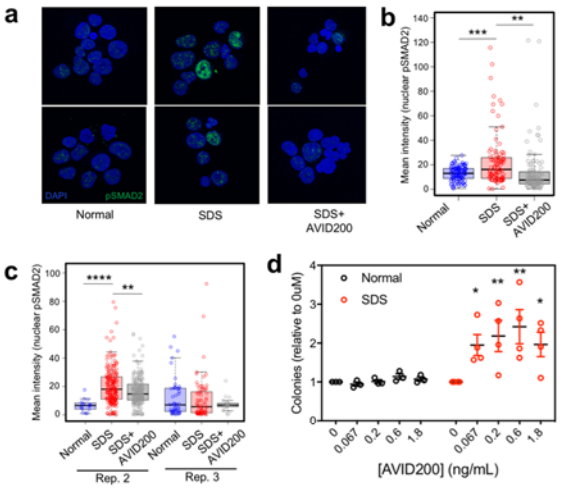

Figure 4. TGFb pathway activation through TGFbR1 suppresses hematopoiesis in SDS BM progenitors. a) Representative images showing DAPI and phospho-SMAD2 staining of primary BM CD34+ cells from normal donor BM and SDS BM, either untreated or treated with AVID200. b) Mean intensity of phospho-SMAD2 staining in individual CD34+ nuclei from samples depicted in panel (a). Significance was determined by two-way ANOVA, with Holm-Sidak's multiple comparisons test. Error bars= minimum and maximum values, excluding outliers that exceed median+1.5*IQR. **p<0.01, ***p<0.001. c) Mean intensity of phospho-SMAD2 staining in individual CD34+ nuclei in two additional pairs of SDS and normal donor BM samples. Error bars= minimum and maximum values, excluding outliers that exceed median+1.5*IQR. **p<0.01, ****p<0.0001. d) Number of colonies formed by normal donor and SDS patient BM-derived mononuclear cells with increasing concentrations of AVID200, normalized to the 0uM treatment. Significance was determined relative to the 0uM treatment by two-way ANOVA, with Holm-Sidak's multiple comparisons test. Error bars=SEM. *p<0.05, **p<0.01.

erythroid colonies, suggesting that TGFß inhibition targets multipotent progenitors. These data support our computational prediction that hyperactivation of TGFß in HSC/MPP promotes bone marrow failure in SDS.

To evaluate microRNA function, we engineered a microRNA activity reporter system. In this system a blue fluorescent protein, tetracycline repressor, and an artificial microRNA precursor are constitutively expressed. Tetracycline blocks the repressor and thereby induces the expression of a red fluorescent protein that contains tandem binding sites for the artificial microRNA. The expression of the red fluorescent protein (relative to the blue fluorescent protein) measures microRNA activity independent of microRNA levels. We plan to assess microRNA activity in normal versus siRNA-mediated SBDS-knockdown CD34+ cells at each step of myelopoiesis.

We also identified 7 lncRNAs (*CRNDE*, *HOXA10-AS*, *CASC15*, *MALAT1*, *HOTAIRM1*, *UCA1*, and *SNHG4)* that were differentially expressed in SDS compared normal bone marrow progenitors. These lncRNAs have been previously associated with TGFß signaling. We demonstrated that overexpressing *CRNDE* and *MALAT1* alters the proliferation rate of K562 an acute myelogenous leukemia cell line. We have begun to identify proteins that interact with these lncRNAs, which may help explain the pathogenesis observed in SDS.

We plan to use these data for future efforts elucidating the role of non-coding RNAs (lncRNAs and microRNAs) in SDS.

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What opportunities for training and professional development has the project provided?

A postdoctoral fellow, Dr. Cailin Joyce, has attended three meetings related to this project: Single Cell Genomics (Utrecht, Netherlands, September 2015), the Harvard School of Public Health Program in Quantitative Genetics Annual Symposium (Boston, MA, November 2015), and the American Society of Hematology Annual Meeting (San Diego, CA, December 2016). For the latter, she was selected for an Abstract Achievement Award and oral presentation, and attended several trainee career development events. Dr. Joyce's contributions to this project resulted in her promotion to Instructor in 2017. This past summer, Dr. Joyce's successfully transitioned to a position at Agenus biotechnology.

How were the results disseminated to communities of interest?

Dr. Novina and Dr. Joyce have presented posters and given talks at national and international meetings. We have also given talks locally for the Department of Cancer Immunology and Virology (DFCI), the Division of Hematology/Oncology (Boston Children's Hospital), the Department of Microbiology and Immunobiology (HMS), and the Division of Immunology (HMS).

Impact

What was the impact on the development of the principal discipline(s) of the project?
This research supported by this grant enabled advanced single cell technologies to perform the first direct analysis of primary human SDS hematopoietic progenitors. We determined that transcriptional targets of TGFß were dysregulated in SDS hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs), but not in lineage-committed progenitors. Whereas most single cell transcriptomic studies have focused on dissecting and characterizing cell types, this study demonstrated the power of single cell transcriptomics to uncover a novel disease mechanism in rare cells. Additionally, inhibitors (AVID200 and SD208) increased hematopoietic colony formation of SDS patient cells, which provided the preliminary data necessary for an Investigational New Drug (IND) application for a clinical trial which will be initiated by our close collaborator Dr. Akiko Shimamura.

What was the impact on other disciplines?
Our data adds to an emerging body of evidence linking inflammation to BM dysfunction, including Fanconi Anemia (FA) where the pathogenic mechanism of TGFß is thought to be suppression of homologous recombination repair. We demonstrate a broader role for TGFß in a mechanistically distinct BM failure syndrome.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

Changes/Problems

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

Products

Publications, conference papers, and presentations

Joyce CE, Saadatpour A, Ruiz-Gutierrez M, Vargel Bolukbasi O, Jiang L, Thomas DA, Young S, Hofmann I, Sieff CA, Myers CK, Whangbo J, Libermann TA, Nusbaum C, Yuan GC, Shimamura A, **Novina CD**. Activated TGFß signaling in early hematopoietic progenitors promotes bone marrow failure in Shwachman-Diamond Syndrome. Twelfth International Workshop on molecular aspects of myeloid stem cell development and leukemia. Oral presentation, Cincinnati Children's Hospital, Cincinnati, Ohio, 2018.

Joyce CE, Saadatpour A, Jiang L, Ruiz-Gutierrez M, Vargel Bolukbasi O, Hofmann I, Sieff CA, Nusbaum C, Shimamura A, Yuan GC, **Novina CD**. Single Cell Transcriptional Profiling Reveals Activation of TNF-alpha Signaling in Hematopoietic Stem and Progenitor Cells from Shwachman-Diamond Syndrome Patients, American Society of Hematology. Oral presentation, American Society for Hematology Annual Meeting, San Diego, CA, 2016.

Joyce CE, Li S, Hofmann I, Nusbaum C, Sieff C, Mason CE, **Novina CD**. “Single cell transcriptomic analysis of hematopoietic dysfunction in Shwachman-Diamond Syndrome”. Poster, Keystone Hematopoiesis, Keystone, CO, 2015.

Joyce CE, Jiang L, Hofmann I, Nusbaum C, Sieff C, Yuan GC, **Novina CD**. “Lineage-restricted signatures of bone marrow failure in Shwachman-Diamond Syndrome revealed by single cell RNA sequencing of patient cells”. Poster, Program in Quantitative Genetics Symposium, Harvard School of Public Health, 2015.

Journal publications

Joyce CE, Saadatpour A, Ruiz-Gutierrez M, Vargel Bolukbasi O, Jiang L, Thomas DA, Young S, Hofmann I, Sieff CA, Myers CK, Whangbo J, Libermann TA, Nusbaum C, Yuan GC, Shimamura A, **Novina CD**. Activated TGFß signaling in early hematopoietic progenitors promotes bone marrow failure in Shwachman-Diamond Syndrome. [Manuscript under review]

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Nothing to report.

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses
DFCI invention 2356 *TGF-beta inhibition to treat hematologic symptoms of Shwachman-Diamond Syndrome* inventors: Carl Novina, Cailin Joyce, Akiko Shimamura. Reported to NIH/DHHS 06/05/2017. Patents: 62/534,414 filed 07/19/17, PCT/US2018/042913 07/19/2018.

Other Products
Nothing to report.

Participants and Other Collaborating Organizations

What individuals have worked on the project?

Name:	Carl Novina, M.D., Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.0 (average over the entirety of the project)
Contribution to Project:	Dr. Novina was responsible for the conception and design of all the studies. He supervised their execution, analyzed data, prepared publications related to this work, and presented the findings to the scientific community.
Funding Support:	N/A

Name:	Cailin Joyce, Ph.D.
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	7.0 (average over the entirety of the project)
Contribution to Project:	Dr. Joyce has worked with Dr. Novina on the conception and design of all the studies. She obtained clinical samples, performed single cell RNA-sequencing, and worked collaboratively with computational biologists to analyze the data. She has prepared publications related to this work, and presented the findings to the scientific community.
Funding Support:	N/A

Name:	Dustin Griesemer
Project Role:	Student
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	4.0 (average over the entirety of the project)
Contribution to Project:	Mr. Griesemer worked on methods to purify and characterize RNA.
Funding Support:	N/A

Name:	Frank Buquicchio
Project Role:	Student

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.0 (average over the entirety of the project)
Contribution to Project:	Mr. Buquicchio worked on methods to purify and characterize RNA.
Funding Support:	N/A

Name:	Dolly Thomas, Ph.D.
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.0 (average over the entirety of the project)
Contribution to Project:	Dr. Thomas performed colony formation assays, and worked on methods to purify and analyze hematopoietic progenitors from bone marrow.
Funding Support:	N/A

Name:	Elaine Yee
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1.0 (average over the entirety of the project)
Contribution to Project:	Ms. Yee worked on methods to purify and characterize RNA.
Funding Support:	N/A

Name:	Chadene Tremaglio, PhD
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	<1.0 (average over the entirety of the project)
Contribution to Project:	Dr. Tremaglio assisted in the analysis of hematopoietic progenitors from bone marrow.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name: Boston Children's Hospital
Partner's contribution to the project (identify one or more): Please highlight the appropriate ones.

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc.,
- available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other

Organization Name: Broad Institute of Harvard and MIT
Partner's contribution to the project (identify one or more): Please highlight the appropriate ones.

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc.,
- available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other

Organization Name: Joslin Diabetes Center
Partner's contribution to the project (identify one or more): Please highlight the appropriate ones.

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc.,
- available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other

Organization Name: Massachusetts General Hospital
Partner's contribution to the project (identify one or more): Please highlight the appropriate ones.

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc.,
- available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other

Special Reporting Requirements

None