

Supplementary Table 1: Treatment protocols short-term oral studies

Short-term (7 hr) study protocols profiling the post initial and repeated dosing effects of orally administered PAL variants on plasma Phe levels of ENU2 (PKU) mice made euphenylalaninemic over 3 days and challenged with 0.15mg L-Phe/g mouse body weight. A number of parameters were varied throughout the studies to determine which form of enzyme protection, loading dose and dose responses were the most effective in reducing the hyperphenylalaninemic state in the ENU2 mouse model. The variation in the plasma profiles were used to determine the time in which the effect was most prominent. This table describes the parameters applied to profile each variant. Time in hours refers time post phenylalanine challenge which is considered 0hr, the asterisk (*) indicates that the blood samples were drawn pre-initial gavage. All equivalent manipulations were conducted with the exact same protocol as indicated in the methods and each variant was tested along with an equivalent BSA control (not included in the table).

PAL Species	n	Group designated Formulation						Dosings and Bleeds						
		PEGylation (NOF) in 0.5M Tris buffer pH=8.5			Hydrogel protected nanoparticles in deionized H ₂ O pH = 6.0	Barium Alginate Microspheres in 0.5M Tris buffer pH=8.5	Amorphous Silica particles in 0.5M Tris buffer pH=8.5	PTD- AvPAL TM fusion protein in 0.5 M Tris, pH 8.5	Aprotinin added (40mg/ml)	Dose/ gavage (I.U.)	[Protein] (mg/ml)	# of Gavages	Dosing - Time: post Phe challenge (hrs)	Plasma [Phe] Sampling Time- post Phe challenge (hrs)
		Ratio 1:3	5 kDa linear	10 kDa branched										
Av- p.C503S/ p.C565S	4							X	0.33	16.4	3	1, 2, 3	1*, 4	
	4			X					0.33	8.0	3	1, 2, 3	1*, 4	
Av- p.C503S/ p.C565S/ p.F18A	4							X	3.78	24.0	3	1, 3, 5	1*, 6	
	4	X							4.78	25.0	3	1, 3, 5	1*, 6	
	4		X						3.18	23.0	3	1, 3, 5	1*, 6	
	4			X					2.53	18.3	3	1, 3, 5	1*, 6	
	4				X				3.78	20.0	3	1, 3, 5	1*, 6	
	4			X				X	2.01	17.3	3	1, 3, 5	1*, 6, 7	

	4					X				1.45	5.5	3	1, 3, 5	1*, 6, 7
	4	X								7.55	28.0	3	1, 3, 5	1*, 7
	4						X			1.06	12.5	3	1, 3, 5	1*, 7
	8	X								6.48	24.0	3	1, 3, 5	1*, 7
	5	X								8.75	46.0	3	1, 3, 5	1*, 7
	5	X								5.83	30.7	3	1, 3, 5	1*, 7
	5	X								2.90	15.3	3	1, 3, 5	1*, 7
	4	X								2.90	15.0	3	1, 3, 5	1*, 7
	5	X								0.97	5.0	3	1, 3, 5	1*, 7
	5	X								0.30	1.5	3	1, 3, 5	1*, 7
	5	X								0.10	0.5	3	1, 3, 5	1*, 7
	5	X								2.90	15.0	2	1, 3	1*, 7
	5	X								2.90	15.0	1	1	1*, 7
	8						X	X		5.75	29.0	3	1, 3, 5	1*, 7
	5							X		6.0	37.0	3	1, 3, 5	1*, 7
Rt-wild type	4							X		0.96	24.8	3	1, 2, 3	1*, 4