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Characterization of the Puerto Rican cohort of Essential Thrombocytemia patients and the use of this disease as the theoretical context of high school students' short scientific interventions.

BY

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Abstract

Essential Thrombocytemia (ET) is part of the spectrum of myeloproliferative disorders that affect the myeloid progenitor cells. This neoplasm is characterized by a cytokine overproduction, persistent thrombocytosis, thrombotic events, and the presence of mutations that constitutively activate the JAK-STAT pathway such as JAK2V617F, CALR, and MPL. Unfortunately, the characterization of the mutational, cytokine, and thrombotic profile of the ET Puerto Rican sub-population is still totally unexplored. For this reason, we determined the mutational background of ET Puerto Rican patients and then we analyzed the levels of 37 analytes including cytokine, thrombotic factors, and growth factors. Because this research topic allows the discussion of a variety of molecular biology concepts, this framework was used as a platform to introduce high school students to a wide range of molecular biology topics and experimentation.

Since CALR mutation is the most recently discovered mutation and its mechanism is not completely understood, we use this topic to introduce high school students to cutting edge science. Students were able to develop an ET CALR cell line using CRISPR-Cas9 gene modification tool. To identify if authentic research experiences were the key to improve students' competences and performance, one group of students were part of the research experience and other group was part of research demonstrations and simulations. As part of the experience, we were able to validate a Spanish Science Identity Survey that was used to determine the influence of scientific experiences on students' science identity. The use of this platform allows us to understand how scientific experiences influence students and which components of scientific experiences are key to developing students' science identity.

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"Commit to the LORD whatever you do,

and he will establish your plans".

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To my lovely son Natanael

Manuscripts

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1. Shui B, **Hernandez-Matias L**, Guo, Y, Peng Y. (2016) The Rise of CRISPR/Cas for Genome Editing in Stem Cell. Stem Cells International.
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List of Abbreviations

AGFI-adjusted goodness-of-fit index

ASXL1- additional sex combs like 1

b-FGF Basic Fibroblast Growth Factor

CAD- Coronary artery disease

CALR-calreticulin

CFI- comparative-fit index

CHF- Congestive Heart Failure

CNS- Central Nervous System

CRISPR-Cas9- clustered regularly interspaced short palindromic repeats- CRISPR associated protein 9

crRNA- CRISPR RNA

CURE- Classroom Undergraduate Research Experience

DBER- discipline-based educational research

DEPR-Department of Education of Puerto Rico

DIC- Disseminated intravascular coagulation

ET- essential thrombocythemia

GM-CSF- Granulocyte-macrophage colony-stimulating factor

GPVI- Glycoprotein VI

GPCR- G protein-coupled receptors

gRNA- guide ribonucleic acid

HGF- Hepatocyte growth factor

HR- Homologous repair

IMF- chronic idiopathic myelofibrosis

IP-10- interferon γ-induced protein 10 kDa

JAK2V617F- mutation on Janus Kinase 2

KMO-Kaiser-Meyer-Olkin, measure

LCAS-Laboratory Course Assessment Survey

LNK- lymphocyte adaptor protein

LOX- lysyl oxidase

MCP-1- Monocyte chemoattractant protein 1

META- Medición y Evaluación para la Transformación Educativa.

MI- Myocardial Infarction.

MIP-B- macrophage inflammation protein

MOSS- McCall Outdoor Science School

MPL- myeloproliferative leukemia protein

MPN- myeloproliferative neoplasms

NHEJ- Non-Homologous End Joining

PAM- Protospacer adjacent motif

PDGF-BB Platelet-derived Growth Factor-BB

PI3K- Phosphoinositide 3-kinases

PMF- Primary Myelofibrosis

POS-Project Ownership Survey

PV- Polycythemia Vera

Ras-MAPK family of related proteins-(mitogen-activated protein kinases

RMSEA- root mean square error of approximation

SIS- Science Identity Survey

SISE- SIS-Español

SOCS- Suppressor of cytokine signaling

SRMR-standardized root mean squared residual

STAT- signal transducer and activator of transcription

sTLT1- soluble TREM-Like Transcript

SURE-Survey of Undergraduate Research Experiences

TALEN- Transcription activator-like effector nucleases

TET2-Ten-Eleven Translocation 2

TLT-1-TREM-like transcript-1

TNF-alpha- Tumor necrosis factor

trRNA- trans-activating crRNA

UPR- Universidad de Puerto Rico

URSSA-Undergraduate Research Student Self-Assessment

VEGF- Vascular endothelial growth factor

VWF- von Willebrand factor

ZFN Zinc-finger nucleases

Chapter I

Introduction

The majority of the human myeloproliferative disorders (MPD) are caused by an acquired somatic mutation of myeloid hematopoietic progenitor cells [1]. MPD includes essential thrombocythemia (ET), polycythemia vera (PV), chronic myelogenous leukemia, chronic neutrophilic leukemia, primary myelofibrosis (PMF), chronic eosinophilic leukemia-not otherwise specified, and MPN unclassifiable [2]. ET is diagnosed in around 47 out of every 100,000 persons in the United States (2008 to 2010) [3]. This disorder mainly affects the mid-fifty year old population and is often associated with a persistent thrombocytosis (platelet count is higher than 450x109/L), thrombotic events, cytokine overproduction and three different somatic mutations: Janus Kinase 2 (JAK2V617F), myeloproliferative leukemia protein (MPL), and calreticulin (CALR) [4-9]. In the following sections a description of each of these ET hallmarks will be provided; starting with a basic definition of each of the concepts followed by the current knowledge of this topics on ET neoplasm. After the description of ET hallmarks, an overview of genetic modification tools and education will be presented as a novel strategy to understand and advance the knowledge of ET neoplasm and science education.

1. Essential Thrombocythemia

1.1 Inflammation

Inflammation is the coordination of different steps against a harmful stimulus such as: a physical or chemical injury, irradiation, infection, cell damage or thrombosis [10, 11]. It is considered a response to any threat to the integrity of the cellular hemostasis [12]. In the event of an infection, immune host cells can recognize harmful stimuli well as the incidence

of any cellular damage by specific transmembrane receptors called pattern-recognition receptors (PRRs) [13]. The activation of PRRs induces the production of pro-inflammatory mediators. These mediators recruit macrophages and neutrophils at the site of injury where they release more pro-inflammatory mediators as cytokines [14]. Subsequently, the production of myeloid cells, or myelopoiesis, is stimulated by the harmful stimuli to produce the sufficient cells to destroy damaged cells or infectious pathogens. In addition to the rise in this population, an increased production of red blood cells and erythroid progenitors cells are seen, as well as a further production of inflammatory cytokines [15]. These series of steps act by removing harmful stimuli and initiating the recovery and healing process [16]. After the harmful stimulus is eradicated a reversion of the inflammatory response takes place where the mononuclear cell population, which include macrophages and lymphocytes, return to normal preinflammation numbers and phenotypes [16]. If infection persists and becomes chronic, then a prolonged production of lymphoid and myeloid cells is seen, which increases inflammatory mediators and the propensity of inflammatory diseases and DNA alteration [14, 15].

1.2 Cytokine and inflammation as key players in ET

Cytokines are small proteins that are released by cells as a response to injury or infection [17]. These proteins interact and communicate between one another in an autocrine, paracrine and/or endocrine manner [17]. Some of these small proteins can be classified as pro-inflammatory or anti-inflammatory [10, 17]. As their name suggests, pro-inflammatory cytokines are involved in the up-regulation of the inflammatory response. In contrast, anti-inflammatory cytokines down regulate the pro-inflammatory response maintaining homoeostasis for proper functioning of vital organs [18].

Patients with ET neoplasms are in a constant chronic inflammatory state that results from disruption of immune signaling pathways leading to overproduction of inflammatory cytokines [9, 19]. Not just the neoplastic clones (cells that harbor JAK2V617F, MPL, CALR, and/or other mutations associated with MPD) but also bystander immune cells are responsible for MPD cytokine overproduction [19]. MPD neoplastic clones enhance their own survival and growth by increasing the production of pro-inflammatory cytokines and creating a resistance to growth inhibitors. Bystander immune cells, as M2 macrophages, platelets, neutrophils, and T-lymphocytes, are also involved in the process of inflammation and cytokine production of IL-6, IL-8, and Oncostatin M (OSM) [6, 9, 15, 20]. The cytokine over-production seen in MPD patients may promote the proliferation of neoplastic clones creating a loop where neoplastic clones can induce inflammation promoting their selective advantage [10, 15].

One of the first lines of evidence pointing to the relevance of cytokines in myeloproliferative disorders was the study conducted by Panteli et al. This study discovered, using enzyme-linked immunosorbent assays (ELISA), that the serum derived from patients with MMM, ET, PV and CML contained significantly higher levels of IL-2 and sIL-2Ra than healthy subjects [21, 22]. Also Panteli et al. stress the correlation between the higher levels of IL-2, sIL-2Ra and IL-6 and disease progression of ET and PV to myelofibrosis [22]. Subsequently, a study conducted by Tefferi et al. revealed the correlation between IL-2, HGF, IP-10 IL-1, b-FGF and MPD leukocytosis [8]. Similarly, studies on ET disorder have revealed the correlation between PDGF-BB and red cell count and IL-6 and platelet count [6, 23].

Cytokine overexpression in MPD, regardless of its mutational background, has been correlated with neutrophil chemotaxis, patient survival, cell count, fibrosis, and angiogenesis among others [24]. It is evident that cytokine overexpression plays a very important role in MPD disorders and research has been focused to characterize ET cytokine profiling. Recent articles have shown that cytokine expression levels and growth factors such as: IL-1β, IL-1α, IL-2, IL-4, IL-6, IL- 8, IL-10, IL-12, GM-CSF, IFN-g, MCP-1, PDGF-BB, TNF- α, VEGF, and HGF are significantly increased in ET patients [24, 25]. Others have focused on the cytokine profile differences of patient's mutational background. For example; in JAK2 positive ET patients, the levels of platelet-derived growth factor (PDGF-BB), IL-8, and IL-9 are higher than in JAK2 negative patients. In contrast, IL-10 and IL-12 levels are higher in JAK2 negative patients than in JAK2 positive patients [6, 25]. In vitro JAK2V617F can increase the production of IL-6, IL-8, IL-9, OSM, CCL3, CCL4, and TNF-α, but if these cytokines are directly under the control of the JAK2 mutation is not known [15].

Although ET cytokine measurements have been reported, the ET profiling of some interferon, thrombotic factors, and interleukins remains undetermined (Appendix 1). Moreover, the characterization of the mutational, cytokine, and thrombotic profile of the Puerto Rican sub-population with ET is still completely unexplored.

1.3.1Thrombotic events

In addition to cytokine overproduction, ET patients also present thrombotic events; which are the primary cause of morbidity and mortality in MPD patients [26, 27]. A thrombotic event results from the abnormal function of the highly regulated system of hemostasis. This regulated system helps to maintain the integrity of the blood and vasculature [28]. Abnormal hemostasis leads to arterial or venous thrombus formation. Arterial thrombi are mainly

composed of platelets and occur in vascular wall injury. In contrast, venous thrombi are rich in fibrin and dependent on coagulant factors and blood flow limitation [29]. In normal conditions, the system inhibits thrombus formation by the release of anti-thrombotic factors such as thrombomodulin, plasmin, and the inhibition of platelet aggregation by prostacyclin and nitric oxide [29]. Upon endothelial damage, platelets can be activated by the interaction with collagen, adenosine diphosphate (ADP) thrombin, and thromboxane A_2 [30]. Platelets interact with collagen through the collagen receptor glycoprotein VI (GPVI) to initiate a strong activation. Platelets release ADP in response to collagen and thrombin. ADP released from damaged endothelial cells and activated platelets act on platelet $P2Y_1$ and $P2Y_{12}$ G protein receptors [32], which further induces platelet activation and release granule content (such as P-selectin, TLT-1, VWF, fibrinogen, ADP, ATP, GDP, 5-HT, pyrophosphate, magnesium, platelet factor 4 and calcium) to further propagate the activation and aggregation of platelets [33]. Also platelets synthesize thromboxane via cyclooxygenase-1 to intensify platelet calcium mobilization [31].

Thrombin has a multitude of actions in regards to platelets. First, it activates platelets by the cleavage of G protein coupled protease-activating receptors, PAR1 and PAR4. Thrombin also cleaves fibrinogen to produce the self-polymerizing fibrin. Upon platelet activation, the GP IIb/IIIa receptor results in the cross-linking of fibrinogen promotes platelet aggregation [30, 34]. To further stabilize platelet plugs, thrombin activates Factor XIII to catalyze the formation of covalent crosslinks between γ glutamyl and εlysyl residues on adjacent fibrin chains in polymerized fibrin [35, 36]. Moreover, the binding of thrombin to thrombomodulin results in the activation of Protein C, which has anti-thrombotic and anti-inflammatory properties.

1.3.2 ET Thrombotic events

Normal platelet counts in healthy individuals are between 150,000–450,000 per microliter of blood [37]. In contrast, ET patients usually have more than 450,000 platelets per microliter of blood. Clinical factors that contribute to increased risk of thrombosis are age, previous history of thrombotic events, obesity, hypertension, and an increase in blood cell count. In addition to these clinical factors, the expression of blood cell procoagulant, pro-inflammatory cytokines, and the increase of adhesion molecules and receptors may induce thrombotic events. In ET patients there is an increase of pro-coagulant microparticles (i.e. GPIX, GPIb, GPIIIa, and P-selectin) and a reduction of protein C [38-40].

In particular, ET neoplasm has hemostatic abnormalities in blood and vascular cells. For example, platelets and neutrophils are in a constant activated state [40]. Platelet activation in MPD patients is detected by the expression of surface P-selectin, tissue factor and the increased number of phagocytosed platelets by neutrophils and monocytes. In addition, platelet activation in MPD patients is detected by B-thromboglobulin and platelet factor 4 in plasma and thromboxane A2 metabolites in the urine [41]. Increased plasma levels of granule-derived proteases (elastase and myeloperoxidase) are detected in ET as a result of neutrophil activation [41, 42]. These series of steps increases granule-derived proteases and reactive oxygen species secreted from leukocytes that induce the detachment and/or lysis of endothelial cells [42-44]. This event further promotes the formation of platelet aggregates and increased levels of thrombin [43].

1.3.3 Lysil oxidase (LOX)

Studies looking to dissect the correlation between molecular markers and thrombotic risk have found that the overexpression of an enzyme called lysyl oxidase (LOX) is implicated in thrombus formation [26, 45]. LOX is a copper amine oxidase enzyme that crosslinks

collagen and elastin to provide connective tissue stability by oxidative deamination of peptidyl lysine or hydroxylysine and peptidyl lysine residues, respectively. Other proteins have been discovered including LOXL1 (LOX like-1), LOXL2, LOXL3, and LOXL4 and all of them have a cooper binding site, and a cytokine like receptor. Specifically, LOX gene encodes a 50 kDa pro-enzyme that is further proteoliticaly cleaved by C-proteinases to a 30kDa LOX enzyme and 18kDa N-terminal propeptide [43]. LOX also promotes the intrapeptide and interpeptide chain cross-linking that contributes to the accumulation of extracellular matrix [26, 27, 45].

LOX is expressed in low ploidy megakaryocytes and induces their proliferation by the oxidation of the PDGF receptor [26]. LOX overexpression can increase platelet adhesion to collagen suggesting that higher levels of LOX may be associated with a higher risk of thrombotic events [26, 27]. To study LOX effects on thrombosis and myelofibrosis without other influences of MPD, Matsuura et al. created a transgenic mice line (Pf4-Lox $^{tg/tg}$) that expressed LOX on megakaryocytes and platelets. Using this line they found that megakaryocyte ploidy is not affected, but the total number of low ploidy megakaryocytes is increased. To further understand if LOX prompts thrombus formation, this group induced endothelial injury using $FeCl_3$. Results showed that Pf4-Lox $^{tg/tg}$ mice had complete occlusion in a shorter time of than WT mice [45]. Indeed, Pf4-Lox $^{tg/tg}$ mice platelets have a greater potential to adhere to collagen than WT platelets. To further explore collagen aggregation, Matsuura et al. treated Pf4-Lox $^{tg/tg}$ mice with BAPN (a LOX inhibitor). They found a restored adhesion of platelets to monomeric collagen as seen in WT platelets. Interestingly, platelet aggregation to thrombin, adenosine 5'-diphosphate and thromboxane A_2 was similar for Pf4-Lox $^{tg/tg}$ and WT platelets as well as the amount of glycoprotein

(GP)VI and integrin $\alpha_2\beta_2$ which adheres to collagen. However, Pf4-Lox $^{tg/tg}$ platelet adhesion to integrin $\alpha_2\beta_2$ substrate was higher than WT platelets. This may indicate the association between LOX and integrin a_2B_2. Interestingly, neither myelofibrosis nor splenomegaly was detected in this model.

In an attempt to understand the biological role of LOX proteins in MPD, Tadmor et al. measured the expression of all 4 LOX genes together in PMF, ET, PV, and PML. Results showed no difference in the expression of different LOX. They further measured LOX levels in serum of PMF patients and they found that PMF patients had higher levels of LOX than healthy patients. Unfortunately, LOX levels in ET patients' serum were not addressed.

1.3.4 TREM like transcript-1 (TLT-1)

Another potential marker involved in thrombus formation is the triggering receptor expressed on myeloid cells (TREMs) transcript-1 (TLT-1) [46]. TLT-1 is a 34kDa protein composed of immunoglobulin-like extracellular domain, a transmembrane region and a cytoplasmic domain. TLT-1 is found in the alpha granules and upon platelet activation it is released to the platelet surface. In human serum, a smaller soluble TLT-1 isoform of 15 to 17 kDa is found (sTLT-1) [47]. This isoform has been defined as a marker for disseminated intravascular coagulation (DIC), a condition that causes thrombosis and bleeding due to an imbalance of platelet and coagulation factor aggregation throughout the body [46, 48].

Higher levels of sTLT-1 have been found in patients with sepsis as compared to healthy patients [48]. Together with this result Washington et al. found that sTLT-1 augments platelet aggregation. Also, Morales-Ortiz et al. found higher sTLT-1 levels in patients with acute respiratory distress syndrome (ARDS) than in the healthy comparison

group [49]. In these experiments, they also found that sTLT-1 had a significant inverse correlation with platelet counts and that TLT-1 was a prognostic factor for ARDS patients' survival [50]. Interestingly, Morales-Ortiz et al. found that patients with systemic lupus erythematous (SLE) had lower sTLT-1 levels in plasma than healthy controls [49]. They hypothesized that SLE patients may produce antibodies against TLT-1 [49]. As well as LOX, sTLT-1 levels in ET patients are unknown as well its association with ET thrombotic events.

1.4 ET patients' mutational background

1.4.1 JAK-STAT pathway

ET is associated mainly with JAK2V617F, MPL, and CALR mutations[4-9]. All of these mutations induce cell proliferation through the **Ja**nus **k**inase- **s**ignal **t**ransducer and **a**ctivator of **t**ranscription (JAK-STAT pathway). The Janus Kinase family comprises 4 different kinases: JAK1, JAK2, JAK3 and TYK2 [51]. JAK kinases possess an active kinase domain (JH1) and a pseudokinase domain (JH2) acts as the negative regulator of the JH1 domain [52]. There are 7 different STAT proteins (STAT1, STAT2, STAT3 STAT4, STAT5a, STAT5b, and STAT6).

The JAK-STAT mechanism starts after the JAK binds to one subunit of a stimulated receptor. As a result of receptor subunits multimerizing as homodimers (erythropoietin and growth hormonse) or heterodimers (interferons and interleukins), JAK kinases are brought into proximity and are able to trans-phosphorylate and phosphorylate the receptor [53]. JAK kinase also phosphorylates STATs in a tyrosine residue near the C-terminus. As a result, STATs dimerize through the interaction of the SH2 domain. After STAT dimerization, it translocates into the nucleus through nucleoprotein or Ran mediated import. Inside the

nucleus STATs bind DNA and regulate transcription to stimulate cell proliferation, differentiation, cell migration and apoptosis [53, 54].

1.5.2 JAK2V617F mutation

JAK2V617F mutation is located in 9p24.1 and was discovered in 2005. This mutation is present around the 57% of the ET patients and is mostly present in its heterozygous nature in ET patients and homozygous in PV patients [4, 55]. JAK2V617F changes a phenylalanine for a valine at the 617 position of the Janus kinase 2, where the negative regulatory domain of this protein is present [56]. This gain of function mutation induces a cytokine-independent activation of the JAK2-STAT3, pathway, which in turn increases cell proliferation and induces hypersensitivity of the hematopoietic progenitor cells [25, 57, 58]. JAK2 specifically attaches to the following receptors: erythropoietin (EPO-R), MPL, and G-CSF (myeloid receptors), prolactin and growth hormone, IL-3, IL5R, and IFN-y R2 [53].

1.4.3 MPL mutation

MPL or the thrombopoietin receptor is encoded by the myeloproliferative leukemia gene that is located on chromosome 1p34 [59]. MPL is responsible for megakaryocyte development, mobilization of hematopoietic stem cells, megakaryocyte proliferation and differentiation [60]. MPL exon 10 mutation, which is present in around 3-5% of the ET patients, encodes for the receptor of thrombopoietin. [4, 61, 62]. The two main mutations on the MPL receptor are MPLW515L/K and MPLS505N mutations. MPL exon 10 mutation MPLW515L/K results in a substitution of tryptophan on the 515 position to a leucine, lysine, asparagine or alanine in the cytoplasmic region near the transmembrane domain and prevents the spontaneous activation of the receptor [60]. In contrast, MPLS505N mutation is found at position 505, where a serine is changed to an asparginase in the transmembrane region [63].

MPLW515L/K and MPLS505N induce an autonomous activation of MPL receptor and work through JAK2-STAT1 and JAK2-STAT3 pathway increasing megakaryocyte proliferation [60]. ET patients with MPL mutations have higher platelet count and megakaryocytic proliferation than JAK2 positive patients [64]. Although not fully understood, ET patients have lower surface expression of MPL receptors than healthy patients and this may influence the hyperactivation of MPL receptors as consequence of MPLW515L/K and MPLS505N mutations.

1.5.4 CALR mutation

The second most common mutation on ET patients is found in Calreticulin (CALR) exon 9. CALR mutation is present around the 25% of ET patients and it is not present in PV[4]. The 36 different CALR mutations on ET patients consist of frameshift mutations that change the C-domain of the CALR protein from negative amino acids to positive amino acids and eliminate the KDEL endoplasmic reticulum retention signal [4, 61]. Mutant CALR is found on the cell surface and induces an activation of the thrombopoietin receptor [65]. Subsequently, this step activates cell proliferation through the JAK2/STAT5 pathway. The most common mutation in ET patients is CALR type I CALR type II, and CALR type III. CALR type-1 is a 52-bp deletion (p.L367fs*46), type-2 is a 5-bp TTGTC insertion (p.K385fs*47), and type- 3 is a 3-bp deletion (L367fs*48).

A comparison between JAK2 positive and CALR positive patients show that CALR patients have lower risk of thrombosis, longer survival, and lower white-cell counts [66]. Lower white-cell counts can be explained because CALR is present in higher concentrations in the membrane when it is mutated and subsequently neutrophils having CALR in the extracellular membrane are phagocytized [67]. Although a wide range of research has been focused on the elucidation of CALR mutant function, not much is known. Using the era of

gene modification tools, a CALR cell line could be engineered to understand which normal functions of CALR are impaired and which are those mechanisms.

1.4.5 Other mutations on ET patients

Other mutations are also found in ET patients such: ten-eleven translocation 2(TET2) [68], lymphocyte adaptor protein (LNK), suppressor of cytokine signaling (SOCS) genes, and additional sex combs like 1 (ASXL1), but these are not specific to MPD disorders since they are present in other blood malignancies [51]. TET2 is located in 4q24 and it is present in 4-7% of ET patients [68]. TET2 regulates gene expression by silencing of DNA methylation. Mutations in LNK are located 12q24 and are found in less than 5% of ET patients. LNK mutations result in the absence of PH and SH2 domains or in a missense of PH domain, which ultimately inhibit partially or totally the capacity of LNK to regulate TPO signaling [69]. SOCS proteins are E3 ubiquitin ligases and they work as negative regulators of JAK signaling. SOCS mutations are rare, but epigenetic modifications as hypermethylation of CpG island on SOCS1 and SOCS3 are seen [70]. It is not clear if this epigenetic silencing is a result of JAK2V617F or if it is one of the drivers of ET. ASXL1 is located in the 20q11.21 and is present in less than the 4% of ET patients [71]. ASXL1 function is chromatin modification, but its role in human hematopoiesis is unknown [51].

2. Genetic engineering tools

2.1 Before CRISPR-Cas9

Different genetic engineering tools facilitate the target manipulation of the DNA. Target gene manipulation, usually, helps researchers to understand the function of what has been manipulated. Some gene editing manipulations consist of deletions, insertions, inversions, and replacements of specific DNA regions. Systems such as transcription activator-like

effector nucleases (TALEN) and Zinc Finger Nucleases (ZFN) have been used to perform gene editing manipulations. Both are composed of two main elements: (1) proteins that recognize specific nucleotides (TALEN) or groups of three nucleotides (ZFN) and (2) endonucleases (FokI) that cut the target region once the proteins recognize the target nucleotides [72]. Although, these systems are effective, a new system called clustered regularly interspaced palindromic repeats or CRISPR-cas9, has been discovered, which have a great advantage over TALEN and ZFN because it is easier to make and less time consuming [72].

2.2 CRISPR system as the bacteria defense system

The CRISPR system was first discovered in bacteria. It works as an "adaptive immune system" where bacteria recognize viral DNA or RNA and keeps a memory of the infection. This "memory system" can destroy viral DNA or RNA if a re-infection occurs in the same bacteria or in its descendants. [73-76] There are three different types of CRISPR but type two CRISPR is the most used by researchers. Type II requires a smaller amount of CRISPR-associated proteins (Cas) to perform its function [73, 77, 78]. It works as follows: When viral DNA enters into the bacteria, Cas1 and Cas2 bacterial proteins recognize the viral DNA where the protospacer adjacent motif (PAM) is present (key identification component that the bacteria use to differentiate between self from non-self) [74, 75]. The piece of viral DNA that has been recognized is inserted in the CRISPR loci of the bacteria.

Once it is inserted, it is transcribed and processed to form a CRISPR RNA (crRNA) that works together with a small sequence of transcript RNA (trRNA) that guides the system to the target homologous sequence [77]. Because of the function of this two RNA sequences together, they are called guided RNA (gRNA). Cas9 is able to hold the gRNA and search for

homologous complementary regions [72]. When the PAM region is recognized in the target DNA a destabilization in the target homology DNA occurs which causes the Watson and Crick base pairing between gRNA and the target DNA (the first 8 to 12 base pairs downstream PAM are the most important homology regions, although mutations have been found when less than 8 base pairs homology is achieved) [17, 78-80]. As soon as the complementary region is found, the endonuclease Cas9 makes a double strand brake. Cas9 HNH domain cleaves the complementary DNA strand and Ruvc domain the non-complementary DNA strand within three base pairs upstream PAM [78-80]. As a result, the viral sequence is destroyed [73].

2.3 CRISPR-Cas9 genetic engineering tool

Researchers have used a similar CRISPR-cas9 approach to manipulate the genome of cells, plants, animals, and humans [81]. The CRISPR-cas9 system can induce double stranded breaks into the programed sequence of interest. Non-homologous end joining (NHEJ) or Homologous Recombination (HR) can fix the double stranded brakes (DSB) [81]. The NHEJ mechanisms can insert or delete regions of the DNA in a non-precise manner. In contrast, Homologous Recombination (HR) requires a donor sequence to repair the damage and usually this method is less error prone than NHEJ mechanism [81]. In order to identify the presence of the desired mutation, cells need to be screened [82].

Since, cell line development using CRISPR-Cas9 technology allows the discussion of a variety of molecular biology concepts and techniques, this framework not only could be used to understand CALR mutation, but could be used as an excellent platform to introduce developing scientists to a wide range of molecular biology topics. The use of this platform to develop an authentic research experience could help us to understand how authentic research

experiences influence students' scientific competence and performance and may shade light about the STEM education problems in Puerto Rico, and possible solutions.

3. Overview of high school education in Puerto Rico

3.1 History of education

Education is the process of the acquisition of knowledge and skills in formal and informal environments. In the 16^{th}-17^{th} century the education in Puerto Rico was just for wealthy families. Those families that were not able to pay for the education of their children had to train their kids to work in agriculture or other family trades [83]. In the 1880, as an attempt to diminish educational disparities, all kids from six to nine years old were obligated to go to school, although the amount of schools and their localization were not accessible for many kids. In 1900, after the American colonization of Puerto Rico, the Foraker law, officially known as the Organic Act, reshaped the organization of the educational public system. This law created the "Departamento de Instrucción Pública". The goal of this reorganization was to make the education available to all socioeconomic classes. Despite this effort, the education of low-income kids was very limited [84]. In 1952, the constitution of Puerto Rico was approved, and it included educational rights and established that education was free and mandatory for all. Since, the resources for education were limited, the government was focused on educational programs as "Escuelas Ejemplares" that were aimed to provide high quality education for larger populations [83]. In the 70s and 80s the administrative structure of the educational system changed leaving aside the efforts for a better education of larger populations. In 1990, after the approval of the "Ley 68 Reforma Educativa" schools had more power to make decisions towards their students' education. As a result of this law the "Departamento de Instrucción Pública" changed its name to the "Departamento de

Educación". Throughout the years, multiple amendments were incorporated to this law to improve the equity and efficiency of the public education.

In 2002, President George Bush signed the No Child Left Behind Act in order to promote academic achievement for every student [85]. As a result of this law every school in the United States, including Puerto Rico, had to test students in math and reading to identify their needs and make the necessary changes to bring student achievements to a proficiency level. It was expected that by 2015, students would be able to demonstrate a solid academic performance and justify their answers with evidence and reasoning. Unfortunately, student achievement in many states did not reach proficiency for the expected 100% of its students. Therefore, in 2011 President Obama gave waivers to several States and PR, in order to no longer require schools to aim for this goal of 100% proficiency by the previously established deadline [85]. Reports from 2007 to 2011 have shown an increase in alphabetization and gender equality. Unfortunately a relationship with students' low academic performance and poverty remains, demonstrating a disparity with regards to education access.

As an effort to promote scientific culture among students, in 2014 "Los estándares y expectativas del programa de ciencias" established a new reform of education that helped students to prepare themselves for the workforce. Six years after President Obama signed these waivers, standardized test results in the United States, including Puerto Rico, are still below the expected value of proficiency. For example, in Puerto Rico just 35% of the high schools have a satisfactory performance [86]. In terms of high school retention, just 68% of Puerto Rican students that start 10th grade, graduate in 3 years [87]. The education and retention of Hispanic students in STEM careers is a challenge, as is the low scores in mathematics, science, Spanish and English (as a second language) of the standardized

META (Measurement and Evaluation for the Academic Transformation of Puerto Rico) test [86].

To overcome educational challenges a new public policy “Ley de Reforma Educativa de Puerto Rico” has been signed. This law “will address the current and future needs of our society; reformulate the education system according to the student as the center and main axis of education; establish a budget based on the average cost per student to ensure that each student receives the same investment of resources in their education; establish the Public Schools Alliance to give access to a greater academic offer to students through specialized non-profit entities that can strengthen the curriculum and teaching, and allow communities, including parents, to play a role more active in the education of their children”[88]. Also this law establishes the collaboration “agreements with the National Center for Astronomy and Ionosphere (NAIC) and the National Science Foundation (NSF), in order that all students of the Puerto Rico Education System have the opportunity to visit the Arecibo Radio Telescope to strengthen their studies in science and astronomy" Also, within this timeframe, science research programs for high school students, as well as tutoring and teacher professional development programs have become available.

Standardized test scores on the transformation and education of Puerto Rico (META test) from Puerto Rican Students in science are below the expected value. In general, secondary education in Puerto Rico is facing (i) an expansion in the number of students and teachers while public funds for education are decreasing (ii) the rigidity and inertia of academic disciplinary structures (iii) the need for institutional and professional constructive evaluation processes and training (iv) the difficult relationship with the world of education and the demands of the workforce [83]. Some of these challenges mirror the science

education challenges reported by Anderman as the availability of appropriate resources and the preparation and training of science teachers [89]. These deficiencies influence students' science competences and performance and there is a need to overcome these challenges not only for the scientific workforce, but to have a society that respects and values our natural resources, a society that looks and studies previous data before making a decision, a society with a scientifically responsible culture.

4. Science Identity

4.1 Identity

Identity can be described as a series of representations that gives meaning to the role of individuals or groups [90]. According to Gee (1991) it is "the kind of person one is seeking to be and enact in the here and now" [91]. Gee defines identity using four different groups: Nature-identity, Discursive-identity, Institutional-identity, and Affinity-identity. He defines Nature identity as an imposed identity powered by nature as genetic traits. Discursive-Identity is described as a trait that the person chooses and is not forced into by law or institution as being enthusiastic or positive, for example. Institutional-Identity is the given positions that are set by authorities, for example a work position. The Affinity identity is described as a set of practices of an individual, where the individual as part of this group participate in specific practices. A combination of these identities in a specific time and place are used to recognize a person as part of a community. It is important to recognize that the time and place where these identities manifest is key since conceptions of identities and connotations change over the time. A key component of an identity to be "identity" is that the community in the given time and place recognize these traits. For this reason,

identity is in constant change, it emerges, evolves, and incorporates societal structures, but at the same time, endures over the time and context [92].

4.2 Science Identity as defined by Carlone and Johnson

Carlone and Johnson's approach to conceptualize science identity uses the Gee model of identity and it is informed by the following question: "How would we describe a person who has a strong science identity?"[92]. They define the science identity concept as the kind of person that "makes visible to (performs for) others one's competence in relevant practices, and, in response, others recognize one's performance as credible"[92]. Carlone and Johnson's (2007) model of science identity had three main components or dimensions: competence, performance, and recognition [92].

4.2.1Competence

The perception of the student's competencies in research is defined as how the student perceives what he learns and understands. Different key points have been shown to influence the academic performance of students as being part of underrepresented groups, stereotype and discrimination, induced anxieties, financial constraints, low family and faculty expectations or support, low social and academic college integration, inculcation of enthusiasm, effective mentoring, and research experiences [93-95]. Competence brings in accountable factors like logical, academic, analytical, cognitive, and retention skills in science disciplines [96]. Experiences within educational and practical settings, like research, enhanced academic outcomes and critical thinking [96] .

4.2.2Performance

Performance is defined by how the student believes that they act in scientific practices such as: speaking in public, doing experiments, and using equipment [92]. Engaging in scientific research such as hands on experience with laboratory technical skills is tied to this component [97]. Learning about science can serve as personal encouragement to go in depth with their acquired skills and pursue their goals as a science person. For example, Hunter and Weston (2009) evaluate the gains and benefits of students in undergraduate research. Their findings include empirical evidence on different obtained skills, intellectual, and professional growth as consequences of the students' exposure to research experiences [98].

4.2.3Recognition

Recognition of self as a scientist is strongly influenced by the recognition from others. It is also tied to social judgment and cultural norms [92, 99]. Recognition can be viewed as an essential component to develop identity, for example, a certain pressure to thrive in studies or career can rely on satisfying a family community, or intrinsic motivation to satisfy their own recognition of self-scientist to pursue their independent goals [92]. In other words, if the student recognizes himself as a scientist and if the scientific community recognizes the student's work consecutively he will do what it takes to continue receiving recognition depending on their innate beliefs [92].

These three dimensions integrate each other together with gender, racial, and ethnic identity to define the science identity that one develops. From all of these dimensions performance and competence are necessary but does not predict science identity. In contrast, recognition of self and recognition among others do influence and predict science identity. Is important to observe that these dimensions are interconnected. Those persons that act, talk,

walk, and speak (competence and performance traits) like scientist can be recognized as such. Science Identity has become one of the best predictors of student retention [92]. As such, it is necessary to understand how teaching strategies and the content given in schools influence students and which are the best practices that develop high school students' science identity. Moreover, it is important to understand how to accomplish this in the face of diminishing resources.

5. Summary

There is a need to characterize Puerto Rican ET patients' cytokine profile, mutational, clinical background as well as LOX and TLT-1 levels. As also shown in this chapter, the educational disparities over the years and in the present has being a problem in Puerto Rico and the strategies that the Department of Education have implemented have just mitigated the problem but have not solved it. There is a need to provide inclusive science experiences and identify the best strategies that enhance students' science competences and performance. The work that is presented in the following chapters is informed by the aforementioned literature and guided by the following aims:

Aim I. Determine the cytokine profiling of a Puerto Rican cohort of Essential Thrombocythemia (ET) patients and compare it with previously reported ET cohorts.

Aim II. Validate a scientific identity survey and evaluate if short research interventions using CRISPR-Cas9 technology could modify participants' scientific identity and career goals.

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Chapter II

Essential Thrombocythemia Characterization: Mutational, Cytokine, and Thrombotic Profiling

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Abstract

Essential thrombocythemia (ET) is characterized by a persistent thrombocytosis associated with mutations in Janus Kinase 2 (JAK2V617F), myeloproliferative leukemia protein (MPL), and/or calreticulin (CALR). Although ET mutational background has been defined for some sub populations, the proportion and diversification of ET mutations have not been defined for the Puerto Rican population. Furthermore, its cytokine profiling and clinical characteristics have also yet to be defined. In order to understand and further provide better treatment for the ET Puerto Rican cohort, this study defines the proportion of common ET mutations, cytokine profiles, plasma levels of lysyl oxidase (LOX), soluble Trem-like transcript-1 (sTLT-1) and, correlates these levels to the patients' clinical background. Results show that JAK2V617F, CALR, and MPL mutations were present in the ET Puerto Rican cohort in the proportions of 52%, 18%, and 4% respectively. It was also found that TWEAK/TNSF12 was significantly lower and, that MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2, and IL-29/IFNλ1 were significantly higher in ET patients when compared to controls. Interestingly, it was found that ET patients had higher concentrations of sTLT-1, a marker for disseminated intravascular coagulation, and that all the interferons tested were lower in non-diabetic ET patients than in non-diabetic control patients. These findings provide insight into the ET profiling and lay the foundations to provide a better treatment for the Puerto Rican ET sub-population.

Key words

Essential Thrombocythemia, cytokines, lysyl oxidase, Puerto Rican cohort, Trem-Like Transcript-1

Introduction

Essential thrombocythemia (ET) myeloproliferative disease is diagnosed in 38-57 out of every 100,000 persons in the United States (2008 to 2010) [1]. This malignant disorder, characterized by thrombocytosis of an autonomous nature, is considered part of the spectrum of entities grouped as myeloproliferative neoplasm (MPN). ET neoplasm is predominantly seen in the mid-fifty-year-old population and is often accompanied with signs and symptoms related to bleeding diathesis, cytokine augmentation, thrombotic disorders, thrombocytosis, constitutional symptoms (asthenia, cachexia, satiety, night sweats), and splenomegaly [1-4]. Although, it has been shown that multiple cytokines are increased in ET patients, ET profiling of some interferons, thrombotic factors, and interleukins remain unknown [2, 5, 6]. ET is associated with three different mutations: Janus Kinase 2 (JAK2V617F), myeloproliferative leukemia protein (MPL), and calreticulin (CALR) [7,8]. These mutations ultimately lead to the constitutive activation of the JAK2 pathway and are responsible for 86% of documented ET cases. The JAK2V617F is responsible for 56%, MPL exon 10 mutation is responsible for 5%, and CALR for 25% of ET mutations [8, 9]. The JAK2V617F causes a direct activation of JAK2, while the MPL and CALR mutations affect JAK2 though activation of the thrombopoietin receptor [7-14]. Although these mutations induce ET neoplasms, thrombotic event risk, survival, hemoglobin, and platelet count, differ based on the patients' mutational background [9, 15]. For example, patients with CALR mutation have a lower risk of thrombosis, longer survival, higher platelet counts and, lower white blood cell (WBC) counts than JAK2 positive patients [9, 16]. Patients treated with JAK inhibitors, show a decrease in some constitutional symptoms and pro-inflammatory cytokines [17]

Since thrombotic events are one of the catastrophic manifestations seen in different

disorders, including ET patients, a growing amount of research has focused on the identification of molecular markers for thrombotic risk. Lysyl oxidase (LOX) overexpression can increase platelet adhesion to collagen suggesting that higher levels of LOX may be associated with a higher risk of thrombotic events and fibrosis [18 - 20]. Another potential marker that is involved in thrombus formation is the soluble form of the triggering receptor expressed on myeloid cells (TREM) – Like transcript-1 (TLT-1) [21]. TLT-1 enhances platelet activation in the presence of low agonist concentrations. Soluble TLT-1 (sTLT-1) is a marker for disseminated intravascular coagulation (DIC), a condition that causes thrombosis and bleeding due to an imbalance of platelet and coagulation factor aggregation throughout the body [21-22]. Soluble TLT-1 is also a prognostic factor for survival in acute respiratory distress syndrome [23]. Unfortunately, sTLT-1 levels in ET patients are unknown.

ET mutational proportions have been defined for patients in other countries, but the proportion and diversification of ET mutations have not been defined for the Puerto Rican population nor has its cytokine profiling. In order to understand and further provide a better treatment for the ET Puerto Rican cohort, this study defines the (1) proportion of common mutations, (2) cytokine profile, (3) plasma levels of LOX, and sTLT-1 of these patients, and (4) correlates these levels to the patients' clinical background.

Material and methods

Study samples

Blood samples were obtained from ET and control patients after they signed an informed consent agreement. The number of human subjects included in this study is 34; 23 ET patients and 11 controls. For TLT-1 and LOX measurements 22 ET patients were included. None of the controls were diagnosed with MPN. The institutional review board (IRB) at the

Universidad Central del Caribe approved all procedures of this study. Background information was compiled using the Essential Thrombocythemia Instrument Evaluation (Appendix 2)

Mutational background identification

Whole blood was collected using EDTA tubes and stored at 4 °C. DNA extraction was performed using Sigma GenElute Blood Genomic DNA kit. The extraction protocol was followed as suggested by the manufacturer, and samples were stored at -20 °C. JAK2V617F, MPL (W515L and S505N), and CALR mutations were identified by polymerase chain reaction (PCR) using primers that took into account each mutation (Table 1). We used primers at 2uM each, 20-30 ng of DNA per reaction, and, NovaTaq Polymerase master mix according to manufacturer's recommendation. Samples were run in duplicate and mutations were confirmed by sequencing. The cycling program was as follows: 40x denaturation at 98ºC for 40 seconds, annealing for 30 seconds, and extension at 72 ºC for 1 minute. Each mutation was identified according to the sequences previously published [24, 25]. For CALR mutations, CALR type-1, 52-bp deletion (p.L367fs*46), type-2, 5-bp TTGTC insertion (p.K385fs*47), and type- 3, 3-bp deletion (L367fs*48) were evaluated in all samples [24]. Identified proportions of mutations were compared to previously described proportions using Fisher Exact Analysis [8].

Cytokine measurement

Plasma from 23 ET patients and 11 controls was extracted and stored at -80ºC until further analysis. The concentration of 37 pro-inflammatory markers was measured with a multiplex solid-based immunoassay (Pro-Human Inflammatory kit, Bio-Rad, Hercules, CA) using the Luminex MAGPIX® system. Each sample was assayed in duplicate following the

manufacturer's protocol. Data was analyzed using a 5-parameter logistic curve. Cytokine profile comparisons were not normally distributed and were analyzed using the Mann-Whitney U Test. Comparisons between cell count and cytokine measurements were done using Spearman correlation test.

LOX measurement

The levels of active enzyme LOX and pro-LOX, its glycosylated precursor, were measured in the ET patient plasma using western blot analysis as described previously [25]. Plasma was diluted 1:5 with PBS and disulfide-bond reduction was done using β-mercaptoethanol (β-ME). The plasma was separated by PAGE gel and was transferred onto a Polyvinylidene difluoride (PVDF, Bio Rad) membrane. Anti-Lysyl Oxidase antibody produced in rabbit (Cat. # L4794 from Sigma Aldrich) was used as the primary antibody and peroxidase donkey anti-rabbit IgG horseradish peroxidase (Jackson Immunoresearch) as the secondary antibody. Total protein was quantified and used for normalization. LOX, and pro-LOX bands (30 kDa, 50 kDa respectively) intensity was determined by ChemiDoc™ XRS+ and analyzed using Image Lab™ and GraphPad Prism Software [19,20]. Statistical analysis was completed using the student T-test.

sTLT-1 measurement

The levels of s-TLT-1 in the plasma of patients were measured by slot blot technique as described previously [22]. Plasma was diluted 1:3 with TBS 1X and dotted onto a nitrocellulose membrane. Soluble TLT-1 was detected using antibody 69 anti-human TLT-1 primary antibody (dilution, 1:1000) and peroxidase donkey anti-rabbit IgG (Jackson Inmuno Research) as the secondary antibody (dilution, 1:10000). Soluble TLT-1 recombinant protein (amino acids 1 – 146; CDI Laboratories) was used to create a standard curve (from 1 ng to 20 ng). Band adjusted intensity measure was determined by ChemiDoc™ XRS+ and

analyzed using Image Lab™ and GraphPad Prism Software. Statistical analysis was done using t-test.

Results

Mutational background

To identify the proportions of JAK2, CALR, and MPL in our cohort of patients, PCR and Sanger sequencing was used. Results revealed the presence of JAK2 V617F mutation in 12 of the ET patients (52%). CALR mutations were identified in 4 patients (18%); type 1 mutation was found in 2 patients, type 2 in 1 patient, and type 3 in 1 patient. MPL S505N and W515L mutant sequences were evaluated in all samples and S505N mutation was found in 1 patient (4%) for a total of 17 (74%) ET patients with common mutations and 6 (26%) ET patients whose mutational background remained unknown (Figure 1). Control patients did not carry any of the three tested mutations. The proportions of mutations in our population were similar to those previously described (Fisher exact test for: JAK2 p-value: 0.081, CALR p-value 0.580, and MPL p-value: 1) [9].

Patient clinical background

From the 23 ET clinical profile evaluations, the most prevalent diagnosis is hypertension (52.2%), followed by hypercholesterolemia (30.4%) and CNS thrombi (30.4%; Table 2). None of the patients suffered hemorrhages, which is considered a more serious effect linked to ET. As to medications, 87% of the patients were taking aspirin. The platelet count was elevated as expected and the gender distribution was 13:10 females to males respectively (Table 1). Noteworthy, 100% of patients with non-common mutations had at least one thrombotic event. Regarding the control group, patients' prevalent diagnosis included

hypertension (58%), hypercholesterolemia (58%), and diabetes (50%). None of the controls were diagnosed with MPD. Gender distribution was 7:4 females to males respectively.

ET patient cytokine profile

In order to define ET patient cytokine profile, 37 analytes were measured in the ET and control patient plasma (Table 2). From the 37 analytes tested, the levels of six (IL-34, TNSF14, IL-2, IL-11, IL-20 and IL-27 (p28)) were out of range and subsequently were not included in the analysis. Using Mann-Whitney U analysis, the following 9 analytes showed a significant difference between ET and control patients: TWEAK/TNSF12, MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2 and IL-29/IFNλ1 (Table 2). Since diabetes induces an overproduction of cytokines, only the non-diabetic ET patients and non-diabetic control patients were analyzed. Results showed that non-diabetic ET patients had lower levels of IFN-α2, IFN-β, IFN-γ, IL-2, IL-12 (p40), IL-12 (p70), IL-19, IL-22, IL-28A, IL-35, and MMP1 when compared to control patients (Table 3).

LOX and soluble TLT-1 levels

LOX protein was recently correlated with thrombosis and MPN, but the levels of LOX protein and its precursor were not defined for ET patients [20]. Western blot band intensity analysis revealed that neither ET patient's LOX nor pro-LOX levels were significantly different from control patients. LOX or pro-LOX levels did not differ from ET thrombotic patients and ET non-thrombotic patients (Figure 2A-B). Using a post hoc power analysis we have found that the power of our study is 0.6829. Then, we performed a priori power analysis with a power of 0.8 and found that we will need 3 more patients of the ET thrombotic group and 5 more patients of the ET non-thrombotic group to reach statistical significance. Because ET patients do not just suffer from thrombotic events, but also from

bleeding, we measured sTLT-1, a marker of DIC [4]. The analysis revealed that indeed ET patients had a significantly higher median value (10.95ng/mL) of sTLT-1 than control patients (3.195ng/mL; $p = 0.0490$) (Figure 2C).

Patients' cytokines levels and clinical background.

Subsequently, cytokine levels were analyzed to identify any correlation with the patients' clinical background. As a result, it was found that IL-26 levels were significantly higher in patients that showed constitutional symptoms fever, weight loss, visual disturbance, fatigue, headache, dysesthesias. $p<0.049$). No significant associations were observed between cytokine profile, TLT-1 or LOX, and thrombosis, WBC count, hemoglobin, platelet count, or bleeding.

Discussion

The purpose of this study is to characterize the ET Puerto Rican population to further provide a better treatment for these patients. With this intention, the present study first characterized the ET Puerto Rican cohort proportion of common mutations and found that the mutational profiling was similar to the previously published populations [8, 9]. However, the ET cytokine profile was slightly different when compared to the previously reported ones. It was found that MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2 and IL-29/IFNλ1 were significantly higher in ET patients than in control patients. Noteworthy, from the eight different analytes that were significantly higher, just IL-8 was known to be higher in ET patients [2]. Even though MMP-1 and IL-22 were increased in the ET Puerto Rican cohort, previous studies found that ET patients had normal levels for MMP-1 and IL-22. MMP-1 is known to regulate thrombus formation and platelet activation [27]. Interestingly, not just MMP-1 but some of the analytes that were highly expressed in ET patients have

been shown to have a direct effect on platelet count. For example, IL-8 was found to impair the proliferation and differentiation of megakaryocyte and myeloid progenitor cells on patients with myelofibrosis. Moreover, the neutralization of IL-8 receptors had been shown to restore megakaryocytes ploidy [28]. However, mice treated with IL-22 showed a significant increase in platelet count [29]. Previous articles have reported that Interferon alpha (IFN) inhibits the growth of megakaryocyte progenitor cells leading to a reduction of peripheral platelet counts on ET patients [30]. Also, IFN had been shown to down-regulate the expression of IL-8 and enhance the expression of IL-22 [31, 32, 33]. Further studies focused on the combination of the effects of IFN, IL-22 and IL-8 on the augmentation of platelet count in ET patients is suggested. Indeed, emphasis should be made on the fact that all the tested interferons were lower in non-diabetic ET patients than non-diabetic control patients. IFNs are produced in response to a viral infection or by a direct response to inflammation. To our knowledge, there is no association between ET and the capacity of these patients to deal with viral diseases, which could be an interesting point for further research.

In order to further characterize ET patients, we not only define their cytokine profile but also analyze two different molecular markers of thrombotic events: LOX and sTLT-1. Even though LOX protein had been associated with thrombotic events, LOX levels were not able to explain ET patients' thrombotic risk. Instead, sTLT-1 levels were significantly higher in ET patients than the control group. We suggest that the augmentation of sTLT-1 levels may be associated with the hyperactivation of platelets in ET patients, but further studies are needed to validate this observation.

We believe that although, ET is a rare disorder and the Puerto Rican population in comparison to other countries is very small, we were able to gather a good representative cross section of ET Puerto Rican patients. This characterization of the ET Puerto Rican cohort described in this study provides multiple insights for further studies and laid the foundations to a better treatment for ET patients.

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Conflict of Interest and Sources of Funding

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Figures

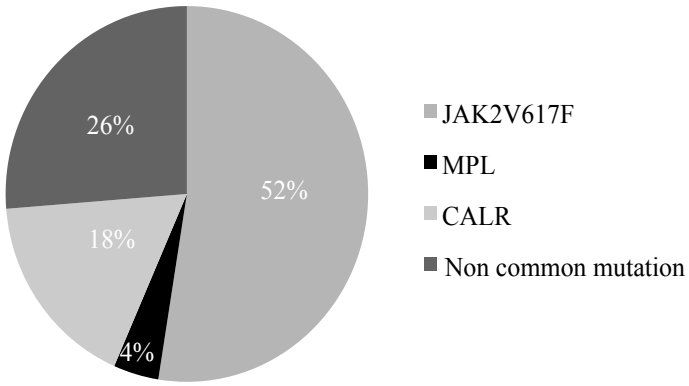

Figure 1. Mutational distribution seen in Puerto Rican ET patients. N=23

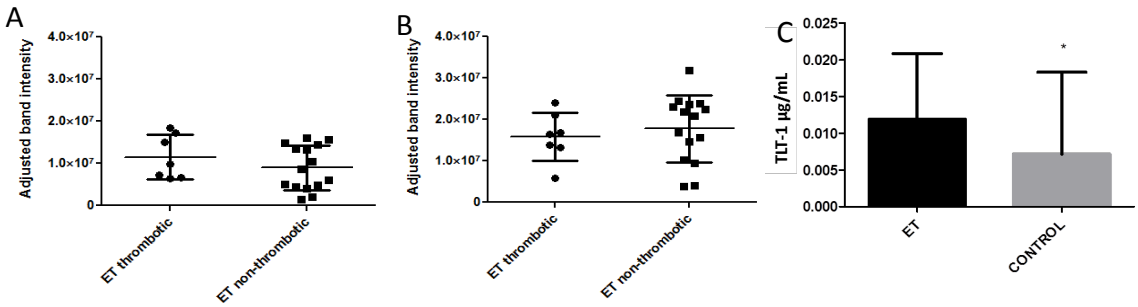

Figure 2. Soluble TLT-1 and LOX levels in ET patients. (**A-B**) Different forms of LOX levels in ET patient plasma. Western blot was used to measure LOX and results were analyzed using T-test. The lines in the scatter dot plot diagrams represent mean with SD, (**A**) pro-LOX (**B**) LOX band intensity is reported. ET thrombotic N= 7 ET non-thrombotic=15. **(C)** Slot-blot technique was used to determine s-TLT-1 levels in ET patient plasma. Optical density of each sample was detected using Image Lab 6.0. Concentration was interpolated from a standard curve. Analysis was done using a Mann Whitney test and p-value was established at <0.05. ET N =22 Controls N=11.

Tables

Mutation	Forward Primer	Reverse Primer
JAK2	5'-ATTGCTTTCCTTTTTCACAAGAT-3'	5'-GTTTTACTTACTCTCGTCTCCACAaAA-3'
CALR	5'-CATACGCTGAGGAGTTTGGC-3'	5'-GAGTGGAGGAGGGGAACAA-3'
MPL	5'-TAGCCTGGATCTCCTTGGTG-3'	5'-GCGGTACCTGTAGTGTGCAG-3'

Table 1. Primer sequences: forward and reverse primer sequences for amplification and sequencing of JAK2, CALR and MPL segments.

Variable	Experimental Group N (Range)	%	Control group N	%
Age Median (range)	68(21-82)	-	63(39-76)	-
Hemoglobin Median (range)	12.8 (6.4-16.4)	-	-	-
WBC Median (range)	7.35 (2.83-31.3)	-	-	-
Platelets Median (range)	651 (366-1284)	-	-	-
Female	13	56.5	7	58
Diabetes	6	26.1	6	50
Hypertriglyceridemia	4	17.4	0	0
Hypercholesterolemia	7	30.4	7	58
Hypertension	12	52.2	7	58
CHF	0	0.0	0	0
CAD	2	8.7	0	0
Hemorrhage	0	0.0	0	0
Petechiae	0	0.0	0	0
Splenomegaly	6	26.1	0	0
Renomegaly	1	4.3	0	0
Hepatomegaly	1	4.3	0	0
CNS thrombus	7	30.4	0	0
Heart thrombus	1	4.3	0	0
MI	0	0.0	0	0
Venous thrombus	2	8.7	0	0
Medications				
Aspirin	20	87.0	3	25
Alkylating	0	73.9	0	0
Hydroxyurea	17	73.9	0	0
Anagrelide	3	13.0	0	0
Clopidogrel	1	4.3	0	0
Coumadin	0	0.0	0	0

Table 2. Essential Thrombocythemia patient profiling, gender, diagnosis and medications. A questionnaire, addressing clinical background, medications, and demographics, was filled out by the participants at the moment of the blood draw. ET N=23 Controls N=11; CHF stands for Congestive Heart Failure; CAD stands for Coronary artery disease; CNS stands for Central Nervous System; MI stands for Myocardial Infarction.

Cytokines	Median ET (range) n=23	Median Control (range) n=11	Mann Whitney test P value
TWEAK TNFSF12	**43.57(27.57-57.9)**	**47.77(41.71-69.86)**	**0.0392**
TSLP	7.05(1.79-25.72)	5.89(1.96-10.84)	0.2039
sTNFR2	1166(439-3054)	732.8(367.2-1760)	0.3256
sTNFR1	387.4(123.1-1011)	307.2 (256.6-602.1)	0.6433
Pentraxin-3	69.61(36.19-174.2	71.32(32.07-103.1)	0.5372
Osteopontin	12360(6307-27972)	13435(9144-20473)	0.3781
Osteocalcin	508.9(199.2-2491)	590.8(283.8-866.90)	0.717
MMP3	1427(595.6-5695)	1503 (680.9-2409)	0.5672
MMP2	5157 (2366-9922)	4114 (2753-7235)	0.3256
MMP1	**664.1(297.4- 2279)**	**399.8 (217-1729)**	**0.006**
IL-35	**36.15 (23.64-99.88)**	**28.34 (17.37-51.74)**	**0.0059**
IL-8	**6.93 (5.07-11.26)**	**6.33 (-5.29-7.69)**	**0.0481**
April /TNSF13	32374 (11095-66721)	27246, (22242-59916)	0.4618
BAFF/TNS13B	2074(844.6-9765)	1665(942.6-2400)	0.0608
sCD30 TNFRSF8	77.89(33.59- 283.3)	73.21 (63.53-122.4)	0.9422
scD163	21379(9011-68511)	25386(12807-54294)	0.4675
Chintase-3 like 1	4378(1629-9420)	5357(2931-7985)	0.1144
gp130 sIL6Rβ	7543(3178-17026)	9103(4964-14364)	0.1427
IFN-α2	**14.38 (10.44-30.59)**	**12.63(9.13-16.56)**	**0.0154**
IFN-β	7.5 (2.55- 19.24)	6.83(4.83-11.59)	0.2032
IFN-γ	4.85(3.03-15.96)	4.36(3.03-7.23)	0.1033
sIL-6Rα	1947(766.9-5206)	1992(1577-4319)	0.2741
IL10	2.79(1.99-3.8)	2.49(1.99-2.81)	0.0509
IL-12(p40)	16.27(7.38-57.08)	13.32(7.38-26.5)	0.066
IL-12(p70)	0.57(0.39-1.12)	0.51(0.48-0.62)	0.1638
IL-19	**6.56(4.15-12.6)**	**5.13(4.15-9.3)**	**0.0162**
IL22(28)	**7.38(4.71-14.21)**	**6.23(5.09-8.34)**	**0.0153**
IL-26	11.7(8.57-15.8)	10.55(7.48-15.45)	0.0886

IL-28A	**3.76(2.24-13.79)**	**2.79(1.9-5.89)**	**0.0334**
IL-29	**12.24(6.44-26.8)**	**10.03(6.44-15.23)**	**0.0421**
IL-32	8.6(2.81-46.13)	4.71(0.38-13.59)	0.0691

Table 3. Profiling of ET patients and controls. Plasma levels of cytokines and other analytes were measured on the ET and control patient plasma using a 37-plex solid-based immunoassay. Analysis was performed using Mann Whitney U test and p-value was established at <0.05. Bold values represent significant values. ET N=23, Controls N=11.

Analyte	P-value
APRIL/TNFSF13	0.9396
BAFF/TNFSF13B	0.2231
sCD30/TNFRSF8	0.8201
sCD163	0.8201
Chintinase-3 like 1	0.1196
gp130/IL-6Rβ	0.3587
IFN-α2	**0.0135**
IFN-β	**0.0233**
IFN-γ	**0.0491**
IL-2	**0.0112**
IL-6Rα	0.3587
IL-8	0.1241
IL-10	0.1958
IL-12(p40)	**0.05**
IL-12(p70)	**0.0432**
IL-19	**0.0371**
IL20	0.883
IL-22(28)	**0.0235**
IL-26	0.3086
IL-28A	**0.0296**
IL-29	0.0739
IL-32	0.0704
IL-35	**0.0019**
MMP1	**0.0373**
MMP2	0.1196
MMP3	0.7616
Osteocalcin	0.4008
Osteopontin (OPN)	0.1636
Pentraxin 3	0.4929
sTNFR1	0.8911
sTNFR2	0.5946
TSLP	0.1404
TWEAK/TNFSF12	0.0608

***Table 4*.** Comparison between non-diabetic ET and non-diabetic control patients. A total of 17 non-diabetic ET patients and 5 non-diabetic controls were analyzed using Mann Whitney U Test. p-value was established at <0.05.

Chapter III

Spanish-language Version of the Science Identity Survey (SISE): Translation, Cultural Adaptation, and Evaluation

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Abstract

Multiple worldwide efforts, including research experiences and internships for students, have been developed to increase diversity in STEM. In order to understand the outcomes of these research experiences, instruments have become available, but surprisingly, Spanish instruments for these purposes are scarce. The evaluation of diverse scientific experiences and their influence on science identity is imperative. For this reason, we aimed to translate, and evaluate a Science Identity Survey for Puerto Rican high school students. A committee of experts evaluated the original survey of Science Identity and it was translated to Spanish using back-translation. Think-aloud results revealed that students' perception of their: (1) science competence is based on their grades, understanding, knowledge, and learning; (2) performance is based on design and completion of a scientific task; (3) recognition is based on the value that others give to science. The survey was analyzed to determine its dimensionality and reliability. A Cronbach's alpha of .857 was obtained, which suggests that the items have a good internal consistency. Exploratory factor analysis was performed and three factors; competence, performance and recognition were retained. This version of the survey was deemed to be an appropriate instrument to address student science identity.

Keywords: science identity, high school students, Spanish translation, quick assessment, scale

Introduction

A recurring global issue in Science, Technology, Engineering and Math (STEM) education is the poor academic performance and retention of students [1,2]. Multiple worldwide efforts, including research experiences and internships for students, have been provided to increase diversity in STEM. [3-6]. In order to identify program outcomes and define gains, multiple surveys have been developed [6-10] and a growing body of research has been reported [9, 11-16].

Although these instruments comprised multiple important factors that influence research experiences and persistence of different populations including Latino/Hispanic populations, the impact of scientific experiences on the science identity of Latino/Hispanic high school remains incompletely defined. Due to the increasing population of students whose first language is Spanish, it is critical to understand the science identity of these high school students taking into consideration culturally-patterned differences, native language, and familiar concepts to obtain a better understanding of their science identity [18]. Since diversity and inclusion of everyone into science, including the Latino/Hispanic population, is important for the nation's economic and social development, the study of science identity and key components for retention is imperative [19]. Unfortunately, in a review of the literature, no single validated Spanish-language assessment instrument of science identity was found.

Identity as described by Gee (1991) is "the kind of person one is seeking to be and enact in the here and now" [20]. When it comes to science identity, researchers agree that there is a component of self or intrinsic factors and a component of fitting into the norms and practice of the scientific community that leads to the recognition of the person in the

specific community. A growing amount of research has argued that the components that build up student's science identity offers "the most complete understanding of students' trajectories and persistence in science related careers" [21, 22]. Although science identity has taken many different meanings, we will focus on the definition given by Carlone and Johnson (2007) because of their methodological and practical implications [23]. This selection does not deny other useful approaches that could be taken using other definitions; it gives us a framework for data analysis and interpretation.

Carlone and Johnson's approach to define and contextualize science identity model is formed by the following question: "How would we describe a person who has a strong science identity?" They define the science identity concept as the kind of person that "makes visible to (performs for) others one's competence in relevant practices, and, in response, others recognize one's performance as credible" [23]. In other words, their science identity model captures the key elements that build and describe a person that belongs to the scientific community. Interestingly, this model is based on the interrelated dimensions of competence, performance and recognition that an individual can envision at different degrees and configurations [23] Competence is defined as "knowledge and understanding of science content"; performance is defined as "social performances of relevant research practices such as: ways of taking and using tools"; and recognition by "recognizing oneself and others as a "science person" [23].

Multiple researchers have developed surveys addressing science identity [17, 24- 28] These instruments attempt to define science identity using the following constructs: self-identification, performance, recognition, students' interests related to science, identity prominence, reflected appraisals, science self-efficacy, science behavior, interest, fascination, values, competency beliefs, project ownership, emotion, and networking.

Among the surveys that study students' science identity and follow the structure and specific dimensions of Carlone and Johnson is Jennifer Schon's Science Identity survey (SIS). The SIS instrument measures intrinsic and extrinsic components of science identity using 15 items. Although the SIS instrument measures competence using knowledge and understanding of science topics, these items are not content-based and therefore can be used for the evaluation of interventions of a wide range of topics. Its length and approach make this instrument suitable for the evaluation of a variety of short interventions and for these reasons we have selected this survey instead of others to study students' high school science identity.

The SIS was translated, contextualized, and evaluated [26] with Spanish-speaking, Puerto Rican high-school students as research subjects. The reason behind this selection is that surveys are quick assessment tools and multiple responses can be collected at the same time [29]. Survey evaluation was conducted following a mixed method approach, as the one performed by the original SIS developers [26].

SIS development and use

The SIS was created to evaluate the impact of students' experiences at informal education centers [26]. Since informal education experiences, such as museums, afterschool programs, and activities in off-school venues differ in style, context, and content, the developers of the survey created a short non-content based survey to evaluate students' experiences based on a mixed method approach [26]. First, interviews were held to gather insight on student's scientific experiences. Items were then constructed, followed by think-aloud and pilot testing [26].

The three different dimensions or constructs of science identity described by Carlone and Johnson: competence, performance, and recognition were included and studied in the

SIS. The competence category consists of 5 items that are related to student perception of knowledge and learning. Performance consists of 5 items based on student perception of science skills as experimental design, making observations, and using the scientific method. Recognition includes 5 items that identify if the students feel like a scientist or if they perceive that friends or relatives see them as scientist. For each of these categories, a 5 to 1 Likert scale from "Strongly agree" to "Strongly disagree" was used.

The original instrument was used for 5th and 6th grade students at the University of Idaho College of Natural Resources' McCall Outdoor Science School (MOSS). For the confirmatory factor analysis they report the following indices: comparative-fit index (CFI) = .934, adjusted goodness-of-fit index (AGFI)= .869, root mean square error of approximation (RMSEA)= .07, standardized root mean squared residual SRMR= .065. This instrument was further used to evaluate students' science identity before and after an informal education experience at MOSS. Also a follow up evaluation was performed after a month. Results showed that the experience at MOSS was a positive influence on the participants' science identity [26].

Methods

Participants

The Institutional Review Board at the University of Puerto Rico approved this study (IRB protocol 1718-036). Participants did not receive any incentive for their participation. Anonymity of all participants is guaranteed. We select participants based on their grade level (10th, 11th and 12th grade), and availability and willingness to complete the survey. Two weeks after the initial approach, an informative brochure of the study together with the

consent/assent form and two weeks after the initial approach, consent/assent forms were collected and participants answered the survey or participated in interviews. Survey content evaluation was addressed using the think-aloud method [30]. One group of three and another of four students participated in this process to confirm that participants understood the intended meaning of the questions. A preliminary evaluation was performed with 32 participants (19 females and 12 males) from one school located in San Juan, Puerto Rico. For the construct evaluation, three different high schools from the San Juan region were approached. The participants' schools where selected according to their specialization (science, sports, or languages) in order to include students with a diverse range of interests. A total of 180 participants completed the survey.

Translation

The SIS was translated from English to Spanish as suggested by the World Health Organization guidelines [31]. A bilingual translator, who was familiar with science identity constructs, and whose mother tongue is Spanish performed the forward-translation step. Once the initial translation was completed, a bilingual panel composed of 4 experts in the field of science, education, translation, and/or instrument development discussed each item. The expert panel evaluated each item for discrepancies between the original version and the translated version, cultural discrepancies, concept translation, jargon, and clarity. Once the expert panel solved discrepancies and reached a consensus on all items, the revised Spanish version was given to an independent translator whose mother tongue is English and did not have any knowledge of the studied concepts of science identity. The independent translator translated the Spanish version of the survey back to English (backward translation). Subsequently, the expert panel compared the English version of the survey to the original

version and discrepancies were discussed until conceptual and cultural equivalence of the survey was achieved. Each panel discussion took approximately 4 hours.

Survey content evaluation

The final version of the translation process was given to groups of 4 participants as suggested by Virzi [32]. Participants were asked to answer: (1) what was their first thought about the item, (2) what was their answer, (3) if something was not clear, and if so, what was not clear to them, and (4) if they had a suggestion to improve the item. Participants evaluated each item and their suggestions were incorporated in the survey. The final version of this process was a consensus among all the participants. At the end of the process, the interviewer read out-loud the survey and final changes or suggestions were incorporated. This process was repeated until it reached saturation of responses [30].

Survey construct evaluation

Think-aloud suggestions were incorporated into the survey and administered to participants. During this process we realized that the numbered Likert scale was not clear to participants. For this reason, we incorporated another session of think-aloud with 4 additional participants, in which two versions of the survey were given, one with a scale labeled with numbers and another one labeled with descriptive word answers. Participants were asked to answer the survey in both formats and talk about their answer selection process allowing us to define and correct any misconception and select the best scale format for our survey.

The survey was administered to 180 participants. Survey descriptive statistics, reliability, and goodness of fit analysis were calculated using IBM SPSS Statistics software package, version 24. Cronbach's alpha was used to estimate the internal consistency of the survey [33]. Measurement criterion was as followed: $\alpha \geq 0.90$ (high internal consistency or items may be redundant) $\alpha \geq .80$ (good internal consistency) $\alpha \geq .70$ (adequate internal

consistency) [34]. Skewness and kurtosis acceptable criterion for normality was set at |2.0| as suggested by George D. and Mallery P [36]. Kaiser-Meyer-Olkin, measure (KMO) of sampling adequacy threshold was set at 0.5 as used by Hanauer and Dolan [17].

Students' perceptions of their competence, performance and recognition are variables that cannot be directly observed (latent variables). To study these unobservable variables, we analyze participants' responses to specific questions (measurable variables) to make inferences about the studied latent variables. Exploratory Factor Analysis (EFA) with principal axis extraction method was selected instead of the Principal Components Analysis, because we wanted to determine interpretable constructs that explain correlations among measurable variables and not in find components that explain as much variance as possible [36, 37]

To identify the best structure to interpret our results we rotated the factor solutions. Among the rotation methods that are available we selected the oblimin method, which allowed correlation among factors [36]. Parallel analysis was performed to determine the number of factors to retain; Principal Axis Factor was used as the method of extraction, 1000 data sets, 95 percentile, and Pearson correlation [38].

Results

Participants

Participants' age ranged from 14 to 17 years old. The proportion of females and males was fairly evenly distributed, but overall more females participated (51.6%). Most of the participants live in the metropolitan area of San Juan, Puerto Rico (86%). Some participants failed to indicate their parent's highest degree obtained (Father: 29%, Mother: 39%), field of study (Father: 38%, Mother: 21%), and/or occupation (Father: 18%, Mother: 17%), because they had no knowledge about this information, declined to answer, or left unanswered

because it didn't apply to their case. From the participants who answered, the category that had the largest number (Father: 32%; Mother 23%) was the postgraduate or professional degree (includes Master's, Doctorate, Medical, or Law degrees). A few participants had parents who graduated from Associate degree programs (3%). Most parents studied in a field (Father: 44%; Mother 59%) and/or have an occupation (Father: 67%; Mother 70%) that is not related to STEM.

Content evaluation

Two groups of four participants discussed each item of the Spanish-translated version of the survey, suggesting a total of 8 changes, all of which were incorporated (Table 1). Changes were mainly focused on verb usage and the inclusion of not just task, but projects in item number 12. Participants also requested to delete "mis" ("my") on the item number 13, (Las personas me ven como un científico cuando comparto "mis" observaciones) since they feel like scientists when they are sharing observations of other scientists as well as their own.

Participants also commented that the numbered scale is subjective and the descriptive scale is clearer to them. From the think-aloud interviews we gathered the following participants' quotes (see translated English version at the bottom of each quote):

"Me enfrento a la escala de palabras y mi humildad toca la puerta. En la escala de palabras valgo menos.

"When I am confronted with the words scale my humility knocks on the door. With the words scale I feel of less value"

"Es más claro (en palabras), número es más subjetivo

"It is clearer (in words), numbers are more subjective"

"Palabras es más claro".

"With words is clearer."

"Con números la interpretación es diferente; es subjetivo."

"With numbers the interpretation is different; is subjective"

Therefore, the following descriptive word scale was incorporated: "Muy de acuerdo", "De acuerdo" Ni en desacuerdo ni de acuerdo", "En desacuerdo", and "Muy en desacuerdo". The modified version was administered to 180 participants for construct evaluation.

Construct evaluation

Answers mean value for the items ranged from 2.8 to 4.3 (Table 2). All the items had a skewness and kurtosis below |1.0|. Intra-subscale correlations ranged from 0.325 to 0.724 and communalities range from 0.463 to 0.785. A Chi-Square Analysis was used to compare the pattern of inter-item correlations against the identity matrix to determine if there is a difference between the observed data and the hypothesized one-factor model. Results showed a Chi-Square value of 1125.633, significance 0.000, which means that the null hypothesis is rejected and the observed data does not fit in a one-factor model. In addition Cronbach's alpha coefficient value of 0.867, and Kaiser-Meyer-Olkin, measure (KMO) of sampling adequacy of .855 was calculated. Also a Bartlett's test of sphericity, was performed to determine whether or not a factor analysis could be conducted. Results showed a significance of 0.000. After the analysis and interpretation of the measurements mentioned above and descriptive statistics, we confirmed that the sample was adequate and that its dimensionality could be explained using a factor analysis (Table 3).

The internal structure of the test items was identified using the exploratory factor analysis. Principal axis factoring using the oblimin with Kaiser normalization rotation was

conducted. Results indicate a 4-factors solution (Table 4). The 4-factor solution explained 53.32% of the variance. Each item was explained by one factor, with the exception of item 11 “Mis amigos me ven como una persona que es buena en ciencia”. Factor 1, which accounts for 33.71% of the explained variance, was constructed of competence and recognition items. Factor 2 (10.174% variance explained) was constructed of recognition items. Factor 3 (6.083% variance explained) was constructed of performance and one item of competence. Factor 4 (3.357% variance explained) was constructed of performance items.

In general, these factors were consistent with what was originally described for the SIS. The first factor, that comprised mainly competence items, included the following items that were previously categorized in the recognition dimension: “Mis amigos me ven como una persona que es buena en ciencia” (SIS original item: “My friends see me as someone who is good at science”) and Puedo ayudar a las personas cuando tienen dudas de ciencia (SIS original item: “I can help others with science related topics”). In the think-aloud, participants commented that in order to help others and to be recognized as a person that is good at science, they needed to know the material and have good grades. Thus, they related these items to their ability to understand and know topics of science, which directly associates to science competencies.

The third and fourth factors are mainly composed of performance items. One item previously included in the competence dimension: “Soy bueno llevando a cabo experimentos científicos” (SIS original item: “I am good in most science experiments”) was incorporated in the third factor. Participants’ interpretation of this item focused on experimental design, methods, and experimentation. Participants emphasized that the item is open enough that it can be interpreted as experimental design or experimentation. The fourth factor was composed of two performance items. According to the parallel analysis and

because of the small number of items in factor 4, just factors 1, 2 and 3 were retained (Figure 1).

Based on the exploratory factor analysis, parallel analysis, and think-aloud comments we recommended a rearrangement of the items on each of the dimensions and the deletion of items 8, 9, and 11. Cronbach's alpha index was re-calculated for the final version of the survey and we obtained a result of .857. The final version of the survey is presented in table 5 and it has incorporated the aforementioned modifications.

Discussion

Although science identity has been mainly studied in undergraduates or higher degrees, it is known that high school students' science identity is influenced by students' persistence, the role of the community, and science classes at school [39]. Unfortunately, the impact of scientific experiences on the science identity of Latino/Hispanic high school remains relatively undefined [40-43]. To characterize the effectiveness of research experiences and identify which components actually are important for STEM retention of high school Latino/Hispanic population, an assessment in Spanish was necessary. This study presents evidence of the translation and evaluation of the Spanish version of the SIS (SISE, for SIS-Español), using Puerto Rican high school students as research subjects and takes in consideration culturally-patterned differences.

After the think aloud process, participants agreed that the numbered Likert scale was subjective, and the descriptive word scale was more informative. For this reason, the original numbered scale on the SIS was replaced and the word descriptive scale was incorporated. This result is consistent with previous research on scales that found that numbered scales are subjective to participant interpretations and are more problematic for respondents that do not

tolerate ambiguity [44]. Interestingly, our results show that participants tend to assign higher scores when using the numbered scale than when they are using the descriptive word scale. This effect may be influenced by the submissive response (*simpatía*) style documented among Latinos and Hispanics [44]. Our results suggest that the use of a descriptive word scale can help participants to think about the best word that describes their answer and not on giving the highest score possible to each item.

After content evaluation and scale changes, an exploratory factor analysis and Cronbach's alpha index were calculated to explore the structure and reliability of the survey. A 4-factor solution was suggested, but one of the factors was not reliable. As a result, this factor was deleted.

Limitations and Suggestions

One limitation of the SIS is that its evaluation was performed using just one informal center. Since, participants of the SIS evaluation were self-selected, they may have a predisposition to science careers and this selection process excluded those students that may not like science and are not interested on a STEM career. To overcome this limitation we chose schools specialized in various areas to have a diverse group of students with different levels of interests in science. We are aware that this selection does not imply or ensure participants' interest for science, but it does gather different student profiles. A potential limitation of the survey for future SISE users is that it has only been validated with Puerto Rican high schools students, and there are cultural differences across Spanish-speaking communities. We encourage future users of the SISE to validate this survey with a similar population to the one that will be further tested, taking into account culturally-patterned differences and scale interpretations.

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Declaration of Interest

The authors declare no conflict of interest.

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Tables and Figures

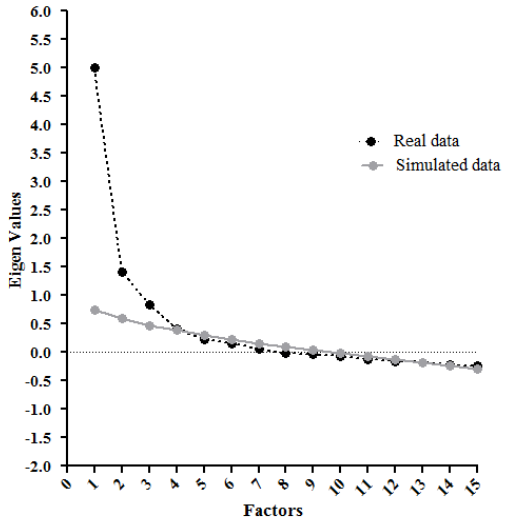

Figure 1. Parallel analysis. Method of extraction: Principal Axis Factor, 1000 data sets, 95 percentile, and Pearson correlation.

Original questionnaire	Translated questionnaire	Incorporation of think-aloud suggestions
1. I am good at science	Soy bueno en ciencia.	Soy bueno en ciencia.
2. I know a lot about science	Se mucho de ciencia.	Se mucho de ciencia.
3. I am good at most science experiments	Soy bueno haciendo experimentos científicos.	Soy bueno llevando a cabo experimentos científicos.
4. I understand science topics	Entiendo fácilmente los temas de ciencia.	Domino los temas de ciencia.
5. I learn new science topics easily	Aprendo fácilmente nuevos temas de ciencia.	Aprendo fácilmente nuevos temas de ciencia.
6. I can use science equipment and/or technology to collect data	Puedo usar equipos científicos y/o tecnología para obtener datos.	Puedo usar equipos científicos y/o tecnología para obtener datos.
7. I know how to use the scientific method/process	Se cómo usar el método científico.	Se cómo usar el método científico.
8. I can talk with others about science related topics	Puedo hablar con otras personas sobre temas de ciencia.	Puedo hablar con otras personas sobre temas de ciencia.
9. I can create my own science experiments	Puedo crear mis propios experimentos científicos.	Puedo diseñar mis propios experimentos científicos.
10. I can use my observations to create a hypothesis	Puedo usar mis observaciones para hacer una hipótesis.	Puedo usar observaciones para hacer una hipótesis.
11. My friends see me as someone that is good at science	Mis amigos me ven como una persona que es buena en ciencia.	Mis amigos me ven como una persona que es buena en ciencia.
12. When giving a science report, I feel like a scientist	Cuando hago mis trabajos de ciencia, me siento como un científico.	Cuando hago trabajos y/o proyectos de ciencia, me siento como un científico.
13. Others see me as a scientist when I share my observations	Las personas me ven como un científico cuando comparto mis observaciones.	Las personas me ven como un científico cuando comparto observaciones.
14. When I share data I've collected, I feel like a scientist	Cuando comparto los datos que he obtenido me siento como un científico.	Cuando comparto los datos que he obtenido me siento como un científico.
15. I can help others with science related topics	Puedo ayudar a las personas cuando tienen dudas de ciencia.	Puedo ayudar a las personas cuando tienen dudas de ciencia.

Table 1: Translation of the items and the result of think-aloud. Translation of the Science Identity questionnaire published by Jennifer Schon was performed using back-translation followed by a committee expert evaluation. Think-aloud was performed twice using a group of 3 to 4 students.

	Mean	Std. Deviation	Skewness	Kurtosis	Corrected Item-Total Correlation
Item 1	3.8	0.84313	-0.528	0.664	0.602
Item 2	3.4	0.7907	-0.015	0.271	0.591
Item 3	3.9	0.82827	-0.402	-0.31	0.438
Item 4	3.6	0.79451	-0.386	0.793	0.615
Item 5	3.7	0.84643	-0.379	0.184	0.568
Item 6	4.2	0.72541	-0.759	0.689	0.473
Item 7	4.2	0.69171	-0.318	-0.501	0.325
Item 8	3.9	0.89872	-0.571	-0.349	0.503
Item 9	3.4	0.92727	-0.089	-0.326	0.406
Item 10	4.3	0.60051	-0.208	-0.573	0.355
Item 11	3.4	1.06871	-0.242	-0.448	0.724
Item 12	3.4	1.14525	-0.265	-0.678	0.374
Item 13	2.8	0.92244	-0.027	-0.033	0.59
Item 14	3.2	1.13551	-0.146	-0.769	0.491
Item 15	3.7	1.01882	-0.651	0.176	0.661

Table 2: Descriptive statistics for each of the items. n=180

Number of items	15
Number of responses	180
Average inter-item correlations	0.306
Standard deviation of Inter-item correlations	0.15
Cronbach's alpha	0.867
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.	0.855
Bartlett's Test of Sphericity	1125.633
Bartlett's Test of Sphericity Significance	0

Table 3: Scale and Reliability Statistics of the survey. 15 items, n=180

Item	Component Matrix 1	2	3	4	Total variance explained
Soy bueno en ciencia.	.873				5.492(33.71%%)
Se mucho de ciencia.	.701				
Domino los temas de ciencia.	.699				
Aprendo fácilmente nuevos temas de ciencia.	.654				
Puedo ayudar a las personas cuando tienen dudas de ciencia.	.590				
Mis amigos me ven como una persona que es buena en ciencia	.565			-.402	
Cuando hago mis trabajos de ciencia, me siento como un científico.		.950			1.834 (10.174%)
Las personas me ven como un científico cuando comparto mis observaciones.		.801			
Cuando comparto los datos que he obtenido me siento como un científico.		.659			
Se cómo usar el método científico.			.704		1.478 (6.083%)
Soy bueno llevando a cabo experimentos científicos			.539		
Puedo usar equipos científicos y/o tecnología para obtener datos.			.494		
Puedo usar observaciones para hacer una hipótesis.			.419		
Puedo hablar con otras personas sobre temas de ciencia				-.643	1.02 (3.357%)
Puedo diseñar mis propios experimentos científicos.				-.431	

Table 4: Exploratory factor analysis. Using Oblimin rotation four factors were found that explained 53.32% of the variance.

	Muy de acuerdo	De acuerdo	Ni en desacuerdo ni de acuerdo	En desacuerdo	Muy en desacuerdo
1. **Soy bueno en ciencia.**					
2. **Se mucho de ciencia.**					
3. **Soy bueno haciendo experimentos científicos.**					
4. **Entiendo fácilmente los temas de ciencia.**					
5. **Aprendo fácilmente nuevos temas de ciencia.**					
6. **Puedo usar equipos científicos y/o tecnología para obtener datos.**					
7. **Se cómo usar el método científico.**					
8. **Puedo usar observaciones para hacer una hipótesis.**					
9. **Cuando hago mis trabajos de ciencia, me siento como un científico.**					
10. **Las personas me ven como un científico cuando comparto mis observaciones.**					
11. **Cuando comparto los datos que he obtenido me siento como un científico.**					
12. **Puedo ayudar a las personas cuando tienen dudas de ciencia.**					

Table 5: SISE Suggested changes

Chapter IV

An exploratory study comparing students' science identity perceptions derived from a hands-on research and non-research-based summer learning experience.

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Running title: Summer Learning Experiences Influence Science Identity Perception

Keywords: high school students, competence perception, performance perception, short scientific experiences, hands-on, CRISPR-Cas9, active learning

Abstract

Although multiple efforts have been initiated to increase students' science proficiency scores, most of the schools in the United States do not reach the expected student academic performance. This study addresses the impact of a one-week summer scientific learning experience on students that worked with experimental procedures and students that did not. We describe and evaluate these two different interventions to explore what components influence high school students' perception of their scientific competence, performance, and recognition, using science identity as an analytical lens. Science Identity score was increased at the end of both interventions. Interestingly, science identity change index was higher for the group that did not work with experimental procedures. Although this group did not perform any hands-on experiments, they report, through reflexive diaries and interviews that working with CRISPR-Cas9 models, being in a research laboratory, and seeing the instrumentation made them feel like scientists. Regarding science competence, both groups report exponential learning gains, although the group that performed the experiments reports more difficulties. Both groups report that mentorship was key in their competence and performance development. These findings suggest that our one-week scientific learning programs influence participants' perception of scientific competence and performance and create an opportunity to develop further studies on short scientific learning experiences using models and active learning activities.

Introduction

Student outcomes of long-term research experiences have been reported [1-4]. Nevertheless, the effectiveness of short scientific learning interventions is not fully understood nor which aspects influence STEM performance, particularly for Latino/Hispanic high school students. The context and cultural aspects of students need to be considered to understand the best practices that positively influence STEM performance. Our work focuses on a group of Puerto Rican students that attend low-performing public high schools. These schools have limited resources to provide their students access to laboratory, research facilities or diverse scientific learning experiences. Furthermore, their exposure to STEM professionals to serve as mentors or role models is limited.

We aimed to understand the impact of scientific learning experiences on a group of Puerto Rican high school students by addressing the following questions: First, how scientific learning experiences influence high school students' perceptions of their scientific competence, performance, and recognition? Second, is it critical to include authentic experiments as part of the intervention or is simulated experimentation sufficient to improve the students' perceptions of their scientific competence, performance, and recognition?

To answer these questions, we have developed and evaluated two different scientific learning experiences conceptualized on cancer research. This topic allows for the discussion of a variety of molecular biology concepts and techniques such as DNA, gene expression, gene modification tools, cell culture techniques, transfection, and ethics. Specifically, we based our scientific learning experience on Essential Thrombocythemia (ET). This type of cancer is characterized by an increase in platelet count, and three different mutations have been associated with this disease: calreticulin (CALR) type I and type II, thrombopoietin receptor (MPL), and Janus Kinase (JAKV617F) [5, 6]. Since CALR mutations were recently found, the molecular mechanisms that lead the CALR mutation to increase platelet count are not well known. Our intervention used the cutting edge CRISPR-Cas9 gene modification tool to create a CALR cell line that will help us to further understand the mechanisms of the CALR mutant protein.

Theoretical Framework

The framework that encompasses our study is science identity. Identity can be described as a series of representations that give meaning to the role of individuals or groups and describe "the kind of person one is seeking to be and enact in the here and now" [7, 8]. A person's identity is in constant change; it emerges, evolves, and incorporates societal structures, but at the same time, endures over time and context [2].

Although many science identity dimensions have been defined [9-12], we have chosen to focus on the three main categories or dimensions described in Carlone and Johnsons' model: competence, performance, and recognition. Although there are other science identity influencers that are not included in this model [9-12], we chose this model because this model takes into account both the intrinsic factors (self- concepts) and the

extrinsic factors, (perception of individuals on how they are recognized by others) that frame science identity.

Competence

The perception of the student's competencies in research is defined as how the student perceives what he learns and understands. Different key points have been shown to influence the academic performance of students such as being part of underrepresented groups, low family and faculty expectations or support, low social and academic college integration, inculcation of enthusiasm, effective mentoring, and research experiences [4]. Competence brings in accountable factors like logical, academic, analytical, cognitive, and retention skills in science disciplines [3]. Experiences within educational and practical settings, like research, enhance academic outcomes and critical thinking [3] .

Performance

Performance is defined by how the student believes that they act in different relevant scientific practices such as: public speaking, doing experiments, and using equipment [2]. Engaging in scientific research such as hands on experience with laboratory technical skills is tied to this component [13]. Learning about science can serve as personal encouragement to go in depth with their acquired skills and pursue their goals as a science person.

Recognition

Recognition of self as a scientist is strongly influenced by the recognition from others. It is also tied to social judgment and cultural norms [2]. Recognition can be viewed as an essential component to develop identity, for example, a certain pressure to thrive in studies or career can rely on satisfying a family community, or intrinsic motivation to satisfy their

own recognition of self-scientist to pursue their independent goals [2]. In other words, if the student recognizes himself as a scientist and he feels recognized as such by the scientific community, then he will do what it takes to continue receiving recognition depending on his innate beliefs [2].

Methods

Design

A mixed-methods design was used to understand the influence of short scientific learning experiences in high school students. Individuals' competence, performance and recognition categories were based on the individual perceptions. A sequential explanatory approach was used to analyze the impact of a hands-on research experience and a hands-on non-research experience. Quantitative data was collected using the Spanish Translated Science Identity Survey (SIS) before and after the experience [14]. A committee of bilingual experts in translation, education, and/or biology translated and evaluated the Spanish Translated SIS. Content validation was achieved using the think-aloud process with high school students as previously reported [14]. Suggested changes were incorporated in the survey. Subsequently, qualitative data were collected using daily-guided reflexive diaries and focal interviews. Both quantitative and qualitative findings were integrated to understand the influence of short scientific learning experiences. Survey data was used to determine a science identity score index change before and after the experience; diaries and focal interviews addressed how the scientific learning experiences influence students' perception of their competence, performance, and recognition. Cronbach's Alpha was used to measure the survey internal consistency. To address trustworthiness, relevant questions were repeated during diaries and interviews, and three independent researchers compared the outcomes.

Participants and intervention

The study was approved by the UPR Río Piedras Institutional Review Board (IRB protocol 1718-036) and the Department of Education in Puerto Rico. All participants provided informed consent to participate and did not receive any incentives for their participation. Anonymity of all participants is guaranteed.

Participants were recruited from eight low academic proficiency high schools in the municipality of San Juan, Puerto Rico [15]. The participants were selected according to their (1) availability of five constitutive days during their summer break, (2) study grade level (11th grade), (3) availability and willingness to complete a pre and post-survey, reflexive diary, an interview, and (4) parent's availability and willingness to fill out the informed consent. Since the hands-on research and hands-on non-research experience where held in two different weeks, the selection of participants for one experience or the other was according to the week in which the participants were available. Group classification (hands-on research or hands-on non-research) was not disclosed to the participants, and groups did not overlap during the intervention. In order to make groups homogeneous, the participants who were available both weeks were distributed among groups, considering the equal participation of the different schools in both groups. We recruited 40 prospect participants. From those prospects, 12 had the hands-on research experience and 13 had the hands-on non-research experience. The hands-on research group was part of an authentic research experience using the CRISPR-Cas9 technique and the hands-on non-research group was part of a scientific learning experience using models and active learning activities (Supplemental Table 1). The scientific learning experience was conducted in the facilities of the College of Natural Sciences at the University of Puerto Rico – Rio Piedras Campus. As part of the learning experience, both groups received orientation on college admission, sources of

funding for college, internships, myths and realities of research, and laboratory safety practices, The instructor-to-participant ratio for both experiences was 1:2. The same instructors (undergraduate and graduate biology students) guided both groups on the same topics, places, amount of time in the laboratory setting, and outside the laboratory area.

Assessments

The participants answered the Spanish Translated SIS survey before and after the intervention. Students wrote daily on a guided reflexive diary to record their perception about the scientific learning experience. The guided questions of the reflexive diary explored project gains, students' perception of a scientist and its workspace, as well as participants' science competence, performance, and recognition. Additionally, the reflexive diary assessed the relevance and pertinence of the scientific project for the participants, the impact of the experience on study plans, and the contribution of the instructors and staff to their scientific learning experience. A focal group interview of one hour took place with 6 participants at the end of both interventions.

Data Analysis

Quantitative data analysis

Reliability was addressed using Cronbach's Alpha, which is a statistical measure of the internal consistency of the survey [16]. Measurement criterion was as followed: $\alpha \geq 0.90$ (high internal consistency or items may be redundant) $\alpha \geq .80$ (good internal consistency) $\alpha \geq .70$ (adequate internal consistency) [17]. Cronbach's Alpha and descriptive statistics were calculated using IBM SPSS Statistics software package, version 24. In order to comply with the safety requirements of the laboratory, the sample size of each of the groups was 12 and

13 students. The data was analyzed using the Mann-Whitney U test and descriptive statistics. Both analysis and figures were performed using GraphPad Prism Software. Since, missing values were less than 10% of the total survey answers, the missing values for each item were replaced according to the median of the particular item.

Qualitative data analysis

The interviews were transcribed at verbatim. Content analysis was used to interpret the participants' experiences expressed in the interviews and daily reflexive diaries. A deductive approach was used to study scientific learning interventions. We focused on manifest content to code the visible and surface content of text [18]. The justification for the selection of content analysis is based on the fact that our research seeks to understand the influence of short scientific learning interventions and recognize whether the pre-selected categories permeate our data. Before beginning the analysis, we proceeded to identify the pre-conceived categories from the literature (i.e., competence, performance, and recognition). Data were coded according to the predetermined categories. The content of each category was compared between groups. Three independent researchers reviewed the data and the data analysis consensus is presented in the following results section. Adjectives for each of the groups were quantified and the proportions of positive and negative adjectives are reported by categories. The reported quotes were taken from diaries and interviews.

Results

Participants

Participants gender distribution was similar for both groups; 3 females: 1 male. Almost all the participants lived in the metropolitan area of San Juan (93%). Age range was 15 to 18

years old and the median was 17. Mother or father highest degree obtained, field of study, or occupation is reported in Supplemental Table 2.

Survey results

A Cronbach's Alpha index of .836 was obtained, confirming that the instrument is reliable (.70 = low, .80 = moderate, .90 high). Using Mann-Whitney test, a significant difference between the pre and post-test of the hands-on non-research group (p-value <0.0001) was found. A comparison between the pre and post-test of the hands-on research group also was performed and a p-value of 0.0560 was calculated (Figure 1).

Interviews and Reflexive Diaries results

Participants report that they felt like scientists when they gained competence, performed like scientists, recognized themselves as scientists, and perceived that others recognized them as scientists.

In the next sections, we report (1) the student's perception of their science competence, performance, and recognition before the scientific learning experiences; (2) perception changes, if any, after the scientific learning experience, and how the perception changed; and (3) which components of the scientific learning experiences influenced student's perception of their science competence, performance, and recognition. To support each of the main points of this article we included translated (Spanish to English) quotes of the participants. Spanish language original quotes are available in the Supplemental Material section.

A. Competence

The 74% of the participants explained that before the scientific learning experience their knowledge about DNA, cancer, and gene modification techniques was either very basic, none, or wrong. Also, they commented that these topics were difficult or confusing to understand at school. When the participants compared our intervention with the school experience, they expressed that the school did not offer demonstrations of the studied material, it only focused on the lecture, and they did not have confidence to clarify concepts with their teacher. On the other hand, through the intervention, they had the opportunity to go to laboratories, ask questions, and understand topics much more easily. Interestingly, participants reported an exponential learning throughout the program.

- "the teachers that I've had are like: we sit to read a book and we start to discuss and discuss. There is no dynamic, it's like they don't demonstrate [the material]."
- "… [Teachers] should take us to the universities, into the laboratories to teach us, to have a bit more demonstration, and make it more of a dynamic experience, it should be much more different, they don't have to lock you up in a classroom with a book, it's not the same, because here, in this program, for example, we had the opportunity to go to a laboratory and be able to be with all of you [instructors] …"
- "I did not have the confidence to ask… and now…, I feel that I can ask about my doubts and I know that they will clear them up."
- "everything that was discussed, was done in a much easier and interactive way for my style of learning… "
- " …here, in only a few hours, they taught me what the school couldn't do in a year, and I'm not saying it just to say it, I'm say it because it's true."

- "this program makes up for all of the years that I have been in school. The school has not properly taught me some things that this program has…"

Mentorship

Participants emphasized the influence of instructors in their gain of competence. They highlighted that the instructors explained concepts in different and creative ways that allowed them to understand very easily. Several participants commented that their interaction with the instructors was very comfortable, which allowed them to have the confidence to ask and clarify their ideas.

- "the instructors explained very well and with lots of patience to understand it."
- "I liked this project because the staff was very well prepared and willing to help us as many times as necessary."
- "That confidence that the staff gave us (instructor name)… since the day that I came… I could ask questions… about his life in the university, how he could control or use money from university scholarships for his studies, his personal things like gasoline, emergencies, food, car, rent, and a lot of advice, it was of great help."
- "In school I felt embarrassed to ask and here I felt comfortable, I felt at ease to ask anything to anyone, be it my instructor or others, or any of my peers. I truly think that the comfort that I felt was the reason I was able to learn."
- "…and then here they taught me, and made sure you understood, that you learned, and they took their time to explain it again if you did not understand."

B. Performance

Participants acknowledged the fact that being in the laboratory influenced their science performance. In the laboratory, they learned what instruments looked like and what their function was. Participants expressed that in the program they were not just part of traditional, static lectures as in school, but they were part of the research and experimentation.

- "from the dynamics, they took you to the laboratory, you could see what was really happening. You could see the cell count, we could see the instruments that were used for the cell culture and that was what I liked."
- "but I think the difference is that there [school] they told you more and that here [project] they explained to us too, but we also did experiments… "

During the course of both programs, participants reported that multiple practices made them feel like scientists such as the: use of the scientific method, experiments, analysis, present their findings, coming up with ideas, explain and discuss what they have learned, and find information. They expressed that seeing and counting cells in the microscope, and the genomic comparison of the human being and the mouse in the database of NCBI were the most significant experiences of the program.

- "talking or sharing my knowledge with my classmates was cool."
- "I felt like a scientist while we were talking and discussing about our research"
- "at the end of the project to be able to explain what was learned”
- "I had many opportunities to do things that a scientist does such as see cells and do research.

Self-instruction

Participants described that the scientific learning experience prompted them to read biology books, to find more information, ask, and learn more about science. The experience aroused an interest and desire in them to keep looking beyond the information that was provided.

- "My performance changed because I am a person that likes to read but not much about science... and when I got here, it was like ‘boom’ and I got home, and I started to see science related things and research on my own different topics. Being able to search for information by myself and elaborate an idea, that was something that changed in me "

C. Recognition

Recognition of self

Participants commented that they felt like ‘scientists’ when they participated in the activities and demonstrations, but more than anything when they entered the lab, used a pipette and observed cells through a microscope. Also, participants described that they felt like scientists when they searched for universities, understood concepts, answered questions, and discussed what they learned. They shared that they felt like professionals and, due to the acquired skills and knowledge in the program, almost all of them felt like scientists.

The 84% of the participants believed that they were capable to contribute to the scientific community. Those who used the following statement, "scientific work is very complicated" were deemed not able to contribute to the community. On the other hand, those who report that they were able to contribute to the scientific community, focused on what they were able to accomplish during the experience, for example, they were capable of following the scientific method, solve problems, and find an answer. Others focused on their

enthusiasm, dedication to their studies, what they like, what they do on their own as scientists, and their careers goals. Interestingly, some of them commented that they were able to contribute to the community because of what they were doing in the learning experience. They felt that they can contribute to science research and they included themselves as part of the research community. Participants understood that what they were doing is relevant to the community because they were able to understand the disease and potentially find a cure so lives could be saved. Other participants commented that they felt like part of the scientific community because their results could be useful to cure the studied disease.

- "the information we obtain can benefit by helping experts to be closer to a cure"
- "if we know more about this disease, we can create a remedy"

Career goals

Before the scientific experience, participants' interest on STEM careers was very diverse. Thirty-six percent of the participants were already interested in STEM and the experience helped them to awaken their interest in science and see other fields in STEM. Before the experience, 16% of the participants were interested in non-STEM fields, and after the scientific learning experience, they wanted to pursue a career in STEM. For other participants (48%) this experience helped them feel empowered and follow their dreams.

- "For what I want to study, a lot of science is needed, and this woke up my interest in science"

- "After I finished high school I wanted to study only cosmetology and after the experience in Science in Action, I want to study or know the world of chemistry, which interests me a lot."
- "After the experience I want to continue and improve my studies in order to graduate from a good University"

Recognition from others

Some participants (48%) reported that they perceive that others see them as scientists and the 52% of the group perceived that they are not seen as scientists. Those that reported that they are not seen as scientists, explained that because they do not see themselves as a scientist, others will not see them as such. In addition, they reported that they do not see themselves as scientists because of their performance, and competence: “it is not my vocation”, “I am not a professional” “I do not act as such”, “It is hard for me to learn science”. Participants that reported that they perceive that others recognized them as scientists point out that it is because of their competence and performance: “I like investigating on my phone” “I am always asking questions about everything. “I love inventing things”, “I like helping people”, and “because I have curiosity, passion, and potential”.

Hands-on research vs. hands-on non-research experience

In order to understand if experimentation is the key component of scientific learning experiences that help high school students to perceive themselves as scientists, we divided our cohort of participants into two groups: one group performed experiments (research) and the other group developed models with crafts (non-research). When science identity change

index scores were compared between groups, we found that the score for the non-research group (median 14) was significantly higher (p-value: 0.0006) than the research group (median 5.5) (Figure 2). To understand why non-research group had a higher science identity score, we compared the diaries, interviews, and the use of adjectives to describe the experience for both groups. Interestingly, the group that performed the experiments described their competence and performance with more negative adjectives (competence:19%; performance 29%) than the comparison group (10%; performance 20%), for example they mentioned that the learning process was complicated or difficult (Table 1).

Participants of the non-research experience report that they were experimenting when they were observing the cells in the microscope and working with models. The group that was part of the authentic research experience highlights its performance describing the techniques that they learned: the use of the pipette and other instruments, and the cell culture work. Both groups report that they were able to experiment. No differences between groups in terms of the participants' self-recognition as a scientist were found.

Discussion

Previous studies have found that summer or long-term research experiences influence students' academic outcomes [1,3,19]. These experiences allow participants to grow professionally, intellectually, and reinforce their critical thinking skills. Similarly, our participants reported that the one-week scientific learning experiences had influenced their critical thinking and learning. Moreover, demonstrations, mentorship, laboratory setting, and the ability of students to ask questions and clarify their doubts (as described by participants in their reflexive diaries) influenced participants' perception of their competence and made

them feel like scientists. Our results demonstrated that the science identity of both groups increased at the end of the learning experience, indicating that they did feel like scientists.

Studies on long-term research experiences also have shown that scientific experiences develop students' critical thinking, their ability to be independent, and develop student's own ideas [1, 3, 19]. Similarly, our results show that participants' performance was influenced by their ability to instruct themselves, find information, develop their own ideas, and explain what they have learned. Noteworthy, both groups claimed that they performed experiments although the hands-on non-research experience group only practiced demonstrations and active learning activities. These results suggest that participants' interpretation of scientific tasks influences participants' perception of their performance and ultimately their science identity. Because our student's performance was based on self-perceptions and not directly tested, it would be interesting to add direct assessment of skills gained. Furthermore, it would be exciting to follow the longer-term effects of this study. We would expect that both performance and retention would be improved in both groups. These are subjects of ongoing studies in our laboratory.

A growing amount of evidence points out that mentorship is key for students' retention and persistence in STEM [21,22,23]. Interestingly, Daniels *et al.* showed that mentoring is more significant and efficient to promote students personal, and skill gains than the time spent doing research [24]. Our results support these findings; although time spent in research is important, mentorship is key to develop students' confidence, competence and performance. Participants' gained confidence in themselves from interactions with mentors and, due to this interaction, they report that their future career plans and educational possibilities in science have broadened. Students also report that being with younger

scientists helped them unravel the paradigm that just older people are scientists; they realized that they could contribute to science too.

These findings suggest that our one-week scientific learning experiences enhance participants' science identity and create an opportunity to develop further studies on short interventions using models and active learning activities. Although engaging in experimentation, as reflected by participants' comments, is very important for the development of their perception on scientific performance, other components such as mentorship, laboratory setting, and active learning activities influence participants' perception of their competence and performance.

Interestingly, through the reflective diaries and interviews, participants of the research learning experience described the experience with more negative adjectives (such as difficult, complicated, and deficient) than the comparison group. We infer that this may be a result of their naivety doing research experiments and their lack of experience in problem solving strategies that are required during authentic-research activities. Problem-solving tasks may be a challenge for those who are not used to laboratory work or have low confidence performing activities that require analysis [20]. We can also not dismiss that this perception was influenced by the short duration of this learning experience, as this observation has not been made in long-term research experiences (1-4).

The effectiveness of hands-on research experiences is well established in the literature [1-4]. Our finding is novel as our work suggests that it may be possible to develop science identity at an early level (i.e., high school) with short term, low-budget, hands-on scientific learning experiences. The significance is highlighted by considering that our population, like many other underrepresented minorities, is normally deprived of significant

scientific learning experiences and/or laboratories due to budget or space limitations. Furthermore, their access to researchers or STEM professionals to serve as mentors or role models is very limited. Hence, we suggest that inexpensive, short, hands-on non-research activities can be implemented and may have a positive impact on student's science identity.

Since both research and non-research scientific learning experiences had a positive influence on students' science identity, we suggest the use of either method to develop science identity in high school students. These findings can guide efforts for the development of low-cost strategies (i.e., hands-on activities and demonstrations) that can be easily implement in high school classrooms. Our findings support, and we encourage, the collaboration between high schools and Universities to provide high quality mentorship to high school students.

Contributions

LHM wrote the manuscript, conducted the interviews, coordinated the program activities, analyzed the data, prepared most of the program lectures and revised all lectures. Also coordinate and offered the instructors training, and create Table 1, Supplemental Table 2. LPD was the co-coordinator of program activities, prepared some of the program lectures, served as instructor of the program, analyzed statistical data, did the Supplemental Table 1, and formatted part of the manuscript for submission. FLT was the co-coordinator of the program and activities and prepared some of the program lectures. NCJ prepared some of the program lectures. SMT coordinated some program lectures and activities, served as instructor of the program, wrote part of the introduction of the article, and translated the participants' quotes. PLR gave remarks in the analysis. AVW assisted on the logistic plan, lectures to the students and writing of the manuscript. MB took part on the logistic plan and program

content coordination. Also, MB assisted on the manuscript writing. All authors reviewed the final manuscript.

Potential conflicts of interest

The authors declare no conflict of interest.

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Figures

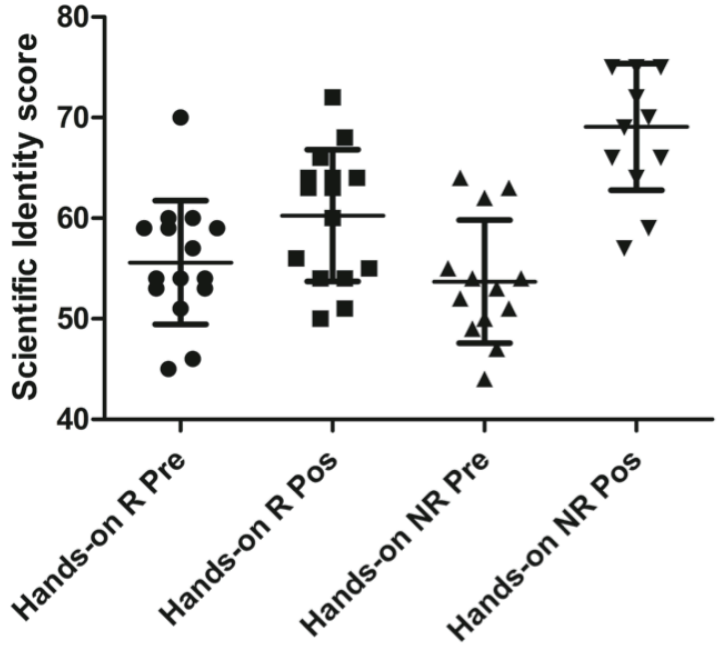

Figure 1. Science Identity Scores after Short-Scientific Experiences. Hands-on R stands for Hands-on Research experience and Hands-on NR stands for Hands-on non-research experience. Hands-on NR n=13, Hands-on R n=12. The lines in the scatter dot plot diagrams represent mean with SD. Significance was established at p-value <0.5; *** p-value: <.0001.

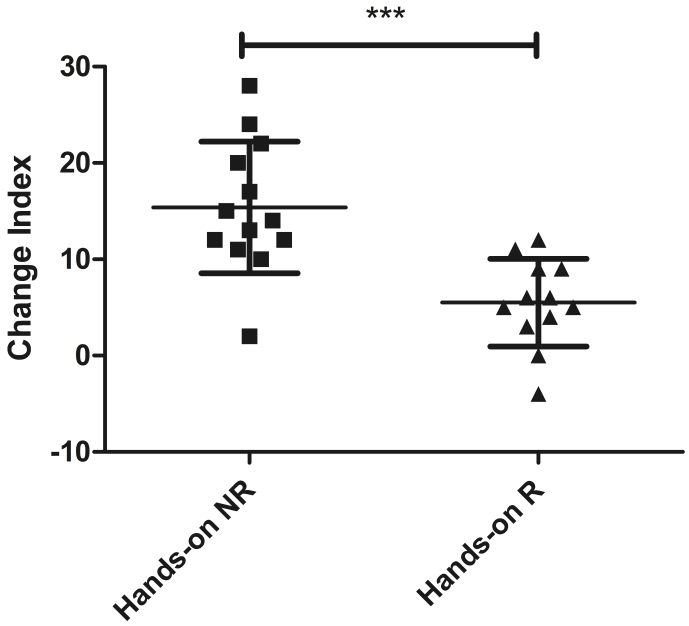

Figure 2. Science Identity Survey Index Score Change. Hands-on R stands for Hands-on Research experience and Hands-on NR stands for Hands-on non-research experience. Hands-on NR: n=13 Hands-on R: n=12. The lines in the scatter dot plot diagrams represent mean with SD. Significance was established at p-value <0.5; *** p-value: 0.0006.

Tables

	Hands-on Non-Research		Hands-on Research	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Competence	90	10	81	19
Performance	80	20	71	29
Recognition	100	0	100	0

Table 1. The students' reported percentages of positive and negative adjectives for pre-determine categories.

Supplemental Materials

	Hands-on	Non hands-on
Day 1	Pre-survey	
	Dynamic and interactive activities: DNA, base pairing combinations, protein codes, DNA modifications	
	Videos: scientific methods and cell culture	
	Cell count with hemacytometer	Demonstration/observe cell count procedure
Day 2	Reflexive diary	
	Lectures: ET, CRISPR-Cas9	
	gRNAs design in Benchling	
	Define problem, hypothesis, and methods	
	CALR cell lines design with different gRNAs by transfection	Create models: cell count, transfection, CRISPR-Cas9...
Day 3	Reflexive diary	
	Two scientists talked about their academic trajectories	
	Lectures: college submission, grant opportunities, internships	
Day 4	Reflexive diary	
	Final presentation preparation	
	Analysis of transfection and transformed cells Debriefing of results	Lecture about flow cytometry
Day 5	Reflexive diary	
	Tour around Molecular Science Research Center	
	Final presentations of results	Final presentation of what they learned in the program
	Post-survey	
	Focal group interview	

Supplemental Table 1. Description of the intervention, activities and topics covered for each group

Socio-demographic information	Scientific Experience	Hands-on experience
Father highest degree obtained		
High School	7	4
Two Year Associate	1	0
Bachelor's Degree	2	5
Postgraduate or professional degree	0	0
N/A	5	5
Mother highest degree obtained		
High School	4	5
Two Year Associate	1	1
Bachelor's Degree	6	4
Postgraduate or professional degree	2	0
N/A	2	4
Father field of study		
N/A	6	5
STEM	5	3
Other	4	6

Father occupation		
N/A	6	6
STEM	1	1
Other	8	7
Mother field of study		
N/A	5	7
STEM	0	0
Other	10	7
Mother occupation		
N/A	2	3
STEM	4	0
Other	9	11

Supplemental Table 2. Mother or father highest degree obtained, field of study, or occupation

Spanish original quotes with English translation

- "the teachers that I've had are like, we sit to read a book and we start to discuss and discuss and there is no dynamic, they do not demonstrate it [the material].

  "los maestros que me han tocado es como que nos sentamos a leer un libro y empezamos a discutir y a discutir y no hay ninguna dinámica como que no te muestran"

- "… [Teachers] should take us to the universities, in to the laboratories to teach us, to have a bit more exposition, and make it more of a dynamic experience, it should be much more different, they don't have to lock you up in a classroom with a book, it's not the same, because here, in this program, for example, we had the opportunity to go to a laboratory and be able to be with all of you [instructors] …"

  "[Los maestros] deberían de tratar de llevarnos a la Universidades, a laboratorios a que nos enseñen a que estemos un poco mas expuestos y sean mucho más dinámicos las experiencias y sea mucho más distintos no solamente te tienen en un salón de clase encerrado con un libro y no es lo mismo, porque aquí en este programa por ejemplo tuvimos la oportunidad de ir al laboratorio y de poder estar con ustedes que tienen mucha más experiencia…"

- "I did not have the confidence to ask… and now…, I feel that I can ask about my doubts and I know that they will clear them up."

"También, no tenía la confianza de preguntar. Soy una persona mega tímida. Como ven estoy hablando ya con más confianza y ahora que siento que venga el año nuevo escolar, siento que puedo preguntar de mis dudas y se que me van a aclarar."

- "everything that was discussed, was done in a much easier and interactive way for my style of learning… "

  "todo lo discutido, fue una manera mucho más fácil e interactiva para mi aprendizaje o como tal reforzar material aunque en este caso la información la capte mucho mejor que la vez anterior que había estudiado esta"

- " …here, in only a few hours, they taught me what the school couldn't do in a year, and I'm not saying it just to say it, I'm say it because it's true.

  "...aquí en unas cuantas horas me enseñaron lo que no me pudieron enseñar en la escuela por un año y no lo dije pues por querer decirlo si no por que fue cierto"

- "this program makes up for all of the years that I have been in school. The school has not properly taught me some things that this program has…"

  (original quote is in English)

- "the instructors explained very well and with lots of patience to understand."

  "los instructores nos explicaron muy bien y con mucha paciencia para entenderlo."

- "I liked this project because the staff was very well prepared and willing to help us as many times as necessary."

  "Me gustó este proyecto ya que el personal estaba muy bien preparado y dispuesto a ayudarnos las veces que fueran necesarias."

- "That confidence that the staff gave us (instructor name)… since the day that I came… I could ask questions… about his life in the university, how he could control or use money from university scholarships for his studies, his personal things like gasoline, emergencies, food, car, rent, and a lot of advice, it was of great help."

  "Esa confianza que el staff nos brindó como [nombre de instructor]… que ya el mismo día que llegué… me dejaba hacerle preguntas… como fue su vida universitaria, como él pudo controlar o usar ese dinero de la beca para la universidad, sus estudios, sus cosas personales como gasolina o algo de emergencia, comida, aquello, carro renta y me dio bastantes consejos y en verdad fue buena ayuda."

- "In school I felt embarrassed to ask and here I felt comfortable, I felt at ease to ask anything to anyone, be it my instructor or others, or any of my peers. I truly think that the comfort that I felt was the reason I was able to learn."

  "Yo en la escuela pues me da ese pacho de preguntar y aquí me sentí cómoda, me sentí en la comodidad de poder preguntar a cualquier persona sea pues mi instructora o otros o a los mismos compañeros. De verdad que fue, creo que fue mucha la comodidad que sentí por la cual yo aprendí"

- "…and then here they taught me, and made sure you understood, that you learned, and they took their time to explain it again if you did not understand."

  "entonces aquí ellos te lo enseñaban, se cercioraban de que te lo aprendieras, que supieras y se tomaban el tiempo de volverte a explicar si no lo entendías.."

- "from the dynamics, they took you to the laboratory, that you could see what was really happening. You could see the cell count, we could see the instruments that were used for the cell culture and that was what I liked."

  "de las dinámicas, te llevaban al laboratorio, que tu podías ver lo que estaba pasando en realidad. Tu podías ver el conteo de células, pudimos ver los instrumentos que se utilizaban para el cultivo de células y eso fue lo que me gustó."

- "but I think the difference is that there [school] they told you more and that here [project] they explained to us too, but we also do the experiments… "

  "pero yo pienso que la diferencia es que allá [escuela] te decían más y que aquí [proyecto] nos explicaban también pero experimentábamos, interactuábamos más con lo que nos estaban explicando."

- "talking or sharing my knowledge with my classmates was cool."

  "hablar o compartir mi conocimiento con mis compañeros fue cool."

- "I felt like a scientist while we were talking and discussing our research"

  "me sentí como un científico mientras hablábamos y discutíamos sobe nuestra investigaciones"

- "at the end of the project to be able to explain what was learned"

  "al final del proyecto poder explicar lo aprendido"

- "I had many opportunities to do things that a scientist does such as see cells and investigate.

  "tuve muchas oportunidades de hacer cosas que un científico hace como ver las células e investigar."

- "My performance changed because I am a person that likes to read but not many things about science... and when I got here and it was like 'boom' and I got home and I started to see science related things and research on my own different topics. Being able to search for information by myself and elaborate an idea, that was something that changed in me"

  "Mi desempeño cambio porque soy una persona que me gusta leer pero no muchas cosas como de ciencia y que se yo y cuando llegue aquí y fue como que boom y llegue a casa rápido y empecé a ver cosas de ciencia y investigar por mi cuenta diferentes temas y me decían algo y yo ok esta bien. Poder buscar yo información y elaborar una idea, eso fue algo que cambió en mi"

- " "the information we obtain can benefit by helping experts to be closer to a cure"

  "que puede beneficiar la información que obtengamos para ayudar a expertos a estar más cerca de una cura"

- "if we know more about this disease we can create a remedy"

  "que si conocemos más sobre esta enfermedad podemos crear un remedio"

- "For what I want to study, a lot of science is needed and this woke up my interest towards science"

  "Para lo que yo quiero estudiar se necesita mucha ciencia y pues esto despertó mi interés hacia la ciencia"

- "After I finished high school I wanted to study only cosmetology and after the experience in Science in Action I want to study or know the world of chemistry, which interests me a lot."

  "Luego de que terminara la escuela superior yo quería estudiar solo cosmetología y a partir de la experiencia en Science in Action deseo estudiar o conocer el mundo de la química, la cual me interesa mucho."

- "After the experience I want to continue and improve my studies in order to graduate from a good University"

  "Luego de la experiencia quiero continuar y mejorar mis estudios para poder graduarme de una buena Universidad."

Chapter V
Conclusion

In our studies we address the need characterize the Puerto Rican cohort of ET patients; its mutational, clinical background and cytokine profile. Also, there was a need to identify the best strategies that enhance students' science competences and performance. To address these gaps we proposed the following specific aims.

> Aim I. Determine the cytokine profiling of a Puerto Rican cohort of Essential Thrombocythemia (ET) patients and compare it with previously reported ET cohorts.
>
> Aim II. Validate a scientific identity survey and evaluate if short research interventions using CRISPR-Cas9 technology could modify participants' scientific identity and career goals.

In order to characterize the cohort of ET Puerto Rican patients we first identified the proportion of JAKV617F, CALR and MPL mutations; we found a proportion of 52%, 18%, and 4%, respectively. Interestingly, the mutational profiling was similar to the previously published populations [1, 2]. However, the ET cytokine profile was slightly different when compared to the previously reported ones. It was found that MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2 and IL-29/IFNλ1 were significantly higher in ET patients than in control patients. Noteworthy, from the eight different analytes that were significantly higher, just IL-8 was known to be higher in ET patients [3]. Even though MMP-1 and IL-22 were increased in the ET Puerto Rican cohort, previous studies found that ET patients had normal levels for MMP-1 and IL-22. MMP-1 is known to regulate thrombus formation and platelet activation [4]. Interestingly, not just MMP-1 but some of the analytes that were

highly expressed in ET patients have been shown to have a direct effect on platelet count. For example, IL-8 was found to impair the proliferation and differentiation of megakaryocyte and myeloid progenitor cells on patients with myelofibrosis. Moreover, the neutralization of IL-8 receptors had been shown to restore megakaryocytes ploidy [5]. However, mice treated with IL-22 showed a significant increase in platelet count [6]. Previous articles have reported that Interferon alpha (IFN) inhibits the growth of megakaryocyte progenitor cells leading to a reduction of peripheral platelet counts on ET patients [7]. Also, IFN had been shown to down-regulate the expression of IL-8 and enhance the expression of IL-22 [8-10]. Indeed, emphasis should be made on the fact that all the tested interferons were lower in non-diabetic ET patients than non-diabetic control patients.

In order to further characterize ET patients, we not only defined their cytokine profile but also analyze two different molecular markers of thrombotic events: LOX and sTLT-1. Even though LOX protein had been associated with thrombotic events, LOX levels were not able to explain ET patients' thrombotic risk in our population. Soluble TLT-1 levels were significantly higher in ET patients than the control group. We suggest that the augmentation of sTLT-1 levels may be associated with the hyperactivation of platelets in ET patients, but further studies are needed to validate this observation. This characterization of the ET Puerto Rican cohort described in this study provides multiple insights for further studies and laid the foundation to a better treatment for ET patients.

ET patient characterization including mutational and clinical background, cytokine profile, LOX and TLT-1 levels covers a wide range of topics in molecular biology. This framework was not only used to understand and characterize ET neoplasm, but to introduce developing scientists to a wide range of molecular biology topics. In order to identify the

outcomes of the students' scientific experiences and identify which components actually are important for STEM retention of high school Latino/Hispanic population we translated and evaluated a Science Identity Survey (SIS).

As described in Chapter 2, the SIS survey was created to evaluate the impact of students' experiences at informal education centers; it measures students' competences, performance and recognition. In order to use this survey to assess the outcomes of short research experiences of a Latino/Hispanic population, we first translated it, then evaluated the content of each item using think aloud process, and then tested its reliability and construct.

After the think aloud process, participants agreed that the original numbered Likert scale was subjective, and the descriptive word scale was more informative. For this reason, the original numbered scale on the SIS was replaced and the word descriptive scale was incorporated. This result is consistent with previous research on scales that found that numbered scales are subjective to participant interpretations and are more problematic for respondents that do not tolerate ambiguity [10]. Interestingly, our results show that participants tend to assign higher scores when using the numbered scale than when they are using the descriptive word scale. This effect may be influenced by the submissive response (*simpatía*) style documented among Latinos and Hispanics [11]. Our results suggest that the use of a descriptive word scale can help participants to think about the best word that describes their answer and not on giving the highest score possible to each item. This finding is not only relevant for the aforementioned survey, but for further instruments administrations since give a strong insight of the answer patterns in this population.

After content evaluation and scale changes, an exploratory factor analysis and Cronbach's alpha index were calculated to explore the structure and reliability of the survey.

A 4-factor solution was suggested, but one of the factors was not reliable. As a result, this factor was deleted. Based on the exploratory factor analysis, parallel analysis, and think-aloud comments we recommended a rearrangement of the items on each of the dimensions and the deletion of items 8, 9, and 11. Cronbach's alpha index was re-calculated for the final version of the survey and we obtained a result of 0.857.

After content and construct validation of the Spanish version of the Science Identity Survey (SISE) we were anxious to accomplish aim 2; to explore the influences of short research experiences on high school students' science identity and identify which were the most influential activities or components of these experiences. Previous studies have found that summer or long-term research experiences influence students' academic outcomes [12, 13]. These experiences allow participants to grow professionally, intellectually, and to reinforce their critical thinking skills. Similarly, the one-week experiences reported in this article also influenced participants' critical thinking and learning. Moreover, demonstrations, mentorship, and laboratory setting, (as described by participants in their reflexive diaries) influenced participants' competences and made them feel like scientists. Our results demonstrated that the science identity of both groups increased at the end of the intervention as shown by the change index score, indicating that they did feel like scientists.

Interestingly, the hands-on research group described their learning experience with more negative adjectives than the comparison group. We infer that this may be a result of their inexperience doing research experiments and the lack of expertise in problem solving strategies that are required during authentic-research activities. Problem-solving tasks may be a challenge for those who are not used to open laboratory work or have low confidence performing activities that require analysis [14]. Students that were able to experience

authentic-research activities had the experience of actually trying to get something to work, as opposed to just theroetically solving the problem.

Studies on long-term research experiences also have shown that scientific experiences develop students' critical thinking, their ability to be independent, and develop student's own ideas [12, 13]. Similarly, our results show that participants' performance was influenced by their ability to instruct themselves, find information, develop their own ideas, and explain what they have learned. Noteworthy, both groups claimed that they performed experiments although the hands-on non-research experience group only practiced demonstrations and active learning activities. These results suggest that participants' interpretation of scientific tasks influences participants' perception of their performance and ultimately their science identity.

A growing amount of evidence points out that mentorship is key for students' retention and persistence in STEM [15-17]. Interestingly, Daniels *et al.* showed that mentoring is more significant and efficient to promote students' personal, and skill gains than the time spent doing research [18]. Our results support these findings; although time spent in research is important, mentorship is key to develop students' confidence, competences and performance. Participants' gained confidence in themselves from interactions with mentors and thanks to this interaction; they report that their future career plans and educational possibilities in science have broadened. Students also report that being with younger scientists helped them unravel the paradigm that just older people are scientists; they realized that they could contribute to science too.

These findings suggest that our one-week scientific programs enhance participants' science identity and create an opportunity to develop further studies on short interventions

using models and active learning activities. As reflected by the participants' comments, engaging in experimentation is very important for their scientific performance development. However, there are other components such as mentorship, laboratory setting, and active learning activities that influence participants' science identity. These findings can guide our efforts, and others, for the development of activities that promote science identity, which in turn impact the persistence in STEM disciplines. Also, these findings may be pertinent to Puerto Rican high school teachers as it suggests that providing students with low-cost strategies (i.e., hands-on activities and demonstrations) that can be implemented in the classroom easily can improve students' science competences and performance. Our findings support, and we encourage, the collaboration between high schools and Universities to provide high quality mentorship to high school students.

Summary

Through this work we determine the cytokine profiling of a Puerto Rican cohort of ET patients and we have compared our results with the previously available data on ET cohorts. Also, we were able to use this framework as a study context of short scientific experiences. In order to determine the outcomes of these experiences we were able to evaluate the content and construct of a scientific identity survey. Finally, we evaluate how research interventions using CRISPR-Cas9 technology modify participants' science identity and career goals and which were the events that influence science identity. We have accomplished the two main goals of this work successfully. Thanks to the information gathered and the conclusions of this study, we have a better understanding of ET neoplasm to further improve patients' treatment. Also, we have a new tool for the evaluation of science identity that results from short scientific (outreach) interventions. Moreover, we now

understand the main problems of science education, and provide tools to improve students' competences and performance. We encourage the students' participation on short research experiences, not only to increase the participation of Latino/Hispanics into the science workforce, but to also guide our society into a scientific responsive culture.

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Chapter VI
Ford Post-doctoral Proposal

How mentorship influences undergraduate mentors' science identity.

Our previous studies on short research experiences found that high school students' gained confidence in themselves and that they had broadened their future career plans as a consequence of their interactions with undergraduate mentors. Interestingly, high school students also report that being with undergraduate scientists helped them unravel the paradigm that just older people are scientists [1]. As the creator and coordinator of the Science in Action research program I have seen the development of undergraduate mentors during the research experience, which made me wonder: Is the mentor's own scientific identity being developed through the act of mentoring?

Mentorship is defined as the influence of someone more experienced (mentor) in the guidance and support of someone less experienced (mentee) [2]. Science mentorship can take different forms such as: instrumental mentoring and socio-emotional mentoring. Instrumental mentorship is focused on providing the mentee with skills and resources that may prompt the mentee to succeed. Socio-emotional mentoring involves the emotional and social support to the mentee. Mentoring has been shown to improve mentees academic productivity, attitude toward the field, professional identity development, and motivation to learn [3]. Indeed, mentors report that mentorship enhances their own personal learning and skill development [4]; but is the mentor's own scientific identity being developed through the act of mentoring? Does being a mentor as an undergraduate help to develop competence and performance skills? In order to understand the influence of mentorship on the scientific identity of undergraduate mentors I propose to address the following specific aims:

Aim I. **Identify the motivations and challenges of the mentorship experience**. The motivation of science professors to offer mentorship is mainly focused on enhancing gender or ethnic equity and/or supporting students access to scientific training [2]; but are these motivations the same for undergraduate mentors? Why do undergraduate students volunteer to be mentors? What are the main challenges and gratifications? I hypothesize that undergraduate mentor's motivation are to improve their research competencies and performance, and also to support high school students' access to scientific training.

Aim II. **Determine how being a mentor influences science identity.** Carlone and Johnson's science identity framework is based on the interrelated dimensions of competence, performance and recognition [5]. Competence is defined as "knowledge and understanding of science content"; performance is defined as "social performances of relevant research practices such as: ways of taking and using tools"; and recognition by "recognizing oneself and others as a "science person" [5]. A person who has a strong science identity "makes visible to (performs for) others one's competence in relevant practices, and, in response, others recognize one's performance as credible". One of the facets of a scientist is being a mentor; for this task, scientists need to develop effective mentorship skills. These include a set of characteristics such as: being enthusiastic, intellectual, skilled, and ability to act as career guides [6]. To what extent do undergraduate students display these characteristics to be mentors of high school students? How does mentorship influence the mentor's science identity? I hypothesize that undergraduate students that volunteer to be mentors might report that they were able to act as career guides and that during the experience their science identity evolves.

Aim III. **How being a mentor and being mentored influences students' science identity?** Mentoring improves mentees academic productivity, development, and competences.

Mentoring also benefits mentors by enhancing their own personal learning and skill development [7]. How do undergraduate students view being a mentor and receiving mentorship? How do these experiences differ from each other in terms of their influences on students feeling like a scientist? How do these functions complement each other and are they effective for the students' scientific development? I hypothesize that being a mentor will positively influence student's science identity, and that these experiences will help students to develop different scientific competencies.

Methodology The research methodology that is going to be used is ethnographic content analysis. Following the IRB accepted protocol, I will recruit 6 students that participated as mentors in the Science in Action Program. Mentors will be contacted via telephone and email for an individual deep interview of 1 hour to 2 hours. Interviews will be audio-recorded and transcribed at verbatum. Pre-selected categories from the current research on

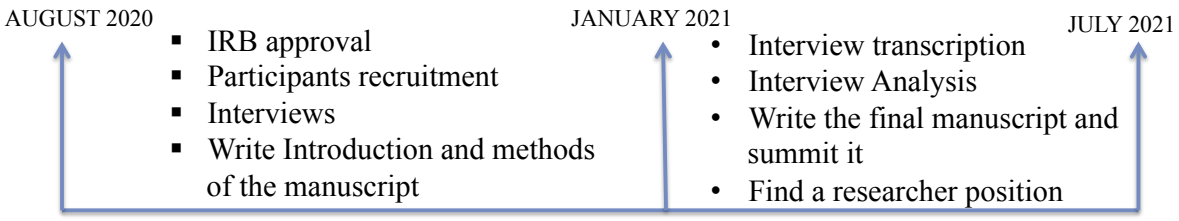

Figure 1. Timeline

mentorship will be used to analyze the content of the interviews, and the science identity theoretical framework will be used to shape our analysis. Results will be reported using pseudonyms. Timeline is detailed in Figure 1.

Broader Impacts The proposed study will define the outcomes of "being an undergraduate mentor" as an effort to develop students' science identity and further persistence in STEM. This work will be carried out with students of the University of Puerto Rico (UPR), Hispanic-serving institution to complement the research of the UPRH Howard Hughes

Medical Institute-funded Puerto Rico Outstanding Undergraduate Diversified (PROUD) program, where I collaborate. Together we will understand what the pitfalls and the necessary improvements are to increase the students' persistence and competence.

As part of this group I am expanding my professional associations not only with the UPR system, but also with evaluators, professors, and administrative staff from all over the world. This post-doctoral position will allows me continue within this amazing group of educators and researchers advancing the science education research, not only for undergraduates but also to a continuum of generations from high school students to professors.

Annotated Bibliography

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This article discusses the influence of short research experiences of high school students through the lens of science identity as defined by Carlone and Johnson. As a result of this experience, high school students highlight the importance of their undergraduate mentors on their competencies and future plans. Through this research project I was able to identify the objectives and research questions for this proposal. The undergraduate students that were part of this short experience will be the participants for the proposed work.

[2] R. D. Robnett, P. A. Nelson, E. L. Zurbriggen, F. J. Crosby, and M. M. Chemers, (2018) "Research mentoring and scientist identity: insights from undergraduates and their mentors," *Int J STEM Educ,* vol. 5, no. 1, p. 41.

This study highlights the importance of mentorship and the different forms of mentorship in the development of students' science identity. For the proposed project I will use the description of the different forms of mentorship explained in this article for data analysis. Also, I will use the mentor motivation and challenges explained in this article as pre-selected categories for data analysis.

[3] P. R. Hernandez *et al.* (2017) "Promoting professional identity, motivation, and persistence: Benefits of an informal mentoring program for female undergraduate students" *PLoS One,* vol. 12, no. 11, p. e0187531.

This study highlihts the benefits of mentorship to develop science identity and interest in science. Specially, talks about informal education strategies and how mentorship is the key in this process.

[4] M. Jacobi, "Mentoring and Undergraduate Academic Success: A Literature Review:," (1991), *Review of Educational Research, vol 61, no. 4 p. 505-532.*

This article discusses a variety of mentorship definitions throughout the area of higher education, managing, and psychology. Explains the role of the mentor and some of its benefits. This article is important for shaping the profile of the mentor and offers a point of contrast to my study.

[5] H. B. Carlone and A. Johnson, "Understanding the science experiences of successful women of color: Science identity as an analytic lens (2007) Journal of Research in Science Teaching .

This article explains a framework model of science identity using student competence, performance, and recognition. Its unique definition and context is used to dismantle the science experiences of women of color. This work effaces (means destroys, obliterates… is this what you want?) in the recognition of others as a key point of identity and a predictor of success. The science identity model explained in this article will be used as the theoretical framework of the proposed research.

[6] C. S. Cho, R. A. Ramanan, and M. D. Feldman, "Defining the ideal qualities of mentorship: a qualitative analysis of the characteristics of outstanding mentors," (2011), Am J Med, vol. 124, no. 5, pp. 453-8.

This study defines the qualities of a good mentor. The authors select the recipients of the Lifetime Achievement in Mentorship Award as participants of the study. They used the mentors descriptions on recommendation letters to define good mentorship characteristics. This list of characteristics is very useful because I will use these descriptions to contrast undergraduate mentor characteristics.

[7] L. J. Liu D., Kwong Kwan H., Mao Y., "What can I gain as a mentor? The effect of mentoring on the job performance and social status of mentors in China (2010) *Journal of Occupational and Organizational Psychology.*

This article explains the gains of the mentors as consequence of the mentorship task. The gains reported on this article will be used as pre-selected categories for the data analysis.

Appendix I

Inflammation Biomarkers	Description	Evidence related to MPD	Ref.	Expressed by
APRIL/ TNFSF13 Cytokine TNF superfamily	• APRIL (ligand) enhances the proliferation and survival of plasma cells. • Is a powerful B cell-activating molecule • APRIL can inhibit pathologic humoral responses as well as autoimmune disorders progression. • Is elevated in many cancers primarily due to expression by tumor-infiltrating neutrophils	Information not found	[1] [2] [3]	Myeloid cells macrophages and dendritic cells
BAFF/TNFSF13B Cytokine TNF superfamily	• Enhances B-cell survival • The overexpression of BAFF results in severe autoimmune disorders in mice • Elevated in some patients who have autoimmune diseases. • Higher in multiple myeloma	The mRNA and plasma TNFSF13B were elevated in active Immune thrombocytopenia patients than healthy patients.	[4] [5]	Macrophagesdendritic cells, astrocytes
sCD30/TNFRSF8 Cytokine TNF superfamily	• Signal transduction that leads to the activation of NF-kappaB. • Is a positive regulator of apoptosis.	Information not found	[6]	Activated T and B cells.
sCD163 Scavenger receptor	• Is a specific marker of activated macrophages. • Associated with fibrosis stages. • A scavenger receptor for the hemoglobin-haptoglobin complex • Shed into blood in a soluble form (sCD163) after stimulation from Toll-like receptors and oxidative stress	Information not found	[7] [8]	Monocytes, macrophages

	• Higher levels in patients with advanced fibrosis and chronic viral hepatitis.			
chintinase-3-like 1/ YKL-40 Secreted glycoprotein	• It is assumed that YKL-40 plays a role in cancer cell proliferation, survival, invasiveness and in the regulation of cell-matrix interactions. • YKL-40 regulates vascular endothelial growth factors and induces tumor proliferation.	Baseline PV patient levels were 2 times higher than in healthy controls ($P<0.0001$) and 1.7 times higher than in ET ($P=0.02$).	[9]	Macrophages, chondrocytes, fibroblast-like synovial cells, vascular smooth muscle cells, and hepatic stellate cells.
Glycoprotein 130 Type I cytokine	• It has been implicated in the regulation of a wide variety of adult tissue systems, including haemopoesis, nervous system, bone, heart, adipose tissue, testes, liver and muscle. • Ubiquitously expressed, signal-transducing receptor • Depending on the cytokine that activates de receptor complex it can promote or suppress inflammation.	Gp130 is increased in patients with essential thrombocythemia (ET) .	[10] [11] [12] [13]	Monocytes
IFN-α2 Type I cytokine IFNs family	• The encoded protein is a cytokine produced in response to viral infection. • Type I IFNs exert potent antitumor activity by several mechanisms such as: o inhibition of the proliferation of cancer cells o activation of the immune system which can eliminate tumor cells	Exhibits a clear platelet reductive effect in patients with Essential Thrombocythemia Levels not found	[14] [15]	Macrophages

Interferon β-1a IFNs family	• Used to treat multiple sclerosis (MS) • It regulates many genes that are involved in antiviral and antiproliferative activities • Binds to the interferon receptor, which consists of two components (IFNAR1 and IFNAR2), and activates the Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway to phosphorylate STAT1 and STAT2. • This in turn activates interferon-stimulated genes and leads to the production of antiviral, antiproliferative, and antitumor products.	Administration reduce the number of platelets	[16] [17]	Macrophages Fibroblast
IFN-y IFNs family	• IFNγ is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression. • Aberrant IFNγ expression is associated with a number of autoinflammatory and autoimmune diseases. • IFNγ binding to the receptor activates the JAK-STAT pathway	Is not an effective treatment for ET	[18] [19]	T-cells natural killer
IL-2 Type I cytokine	• Accordingly, a major function of IL-2 is to promote proliferation of both CD4+ and CD8+ T cells. IL-2-induced proliferation occurs via pro-proliferative signals through the proto-oncogenes c-myc and c-fos, in combination with	The serum derived from patients with MMM, ET, PV and CML contained significantly higher IL-2 and sIL-2Ra than healthy	[20-23]	Lymphocytes. T-cells

	anti-apoptotic signals through Bcl-2 family members	subjects.		
sIL-6R alpha	• Its level is increased in patients with multiple myeloma and human immunodeficiency virus infection.	Information not found	[24, 25]	Fibroblast
Il-8 Chemokine attached to G protein coupled receptor family	• It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. • Induces phagocytosis • Potent promoter of angiogenesis. • In target cells, IL-8 induces a series of physiological responses required for migration and phagocytosis, such as increases in intracellular $Ca^{2+}$and exocytosis	There is a relationship between individual MPN symptoms (fatigue, abdominal complaints, microvascular symptoms, and constitutional symptoms) and IL-1, IL-6, IL-8, and TNF-α	[26] [27]	Macrophages Epithelial cells, airway smooth muscle cells and endothelial cells.
Il-10 Th2 (anti-inflammatory) IL-10 family	• Is an important immunoregulatory cytokine that inhibits T cell function by suppressing the expression of proinflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, and IL-12	Excess production of IL-4, IL-10, and TNF-α has been reported in ET	[28]	T cells
IL-11 gp130 family of cytokines	• Stimulation of megakaryocyte maturation	Higher only in PV. Not elevated in patients with thrombocytosis	[28] [29] [30]	Endothelial cells
Il-12	• Promotes the development of Th1 responses and is a powerful inducer of IFNγ production by T and NK cells	PMF patients were studied; comparison with normal controls revealed significantly increased IL-	[26]	T-cells Dentritic cells Macrohages

		12		
Il-19 **IL**-10 cytokine subfamily. type II cytokines	• It can bind the **interleukin**-20 receptor complex and lead to the activation of the signal transducer and activator of transcription 3 (STAT3).	Information not found	[31]	Monocytes
Il-20 type II cytokines	• Facilitate the communication between leukocytes and epithelial cells, thereby enhancing innate defense mechanisms and tissue repair processes at epithelial surfaces. • IL-20 subfamily cytokines are induced during wound healing in the skin and contribute to several stages of the wound-healing process, including inflammation, angiogenesis, re-epithelialization and remodeling.	Information not found	[32]	Keratinocytes and monocytes
Il-22 IL-10 family	• Can contribute to immune disease through the stimulation of inflammatory responses	No differences	[33] [34]	T-cells
Il-26 IL- 10 family	• Natural human antimicrobial that promotes immune sensing of bacterial and host cell death. • Amphipathic protein that kills extracellular bacteria via membrane-pore formation. • Acts mainly by JAK1 and TYK2	Information not found	[35]	
IL-27 IL-12 cytokine family.	• Signaling pathways including JAK-STAT and p38 MAPK pathways are turned on. • Acts as an anti-	Information not found	[36] [37]	T-cells

	inflammatory by suppressing inflammatory responses • Antitumor agent			
IL-28/ IFN y2 IFN type III	• Plays a role in immune defense against viruses, vaccines with IL-28 anti H1N1 • Increase the cellular synthesis of proteins that directly hinder virus replication and enhance the readiness to present viral antigens to immune cells.	Information not found	[38]	Virus-infected cells Maturing dendritic cells (DCs) Regulatory T-cells
IL-29/IFN-λ1 type III interferon	• IL-29 can either induce tumor promoting effects or tumor inhibiting effects depending on the cancer cell type being affected.	Information not found	[38]	Virus-infected cells Maturing dendritic cells (DCs) Regulatory T-cells Macrophages
IL-32 Pro-inflammatory Does not share sequence homology with known cytokine families	• Is a pro-inflammatory cytokine that can induce cells of the immune system (such as monocytes and macrophages) to secrete inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and IL-6. In addition, it can also induce the production of chemokines such as IL-8 and MIP-2 / CXCL2.	IL-32 mRNA levels in primary adherent cells from patients with Myeloid Malignancies are 14- to 17-fold higher than in controls.	[39] [40]	T-cells
IL-34 No apparent consensus structural domains, motifs, or sequence homology with other cytokines.	Cytokine that promotes the proliferation, survival and differentiation of monocytes and macrophages.	Information not found	[41] [42]	Macrophages Fibroblast

IL-35	• IL-35 inhibit the LPS-induced up-regulation of endothelial cell (EC) adhesion molecule VCAM-1 through IL-35 receptors gp130 and IL-12Rβ2 via inhibition of the MAPK-activator protein-1 (AP-1) signaling pathway. • It is an anti-inflammatory cytokine suppressing the immune response through the expansion of regulatory T cells and suppression of Th17 cell development.	Information not found	[43] [44]	Endothelial cells Smooth muscle cells Monocytes T-cells
LIGHT / TNFSF14 TNF ligand family	• Stimulate the proliferation of T cells, and trigger apoptosis of various tumor cells. • Platelet-associated LIGHT (TNFSF14) mediates adhesion of platelets to human vascular endothelium	Information not found	[45]	
MMP-1 Peptidase M10 family	• Involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. • This secreted protease breaks down the interstitial collagens, including types I, II, and III	Elevated levels of plasma TIMP-1, but not MMPs, were found in ET and PV.	[46] [47]	Fibroblast
MMP-2	• Cleave IV collagen. • These include the breakdown of the uterine lining (endometrium) during menstruation, formation and growth of new blood vessels, repair of damaged tissues, and	Information not found	[48]	Fibroblast

	inflammation. • Bone remodeling			
MMP-3	• This gene encodes an enzyme which degrades fibronectin, laminin, collagens III, IV, IX, and X, and cartilage proteoglycans	Information not found	[49]	Fibroblast
Osteocalcin	• It is important in bone metabolism and is used as a clinical marker for bone turnover, but its precise function remains elusive. • Is the most abundant non-collagenous protein in bone, comprising comprising almost 2% of total protein in the human body.	Not clear. Measurements are compared before and after treatment in ET, but no comparisons to healthy controls	[50]	Osteoblasts
Osteopontin	• Mediates cell migration, adhesion, and survival in many cell types. • Promotes cell-mediated immune responses, and plays a role in chronic inflammatory and autoimmune diseases.	Information not found	[51]	Macrophage T cells Neutrophils
Pentraxin-3 Superfamily of phylogenically conserved multimeric proteins	• Influence a variety of phenomena such as inflammation, angiogenesis, tumorigenesis, cell adhesion	PTX3 levels were significantly increased in carriers of homozygous JAK2V617F mutation compared to all the other genotypes and triple negative ET patients, The risk of haematological evolution and death from any cause was	[52] [53]	Dendritic cells Macrophages

		about 2- and 1.5-fold increased in individuals with high PTX-3 levels, while the thrombosis rate tended to be lower.		
sTNF-R1 TNFR2	• Triggers apoptosis and caspase activation (programmed cell death) • Interaction between TNFα and mTNFR1 leads to a pro-inflammatory stimulus via activation of nuclear factor kappa B (NF-κB) or activator protein 1 (AP-1)	Information not found	[54]	Detected on nearly all kinds of cells and predominantly sequestered in the Golgi apparatus.
sTNF-R2	• Essential role in cell proliferation and survival, and in the activation of regulatory T cells	Information not found	[54] [55]	T-lymphocytes Cardiomyocytes Mesenchymal stem cells Microglia Oligodendrocytes Thymocytes
TSLP (thymic stromal lymphopoietin) IL-2 cytokine family	• An important role in the maturation of T cell populations through activation of antigen presenting cells.	Information not found	[56]	Epithelial cells, Keratinocytes Stromal cells, Dendritic cells Mast cells
TWEAK / TNFSF12	• Promote proliferation and migration of endothelial cells, and thus acts as a regulator of angiogenesis, induction of inflammatory cytokines, and under some experimental conditions, stimulates apoptosis. • TWEAK suppresses	Information not found	[57] [58]	Leukocytes Monocytes Dendritic cells Natural killer cells

	production of IFN-gamma and IL-12, curtailing the innate response and its transition to adaptive TH1 immunity.			

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Appendix 2

Instrument for Evaluation of Essential Thrombocythemia

UPN

DATE (MM-DD-YY)

Section A

PRE-DIAGNOSIS (S&S One year prior to DX)

Bledding:

Hemorrhage: _____ 1) yes 2) No ☐

Petechia: _____ 1) yes 2) No ☐

Hematoma: _____ 1) yes 2) No ☐

Other: _____ 1) yes 2) No ☐

Onset:_____(Of above)

Dx._____(*IF ANY)

Thrombotic:

CNS 1) Yes 2) No ☐

Heart 1) Yes 2) No ☐

MI 1) Yes 2) No ☐

Venous 1) Yes 2) No ☐

Other: _____ 1) yes 2) No ☐

Onset:_____(Of above)

Dx._____(*IF ANY)

Past Hospitalizations or surgery

DX 1_____ 2_____ 3_____ 4_____ 5_____ 6_____

Family History

Leukemia: _____ 1) yes 2) No ☐

MDS: _____ 1) yes 2) No ☐

Thrombosis:_____ 1) yes 2) No ☐

Lymphoma: _____ 1) yes 2) No ☐

MPD:_____ 1) yes 2) No ☐

Cancer: _____ 1) yes 2) No ☐

Other: _____ 1) yes 2) No ☐

SECTION B

DIAGNOSIS (1-0)

Age: ____

Height: ____

Current wt: ____

BMI: _____

BSA: _____

Date OF DX:_____

Sex: F / M

Jak-2: -/+ Heterocygotic 1) yes 2) No ☐

Homocygotic 1) yes 2) No ☐

Mutation: _____

Date: _____

Systemic symptoms:

Fever 1) yes 2) No ☐

Weight loss (>10) 1) yes 2) No ☐

Current wt: ____

Basal wt: _____

Visual disturbance 1) yes 2) No ☐

Fatigue 1) yes 2) No ☐

Headche 1) yes 2) No ☐

Tiredness 1) yes 2) No ☐

Dyesthesias 1) yes 2) No ☐

Other:_____ 1) yes 2) No ☐

Comorbidities and their treatment:

Diabetes: _____	1) yes	2) No		Hypertension	1) yes	2) No	
Hypertriglycertidemia	1) yes	2) No		CHF	1) yes	2) No	
Hypercholesteroremia	1) yes	2) No		CAD	1) yes	2) No	
Other: ____	1) yes	2) No					
Statins: _____	1) yes	2) No		Ca^{+2}blockers: _____	1) yes	2) No	
Nitrates: _____	1) yes	2) No		Beta blockers: _____	1) yes	2) No	
Sulfonylureas: _____	1) yes	2) No		Diuretics: _____	1) yes	2) No	
Biguanides: _____	1) yes	2) No		ACEI: _____	1) yes	2) No	

Bleeding or Hypercoagulability:

Bleeding:				**Thrombi:**			
Hemorrage	1) Yes	2) No		CNS	1) Yes	2) No	
Petechia	1) Yes	2) No		Heart	1) Yes	2) No	
Other: _____	1) yes	2) No		MI	1) Yes	2) No	
				Venous	1) Yes	2) No	
				Other:_____	1) Yes	2) No	

Therapy E-1

			Dose-Onset	Dose-Last
Steroids	1. Yes	2. No	_____	_____
Aspirin	1. Yes	2. No	_____	_____
Radioactive phosphorus	1. Yes	2. No	_____	_____
NSAID	1. Yes	2. No	_____	_____
IFN	1. Yes	2. No	_____	_____
Ankylating agents	1. Yes	2. No	_____	_____
Hydrozxyurea	1. Yes	2. No	_____	_____
Anagrelide	1. Yes	2. No	_____	_____
Other: _____	1. Yes	2. No	_____	_____

Imaging Studies

Splenomegaly	1. Yes	2. No	
Renomegaly	1. Yes	2. No	
Hepatology	1. Yes	2. No	
PE	1. Yes	2. No	
Other: _____	1. Yes	2. No	

Other

Hospitalizations:_____ 1. Yes 2. No

First:	Thrombosis	1. Yes	2. No	**Second:** Thrombosis	1. Yes	2. No	
	Bleeding	1. Yes	2. No	Bleeding	1. Yes	2. No	
	Infectious	1. Yes	2. No	Infectious	1. Yes	2. No	

Other: _____ 1. Yes 2. No ☐ Other: _____ 1. Yes 2. No ☐

Blood transfusions 1. Yes 2. No ☐ Date: _____
Platelet transfusion 1. Yes 2. No ☐ Date: _____
Surgery 1. Yes 2. No ☐ Date: _____ Outcome: _____

SECTION C

LAST SEEN (6 months to one year after Dx)

Death 1. Yes 2. No 3. Unkown ☐ Date of death: _____ Cause of death: _____

New Diagnosi: 1. Yes 2. No ☐
A. _____
B. _____
C. _____
Other: _____

LABS:

	Pre-Diagnosis > 1 year	Diagnosis 0-1 year	Post Diagnosis > 6 months - 1 f/u
CBC	Date: _____	Date: _____	Date: _____
WBC	Date: _____	Date: _____	Date: _____
PMN	Date: _____	Date: _____	Date: _____
Lymph.	Date: _____	Date: _____	Date: _____
Neut.	Date: _____	Date: _____	Date: _____
Eos.	Date: _____	Date: _____	Date: _____
Baso.	Date: _____	Date: _____	Date: _____
Hgb	Date: _____	Date: _____	Date: _____
Hct	Date: _____	Date: _____	Date: _____
MCV	Date: _____	Date: _____	Date: _____
RDW	Date: _____	Date: _____	Date: _____
Platelets	Date: _____	Date: _____	Date: _____
MPV	Date: _____	Date: _____	Date: _____
LDH	Date: _____	Date: _____	Date: _____
AST	Date: _____	Date: _____	Date: _____
ALT	Date: _____	Date: _____	Date: _____
A/APO	Date: _____	Date: _____	Date: _____
TB	Date: _____	Date: _____	Date: _____
PT	Date: _____	Date: _____	Date: _____
PTT	Date: _____	Date: _____	Date: _____
BT	Date: _____	Date: _____	Date: _____

Bone Marrow aspiration:

Date: _____
Megakaruocyte number: _____
W: E: _____

SECTION D

I. In the last 3 weeks have you had any:

	Yes	No
Infection		
Hospitalization		
Surgery		
Steroid use		
Antibiotic use		

II. Current medication

	YES	NO
Hydroxyurea		
Aspirin		
Plavix		
NSAIDS		
Coumadin		
Other medication		

III. Recent labs

WBC	Results/unit
Hb	
Hct	
MCV	
MCHC	
RDW	
Platelets	
Neutrophils	
lymphocytes	

Monocytes	
Eosinophils	
Basophils	
Creatinine	
Total Bilirubin	