AWARD NUMBER: W81XWH-20-1-0574

TITLE: Selective Clonal Growth in Myelodysplastic Syndrome

PRINCIPAL INVESTIGATOR: Dr. Marshall Horwitz, MD, PhD

CONTRACTING ORGANIZATION: University of Washington

REPORT DATE: SEPTEMBER 2023

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2023	Final Report	1JUL2020 - 30JUN2023

4. TITLE AND SUBTITLE
Selective Clonal Growth in Myelodysplastic Syndrome

5a. CONTRACT NUMBER W81XWH-20-1-0574

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Marshall S. Horwitz, Dong-Hui Chen, Wendy Raskind

E-Mail: horwitz@uw.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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 confer toxic gain-of-function. Hematopoietic stem and progenitor cells that undergo somatic mutation that eliminates the mutant allele through one of three mechanisms gain 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 drug combinations 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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	16	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	10
5. Changes/Problems	11
6. Products	12
7. Participants & Other Collaborating Organizations	14
8. Special Reporting Requirements	15
9. Appendices	16

1. **INTRODUCTION:** Narrative *that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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). The effect of these SAMD9L mutations is to increase its suppression of hematopoiesis. Remarkably, bone marrow cells in individuals with ATXPC can undergo subsequent somatic mutations that counteract this toxic growth suppressive effect but the overall result 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:** *Provide a brief list of keywords (limit to 20 words).*

Ataxia-pancytopenia syndrome (ATXPC), SAMD9L, MDS, genetic recombination, induced pluripotent stem cell disease (IPSC) models, reversion mutations

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1 was to "Establish in vitro models of clonal heterogeneity from patients with pathogenic *SAMD9L* mutations". We found that iPSCs are very resistant to the drugs we proposed to use. In addition, iPSC bearing pathogenic *SAMD9L* variants are very difficult to differentiate to committed hematopoietic lineages and we were not able to generate sufficiently robust cultures to proceed. Therefore, we focused on EBV transformed lymphocyte lines (LCLs) from several subjects without a detected revertant somatic mutation ~~in the EBV line~~ (germline H880Q or V1551L) and from one who was found to be mosaic for a hematopoietic clone that had lost the pathogenic H880Q variant by copy neutral loss of heterozygosity for chromosome 7q. As explained below, we later concluded that these cell lines actually did contain revertant subclones that were below detectable levels but that became apparent as they expanded upon further cell culture growth.

For Aims 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" we repeated studies to determine the IC50 doses for docetaxel and irinotecan. In an initial experiment, in control LCLs the IC50 concentrations for docetaxel and irinotecan were 1.7 nM and 450 ng/ml, respectively, and they were lower in LCLs from 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. 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 4 weeks in a range of drug concentrations lower than the IC50s. At the lowest tested concentrations, docetaxel at 0.6 nm and irinotecan at 20 ng/μl, no response to the treatment was observed by cell count and proliferation assays. At docetaxel 16 nM and irinotecan 50 ng/μl, untreated and treated cells from control subjects survived to the end of the testing period, and treated cells showed lower proliferation compared to untreated cells, documenting a response to the drug. To enable the longer course experiments involving samples from affected subjects, we selected an irinotecan concentration of 30 ng/μl.

In this larger scale experiment, LCLs were cultured in wells and sampled periodically. The samples included the affected subject with a p.V1551L pathogenic variant who had fluctuating percentages of monosomy 7 cells and an affected subject who had not manifested copy neutral LOH for chromosome 7q but had a benign p.V266I *SAMD9L* polymorphism *in trans* with the pathogenic p.H880Q variant. LCLs from two normal controls were also included. The plates were cultured for more than four months. Figure 1 shows that there was no significant difference by droplet digital PCR (ddPCR) in loss of the mutant allele regardless of treatment conditions. Although Figure 1A shows a lower mutant percentage in untreated cultures than in treated ones, it should be noted that a low mutant allele percentage in the non-treated cells was observed from the initial stage. To provide a more accurate representation of the effect of drug treatment, we have adjusted the changes in mutant allele percentage in each group during the treatment course by normalizing them with their own baseline measurements taken at the initial time point of treatment at 2 weeks. This adjusted data is depicted in Figure 1B. While the treated cells showed a greater decrease in mutant allele percentage at later time points, this was not significant by one-way ANOVA post-hoc test. The large error bars suggest significant variability, which may be attributed to the variable number of cells carrying the mutation from the initial stage. This variability is further demonstrated by analysis of four individual culture wells (Figure 2). We conclude that there is no dosage effect between 30ng/ml and 50ng/ml on selective advantage for loss of the mutant allele and retention of the wild-type one.

However, these results do not reflect the presence of any other revertant mutation. To address this issue, we assessed the entire *SAMD9L* coding region by whole genome sequencing (WGS) (Table 1).

In the case of Patient-1, within the four distinct H2132 cultures the disease-causing variant H880Q exhibited loss in two of the samples, one of which was subjected to treatment with 30 ng/ml irinotecan while the other underwent

treatment with 50 ng/ml. These two samples also displayed loss of the V226I polymorphism, an observation that aligns with our prior discovery of loss of heterozygosity (LOH) in a broad region on chromosome 7q. The other two LCL samples from this subject, treated with 50 ng/ml, demonstrated a reduction in the variant allele H880Q without complete loss. This outcome suggests that the higher drug dosage did not achieve the intended effect of augmenting LOH.

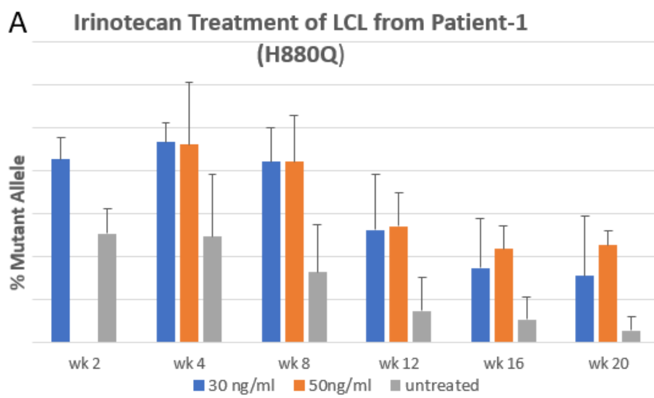
In the case of Patient-2 with H880Q, no clear decrease in variant allele reads was evident in LCL samples treated with either 30 ng/ml or 50 ng/ml irinotecan.

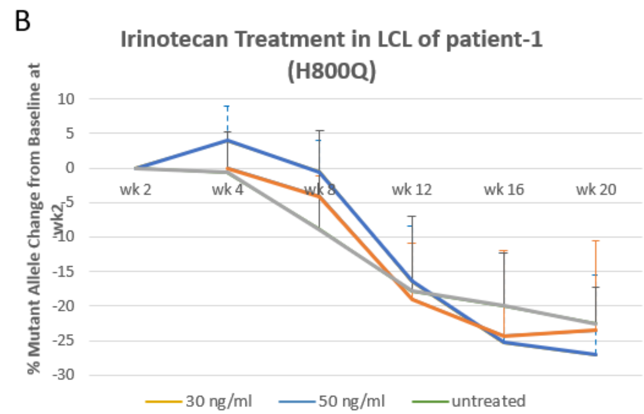
For Patient-3, carrying the V1551L variant, there was no notable reduction in variant allele reads at either 30 ng/ml or 50 ng/ml dosage levels. Interestingly, in all four samples, three additional heterozygous variants were detected, a synonymous nucleotide change G1337A and two missense changes, A868A, and N24S. A bioinformatic evaluation by *in silico* functional prediction and gnomAD annotation, supports the possibility that the N24S variant is deleterious, likely acting as a reversion mutation. For more support of this conjecture, we would need to show that it is *in cis* with the V1551L allele. All other identified variants are deemed likely to be benign.

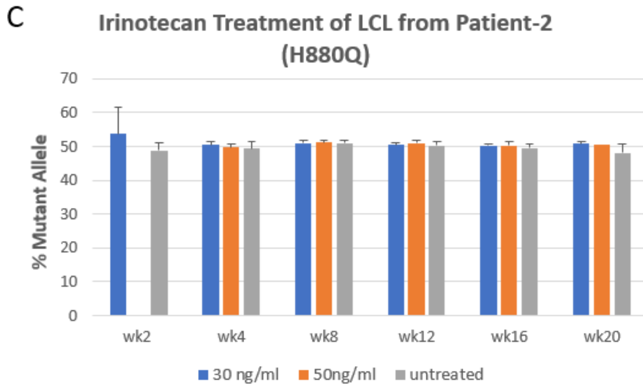
The WGS data provided evidence that irinotecan had no noticeable effect on loss of the mutant allele, consistent with the findings we derived from ddPCR analyses. However, within this dataset, we were able to identify three previously unreported variants in H5055 that had not been documented in the clinical exome sequencing conducted at an outside lab.

To gain an understanding of the origin and development of these newly identified variant clones, we assessed their presence in uncultured blood DNA, untreated LCLs, and LCLs at various stages of culturing. PCR-Seq detected all three variants in the untreated LCL samples derived from Patient-3 (Fig. 3A). In blood DNA, two variants (G1137A and A868A) were observed, but the N24S variant was not present (Fig. 3B). To investigate the progression of the N24S variant, we established a duplex allele-specific PCR (AS-PCR) method (Fig. 4A) in our laboratory. This enabled the concurrent and specific amplification of both the wild-type and variant alleles in a single reaction to analyze the variant in blood DNA. Semi-quantitative assessment of the variant/WT allele ratio revealed a low presence of the variant allele in the patient's blood DNA and a minor variant presence at the initial culturing stage, with a significant increase over the culture duration within the treated and the untreated groups (Fig. 4B). By 16 weeks the WT and variant alleles were present in equal frequency in all three treatment conditions at the 16-week mark (Fig. 4C).

Although we faced obstacles posed by the unusual biological features of SAMD9L that were not anticipated at the outset of the project, there were several outcomes: 1. IPSC are relatively resistant to irinotecan; 2. Growth of lymphocyte lines in the presence of irinotecan did not increase the selective advantage of beneficial revertant clones at the concentration and duration of culture in our experiment; 3. Multiple revertant clones coexist in the hematopoietic system of people with pathogenic variants in SAMD9L, and these clones may escape detection by clinical sequencing. 4. The revertant variant may initially be present in low levels in the blood and during initial culture stages but can undergo significant increase during subsequent culturing, eventually reaching an equal ratio with the WT allele. A better understanding of the selective forces influencing the growth of revertant subclones may help lead to potential new therapies for ATXPC.







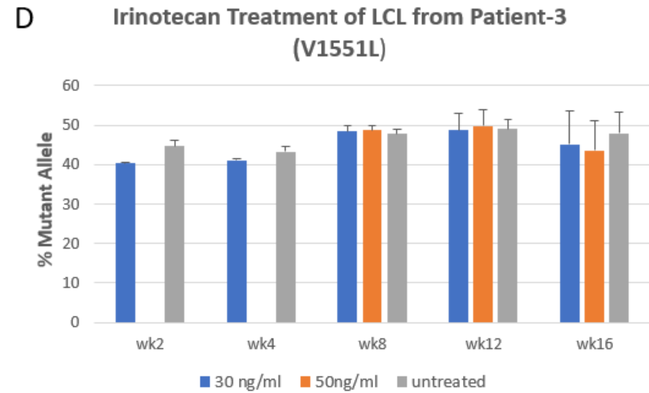


Figure 1. Mutant allele percentage detected by droplet digital PCR (ddPCR) in LCLs grown with and without irinotecan for 5 months. A and B: Patient-1 with H880Q, who demonstrated copy neutral LOH for 7q in a previous study; B: Adjusted changes in mutant allele percentage in each group by normalizing with their own baseline measurements taken at 2 weeks; C: Patient-2, a relative with H880Q who did not show cnLOH; and D: Patient-3, with V1551L who had fluctuating mosaic monosomy 7. Error bars represent the standard deviation of the mutant allele percentage measured in four culture wells. There is no significant difference among treatment conditions and non-treatment by one-way ANOVA post-hoc method.

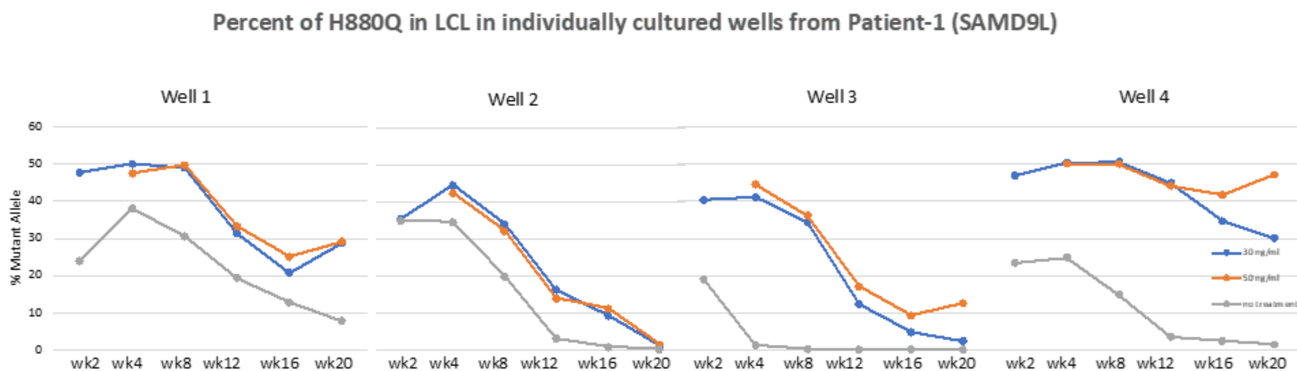


Figure 2. Significant variability of the proportion of the mutant allele is independent of culture conditions but may be related to the fraction of cells with LOH 7q at initiation of the culture.

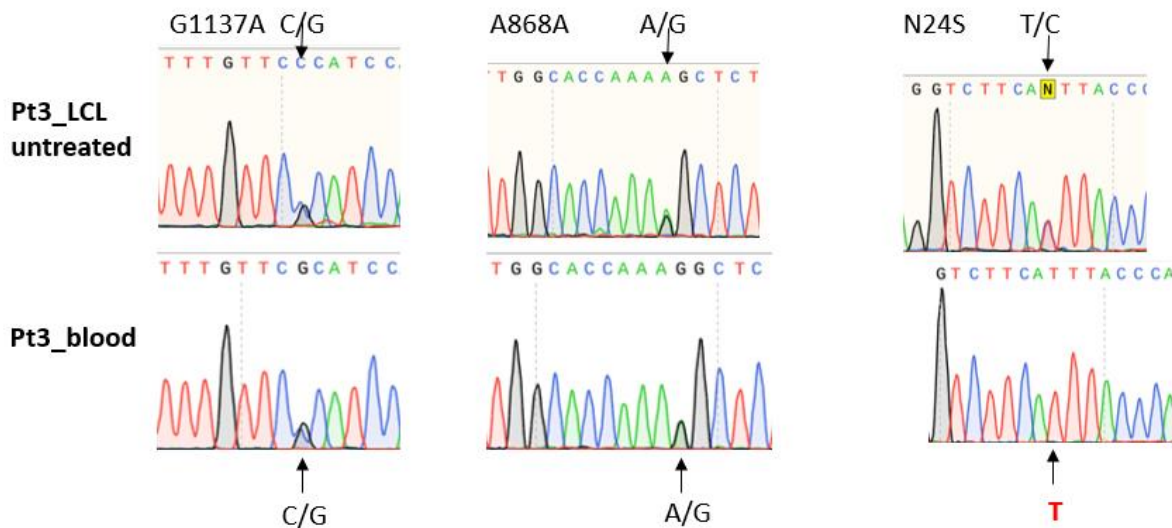


Figure 3. PCR-Seq analysis of three variants in Pt-3 LCL without treatment and in blood DNA. Notably, all three variants were detected in the untreated LCL samples. However, only two of the variants were observed in Pt-3 blood DNA; the N24S variant was not detected.

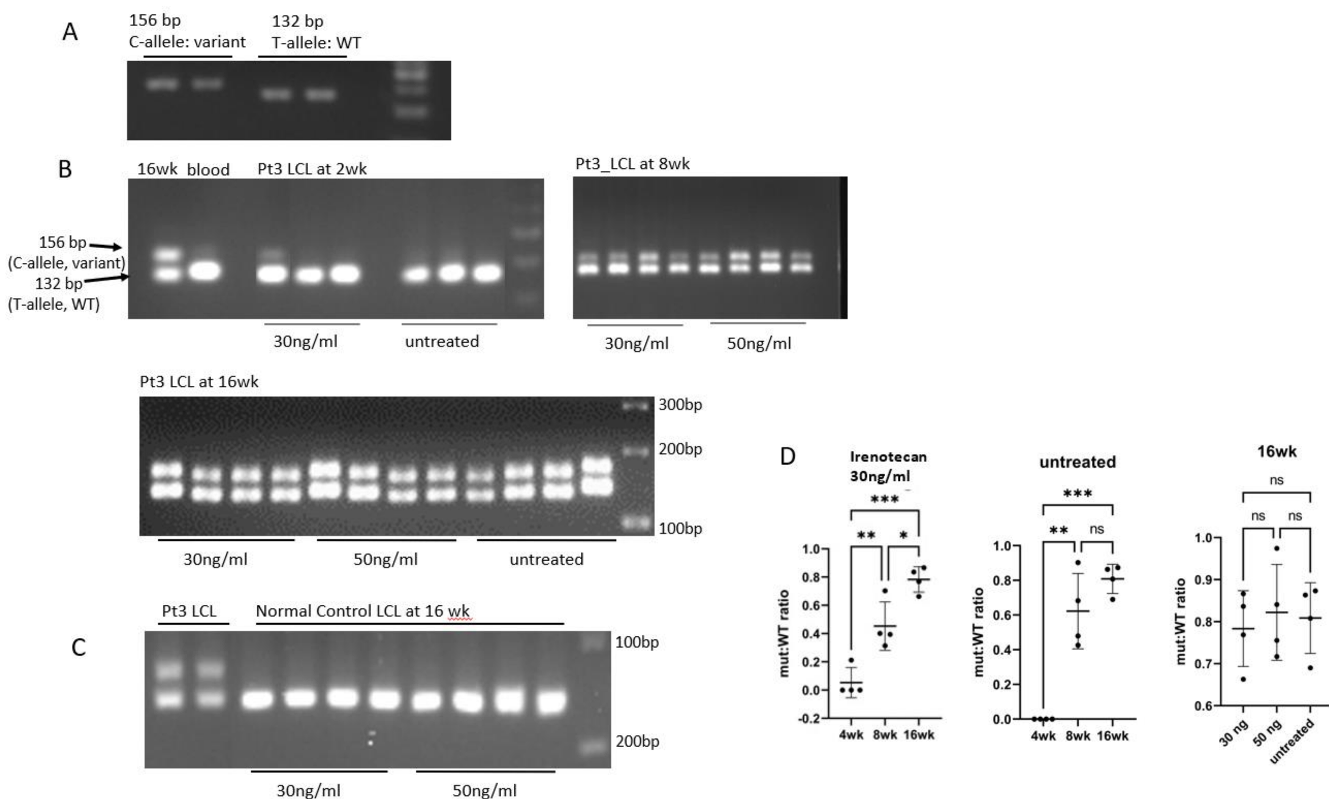


Figure 4. Irinotecan treatment effect on alternate allele frequency. A. PCR using allele-specific primers selectively amplified the wild-type T-allele and the variant C-allele of N24S in individual PCR reactions. B. In multiplex allele-specific PCR (AS-PCR), the WT T-allele (132 bp) and variant C-allele (156 bp) were simultaneously amplified within a single reaction for a semi-quantitative assessment of the T/C allele ratio in blood and at specific time-points during culture. PCR bands visualized on an agarose gel revealed low presence of the variant allele in the patient's blood DNA. This analysis also indicated a low level of the variant at the initial culturing stage, with its increase with increased culture time. C. Multiplex allele-specific PCR detected only the wild-type allele in the LCL derived from a normal control, confirming both the specificity of the AS-PCR method and the exclusive presence of the N24S variant in patient 3. D. The intensities of AS-PCR bands in patient 3 LCL (visualized on the gels in figure X panel B) corresponding to the wild-type T-allele and the variant C-allele were quantified. The allelic ratios were then mapped in scatter plots (mean ± SD). Within the groups treated with

30 ng/ml and the untreated groups, the variant alleles exhibited a significant increase throughout the culturing process. No significant differences were observed among the three treatment conditions at the 16-week mark. Statistical analysis was conducted using a one-way Anova post-hoc method, with significance denoted as follows: * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

Table 1. WGS reads of SAMD9L variants in patient-derived LCL at the end point of Irenotecan treatment (30ng/ml or 50 ng/ml)

Pt1					
	H880Q (causative)			**V266I**	
	WT	Variant		WT	Variant
LCL-30ng	60	0		1	80
LCL-50ng	41	0		2	89
LCL-50ng	42	9		24	36
LCL-50ng	35	15		40	26
Pt2					
	H880Q (causative)			**V266I**	
	WT	Variant		WT	Variant
LCL-30ng	35	24		29	36
LCL-30ng	27	30		51	44
LCL-50ng	24	40		43	36
LCL-50ng	31	29		42	30

Pt3											
	V1551L (causative)			**G1137A**			**A868A**			**N24S**	
	WT	Variant		WT	Variant		WT	Variant		WT	Variant
LCL-30ng	29	21		22	23		29	37		40	43
LCL-30ng	33	33		34	27		28	33		45	21
LCL-50ng	31	23		27	26		36	25		26	47
LCL-50ng	30	38		23	33		20	28		35	16

Table 2. Bioinformatic assessment of SAMD9L variants detected in patients' LCLs.

Protein	cDNA	CADD	DANN	SIFT	PolyPhen	PhastCons	PhyloP	gnomAD
p.N24S	c.71T>C	9.6	0.65	0.03 (deleterious)	0	0.08	0.005	0
p.G1137A	c.3410C>G	0.45	0.38	0.58	0	0.1	0.89	0.02
p.A868A	c.2604A>G	3.39	n/a	n/a	n/a	n/a	n/a	0.21
p.V266I	c.796C>T	0.001	0.48	0.19	0	0	-0.32	0.11

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Co-I Dr. Dong-Hui Chen mentored an undergraduate student in laboratory research. The student took required courses in bloodborne pathogens, human subjects research and was taught basic tissue culture techniques. The student then assisted with cell culture and harvesting.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We tested a high-risk, high-reward hypothesis, based on the reasonable supposition that we could direct clonal growth of revertant clones by taking advantage of the genomic neighborhood of the *SAMD9L* gene. At least for the drugs and concentrations that we evaluated, we could identify no discernable effect of treatment upon revertant clone growth. We did unexpectedly discover that initial clinical and laboratory testing of patient samples failed to small and diverse subclonal cell populations containing SAMD9L revertant and secondary mutations that were below the level of detection.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

We think that in the initial clinical evaluation of patients with ATXPC syndrome, it may be of importance to perform deep sequence to initially survey the clonal landscape and identify rare revertant and secondary mutations, thereby leading to an approach in which patients can be prospectively followed by using specific PCR assays to determine if deleterious clones have risen to levels necessitating further treatment interventions, such as allogenic bone marrow transplant. In some ways, our studies suggest that ATXPC patients should be surveilled similarly to leukemic patients in remission, in which evidence of minimal residual disease is assayed for.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

As noted above, we believe these studies will help open up the possibility of directing the clonal evolution of preleukemic disorders and lead to development of new strategies for screening for malignant transformation, along with the optimal timing of allogenic bone marrow transplantation.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project*

or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

As noted, patient IPSC proved difficult to culture and unusually resistant to the drugs we tested, necessitating that we fall back to evaluating LCLs and fibroblasts.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The COVID pandemic continued to have a negative impact on our research progress. People with any manifestation suggestive of COVID were required to isolate. Compliance with the University of Washington's rules for social distancing hampered efficient use of tissue culture facilities and access to our lab's DNA sequencing equipment and the ddPCR apparatus in another department. As the rules relaxed during the no-cost extension year, we were able to culture the lines and compete the molecular analyses.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page*

numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*

- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Marshall Horwitz
Project Role:	PI
Researcher Identifier	ORCID ID 0000-0002-1683-1680
Nearest person month worked	.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	ORCID ID 0000-0001-8141-9054
Nearest person month worked	.6
Contribution to Project	Shared responsibility for overall design of experiments, lab management, and interpretation of data.
Funding Support	N/A

Name:	Dong-Hui Chen
Project Role:	co-I
Researcher Identifier	ORCID ID 0000-0001-8141-9054
Nearest person month worked	2
Contribution to Project	Dr. Chen supervised the technical staff in culturing the LCLs, determining the ID50s and harvesting the samples, performed the ddPCR assays, mentored the undergraduate student.
Funding Support	N/A

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

Name Project Role Researcher Identifier Nearest person month worked Contribution to Project Funding Support

Name	John Wolff
Project Role	Research Scientist
Researcher Identifier	None
Nearest person month worked	1
Contribution to Project	Processed samples and established LCLs
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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *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.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable;*

however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: ***N/A***

9. APPENDICES: *N/A*