A splice blocking anti-sense oligonucleotide as a novel precision therapy for vascular dysfunction

Steven A. Fisher*[1], Laureano D. Asico, Celio D. deAngelis

[1]Department of Medicine and Physiology, University of Maryland School of Medicine, and Baltimore VA Hospital, Baltimore MD 21201; email: SFisher1@umaryland.edu

Introduction

- Oligonucleotides (ONs) as therapies in clinical medicine is exploding:
- 1- mRNA vaccines : COVID19
- 2- RNAi: high cholesterol (Eliquis)
- 3- splice modifying anti-sense ONs (SMASOs) for genetic diseases Spino Muscular Atrophy (SMA); Duchenne's Muscular Dystrophy (DMD)
- 4- As n=1 treatments: Batten's disease: SMASO to suppress cryptic splice site in MFSD8 E7 N Engl J Med 2019;381:1644-52

Background:

- We are developing SMASO targeting VSM as a novel approach to treating vascular dysfunction of hypertension and heart failure
- 1- Use SMASO to modify splicing of alternative exons (AEs), thereby causing a "therapeutic shift" in the expression of naturally occurring protein isoforms
- 2- AEs are nearly universal = thousands of potential targets in VSM. Llorian et al. Nucleic Acids Res 2016, 44:8933-50.
- 3- We are testing SMASO to Myosin phosphatase (MP) AE24 of the regulatory subunit (Mypt1 (PPP1R12a) E24; Fig. 1)
- 4- MP mediates VSM relaxation and is the end-effector hub upon which all dilator + constrictor signals converge to regulate vessel tone

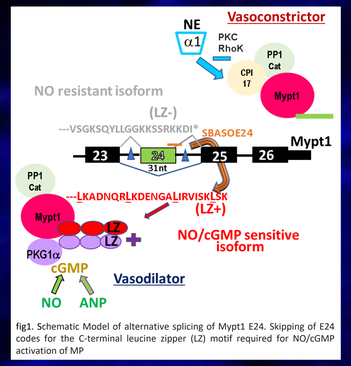

fig1. Schematic Model of alternative splicing of Mypt1 E24. Skipping of E24 codes for the C-terminal leucine zipper (LZ) motif required for NO/cGMP activation of MP

Results

- SMASOE24 nearly completely and specifically suppressed splicing of Mypt1 E24 with EC_{50} = 6.25 mg/kg (Fig 2a)
- Loss of LZ- isoform of Mypt1 with no change in total level (Western blot) (Fig 3)
- Effect persisted for at least 1 month (Fig 2b)
- No inflammatory response/ toxicity in males; mild effect in females. Table 1
- BP- suppressed HTN in one mouse but significant variability and more studies needed. Fig 4)

Previous Results:

- The Fisher lab has studied MP/Mypt1 E24 for >20 years
- Their work supports a model in which Mypt1 E24 tunes VSM sensitivity to NO/cGMP/vasodilators (Fig. 1)
- ~30% of VSM MP pool is E24-/LZ+ = NO/cGMP-sensitive
- They developed Cre-Lox mouse for cKO of E24. The mesenteric arteries of cKO mice had increased sensitivity to NO/cGMP, reduced sensitivity to vasoconstrictors, and suppressed hypertensive response to chronic AngII infusions. Reho et al AJP- Heart I2016, 310:H1715-H24; Htet et all. Pflug Arch 2021, 473:611-22.
- These studies provide foundation for SMASO translational approaches targeting Mypt1E24 to reverse vascular dysfunction in HTN and HF

Hypothesis

- SMASO targeting Mypt1 E24 will restore the balance of NO/vaso-dilator vs constrictor pathways and improve vascular function in HTN and HF(pEF).

Methods

- SMASOE24 targeting the 5' splice site of Mypt1 E24 (Fig. 1) was synthesized by Gene Tools LLC: octo-guanidine conjugated for improved uptake and Phosphodiamidate backbone for stability
- C57B6J mice were injected IP qod x 3 with SMASOE24 for :
- 1)dose-response & time course: Mypt1 E24/LZ +/- variants were assayed by PCR/Western blot
- 2) effect on BP in AngII slow pressor model of HTN 3) toxicology: cytokines, liver and kidney enzymes were measured.

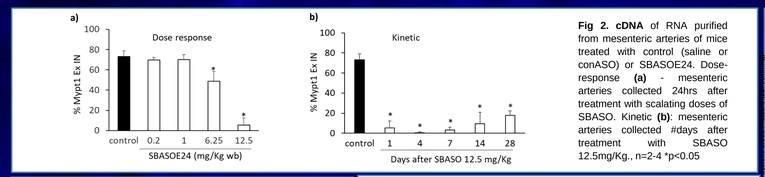

Fig 2. cDNA of RNA purified from mesenteric arteries of mice treated with control (saline or conASO) or SBASOE24. Dose-response **(a)** - mesenteric arteries collected 24hrs after treatment with scalating doses of SBASO. Kinetic **(b)**: mesenteric arteries collected #days after treatment with SBASO 12.5mg/Kg., n=2-4 *p<0.05

		mouse #	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)	IL-1B (pg/mL)	IL-6 (pg/mL)	TNF-A (pg/mL)
F	control	1	241.0	34.0	0.3	23.7	14.9	7.3
		2	-	-	-	30.0	10.8	9.6
		3	41.0	17.0	0.3	945.9	99.4	233.3
	ASO 12.5	4	52.0	20.0	0.4	ND	4.1	ND
		1	483.0	40.0	0.2	ND	18.7	ND
		2	57.0	33.0	0.3	46.6	25.5	ND
		3	60.0	27.0	0.4	15.9	13.7	7.0
M	control	1	39.0	16.0	0.7	-	-	-
		2	94.0	17.0	0.4	ND	1.2	ND
		3	-			ND	ND	ND
	ASO 12.5	4	35.0	15.0	0.4	ND	14.9	ND
		1	53.0	17.0	0.4	ND	16.2	ND
		2	45.0	26.0	0.4	ND	9.8	ND
		3	56.0	15.0	0.3	ND	ND	ND

Table 1. toxicity parameters in serum from C57BJ background mice after treatment with SBASO 12.5 mg/Kg 3x qod/week for two weeks. ND=not detectable

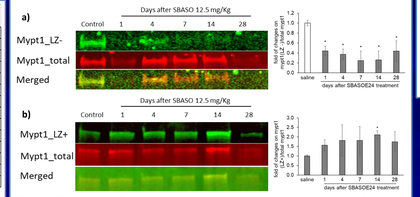

Fig 3. WB to Mypt1 LZ+(a) and LZ- (b) isoforms normalized by toral mypt1 from mesenteric arteries of mice treated SBASO 12.5mg/Kg., n=2-4 *p<0.05

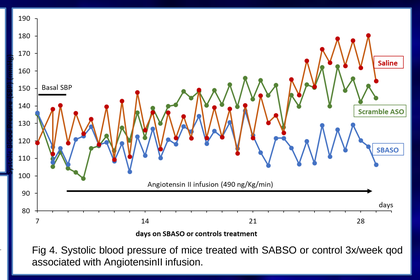

Fig 4. Systolic blood pressure of mice treated with SABSO or control 3x/week qod associated with AngiotensinII infusion.

Conclusion:

- Splice modifying ASOs have potential as a novel approach in precision medicine to reverse the vascular dysfunction of hypertension and heart failure