Mitapivat Improves Red Blood Cell Deformability and Sickling Kinetics in Adult Patients with Sickle Cell Disease

M. Lundt[1], N. Asomaning[1], I. Frey[1], X. Wang[1], A. Conrey[1], J. Xu[2], Q. Li[3], E. Dunkelberger[3], N. Jeffries[4], W. Eaton[3], S. Thein[1]

1. Sickle Cell Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD
2. Department of Medicine, University of Pittsburgh, Pittsburgh, PA
3. National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD
4. Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

INTRODUCTION

A hallmark of Sickle Cell Disease (SCD) is compromised red blood cell (RBC) deformability due largely to 'sickling' caused by polymerization of hemoglobin S (HbS). There have been exciting clinical developments in anti-sickling therapies which include activating red cell pyruvate kinase (PKR). Importantly, deformability measurements of RBCs via ektacytometry are increasingly used to aid the assessment of these agents' therapeutic efficacy. Here we employed the Laser assisted Optical Rotational Red Cell Analyzer (LORRCA, RR Mechatronics) to evaluate RBC deformability as a function of shear stress, osmotic pressure, and oxygen tension in HbSS subjects undergoing treatment with mitapivat (AG-348), a PKR activator under clinical development for SCD (Xu et al 2022). We analyzed changes in subjects' RBC deformability measurements after 4 weeks and 12 weeks of mitapivat therapy and compared them to t50 - a single parameter measure of sickling kinetics (Dunkelberger et al, 2018).

METHODS

Who: Fifteen subjects enrolled in a trial evaluating long-term safety and tolerability of mitapivat (pyruvate kinase activator) in SCD (NCT04610866)

- 5 females, 10 males
- 25 – 57 years old
- African/African-American
- HbSS genotype

What: Fresh whole blood collected prior to drug initiation, after 4 weeks of therapy, and after 12 weeks of therapy

Assay: Same-day measurements of the cells' ability to undergo deformation (given by an Elongation Index (EI)) as a function of different physiologically relevant conditions (obtained via the LORRCA). EI was assessed as a function of the following:

- Increasing levels of shear stress (elongation assay)
- Continuous osmotic changes (osmoscan)
- Decreasing oxygen tension (oxyscan)

Endpoints: Software generated parameters of interest derived from the 3 assays include

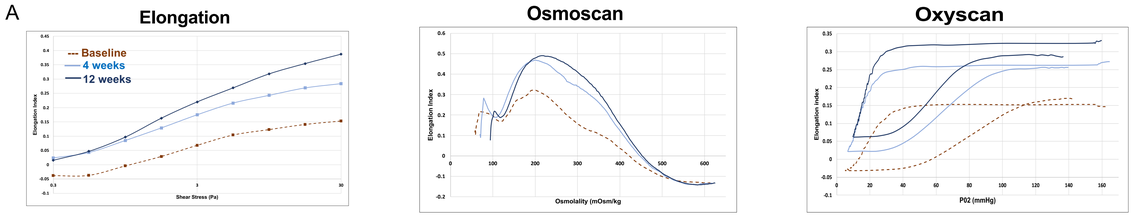
- **EI at all 9 shear stress levels** (elongation assay)
- **O min** (informs on RBC fragility), **O Hyper** (informs on hydration state), **EI max** (maximum deformability - informs on RBC cytoskeletal mechanics), and **O Max** (osmolality at which EI Max is achieved) (osmoscan)
- **Point of Sickling (POS) (**the oxygen tension at which the cells start to sickle (>5% EI change) during nitrogen facilitated deoxygenation of the sample), **EI Max** (maximal deformability), and **EI Min** (minimal deformability) (oxyscan)

Analysis: Percent change from baseline after 4 weeks and 12 weeks of therapy was analyzed for each end point using Wilcoxon signed-rank test (R v1.4.1106) and these changes were compared to those in t50 via a Spearman correlation test (Prism v9).

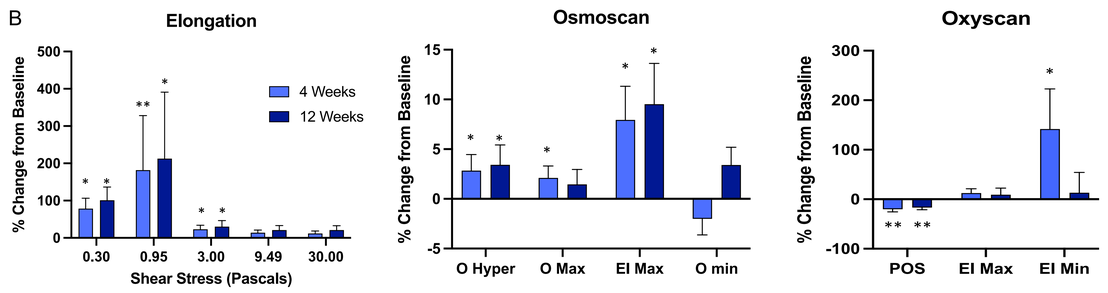
RESULTS

SUBJECTS UNDERGOING MITAPIVAT THERAPY SHOW IMPROVED RBC DEFORMABILITY AND SICKLING KINETICS AFTER TREATMENT

A



B



C

		4 Weeks						12 Weeks					
	LORRCA Derived Endpoint	% Change from Baseline			Correlation with t50			% Change from Baseline			Correlation with t50		
		Mean (min, max)	P value	n	P value	r	n	Mean (min, max)	P value	n	P value	r	n
Elongation	0.3 Pascals	78.45(-90.22, 285.71)	0.025	15	0.0107	0.6703	14	101.22(-42.11, 378.57)	0.013	14	0.1150	0.4615	13
	0.95 Pascals	181.83(-18.96, 2225.0)	0.005	15	0.0008	0.8066	14	212.85(-27.01, 2525.0)	0.024	14	0.0360	0.5934	13
	3 Pascals	23.01(-13.66, 157.35)	0.018	15	0.0001	0.8901	14	30.09(-21.73, 223.52)	0.041	14	0.0067	0.7253	13
	9.49 Pascals	14.10(-11.40, 97.56)	0.055	15	0.0002	0.7187	14	21.04(-19.68, 158.53)	0.104	14	0.0251	0.6813	13
	30 Pascals	11.85(-9.75 11.43)	0.207	15	0.0050	0.8549	14	19.0(-16.58, 152.94)	0.172	14	0.0127	0.6264	13
Osmoscan	O Hyper	2.84(-4.60, 21.88)	0.02	14	0.1405	0.4341	13	3.43(-2.98, 25.25)	0.045	13	0.1767	0.4196	12
	EI Max	7.94(-3.11, 45.5)	0.017	14	0.0006	0.8407	13	9.52(-3.74, 52.0)	0.016	13	0.0278	0.6434	12
	O Min	-2.00(-12.76, 10.20)	0.234	14	0.1517	-0.4231	13	3.40(-10.63, 12.74)	0.077	13	0.0373	-0.6154	12
	O Max	2.10(-8.44,12.76)	0.025	14	0.5786	0.1703	13	1.46(-6.17, 11.70)	0.684	13	0.4303	0.2517	12
Oxyscan	Point of Sickling	-20.15(-49.41, -0.92)	0.007	8	0.8397	0.1071	7	-16.80(-43.54, 1.36)	0.007	9	0.3894	-0.3571	8
	EI Max	12.3(-9.17, 72.66)	0.25	8	0.0067	0.9286	7	9.24(-21.25, 112.0)	0.91	9	0.1150	0.6190	8
	EI Min	141.95(-0.45, 716.66)	0.039	8	0.1389	0.6429	7	13.02(-125.0, 293.75)	0.652	9	0.7033	0.1667	8

Figure: Whole blood obtained at baseline (prior to drug initiation) and again after 4 and 12 weeks on treatment was subjected to distinct levels of shear stress (elongation), continuous osmotic changes (osmoscan), and gradual deoxygenation (oxyscan) via the LORRCA ektacytometer. **(A)** Representative curves of the 3 assays from one trial participant demonstrating shifts from baseline to 4- and 12-weeks treatment. For endpoints of interest, percent change from baseline was calculated per subject and averaged **(B, C).** Increased deformability was observed in response to each level of shear stress for both treatment time points (**B, left),** while subjection to continuously increasing osmotic pressure yielded improvements in O hyper and maximum deformation ability (**B, center**). Results from the oxyscan show that after both 4 and 12 weeks of treatment, there was a marked decrease in the average oxygen tension at which cells start to sickle (point of sickling, **B right**). Mean percent change alongside range and p value are listed (**C**). Spearman correlation analysis between LORRCA endpoints of interest and the time to 50% sickling (t50) showed significant correlation between shear stress levels of .95, 3, 9.49, and 30 pascals and EI max of both the osmoscan and oxyscan assays for the 4 week and 12 weeks times points **(C).** * = p < .05, ** = p< .01. Data displayed as mean % change from baseline + SEM **(B).** Significance for average percent change calculated via R, v1.4.1106 using Wilcoxon signed rank test. Correlation analysis performed using Prism, v9.

AIM

To explore the effects of mitapivat therapy on red cell deformability and sickling kinetics in HbSS subjects undergoing treatment

CONCLUSIONS

+ Mitapivat therapy results in improved ability of RBCs to withstand deformational changes in response to shear stress, osmotic pressure, and decreasing oxygen tension, likely via bolstered membrane integrity.

+ Compared to t50 values, significant correlation was observed at all levels of shear stress and at the maximal deformability (EI max) achieved during both the osmoscan and oxyscan assays.

REFERENCES

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CONTACT

Maureen.Lundt@NIH.gov

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