EVALUATION OF MYELOPROLIFERATIVE NEOPLASMS IN PEDIATRIC PATIENTS

A Thesis

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by

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ABSTRACT

OBJECTIVE: Myeloproliferative Neoplasms are rare, poorly understood diseases in children, and the outcomes, causative lesions, and management strategies for children with these diseases are not clearly defined. The purpose of this study was to evaluate clinical findings and management practices in children with MPN, and identify alternative genetic lesions that could contribute to disease pathogenesis in these patients.

METHODS: Patients were identified as eligible if they were diagnosed with a BCR-ABL negative classical MPN. They were enrolled and provided our team with clinical information, including age of presentation, bone marrow pathology results, and treatments utilized. Blood and buccal samples were collected (and bone marrow if available.) Monocytes and granulocytes were separated from blood and marrow samples, and DNA was extracted from all three types of tissue (tumor samples = granulocytes and monocytes; normal tissue = buccal cells.) Next-generation sequencing was performed on these samples for a pre-determined panel of genes known to be relevant in hematologic malignancies and myeloproliferative neoplasms.

RESULTS: Ten patients were enrolled on study but two were removed when their bone marrow biopsies ruled out a myeloproliferative neoplasm. Of the eight children eligible to remain in the study, known causative mutations were identified on clinical testing in three of them to date. Next-generation sequencing did not show alternative mutations in *JAK2*, *MPL*, or *CALR*. No children have yet had major thrombotic or hemorrhagic complications. Six children received hydroxyurea for their symptoms. None of the 8 subjects have developed leukemia at this time.

CONCLUSIONS: Children with myeloproliferative neoplasms may have lower rates of severe complications and known genetic mutations than adults with similar diseases. More study is needed of this rare population of patients to better quantify clinical outcomes, genetic lesions, and allow for development of appropriate treatment algorithms.

BIOGRAPHICAL SKETCH

Dr. Nicole Kucine received her undergraduate degree, a Bachelor of Arts in Biological Sciences, from Wellesley College. She received her MD degree from the State University of New York - Health Science Center at Brooklyn. From there, she remained in the tri-state area and completed her residency training in Pediatrics at Robert Wood Johnson University Hospital/Bristol Myers Squibb Children's Hospital. She was selected to stay on to complete an additional year as Chief Resident in Pediatrics. Dr. Kucine then returned to her hometown of New York City to receive her subspecialty fellowship training in Pediatric Hematology/Oncology at the combined program of New York Presbyterian/Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center. She participated in an additional Special Fellow year at Memorial Sloan-Kettering Cancer Center in the laboratory of Dr. Ross Levine. After completing her year as special fellow, she joined the faculty at Weill Cornell Medical College in the Department of Pediatrics, Division of Hematology/Oncology. Dr. Kucine is working to develop an academic career in pediatric hematology focusing on clinical and translational research, and to develop expertise in the area of Pediatric Myeloproliferative Neoplasms, which is an area in great need of leadership.

ACKNOWLEDGEMENTS

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I would like to thank all my mentors, and the members of the Levine Lab at MSKCC for their help and support with this work.

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INTRODUCTION

Myeloproliferative Neoplasms (MPN) are defined as clonal disorders leading to overproduction of hematopoietic cells[1]. Chronic Myelogenous Leukemia (CML), known to be associated with the BCR-ABL translocation (also called the Philadelphia Chromosome – Ph) is one of the best recognized of the MPN. Amongst the Ph (-) MPN, the three classic disorders are Essential Thrombocytosis (ET), Polycythemia Vera (PV) and Primary Myelofibrosis (PMF). There are a number of different criteria used for the diagnosis of these diseases, including those put out by the WHO and the Polycythemia Vera Study Group. They include genetic findings (such as absence of the *BCR-ABL* translocation), clinical and lab features, and specific findings in bone marrow (such as comments on cellularity, fibrosis, and appearance of precursor cells), and the exclusion of reactive causes[2,3].

The clinical course of these diseases is well studied in adult patients, and symptoms can include headache, pruritus, erythromelalgia, and splenomegaly[4]. Dangerous complications include severe bleeding, thrombotic events, and transformation to Myelodysplastic Syndrome (MDS) or Acute Myelogenous Leukemia[5,6] (AML). The genetic landscape of these diseases is also well understood. In 2005, the Janus Kinase (JAK) 2-gene mutation *JAK2V617F* was found to be a key driving force in MPN[7,8,9]. We know now that a large majority of adults with PV harbor this mutation, as well as ~ 50% of patients with ET or PMF. It was then discovered that the thrombopoietin receptor gene *MPL* also played a role as a causative mutation, with ~3-5% of patients with ET and 5-10% of patients with MF having mutations in the MPL gene, the most common of which is the MPLW5151K/L mutation[10,11]. More

recent studies have identified mutations in *CALR* in a significant number of JAK2 and MPL-negative patients[12,13]. Additional genes have been shown to play a role in pathogenesis as well, including *TET2*, *ASXL1*, and *EZH2*[14,15,16].

There are non-sporadic forms of MPN as well. Familial forms of MPN have similar clinical features as the classical sporadic MPN, and members of the affected family can have the same or different MPNs. These generally involve mutations in *JAK*, *MPL* or *TERT2* genes. Hereditary forms of MPN (hereditary thrombocytosis or erythrocytosis) are polyclonal and have milder clinical phenotypes with low likelihood of transformation. Erythrocytosis is typically due to mutations in *EPOR*, *VHL*, *HIF1α* and *HIF2α* and thrombocytosis is generally due to mutations in the *TPO* or *MPL* genes[17].

Management of MPN in adults is based on clinical findings and risk stratifications. Higher risk features for various MPN include older age, prior history of thrombosis, severity of anemia, presence of constitutional symptoms, and high white blood cell count, depending on the type of MPN. Treatments may involve phlebotomy (for PV), aspirin, or cytoreductive therapy for higher risk patients, such as Hydroxyurea, Anagrelide (for ET), or Interferon. Targeted therapies with JAK inhibition have recently been approved for PMF and PV[18].

While there have been significant advances in our understanding of adult MPN, there is a paucity of knowledge regarding pediatric MPN. The limited available literature suggests that children with MPN do not harbor the known pathogenic mutations at the same frequency as adults[19,20]. While current literature suggests that complication rates are lower in children, the existing data is limited. No accepted treatment guidelines exist for children

with MPN, and recommendations on management of these disorders in children are limited[2,21,22]. The clear lack of expertise surrounding the causes and management of MPN in children is the driving force behind this research.

MATERIALS AND METHODS

Setting

This study was conducted at the New York Presbyterian/Weill Cornell Medical Center, in both the pediatric inpatient unit and outpatient pediatric hematology/oncology clinic. The Institutional Review Board of the Weill Cornell Medical College approved this study. Participants were either primary hematology patients of the center, patients who were interested in participating in pediatric MPN research, or patients primarily based at other centers that were being cared for by our pediatric hematology service. Clinical information was obtained in the inpatient and outpatient units. Processing of de-identified samples for DNA extraction and genetic sequencing took place in the Levine lab and in the Integrated Genomics Operation core facility at the Memorial Sloan-Kettering Cancer Center.

Participants

This study was a prospective observational study of pediatric MPN patients (as defined by the WHO 2008 Diagnostic Criteria). Anyone aged 0-21 years who was diagnosed with a Philadelphia-chromosome negative MPN prior to his or her 18th birthday is eligible. Consent was provided for minor subjects by their parent or legal guardian. Children aged 7 years to 17 years also provided assent for the study. Any subject enrolled at the time of work-up who was then determined not to have an MPN was removed from the study and samples were discarded. Upon study enrollment, participants are given a subject number to ensure data is de-identified.

Clinical Information

Participants' medical records were reviewed to ensure they were diagnosed with an MPN or were being evaluated for one after consent was signed. All available relevant information regarding the diagnosis, including past medical history, history of presenting illness, complete blood counts, bone marrow examinations, and supporting laboratory studies were documented. The REDCap database system will be utilized as the primary database tool to store pertinent clinical information.

Sample Collection

At the time of scheduled clinical blood draws, 1-3 additional EDTA tubes of peripheral blood was collected for future isolation of white blood cells, depending on the age and size of the patient. If a bone marrow aspiration was being performed as part of routine clinical care, 1-2 additional tubes of bone marrow aspirate was collected. To obtain germline sample, oral buccal mucosal cells were collected by having the subject rinse and spit with 10 milliliters of original Scope™ mouthwash.

DNA Extraction from Blood and Buccal Samples

Utilizing a Ficoll-Paque™ density gradient separation technique (per manufacturer's recommended protocol), mononuclear cells and granulocytes were separated and extracted from peripheral blood and bone marrow samples. DNA was then extracted from these cells utilizing a Qiagen™ DNA extraction kit according to the manufacturer's recommended protocol. A Qiagen™ DNA extraction kit was also utilized to extract DNA from buccal cells in mouthwash.

Sequencing of Potential Genes of Significance

A multi-gene MPN/Leukemia panel (close to 400 genes) was designed incorporating known genes of relevance or interest in MPN, and lymphoid and myeloid malignancies. DNA from subject samples were plated and submitted for next-generation sequencing to evaluate for mutations in the genes of interest. Nimblegen capture was utilized to obtain libraries of sequence correlating to the protein-coding exons of the included genes. Captured DNA was then sequenced using an Illumina HiSeq 2500. The Burrows-Wheeler Aligner was used to align sequence reads to the reference genome[23] and post-processing occurred utilizing the Genome Analysis Toolkit[24]. Paired analysis was done if adequate buccal sample was available. Copy number and sequence variants were identified and reviewed.

Cell Lines

293T cells were grown in Dulbecco's modified Eagle's medium with 10% FBS. 293T cells were transiently co-transfected and retroviral supernatant was produced using Fugene (Roche, Nutley, NJ, USA) according to manufacturer's procedure. Ba/F3 cells (grown in RPMI-1640 + 10% fetal bovine serum (FBS) + 1U/ml IL-3) were transduced with MSCV-*hJAK1V658F*-GFP. Ba/F3 *hJAK1V658F* GFP cells were selected out by withdrawl of IL-3 from the growth media.

In vitro inhibitor assays and Western blot analysis

Viable cells were plated at 10,000 cells/well in 96 well tissue culture treated plates in 200uL media with increasing concentrations of INCB18424 inhibitor or PU-H71 inhibitor in triplicate. 48 hour inhibitor assays were

assessed using the Cell viability luminescence assay (CellTiter-Glo®, Promega, Cat. No. G7571). Analysis based on normalization to cells treated with DMSO, media with DMSO, and media only. The effective dose for 50% death was determined using Graph Pad Prism 5.0 software. Western blot analysis of the cell signaling pathway were evaluated at steady state, and then determined at either 4 hour or 16h time points with inhibitor treatment. Cells were collected in lysis buffer containing Protease Arrest (Geno Technology), Phosphatase Cocktail II (EMD Chemicals), PMSF, and PAO, protein normalized using the Bio-Rad Bradford protein estimation, and separated using 4-12% Bis-Tris electrophoresis gels (Invitrogen). Nitrocellulose membranes were blocked in TBS-T with 5% milk and incubated with appropriate dilutions of primary and secondary antibody

RESULTS

Subject Demographics

Ten children were enrolled during the study period based on a current or working diagnosis. On evaluation, two patients were identified as not having a classical MPN and are not included in the analysis. Of the remaining eight children, all had a diagnosis of MPN. Their ages ranged from 8-21 years old. Three males and five females enrolled (Table 1.) Three children were followed at our center, one was followed at a local center, and four received their primary hematologic care at distant medical centers.

Table 1 – Summary of Enrolled Subjects

Subject #	Diagnosis	Age at Diagnosis (yrs)	Gender
1	ET	0.5	M
2	ET	6	M
3	ET	12	F
4	ET	5	F
5	ET	7	F
6	PMF	13	M
7	ET	11	F
8	ET	19	F

yrs = years

Clinical Analysis

Diagnosis

Seven subjects had a diagnosis of ET and one patient had a diagnosis of ET that was later corrected to a diagnosis of PMF. Seven of the eight subjects had bone marrow evaluations done as part of their diagnostic work-

up, and only one (subject #8) did not have a bone marrow done (her physician made her diagnosis based on clinical and genetic findings.) Therefore, except for subject #8, all subjects were diagnosed in accordance with WHO criteria. No children with PV were enrolled. Age at diagnosis ranged from 6 months of age to 19 years old (Table 1.) One subject, #1, was diagnosed at an early age because of known family history of ET and likely has hereditary and not sporadic disease (see below.) Initial symptoms for subject #6 included abdominal pain and splenomegaly. Subject #8 initially presented with fatigue and unexplained itching. Subject #5 presented with nose bleeding. The remaining four subjects all had presentations that included headache as one of their main complaints.

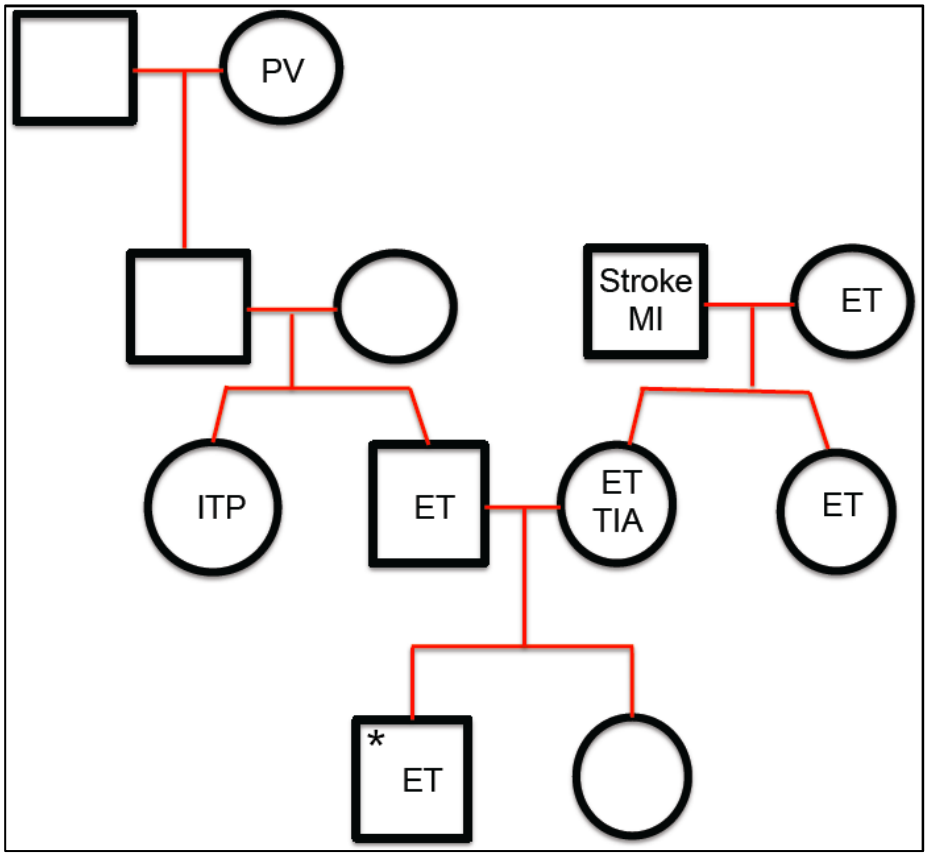
Family History

Because of the differences between hereditary and sporadic disease, a detailed family history was obtained from each subject. As previously mentioned, Subject #1 has a positive family history for MPN (Figure 1.) His father has a diagnosis of ET (*JAK2V617F* negative), made on routine testing, and his paternal great grandmother has a reported history of PV. His mother (diagnosed after having a transient ischemic attack) and maternal aunt both have ET (mother is *JAK2V617F* negative as well.) His maternal grandmother also has a diagnosis of ET, and his maternal great-grandfather had an MI at an early age and died of a stroke. He has a healthy younger sister with normal blood counts. There is no consanguinity between the parents.

All other subjects have negative family histories regarding possible MPN. Questioning regarding diagnoses such as a known diagnosis of MPN,

significant thrombotic or bleeding events, or AML were asked. The seven additional subjects appear to have sporadic disease.

Figure 1. Family Tree for Subject #1



*Subject #1 is identified by **

ITP= Idiopathic Thrombocytopenic Purpura, TIA=Transient Ischemic Attack, MI=Myocardial Infarction

Common Clinical and Laboratory Features

MPN are often associated with a variety of constitutional symptoms, such as erythromelalgia (a neurovascular syndrome leading to painful redness and inflammation, generally in the hands and feet), abdominal discomfort,

itching, and headache. One subject experienced erythromelalgia during her illness, while two additional subjects experienced episodes of discoloration and paresthesias during the course of the illness. All of the subjects have experienced periods of persistent headache during their illness (Table 2.) One subject has had splenomegaly and abdominal discomfort during his illness, one subject experienced significant itching, and one subject has had difficulty sleeping.

Extreme thrombocytosis is defined as a platelet count > 1,000,000 x 10^9/L. All of the subjects enrolled have had extreme thrombocytosis at one point during their illness. Acquired von Willebrand's disease can occur in the setting of extreme thrombocytosis, and three subjects developed this during the course of their illness.

Table 2 – Clinical Findings in Pediatric Patients

Subject #	Symptoms Experienced	Extreme Thrombocytosis	Adverse Event
1	Weakness, Headache, Paresthesias	Yes	Laryngospasm
2	Headache	Yes	---
3	Headache, Fatigue, Paresthesias	Yes	---
4	Erythromelalgia, Headache	Yes	---
5	Nose bleeding	Yes	---
6	Abdominal Discomfort, Splenomegaly	Yes	---
7	Headache	Yes	Pseudotumor Cerebri
8	Fatigue, Itching	Yes	---

Serious Adverse Clinical Events

The most concerning outcomes associated with MPN include severe hemorrhage, thrombotic events, or conversion from another MPN to secondary myelofibrosis, or transformation to acute myeloid leukemia (AML.) None of the eight subjects had a severe thrombotic or hemorrhagic event, and none have transformed to AML.

Subject #1 experienced three episodes of severe laryngospasm, associated with difficulty breathing, anxiety, and fecal incontinence. Neurological evaluation showed no evidence of seizure, stroke or vascular events. He is doing well with dietary modification, speech therapy, psychotherapy, and monitoring by ENT. Subject #7 had an episode of worsening headache and was ultimately found to have pseudotumor cerebri. CNS imaging revealed no evidence of stroke or vascular event and she is doing well symptomatically with medication.

Therapeutic Management

Limited data exists regarding suggested management for pediatric patients with MPN and there is some variation in recommendations regarding when to treat and what agents are first line. Among these subjects, four of them received aspirin during the course of their illness, provided at 2 different medical centers (Table 3.) Six of these subjects received hydroxyurea therapy at some point from six different treating institutions. None of the subjects received alternative cytoreductive agents.

Table 3 – Therapies Prescribed to Pediatric MPN Patients

Subject #	Aspirin	Hydroxyurea	Alternative Agent
1	Yes	--	No
2	Yes	--	No
3	Yes	Yes	No
4	Yes	Yes	No
5	--	Yes	No
6	--	Yes	No
7	--	Yes	No
8	--	Yes	No

Clinical Genetic Evaluation

As part of their diagnostic work-up, genetic testing was performed on all subjects for *JAK2V617F* mutation. Three subjects (#3, #9, and #10) are positive for the *JAK2V617F* mutation, giving a prevalence rate of 37.5%. Of the *JAK2V617F* negative patients, all five were negative for mutations in *MPL*. An additional three patients have been tested for *CALR* mutations and were negative as well, placing them in the "triple negative" MPN category (Table 4.)

Table 4 – Clinical Genetic Testing of Pediatric MPN Subjects

Subject #	JAK2	MPL	CALR
1	*(-)*	*(-)*	*(-)*
2	(-)	(-)	*NT*
3	**(+)**	*NT*	*NT*
4	*(-)*	*(-)*	*(-)*
5	(-)	(-)	*NT*
6	*(-)*	*(-)*	*(-)*
7	**(+)**	*NT*	*NT*
8	**(+)**	*NT*	*NT*

NT = not tested

Genomic Analysis

DNA samples for an initial portion of the subjects were submitted for additional sequencing utilizing a gene panel containing close to 400 genes with potential relevance to MPN and hematologic malignancies (subjects #1,2,4, and 7.) As was seen in commercial testing, patients tested were not found to have any *JAK2*, *MPL*, or *CALR* mutations.

Sequencing demonstrated mutations in various genes of interest in cancer and MPN. Table 5 summarizes a group of mutations identified that may be of interest in this population, including TET2 and EZH2, previously identified in MPN. Of great interest are *JAK1V658F* and *NRASQ61K* (Figure 1), two mutations identified as pathogenic in other malignancies (ALL and Melanoma, respectively.) These particular mutations have not been previously reported in MPN patients and mutations listed below were each only identified in 1 subject. However, some of these were identified in AML patient samples that were sequenced on the same panel of genes.

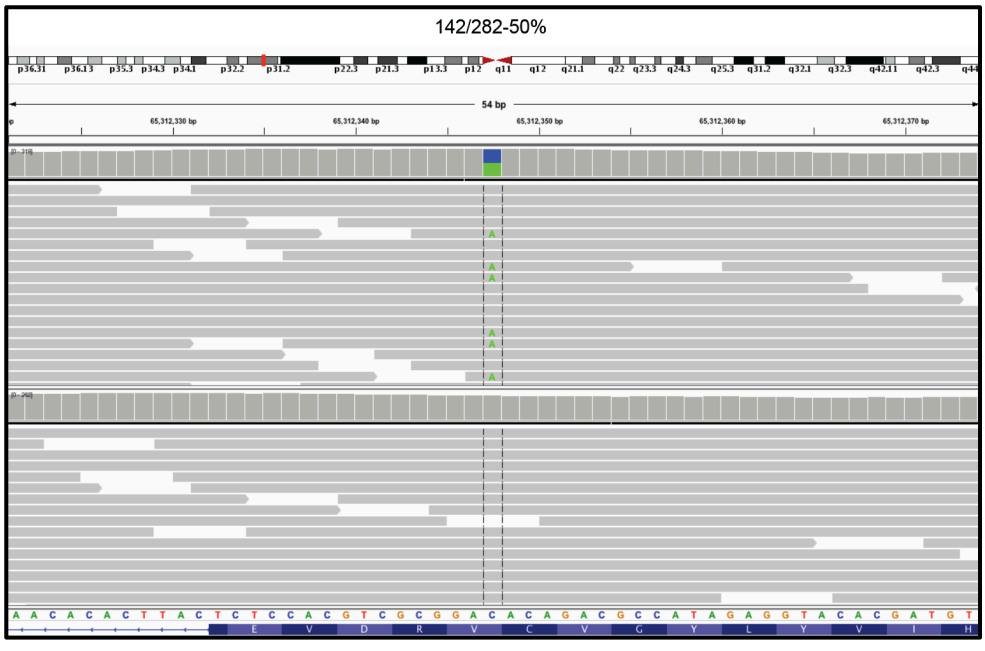
Table 5 – Mutations of Interest in Initial Sequencing Data

Gene	AA Alteration	Tumor Var Frequency	Normal Var Frequency
JAK1	V658F	0.504	0
NRAS	Q61K	0.51	0
EZH2	R207L	0.143	0
JAK3	P151R	0.401	0
TET2	Q1084P	0.345	0
TP53	V274D	1	0
CSF3R	D320N	0.524	0
STAT5B	R294C	0.689	0
ASXL1	E1023D	0.513	0
IL7R	I122V	0.532	0

var = variant

Figure 2. Previously Identified Oncogenic Mutations Found in Pediatric MPN Patients

***NRASQ61K* – Subject #2**

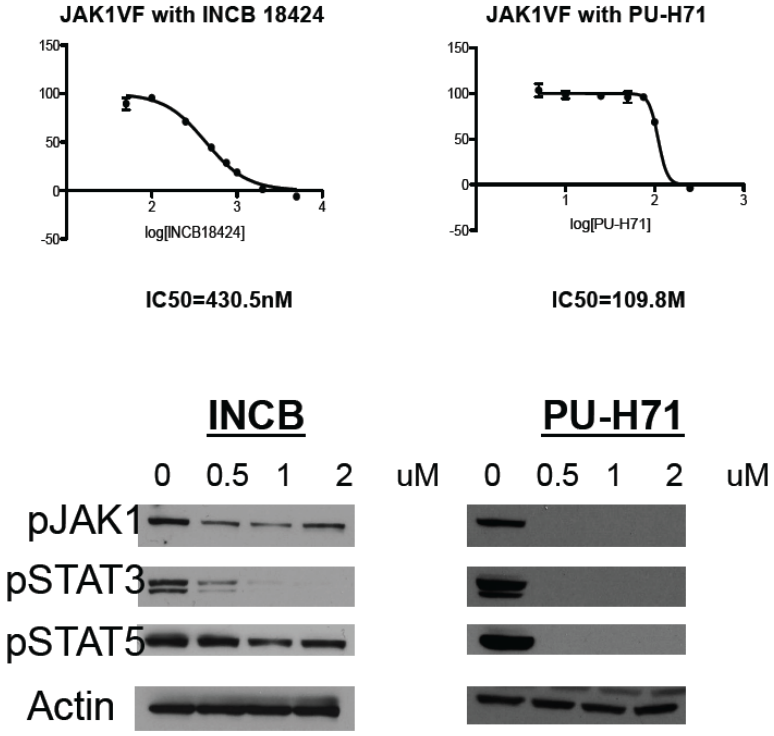


***JAK1V658F* – Subject #1**

Assessment of Individual Mutations

Mutations of interest will be assessed for their potential to be pathogenic, as well as their ability to be inhibited with targeted therapeutics, with *JAK1V658F* being the first mutation evaluated. Ba/F3 cells, a murine interleukin-3-dependent cell line, were successfully transduced with *hJAK1V658F*. Expression of *JAK1V658F* allowed for cytokine independent growth in the transduced cells, and showed constitutive activation of JAK/STAT signaling. Ruxolitinib is a JAK1/2 selective inhibitor that has been evaluated in patients with MPN and malignancies. Treatment of the Ba/F3-*JAK1V658F* cells with INCB18424, the laboratory-grade Ruxolitinib compound, showed dose-dependent inhibition of cell growth in the Ba/F3-*JAK1V658F*. While phosphorylation of JAK1 is not inhibited by treatment with specific JAK inhibition, loss of activation of downstream mediator STAT proteins is observed. HSP90 inhibition has been shown to combat resistance to targeted therapeutics in JAK-mutated disease models, and PU-H71, a purine scaffold HSP90 inhibitor is in clinical trial for patients with MPN and leukemia. Treatment of the Ba/F3-*JAK1V658F* cells with PU-H71 also showed inhibition of cell growth and also inhibited activation of JAK/STAT signaling, including elimination of phosphorylated JAK1.

Figure 3 – Transformation of Ba/F3 Cells With *JAK1V658F*



DISCUSSION

Our results show the spectrum of disease in a small cohort of pediatric MPN patients. It is clear MPN can occur in children of any age. Hereditary disease is less common than sporadic disease. Diagnosis in children with MPN almost always involved a bone marrow evaluation, which is important as the diagnostic criteria of the World Health Organization, the Polycythemia Vera Study Group, and the British Committee for Standards in Haematology all include bone marrow findings as part of their diagnostic criteria. It was recommended to Subject #8 that she undergoes a bone marrow evaluation to ensure she had the proper MPN diagnosis.

Children with MPN show similar symptoms as adults with MPN, such as headache, fatigue, and erythromelalgia. However, none of the subjects have yet experienced any traditional “high-risk” events, including thrombotic events, hemorrhagic events, or transformation to AML. The laryngospasm episodes experienced by Subject #1 and the development of pseudotumor cerebri in subject #8 are not clearly linked to their underlying MPN diagnoses.

Use of aspirin is standardly accepted in patients with PV and is often used in ET patients with microvascular symptoms. Here, two centers utilized low-dose aspirin in four subjects. The use of cytoreductive therapy is generally reserved for “high-risk” patients or those with significant symptoms refractory to aspirin therapy. In our cohort, six different centers started pediatric subjects on hydroxyurea therapy with six of the eight being treated with hydroxyurea. At the time of starting hydroxyurea, all children had extreme thrombocytosis and varied clinical symptoms but no serious clinical adverse events had occurred. Practitioners treating children seem more comfortable

starting cytoreductive therapy in the setting of extreme thrombocytosis rather than not treating.

Our cohort seemed consistent with published literature that rates of known MPN mutations in children are lower than in adults, with lower rates of JAK2, MPL, and CALR mutations. Initial sequencing showed mutations in a variety of genes that have been associated with MPN and myeloid disease, as well as alternative genes that have been associated with other malignancies. The mutations listed above are in genes that can directly or indirectly interact with the JAK/STAT signaling pathway and common downstream mediators. It is possible to assess these genes for their transformative potential and study various potential therapeutics using in vitro models. This implies that alternative genes from those identified in adults may be involved in disease pathogenesis but they can ultimately act through similar and interacting downstream mediators.

FUTURE DIRECTIONS

This project served as a successful pilot to determine if pediatric MPN can be explored further. It provided baseline clinical and genomic data on a small cohort of patients and showed the feasibility of genetic evaluations. Moving forward, there are important questions to answer and additional steps to be taken in order to develop a comprehensive pediatric MPN research program.

The key, principal question to be explored in detail is what type of disease these children actually have. Are pediatric MPN the same diseases as those that affect adults, or are they different? Based on this, I would ask whether the same diagnostic and assessment tools apply to children? Additionally, can management of adults then be extrapolated to children?

The first element of answering this line of questioning is expanding my clinical analysis to tease out hereditary from sporadic diseases. While cohorts of children with both categories of disease will be important to study and will be included in the database, they may prove to show different phenotypes and genotypes over time. Being able to distinguish between these classes of MPN may ultimately be determined by these differences but must initially begin with a thorough family history. To ensure a uniform and detailed analysis of each future subject, I will develop a family history questionnaire that will not only capture those clearly diagnosed with an MPN but will also identify any potential relatives who have gone undiagnosed based on their histories of relevant symptoms, clinical findings, and additional diagnoses (such as unexplained splenomegaly, unexplained thrombosis or hemorrhage, and episodes of MDS or AML.)

To gain insight regarding hereditary versus familial ET, I will confirm an even more detailed family history of subject #1 and will pursue testing of as many of his family members as possible, include germline testing of any healthy relatives (including his sister and paternal aunt) in order to increase my chances of identifying the causative mutations in this family. I will work with the family and the assistance of a statistician and genetic counselor to determine the family members who will need to be included in the evaluation and numbers needed to make significant assessments. Whole exome sequencing and cytogenetic evaluation, rather than a more targeted panel, will be pursued in available family members, and will be analyzed in a similar fashion to the subjects who underwent gene-panel sequencing. Tumor samples will be compared to germline to assess for loss of heterozygosity. Public mutational and SNP databases will be queried to identify potential new mutations. The maternal side of the family has a high penetrance of patients only with ET, while the paternal side has various MPN. Both parents were negative for *JAK2V617F* and this family provides a potentially unique genetic situation that has a high potential for identifying a novel mutation and informing on the pathogenesis of inherited MPNs.

The next step in answering these questions is to obtain more significant information on MPN in children. There are clear criteria for defining MPN in adults, yet as we identify new causative lesions, experts call for revisions of diagnostic criteria[25]. While bone marrow findings seem similar in pediatric and adult patients, the mutations that define disease are likely different, and laboratory values may also have different parameters for diagnosis. By meticulously tracking the symptoms and laboratory findings present at

diagnosis and correlating them with bone marrow and genomic findings, we may identify alternative criteria that define true pediatric disease.

Teasing out the symptoms that are specific to pediatric patients is crucial to allow this to happen. Symptom assessment tools exist for adult patients with MPN, which are important for assessing symptomatic disease burden and treatment effectiveness[26]. Utilizing pilot data obtained from this study, as well as published data on pediatric MPN, I will design a pediatric symptom assessment tool to administer to subjects. It will likely require parental involvement and will incorporate certain pediatric-specific tools such as pain/discomfort assessment scales designed for use in children.

An additional area that can be queried is cytokine profiling in children with MPN. Cytokine up-regulation is a known element of MPN in adults, and is thought to contribute to symptom burden. This area has not previously been explored in children diagnosed with MPN. Therefore, I will evaluate cytokine expression (including IL-6, IL-10, and TNF-α) in both serum and through single-cell assays of harvested blood and, if available, bone marrow cells, utilizing techniques that have previously been described by members of our lab[27].

In addition to determining the similarities and differences in clinical findings between children and adults, additional disease elements will need to be assessed. The genomic analysis will need further evaluation to identify potential driver mutations, as well as co-occurring mutations that may affect disease pathogenesis or progression. Utilizing SNP and mutational databases and assessing transformative ability (as demonstrated above) will be important aspects of identifying those mutations that are of actual relevance. If the gene

panel does not provide answers, whole exome sequencing will likely be the necessary next step.

Evaluating transcriptional profiles will be an important tool to understand disease phenotypes in children and examine the influence of newly identified mutations. I will explore transcriptional signatures in pediatric samples to determine if they show up-regulation of JAK/STAT target genes, as has previously been shown in our lab with adult MPN patients[28]. I will also assess the effect of various mutations I identify on the transcriptional profiles in the in-vitro cell line models I develop to determine how these mutations affect wild-type gene expression to evaluate their potential to cause or alter disease. This will also need to be done on the familial samples for subject #1's family.

Targeted therapy with Ruxolitinib, while not leading to significant decreases in allele burden, has shown significant symptomatic improvement in adults with MPN. It is possible that it can have symptomatic benefits in children without the potential risks associated with Hydroxyurea treatment in these pre-malignant conditions. The Children's Oncology Group has conducted a small Phase I study with Ruxolitinib in children with various malignant and pre-malignant conditions and has shown that it can safely be given. I will plan to set up talks with Novartis, the makers of Ruxolitinib, to develop a small Phase I trial of Ruxolitinib in children with MPN to determine if count improvement and symptom burden improve with a short course of treatment.

To allow me to accomplish any of these goals, I will need to drastically increase my subject recruitment. This will require a multi-faceted approach since these are rare diseases in children. One key resource I have available

to me to reach my pediatric hematology/oncology colleagues is the numerous professional societies I have membership in. I will work with leaders from the Children's Oncology Group, the American Society of Hematology, and the American Society of Pediatric Hematology and Oncology, as well as my mentor Dr. Bussel, to present my research plan to fellow members who likely have MPN patients in their programs. This will also allow me to identify any sample banks that may exist (such as within the Children's Oncology Group) of pediatric MPN or post-MPN samples that I can use in my research.

Direct contact with colleagues will be an important way to make a direct appeal for the importance of including as many pediatric MPN patients as possible in this research program. Working with my mentor Dr. Bussel, I will develop a list of the major pediatric hematology/oncology programs in the country. I will develop a letter explaining the research program, the goals, and what patients are needed. I will also identify key groups from which to request collaborators to help tackle some of the questions we want answered and to help grow the research program, which will be mutually beneficial. I will also identify the appropriate person from each of the large New York programs for me to make a personal appeal to in order to rapidly start acquiring patients and disseminating information about our program. Using the contacts of both Drs. Bussel and Levine and our knowledge of other major MPN research programs, I will reach out to any potential collaborators in major academic centers in the US, as well as in Europe and Asia, to identify any sample banks of patients who were diagnosed with MPNs before the age of 21 years old but may be seen by adult practitioners. It is likely that there are a number of patients with MPNs who were diagnosed earlier in life but due to lack of available experts are followed by adult providers.

Another key area for recruitment will be to appeal to patients themselves. I will do this in a number of different ways. First, I will meet with the leadership of my department and the NYP/WCMC public relations group to help develop a web page for our pediatric MPN program. Social media is a very successful tool to recruit patients for clinical research, and I will make sure that we have a website explaining our goals and plans and providing the appropriate information for interested patients and families to reach us. I will also work with patient organizations, such as the MPN Research Foundation. I have begun speaking to the head of this organization, which is a wealth of resources for patients and researchers. Utilizing their membership and social media sites and meetings, I will plan to disseminate information to patients and providers regarding the opportunities for research. Lastly, I will appeal directly to the families of my patients. A number of them have expressed interest in the research program and often have contact with other families whose children have MPN. With their help I will be able to spread the word to additional families in various parts of the country. Utilizing all these outlets will hopefully allow me to gather a significant number of samples to answer the questions laid out above and develop my expertise in this neglected area of pediatric hematology/oncology.

CONCLUSIONS

Children with MPN have similar common clinical symptoms to adults, yet have less frequent episodes of serious adverse events. Pediatric hematologists frequently utilize hydroxyurea in the management of MPN in children in the absence of adverse clinical events. These children have lower rates than adults of known pathogenic mutations, but may have driver mutations in genes that interact with similar pathways. Further evaluation of both phenotypic and genotypic outcomes in this population is needed to allow for true characterization of the nature of these diseases in children, and to ultimately allow for the development of comprehensive pediatric MPN programs and accepted management guidelines.

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