SUPPLEMENTARY APPENDIX

Eltrombopag monotherapy can improve hematopoiesis in patients with low to intermediate risk-1 myelodysplastic syndrome

Alana Vicente,[1*] Bhavisha A. Patel,[1*] Fernanda Gutierrez-Rodrigues,[1] Emma M. Groarke,[1] Valentina Giudice,[1] Jennifer Lotter,[1] Xingmin Feng,[1] Sachiko Kajigaya,[1] Barbara Weinstein,[1] Evette Barranta,[1] Matthew J. Olnes,[1] Ankur R. Parikh,[1] Maher Albitar,[2] Colin O. Wu,[3] Ruba Shalhoub,[3] Katherine R. Calvo,[4] Danielle M. Townsley,[1] Phillip Scheinberg,[5] Cynthia E. Dunbar,[1] Neal S. Young[1] and Thomas Winkler[1]

[1]Hematology Branch, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, MD, USA; [2]Genomics Testing Cooperative Laboratories, Irvine, CA, USA; [3]Office of Biostatistics Research, National Institutes of Health (NIH), Bethesda, MD, USA; [4]Hematology Section, Department of Laboratory Medicine, Clinical Center, National Institutes of Health (NIH), Bethesda, MD, USA and [5]Hematology Department, Hospital A Beneficiencia Portuguesa, Sao Paulo, Brazil

**AV and BAP contributed equally as co-first authors.*

©2020 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2020.249995

Received: March 24, 2020.

Accepted: May 21, 2020.

Pre-published: May 21, 2020.

Correspondence: *NEAL S. YOUNG* - youngns@nhlbi.nih.gov

SUPPLEMENTARY DATA

Eltrombopag monotherapy can improve hematopoiesis in patients with low to intermediate risk-1 myelodysplastic syndrome

Alana Vicente,[1]* Bhavisha A. Patel,[1]* Fernanda Gutierrez-Rodrigues,[1] Emma M. Groarke,[1] Valentina Giudice,[1] Jennifer Lotter,[1] Xingmin Feng,[1] Sachiko Kajigaya,[1] Barbara Weinstein,[1] Evette Barranta,[1] Matthew J Olnes,[1] Ankur R. Parikh,[1] Maher Albitar,[5] Colin O. Wu,[2] Ruba Shalhoub,[2] Katherine R. Calvo,[3] Danielle M. Townsley,[1] Phillip Scheinberg,[4] Cynthia E. Dunbar,[1] Neal S. Young,[1] and Thomas Winkler[1]

Table of contents

Methods

Patients and Eligibility

Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The change in inclusion criteria to include patients with any cytopenia was based on our concurrent observation of multilineage responses in patients with AA. IPSS score was assigned to all patients at enrollment regardless of previous treatments. Prior therapy with alemtuzumab or horse/rabbit antithymocyte globulin within 6 months of study entry was also an exclusion criterion.

Treatment Plan and Study Endpoint

Initial and maximum EPAG dosing was adjusted by 50% for patients with East or Southeast Asian ancestry. Blood counts and chemistries were monitored weekly till the primary endpoint. BM aspiration and biopsies, and cytogenetic analyses were performed at baseline, 16 weeks, and at least every 12 months while on the extension phase. BM assessments were conducted at six months after the end of treatment. Patients could receive supportive care during study, including blood product transfusions as clinically indicated. G-CSF was held for 3 weeks prior to enrollment, and BM biopsy. After EPAG discontinuation for robust response (RR), blood counts were monitored for 2 years. All adverse events occurring during the study were recorded. Treatment related adverse events were considered if they were possibly, probably, or definitely attributed to the drug. Treatment related serious adverse events were defined as death, any grade IV toxicities, grade IV thrombosis/embolism, progression to AML, and increase in reticulin fibrosis grade by 3 points above baseline. Secondary endpoints higher bleeding events were according to CTCAE v4.0.

Per International Working Group (IWG) criteria, major cytogenetic response was defined as disappearance of a previous abnormality and a minor cytogenetic response was reduction of abnormal metaphases by 50% or more. Progression of disease per modified IWG criteria 2006 were considered: if patients with baseline BM blasts of $< 5\%$ increase by $\geq 50\%$ to $>5\%$ blasts; and either at least 50% decrease in granulocytes or platelets from maximum response, decreasing in Hb by

2g/dL or transfusion dependence. The acquisition of new cytogenetic abnormality without meeting other IWG criteria for progression was not considered disease progression.

Statistics

In this intention-to-treat study, sample size was calculated using Simon's Two-Stage Minimax Design for testing the null hypothesis that the 16-20 week response rate was 10% or lower versus the alternative that the response rate was 30% or higher, at a significance level of 0.05 and 80% power. The first stage involved accrual of 15 patients, and required 2 or more subjects to respond in order to proceed to the second stage. An additional 10 patients were accrued in the second stage, making the total number of patients required using this design 25. To account for loss to follow-up, an assumed dropout rate of 15-20% was adopted, and an additional 5 subjects could be enrolled. Summary statistics for patient demographics and laboratory measurements were presented using the medians and ranges for continuous variables and counts and proportions for categorical variables. Covariate effects on the response rates and the distributions of survival time were evaluated using the univariable logistic regression and Cox Proportional Hazard models, respectively, with statistical inferences presented using the corresponding standard errors, 95% confidence intervals and p-values for testing the null hypotheses of zero coefficients. Numerical results were calculated using the R and SAS software packages.

Targeted next-generation sequencing

We screened all patients for somatic variants in 63 candidate genes known to be related to myeloid malignancies by targeted NGS as previously described (Supplemental Table 2).[1] BM cells and peripheral blood cell-free DNA (cfDNA) collected at baseline, 16 weeks, and at the time of disease progression were used. Five patients who lacked BM samples were only screened in cfDNA. These data were included in the study as the variants identified in cfDNA correlated with the BM in paired analysis ($R^2 = 0.72$; Supplemental Figure 1).

Briefly, DNA was extracted from paired BM cells and cfDNA samples as previously described.[1] For sequencing, samples were enriched for target exons using the QIAseq Single Primer Extension

system (Qiagen, CA) in which an original DNA molecule is assigned to a unique molecular barcoding (UMI). Genomic DNA was first fragmented, end repaired, and A-tailed within a single controlled multi-enzyme reaction. DNA fragments were then ligated at their 5' ends with a sequencing platform-specific adapter containing an UMIs and sample index. For enrichment, ligated DNA molecules were subjected to several cycles of targeted PCR using one region-specific primer and one universal primer complementary to the adapter. Libraries were pooled and paired-end sequenced using 150 bp reads on NextSeq 500 instrument (Illumina, CA). NGS data analysis was conducted remotely in the Cloud (Illumina BaseSpace) and complemented locally with the DRAGEN bioinformatics pipeline to ensure maximal accuracy. Called variants were included in the analysis if variant allele frequency (VAF) was higher than 2.5% and if was classified as "pathogenic" in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) or "confirmed Somatic" in Cosmic (https://cancer.sanger.ac.uk/cosmic) databases. Variants of unknown significance were not reported. Variants with VAF ≥ 40% were further confirmed as somatic by the sequencing of sorted CD3 positive cells used as germline control.

Supplemental Table 1A. EPAG dose adjustments or discontinuation for hematologic side effects

Platelet Count ≤ 30,000/μL or transfusion-dependent at baseline	**Dose Adjustment or Response**
< 20,000/μL above baseline or platelet transfusion requirement has not decreased following at least 2 weeks of eltrombopag	Increase dose by 25 mg (25 mg for East Asians) every 2 weeks to 150 mg for non East Asians (75 mg for East Asians).
≥ 20,000/μL above baseline but ≤200,000/μL following at least 2 weeks of eltrombopag	Keep at current dosage
> 200,000/μL (untransfused) at any time on study	Decrease dose by 25 mg (25 mg for East Asians) every 2 weeks to lowest dose that maintains platelet count ≥ 20,000/μL above baseline.
> 400,000/μL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets < 20,000; restart at 50% of current dose.
Hemoglobin < 9gr/dL or transfusion dependent at baseline	**Dose Adjustment or Response**
Hemoglobin rise of < 1.5 g/dL.	Increase dose by 25 mg (25 mg for East Asians) every 2 weeks to maximum 150 mg for non-East Asians (75 mg for East Asians).
≥ 1.5 g/dL above baseline but ≤13 g/dL following at least 2 weeks of eltrombopag	Keep at current dosage
> 13 g/dL (untransfused) at any time on study	Decrease dose by 50% to lowest dose that maintains Hb ≥ 1.5 g/dL above baseline.
15 g/uL (untransfused) at any time on study	Discontinue eltrombopag for one week, if Hb < 13 g/dL restart at 50% of current dose. Phlebotomy may be performed if clinically indicated as determined by the investigator.

Supplemental Table 1B. EPAG dose adjustments or discontinuation for non-hematologic side effects

Infection	Eltrombopag will be held if patients experiences infection that required vasopressors or intubation, the drug can be withheld until the patient is stable.
Liver function abnormalities	Eltrombopag will be held if ALT remains > 6 times on a second blood test and will be discontinued until ALT is < 5 times.The drug will be restarted at a dose level 25 mg/day lower than the prior dose.
Thrombosis/Embolism	Eltrombopag will be discontinued if patients experience a deep vein thrombosis, pulmonary embolus, stroke, or a myocardial infarction and go off study.
Peripheral blood smear shows new morphological abnormalities	The presence of persistent morphologic abnormalities or the development of significant worsening of anemia or neutropenia will require discontinuation of the drug and performance of a bone marrow exam.

Supplemental Table 2. Candidate genes screened in our cohort for somatic variants by next-generation sequencing

Splicing factors	*SF3B1, U2AF1, U2AF2, SRSF2, ZRSR2*
DNA methylation	*DNMT3A, TET2, IDH1, IDH2*
Chromatin and Histones modifiers	*ASXL1, ATRX, BCOR, BCORL1, EZH2, KDM6A, KMT2A*
Cohesins	*RAD21, SMC3, STAG2*
Signaling	*ABL1, BRAF, CALR, CBL, CBLB, CBLC, FLT3, GNAS, HRAS, JAK1, JAK2, JAK3, KIT, KRAS, MPL, NRAS, PTPN11*
Transcription regulation	*CEBPA, CUX1, ETV6, GATA1, GATA2, NPM1, PHF6, RUNX1, WT1*
Others	*CDKN2A, CREBBP, CSF3R, CTCF, EP300, FBXW7, IKZF1, IKZF3, MYD88 NF1, NOTCH1, PPM1D, PTEN, SETBP1, SETD2, SMC1A, TP53, SUZ12*

Supplemental Table 3. Adverse events attributed to EPAG

Patients (n = 30)		
Adverse events attributed to EPAG	**Event number (%)**	
	Grade 1 - 2	**Grade 3**
Increased liver transaminases	1 (3)	3 (10)
Increased bone marrow fibrosis		1 (3)
Skin lesions	6 (20)	
Nausea and vomiting	6 (20)	
Sclerae discoloration	5 (17)	
Headache	5 (17)	
Abdominal pain	4 (13)	
Joint Pain	4 (13)	
Dyspepsia	2 (7)	
Pruritius	2 (3)	
Diarrhea	1 (3)	
Myalgia	1 (3)	
Paresthesia	1 (3)	
Increased vitiligo	1 (3)	
Flatulence	1 (3)	
Ankle edema	1 (3)	

Evaluated were 30 patients. Events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4).

Supplemental Table 4. Severe adverse events not attributed to EPAG

Patients (n = 30)		
Severe adverse events	**n (%)**	
	Grade 1 - 2	**Grade 3**
Osteonecrosis of the humeral		1 (3)
Urothelial carcinoma		1 (3)
Dysphasia	1 (3)	
Hematuria		1 (3)
Neutropenic fever	2 (7)	
Cellulitis (PICC/line)	1 (3)	
Subdural hematoma		1 (3)
Bleeding	4 (13)	2 (7)

PICC, Peripherally inserted central catheter

Supplemental Table 5. Patients with cytogenetic response to EPAG treatment

UPN	Age	Sex	WHO subtype	IPSS	Baseline	Primary endpoint	Cytogenetics response*	Time to cytogenetic response (months)	Present Status
17	54	F	RCUD	Int-1	47,XX,+6[1]/ 46,XX[19]	47,XX,+6[1]/ 46,XX[19]	46,XX[20]	21	RR
18	59	F	RCUD	Int-1	47,XX,+15[4]/46,XX [16]	47,XX,+15[4]/ 46,XX[16]	46,XX[20]	20	RR
26	36	F	MDS-U	Int-1	46,XX,del(13)(q12q2 2)[5]/ 46,XX[15]	46,XX,del(13) (q12q22)[5]/ 46,XX[15]	46,XX[20]	9	RR

Of the 3 patients, only UPN-18 had a pathogenic somatic variant in IDH1 identified at baseline and primary endpoint at similar frequencies (40.3% and 37.4%, respectively). The other two patietns had no pathogenic somatic mutations at any timepoint. *Per modified 2006 IWG criteria. Abbreviations: UPN, unique patient number; IPSS, International Prognostic Scoring System; Int-1, intermediate 1; Int-2, intermediate 2; RCUD, refractory cytopenia with unilineage dysplasia; MDS-U, myelodysplastic syndrome-unclassifiable; RR, robust response.

Supplemental Table 6A. Univariable logistic model for response using continuous variables

Baseline Risk	Full Cohort (n = 30)			Revised Cohort (n = 25)		
	Coefficent (β)	SE	*P*-value	Coefficent (β)	SE	*P*-value
Age	-0.0629	0.0326	0.054	-0.0657	0.0363	0.070
ANC	-0.2530	0.4281	0.555	-0.3332	0.4617	0.471
ARC	0.0211	0.0132	0.109	0.0269	0.0145	0.063
TPO	0.0007	0.0003	0.027	0.0006	0.0003	0.063
BM Cellularity	-0.0314	0.0175	0.072	-0.0202	0.0184	0.272

Supplemental Table 6B. Univariable logistic model for response using categorical variables

Baseline Risk	No.	Full Cohort (n = 30)			Revised Cohort (n = 25)		
		Coefficient (β)	SE	*P*-value	Coefficient (β)	SE	*P*-value
PNH							
<1%	19	-	-	-	-	-	-
≥1%	11	1.7540	0.8378	0.036	1.8589	0.9039	0.040
ARC*							
<42.2	15	-	-	-	-	-	-
≥42.2	15	0.5390	0.7387	0.466	1.1474	0.8390	0.171
TPO*							
<2219	14	-	-	-	-	-	-
≥2219	15	2.3109	0.8747	0.008	2.1848	0.9443	0.021
BM Cellularity							
Hypocellular	11	1.7540	0.8378	0.036	1.4816	0.8893	0.096
Hypercellular**	19	-	-	-	-	-	-
Diagnosis							
h-MDS/AA ***	18	1.5506	0.8235	0.060	1.7918	0.9501	0.059
MDS	12	-	-	-	-	-	-
Baseline Cytopenia							
Thrombo§	20	2.8160	1.1540	0.015	2.8900	1.1880	0.015
No thrombo	10	-	-	-	-	-	-

Abbreviations: PNH, paroxysmal nocturnal hemoglobinuria; ARC, absolute reticulocyte count; TPO, thrombopoietin; SE, standard error; Thrombo, thrombocytopenia.
*Median is used as the categorical cutoff;
**Hypercellular includes those with normal cellularity and hypercellularity.
***Diagnosis of h-MDS or MDS progressed from AA.
§Presence of thrombocytopenia vs. absence of thrombocytopenia.

Abbreviations: ANC, absolute neutrophil count; ARC, absolute reticulocyte count; TPO, thrombopoietin; SE, standard error.

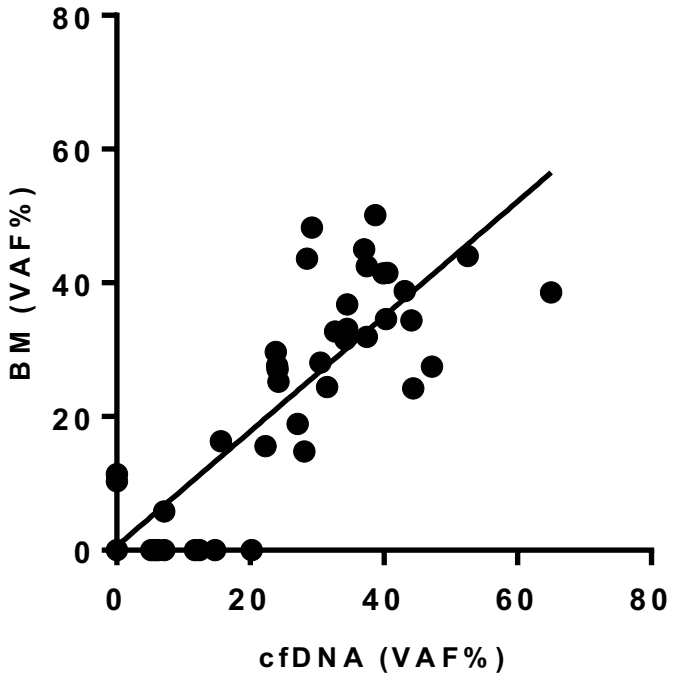
Supplemental Table 6C. Regression coefficient and the hazard ratio obtained from the univariable Cox Proportional Hazards models of survival time

Baseline Risk	Coefficient		HR	95% CI	*P*-value
	β	SE			
Age	0.006	0.0171	1.0060	(0.9728, 1.0400)	0.727
PNH					
<1%	-	-	1	-	
≥1%	-0.0364	0.4435	0.9643	(0.4043, 2.3000)	0.935
ANC	-0.1952	0.3490	0.8226	(0.4151, 1.6300)	0.576
ARC	-0.0081	0.0069	0.9919	(0.9787, 1.0050)	0.237
TPO	-0.0004	0.0002	0.9996	(0.9992, 0.9999)	0.024
Diagnosis					
h-MDS/AA*	-0.3237	0.4741	0.7235	(0.2856, 1.8320)	0.495
MDS	-	-	1	-	

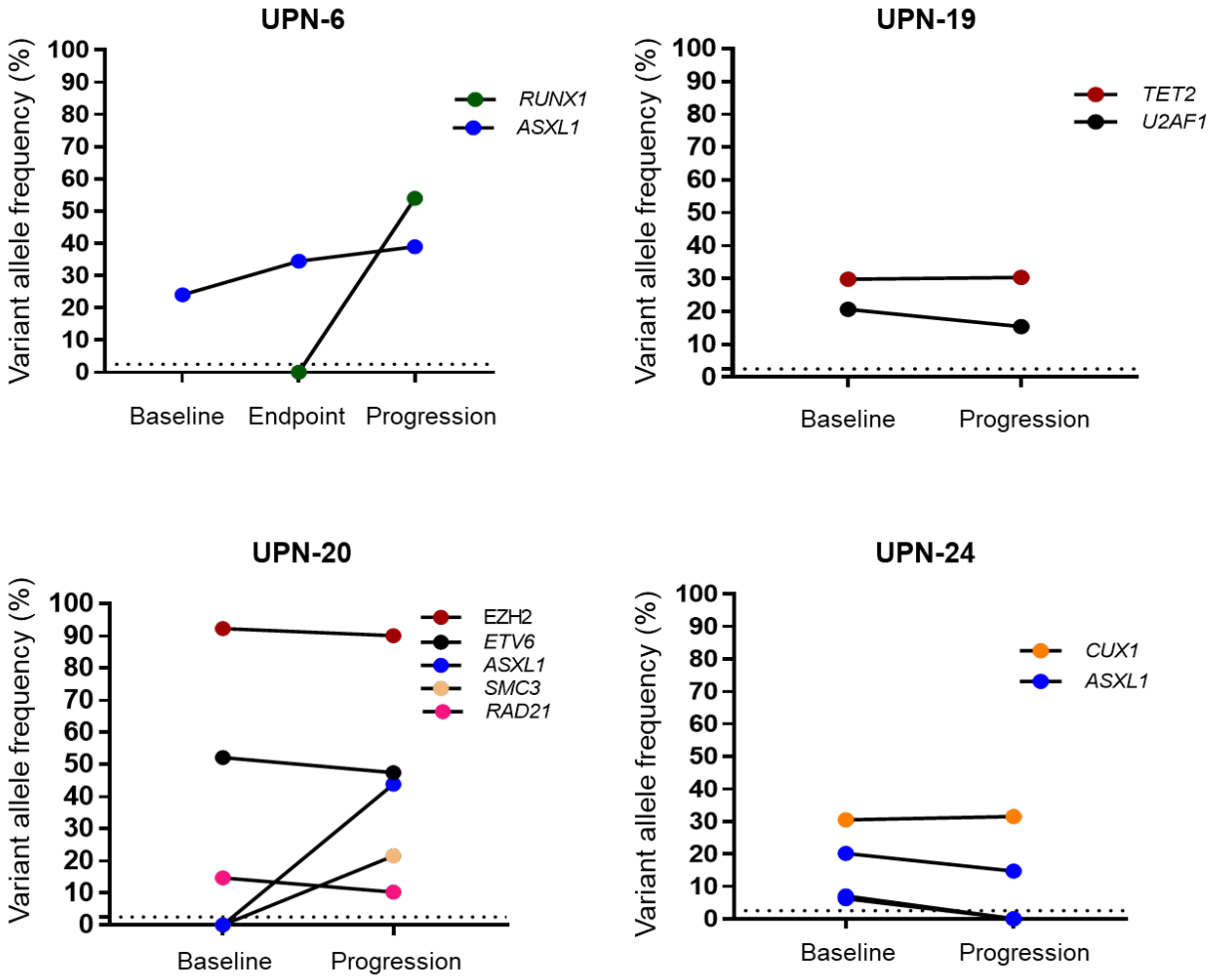
Abbreviations: PNH, paroxysmal nocturnal hemoglobinuria; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; TPO, thrombopoietin; HR, hazard ratio; SE, standard error; CI, confidence interval**.** * Diagnosis of h-MDS or MDS progressed from AA.

Supplemental Table 7. Previous treatment for MDS of patients enrolled in the study							
UPN	**Alemtuzumab**	**ESA**	**HMA**	**CSA**	**G-CSF**	**Lenalidomide**	**Danazol**
Responders							
1	x	x					
4		x	x				
5		x	x				
11					x		
16		x				x	
25		x					
26		x					x
27				x			
Non-responders							
3			x				
8	x						
9		x			x	x	
10	x	x			x		
12		x	x				
19						x	
20	x						
21	x						x
22		x	x		x	x	
23	x			x		x	
24		x					
28		x					
29		x					

Abbreviations: UPN, unique patient number; HMA, hypomethylating agents; ESA, erythropoietin stimulating agents; CSA, cyclosporine; G-CSF, granulocyte-colony stimulating factor. UPN-2, UPN-6, UPN-13, UPN-14, UPN-15, UPN-17, UPN-18, UPN-30 had prior diagnosis of aplastic anemia and had failed immunossupressor therapy. UPN-7 received EPAG as first line of therapy.



Supplemental Figure 1. Correlation between the variant allele fraction (VAF) of somatic clones identified in 46 paired samples of bone marrow (BM) and cell free DNA (cfDNA). (R^2 = 0.72; *P* value < 0.0001).



Supplemental Figure 2. Somatic variants identified in patients whose disease progressed on study. UPN-6 was found to have an *ASXL1* clone at baseline and also acquired a new somatic variant in *RUNX1* in the bone marrow at the time of disease progression. **UPN-19**, who progressed according to IWG criteria before reaching the primary endpoint, had the a *TET2* and *U2AF1* clones in bone marrow at presentation and time of progression in similar frequencies. **UPN-20,** who progressed at 12 weeks while receiving EPAG, acquired new somatic *ASXL1* and *SMC3* clones in cfDNA at disease progression. Other somatic clones remained stable. **UPN-24,** who progressed at 16 weeks while receiving EPAG, had *ASXL1* clones in the bone marrow that decreased in size at the time of disease progression. The limit of detection of somatic variants is indicated in the graphs by a dashed line (cutoff of the variant allele frequency = 2.5%).

References

1. Albitar A, Townsley D, Ma W, et al. Prevalence of somatic mutations in patients with aplastic anemia using peripheral blood cfDNA as compared with BM. *Leukemia.* 2018;32(1):227-229.