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PRINCIPAL INVESTIGATOR: Marshall S. Horwitz

CONTRACTING ORGANIZATION: University of Washington, Seattle, WA

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Marshall S. Horwitz

E-Mail: horwitz@uw.edu

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UNIVERSITY OF WASHINGTON
4333 BROOKLYN AVE NE
SEATTLE WA 98195-0001

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14. ABSTRACT

Loss of all or part of one copy of chromosome 7(7q$^-$)is frequent in MDS and portends a poor prognosis. The recent identification of germline mutations in *SAMD9L* in individuals with ataxia-pancytopenia syndrome has helped elucidate the role of 7q$^-$ in promoting MDS. The mutations are toxic gain-of-function. Hematopoietic stem and progenitor cells undergoing somatic mutation that eliminate the mutant allele through one of three mechanisms confers a selective growth advantage. The first mechanism involves loss of all or part of the chromosome 7q region containing *SAMD9L* and is deleterious. A second mechanism involves *cis* suppressor point mutations and is better tolerated. A third and potentially beneficial mechanism involves auto-correction of the underlying germline mutation through interhomolog recombination. Our project is aimed at modeling this phenomenon in vitro and identifying therapeutic factors that may both promote auto-correction and confer a selective advantage to cells retaining two intact copies of chromosome 7.

15. SUBJECT TERMS

Ataxia-pancytopenia syndrome, SAMD9L, MDS, genetic recombination, induced pluripotent stem cell disease models

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1. Introduction

MDS is frequently accompanied by loss of all or part of chromosome 7q (7q$^-$), typically including *SAMD9L*, toxic gain-of-function mutations which our group identified as the cause of ataxia-pancytopenia syndrome (ATXPC). Remarkably, bone marrow cells in individuals with ATXPC undergo subsequent somatic mutations that can be either deleterious or beneficial. Deleterious somatic mutations involve loss of all or part of the region of chromosome 7 containing the germline mutant-*SAMD9L* allele and confer a proliferative advantage that leads to bone marrow failure and MDS. Beneficial somatic mutations include both *SAMD9L cis* suppressor mutations and—even better—interhomolog recombination that creates uniparental disomy, the net effect of which is to completely correct the underlying germline mutation. Our proposal has three specific aims. The first is to establish in vitro cellular models of ATXPC. The second is to promote autocorrection of *SAMD9L* by suppressing DNA repair pathways that ordinarily lessen its occurrence. The third is to take advantage of the fact that genes adjacent to *SAMD9L* are involved in detoxification of xenobiotics, suggesting that a cocktail of drugs may be identified that can confer a growth advantage to cells retaining two copies of chromosome 7. The overarching goals of our research are to identify factors promoting 7q$^-$ and develop new therapies to both treat it and prevent its occurrence.

2. Keywords

Ataxia-pancytopenia syndrome, SAMD9L, MDS, genetic recombination, induced pluripotent stem cell disease models

3. Accomplishments

- **What were the major goals of the project?**

1. Establish in vitro models of clonal heterogeneity from patients with pathogenic *SAMD9L* mutations.
    a. Establish EBV-transformed lymphocyte lines.
    b. Derive iPSC and differentiate them to CD34+ hematopoietic cells.
2. Promote autocorrection of SAMD9 and SAMD9L germline mutations.
3. Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies.

- **What was accomplished under these goals?**

1. Establish in vitro models of clonal heterogeneity from patients with pathogenic SAMD9L mutations.
    a. Establish EBV-transformed lymphocyte lines.

We have successfully accomplished all items under Aim 1a and achieved the milestone related to IRB approvals. We generated a new UW IRB protocol for this project and obtained DOD IRB approval.

We have continued to ascertain new SAMD9L patients and to establish EBV-transformed lymphoblastoid cell lines (LCL). We now have frozen mononuclear cells (MNC) and LCL from a total of 12 subjects from 6 pedigrees, who are heterozygous for germline *SAMD9L* p.H880Q, p. H880R, p.C1196S, p.V1551L, or compound p.G1027R/p.I876T missense variants. This exceeds our estimated target enrollment of 10.

We sequenced the entire coding region of *SAMD9L* in LCLs from an affected person in each of three families to evaluate retention of the germline pathogenic variant and possible acquisition of a somatic revertant mutation. Each of these three lines did retain the pathogenic variant. In the UW-AP family, evaluation of the subject and her parents revealed that the p.V266I variant was inherited from her unaffected mother and therefore is in trans with the pathogenic p.H880Q variant and is not a revertant mutation.

The subject from a second family was also found to carry the p.V266I variant in addition to the pathogenic p.880R variant, but in this case the subject's affected sibling was homozygous for it and their affected mother did not carry it. The most parsimonious way to explain this seeming Mendelian inconsistency is that both parents of this sibship carried the p.V266I variant and it was in *cis* with the pathogenic variant in the mother. A uniparental disomy (UPD)/copy neutral LOH reversion event eliminated both variants in her hematopoietic

system. Both affected children inherited both the pathogenic variant and V266I in *cis* from the affected mother, and the homozygous sib also inherited an allele with the p.V.266I variant from the unaffected father. We will obtain a sample from the father to confirm this interpretation. The presence of the variant in three progenitors in two families would show this is a benign polymorphism.

In the third family with a p.C1196S pathogenic variant, the second variant, p.C883Y, was found in the LCL from the subject but not in the fibroblasts from her siblings. She has had very mild hematopoietic manifestations compared to her affected siblings who all died of marrow failure or hematologic malignancies in childhood. This strongly suggests it is a revertant mutation. Therefore, we will not use this family in further studies.

b. Derive iPSC and differentiate them to CD34+ hematopoietic cells.

We remain on track with respect to work items for this subaim, including ongoing reprogramming, *SAMD9L* genotype determination, chromosome 7q ploidy content determination via ddPCR and CGH array. We have preliminarily achieved the milestone of having sufficient iPSC lines to evaluate for Aims 1 and 2, though collection of additional patients is likely to prove useful.

We are therefore continuing to derive iPSC cells from newly acquired patient samples. We are additionally identifying subclones using karyotype and genomic microarrays to dissect the clonal complexity of bone marrow failure evolution in ATXPC patients, paying particular attention to clones that have undergone revertant mutations including 7q-, *cis* suppressor mutations (as described above), and uniparental disomy.

2. Promote autocorrection of *SAMD9* and *SAMD9L* germline mutations.

We remain on track with work items related to double-strand break targeting of *SAMD9L* with CRISPR. We have not yet evaluated irinotecan's effects on double-strand break targeting, as this is dependent on finalizing appropriate concentrations of this drug, as described for aim 3.

In light of a recent exciting discovery from the D'Andrea lab that a currently FDA-approved antibiotic, novobiocin, inhibits POLQ and phenocopies POLQ depletion (Zhou et al. Nature Cancer 2021;2:598), we are presently modifying plans to include this drug in testing if intrahomolog recombination frequencies can be increased. We are currently at the design and evaluation stage for CRISPR guides targeting *SAMD9L*. We are therefore revising plans, as described below for the work item related suppression of POLQ with RNAi and small molecule inhibitors.

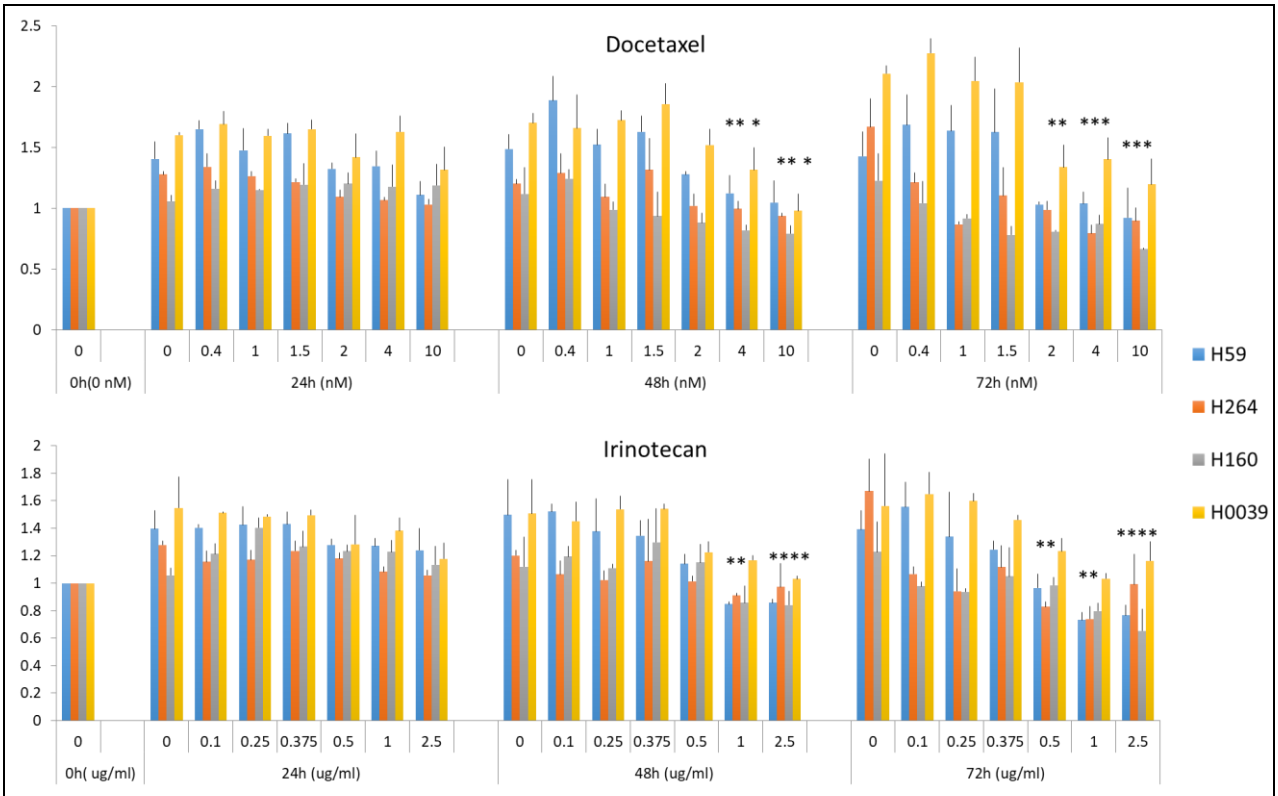

Fig. 1 Drug sensitivity testing of LCLs. Proliferation assay, based on cell counts. * p <0.05

3. Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies.

We are close to completing the first two work items and attendant milestone related to this aim.

We have now evaluated control, wild type LCL, and iPSC with both irinotecan and docetaxel to select appropriate concentrations of drugs suitable for testing the underlying hypothesis that xenobiotic detoxification genes (*ABCB1* and *CYP3A4*) adjacent to *SAMD9L* in the deleted region of chromosome 7 can selectively promote the growth of cells retaining two copies of chromosome 7. In an initial experiment, we determined the IC50 of docetaxel and irinotecan in LCLs from patients and controls. In control LCLs, the IC50 concentrations were 1.7 nm and 450 ng/ml, respectively, and they were lower in ATXPC patients, suggesting greater drug sensitivity, although the difference was not significant by t-test. To validate the IC50s, we repeated the dose-response in newly established LCLs from 4 normal controls (Fig 1). The results were nearly identical to those previously detected (1.5 nm for docetaxel and 536 ng/ml for irinotecan). We then grew the LCLs for up to 3 weeks in a range of drug concentrations lower than the IC50s. At the lowest tested concentrations, docetaxel at 0.4nm and irinotecan at 100 ng/μl, most cells died by week two, while untreated cells survived well to the end of the testing period. Therefore, we will repeat this experiment with even lower dosages to determine conditions that will allow long-term culture. For iPSC, we found docetaxel to be more discriminatory than irinotecan (Fig. 2).

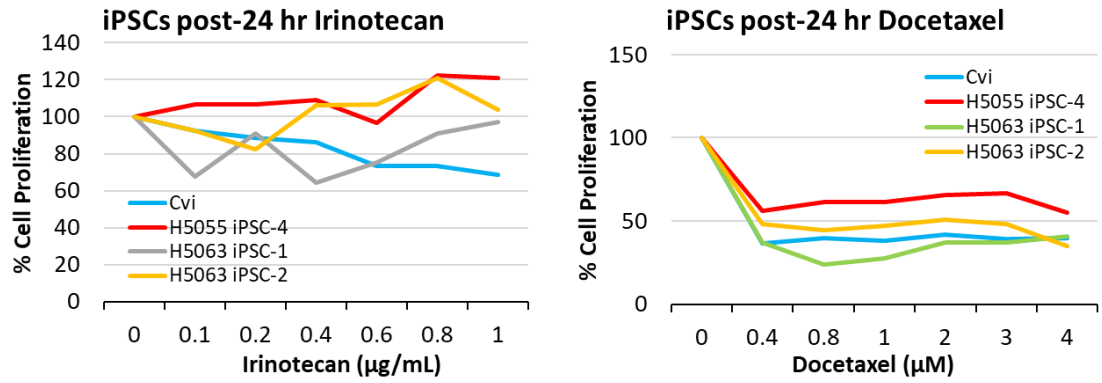

Fig. 2 Drug sensitivity testing of iPSC, normal controls from unaffected family members.

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

Nothing to Report. The project was not intended to provide training and professional development opportunities.

- **How were the results disseminated to communities of interest?**

Nothing to Report. The project is still somewhat early in development and COVID-19 has diminished ability to participate in relevant meetings.

- **What do you plan to do during the next reporting period to accomplish the goals?**

We plan to largely abide by the detailed research plan contained within the original proposal and feel that we are on track at this stage of the project.

4. Impact

The impact largely pertains to the development of the principal disciplines of the project, which are focused on bone marrow failure occurring in the context of $7q^-$ syndrome. Germline mutations in *SAMD9L* and its immediately adjacent chromosome 7q paralog, *SAMD9*, are increasingly well described in a sizeable proportion of patients with bone marrow failure and MDS, particularly among pediatric patients, where the burden of disease is most profound. Additionally, $7q^-$ syndrome tends to occur in younger populations of MDS patients, where germline mutations in other genes, such as *GATA2* are also disproportionately more common.

The goal of the project will have potentially broad applicability to bone marrow failure accompanied by chromosome 7 loss, regardless of underlying hereditary risk factors.

5. Changes/Problems

As noted above, we are excited by the recent finding from the D'Andrea lab that an existing approved antibiotic, novobiocin (whose clinical use as an antibacterial agent has largely been supplanted by fluoroquinolones) has been found to be a potent and reasonably selective POLQ inhibitor. We plan to evaluate novobiocin in specific aim 2.

One problem was the COVID-19 pandemic. It severely affected our access to laboratories, disrupted supply lines, imposed barriers to accessing patients and obtaining samples, and diverted attention of staff to attend to personal and family illnesses and other issues. A related issue was the pandemic's impact on delaying full human subjects approval and initial release of funds.

6. Products

Nothing to report, as of yet.

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	Marshall Horwitz
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-1683-1680
Nearest person month worked:	0.6
Contribution to Project:	Overall design of experiments, lab management, and interpretation of data.
Funding Support:	N/A

Name:	Wendy Raskind
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0001-8141-9054
Nearest person month worked:	0.6
Contribution to Project:	Shared responsibility for overall design of experiments, lab management, and interpretation of data. Prepared and submitted the IRB protocol. Supervised technical staff in generating LCLs.
Funding Support:	N/A

Name:	Dong-Hui Chen

Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0003-2584-8609
Nearest person month worked:	0.6
Contribution to Project:	Dr. Chen supervised the technical staff for determination of ID50.
Funding Support:	N/A

Name:	Sudeshna Seal
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	none
Nearest person month worked:	6
Contribution to Project:	Established iPSC hematological differentiation assays.
Funding Support:	N/A

Name:	Jun Xu
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	none
Nearest person month worked:	5
Contribution to Project:	Performed iPSC hematological differentiation and *SAMD9L* expression assays.
Funding Support:	N/A

Name:	John Wolff
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	none
Nearest person month worked:	1
Contribution to Project:	Processed samples and established LCLs

Funding Support:	N/A

Name:	Prasanthi Karna
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	none
Nearest person month worked:	1
Contribution to Project:	Cultured LCLs for ID50 assays
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?**

Horwitz, Marshall
Completed:
NIH R01 HL130472- (Horwitz) 1/15/16 – 11/30/20 NCE
Pathogenesis of ELANE-Associated Neutropenia

Edward P Evans Foundation DRG2018 (Horwitz, Raskind) 9/1/18 – 2/28/21 NCE
Selection Against Cells Containing Monosomy 7

Raskind, Wendy
Completed:
NIH R01NS069719 (Raskind) 4/1/10 – 3/31/20
Next Generation Gene Discovery in Neurogenetics

Edward P Evans Foundation DRG2018 (Horwitz, Raskind) 9/1/18 – 2/28/21 NCE
Selection Against Cells Containing Monosomy 7

Chen, Dong-Hui
Completed
NIH R01NS069719 (Raskind) 4/1/10 – 3/31/20
Next Generation Gene Discovery in Neurogenetics

- **What other organizations were involved as partners?**

Nothing to Report

8. Special Reporting Requirements

NA

9. Appendices

Award chart

Award Log Number: BM190019 **Award Title** Selective Clonal Growth in Myelodysplastic Syndrome
PI: Marshall S. Horwitz, University of Washington, WA **Budget:** Total Award Cost
Topic Area: Bone Marrow Failure Research Program, Idea Development Award **Mechanism:** W81XWH-19-BMFRP-IDA

Research Area(s): SCS Coding Award Status: 7/1/20 – 6/30/22

Study Goals:

1. Establish in vitro models of clonal heterogeneity from patients with pathogenic SAMD9L mutations.
    a. Establish EBV-transformed lymphocyte lines.
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2. Promote autocorrection of SAMD9 and SAMD9L germline mutations.
3. Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies.

Specific Aims:

1. Establish in vitro models of monosomy-7 clonal heterogeneity from patients with ATXPC
2. Promote autocorrection of SAMD9 and SAMD9L germline mutations and
3. Identify drug combinations that specifically inhibit growth of cells deficient for part or all of chromosome 7q while favoring growth of blood cells retaining two intact chromosome 7 copies.

Key Accomplishments and Outcomes:

Publications: none to date
Patents: none to date
Funding Obtained: none to date