

## **Supplemental Information**

### **Iduronate-2-sulfatase fused with anti-hTfR antibody, pabinafusp alfa, for MPS-II: A phase 2 trial in Brazil**

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Table S1. Plasma half-life of pabinafusp alfa at three doses. Standard deviations are available only when the sample size was larger than 3 (4.0mg/kg group at week 1). Actual maximum and minimum values of half-life were shown when N=2.

(a) Week 1

	Dose Group (mg/kg)	N	Mean	SD	Min	Median	Max	Geometric Mean
$t_{1/2}$ (hr)	1.0	0	--	--	--	--	--	--
	2.0	2	--	--	3.16	--	4.31	3.690
	4.0	3	2.845	0.494	2.55	2.570	3.42	2.819

(b) Week 26

	Dose Group (mg/kg)	N	Mean	SD	Min	Median	Max	Geometric Mean
$t_{1/2}$ (hr)	1.0	1	--	--	11.96	--	11.96	11.964
	2.0	2	--	--	3.64	--	9.19	5.784
	4.0	1	--	--	7.53	--	7.53	7.535

Table S2. Investigators' and families' reports on 19 patients with MPS-II regarding their observations of changes at 26 week treatment with pabinafusp alfa.

Subject	Dosage (mg/kg)	Age (y/m)	Subtype	Clinical Findings			
				Speech	Motor skills	Liveliness/ Expression	Other comments
01-01	4.0	24 y 7 m	A	<b>Vocabulary:</b> slightly improved <b>Comprehension:</b> slightly better	<b>Joint stiffness:</b> slightly better <b>Walking time:</b> much longer <b>Walking distance:</b> much farther Becoming able to perform activities not possible before	<b>Smiling:</b> greatly increased <b>Mood:</b> much more stable	Sleep quality: much better Body pain: slightly better Plays soccer Patient says he is breathing better, his skin is softer, and his hair less dry.
01-02	2.0	37 y 11 m	A	<b>Vocabulary:</b> slightly improved <b>Comprehension:</b> slightly better	<b>Joint stiffness:</b> no change <b>Walking time:</b> slightly longer <b>Walking distance:</b> slightly farther	<b>Smiling:</b> greatly increased <b>Agitation:</b> much less <b>Mood:</b> much more stable	Patient has taken up new sports Patient says he has been feeling willing to work longer and that he can study more – feeling more focused.
01-03	1.0	10 y 0 m	S	<b>Vocabulary:</b> slightly improved <b>Comprehension:</b> much better	<b>Joint stiffness:</b> much better <b>Walking time:</b> slightly longer <b>Walking distance:</b> no change Becoming able to perform activities not possible before	<b>Smiling:</b> slightly increased <b>Agitation:</b> no change <b>Irritability:</b> slightly better <b>Mood:</b> slightly more stable	Much less body pain Mother says that the patient helps her with chores and that he brushes his teeth by himself.

01-04	1.0	29 y6 m	A	No change	<p><b>Joint stiffness:</b> slightly better</p> <p><b>Walking time:</b> slightly longer</p> <p><b>Walking distance:</b> slightly farther</p> <p>Becoming able to perform activities not possible before</p>	<p><b>Smiling:</b> greatly increased</p> <p><b>Agitation:</b> no change</p> <p><b>Mood:</b> no change</p>	<p>Less body pain</p> <p>Patient says his skin is softer, and his hair less dry.</p>
01-05	2.0	6 y2 m	S	<p><b>Vocabulary:</b> slightly improved</p> <p><b>Comprehension:</b> slightly better</p>	<p><b>Falls:</b> far fewer</p> <p><b>Joint stiffness:</b> slightly better</p> <p><b>Walking time:</b> much longer</p> <p><b>Walking distance:</b> much farther</p>	<p><b>Smiling:</b> greatly increased</p> <p><b>Agitation:</b> much less</p> <p><b>Irritability:</b> much less</p> <p><b>Mood:</b> much more stable</p>	<p>Control of urinary sphincter: no change</p> <p>Mother says her son is progressing, mainly in behavior.</p>
01-06	4.0	4 y11 m	S	<p><b>Vocabulary:</b> greatly improved</p> <p><b>Comprehension:</b> much better</p>	<p><b>Falls:</b> far fewer</p> <p><b>Joint stiffness:</b> slightly better</p> <p><b>Walking time:</b> much longer</p> <p>Becoming able to perform activities not possible before (e.g. takes off his socks by himself)</p>	<p><b>Smiling:</b> no change</p> <p><b>Agitation:</b> no change</p> <p><b>Irritability:</b> no change</p> <p><b>Mood:</b> no change</p>	<p>Control of urinary sphincter: slightly better</p> <p>Control of anal sphincter: no change</p> <p>Body pain: slightly better</p> <p>Mother says that the patient feels less pain in hands.</p>
01-08	1.0	5 y5 m	S	<p><b>Vocabulary:</b> slightly improved</p> <p><b>Comprehension:</b> much better</p>	<p>Patient has started to walk and run a lot.</p> <p><b>Falls:</b> slightly fewer</p> <p><b>Walking time:</b> slightly longer</p> <p><b>Walking distance:</b> much</p>	<p><b>Smiling:</b> much more</p> <p><b>Agitation:</b> slightly better</p> <p><b>Irritability:</b> slightly better</p> <p><b>Mood:</b> no change</p>	

					farther Becoming able to perform activities not possible before (patient takes his clothes off by himself)		
01-10	1.0	2 y3 m	S	<b>Vocabulary:</b> greatly improved <b>Comprehension:</b> much better	Started running <b>Falls:</b> far fewer <b>Joint stiffness:</b> no change <b>Walking time:</b> much longer <b>Walking distance:</b> much farther	<b>Smiling:</b> greatly increased <b>Agitation:</b> slightly better <b>Mood:</b> slightly more stable	Control of urinary /anal sphincter: no change
01-11	4.0	2 y8 m	S	<b>Vocabulary:</b> greatly proved <b>Comprehension:</b> much better	<b>Falls:</b> far fewer <b>Joint stiffness:</b> no change <b>Walking time:</b> slightly longer <b>Walking distance:</b> slightly farther Becoming able to perform activities not possible before	<b>Smiling:</b> greatly increased <b>Agitation:</b> much less <b>Mood:</b> no change	Control of urinary/anal sphincter: slightly better Dresses by himself.

01-12	4.0 y	2 y8 m	S	<p><b>Vocabulary:</b> slightly improved</p> <p><b>Comprehension:</b> much better</p>	<p><b>Falls:</b> slightly fewer</p> <p><b>Joint stiffness:</b> no change</p> <p><b>Walking time:</b> much longer</p> <p><b>Walking distance:</b> much farther</p> <p>Becoming able to perform activities not possible before (can take his clothes off by himself)</p>	<p><b>Smiling:</b> slightly more</p> <p><b>Agitation:</b> slightly better</p> <p><b>Irritability:</b> no change</p> <p><b>Mood:</b> slightly more stable</p>	Control of urinary/anal sphincter: no change
02-01	2.0	9 y5 m	S	<p><b>Family perception:</b> some improvement. Patient pronounces more syllables and vowels (I, O, U)</p>	<p><b>Family perception:</b> no change</p>	<p><b>Family perception:</b> no change in liveliness</p> <p>Improvement in expression: smiling more, and more attentive</p>	<p><b>Family perception:</b> thinner and silkier hair; more friendly expressions, and milder, calmer and quieter</p> <p>Patient understands better, and interacts better with family members.</p> <p>Has acquired muscle mass.</p>
				<p><b>Perception of the neurologist:</b> the patient better understands some verbal orders.</p>	<p><b>Perception of the neurologist:</b> improvement of left lower limb spasticity</p>	<p><b>Perception of the neurologist:</b> less agitation, and calmer than before; patient can fix his gaze on the examiner; making better contact and understanding simple orders</p>	

02-02	4.0 y	44 y4 m	A	<b>Patient's own perception:</b> no change	Patient noted that the left arm is straighter.	<b>Patient's own perception:</b> no change	Patient noted thinner hair and a smaller abdomen.
				<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no change	
02-03	4.0	38 y2 m	A	<b>Patient's own perception:</b> no change	Patient reports decreased body aches.	<b>Patient's own perception:</b> improved; feeling more willing	Patient observed thinner and softer hair, but reported flatulence and constipation..
				<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no change	
02-04	1.0	8 y11 m	S	<b>Family perception:</b> Mother reports that the patient sometimes speaks more, sings, and completes sentences. Usually, however, he speaks little, even when stimulated.	<b>Family perception:</b> finer motor coordination in hands and fingers; worse gross motor coordination worse; difficulty climbing stairs and walking for long periods	<b>Family perception:</b> better participation in daily activities; often calmer; shows more emotions like joy, sadness, and shame; sleeps better, gets less congested, and has less flu; less coarse facies	<b>Family perception:</b> smaller abdomen; hands more open, less hard, and more malleable – can make tweezer movements to eat (not done before); constipated and needs to use a suppository (bowel movements once or twice a day before the study)

				<b>Perception of the neurologist:</b> Patient understands verbal orders better, and in the last evaluation showed interest in some play materials.	<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> better interaction	
02-05	1.0	7 y7 m	S	<b>Family perception:</b> Patient resumed saying some words such as water, juice, stop, cold, sick, and numbers up to 10.	<b>Family perception:</b> no change	<b>Family perception:</b> smiles more, understands better, repeats words, and sings when watching videos.	
				<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> gait improvement with plantar support; at the last evaluation toe walking was impossible with plantar support.	<b>Perception of the neurologist:</b> no change	
02-06	1.0	5 y8 m	S	<b>Family perception:</b> Patient tries to pronounce words.	<b>Family perception:</b> Patient runs, walks, and can go up and down stairs.	<b>Family perception:</b> more affective.	<b>Family perception:</b> Mother reports improved breathing and sleep, and reduced tiredness.



				<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> slight improvement, starting to show fine motor skills	<b>Perception of the neurologist:</b> slight improvement on neurological examination	
02-07	2.0	7 y11 m	S	<b>Family perception:</b> Patient tries to pronounce words.	<b>Family perception:</b> Patient has difficulty moving but tries.	<b>Family perception:</b> less tiredness and improved walking skills.	
				<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no change	<b>Perception of the neurologist:</b> no changes.	
02-08	2.0	3 y3 m	S	<b>Family perception:</b> Patient can pronounce some new words like mommy, daddy, aunt, grandma, give, come, no and water.	<b>Family perception:</b> Patient can go up and down stairs, run without falling, and hold a glass with one hand.	<b>Family perception:</b> stronger, better complexion (disappearance of pimples), smaller abdomen, less tired, fewer runny noses	<b>Family perception:</b> Patient recognizes people better, listens more, speaks more clearly, sometimes asks to go to the bathroom, cries for things, and asks for volume to be turned down if it is too loud. He is also more focused, communicative, and happy. He is calmer, more agile, walks better, and plays with other children. Patient can hang from bars, balance on skates for a few feet, dance, and
				<b>Perception of the neurologist:</b> Patient understood some verbal orders at the last assessment.	<b>Perception of the neurologist:</b> Patient grasps objects, and at last assessment climbed 5 flights of stairs unassisted.	<b>Perception of the neurologist:</b> no change	

							play soccer.
02-09	1.0 mg/kg weekly	0 y9 m	S	<p><b>Family perception:</b> not available</p> <p><b>Perception of the neurologist:</b> Patient maintained speech and language acquisition. In the first evaluation at 9 months of age, he could still not vocalize. In the assessment at 1 year and 1 month, he spoke isolated words.</p>	<p><b>Family perception:</b> can sit without support, crawl on his buttocks, grasp objects, and make quick body turns</p> <p><b>Perception of the neurologist:</b> improvements found to have been maintained at the last assessment; sits without support, and grasps objects</p>	<p><b>Family perception:</b> more agile, shows more liking or disliking of things</p> <p><b>Perception of the neurologist:</b> good interest in the environment and interpersonal contact</p>	<p><b>Family perception:</b> Family noted decreased nasal discharge and shortness of breath, and on medical examination a large adenoid was identified. Patient has a thinner face, softer, straighter hair, a lower abdomen, and a less arched back. Family has observed that the patient is attentive, responds quickly when called by his name, learns more easily, and accepts food more readily.</p>

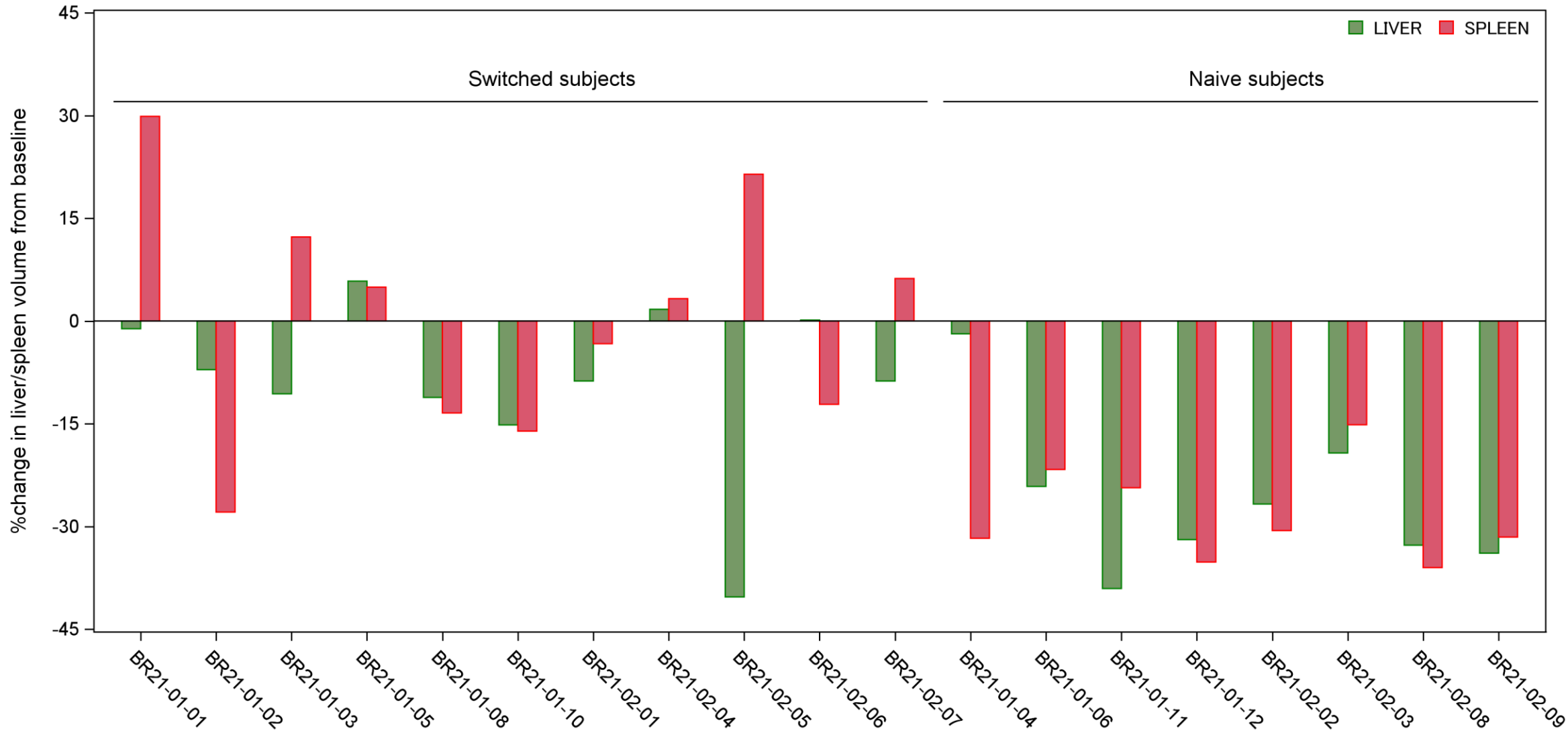
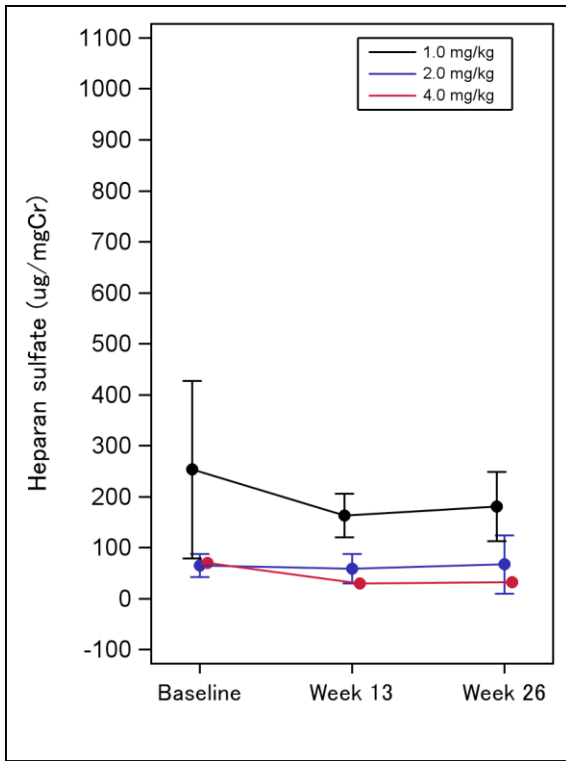
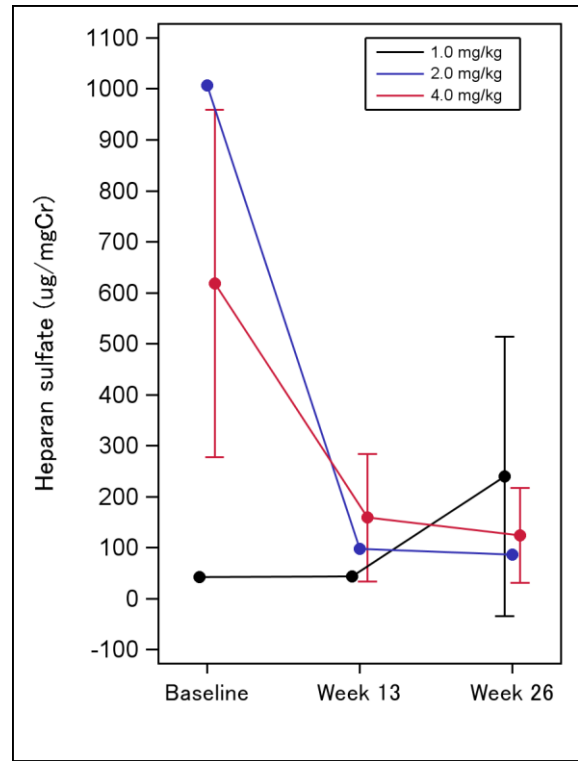


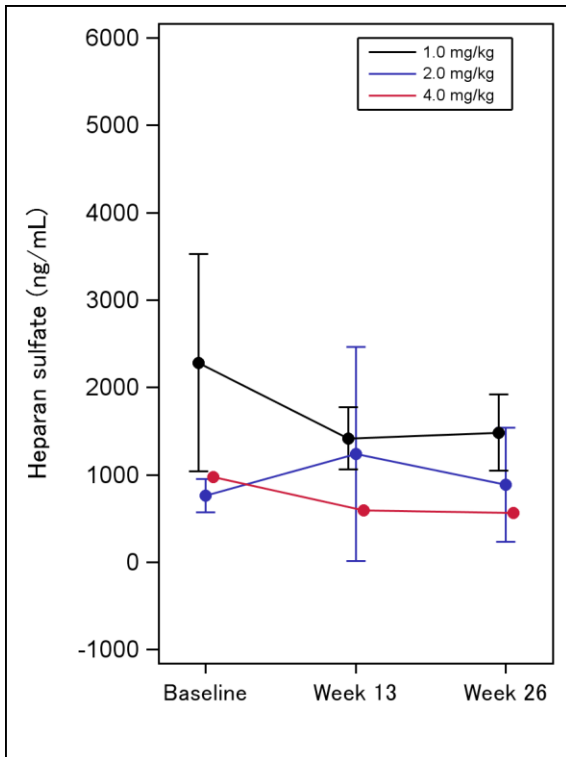
Figure S1. Individual relative volume changes in liver and spleen by subjects at week 26.



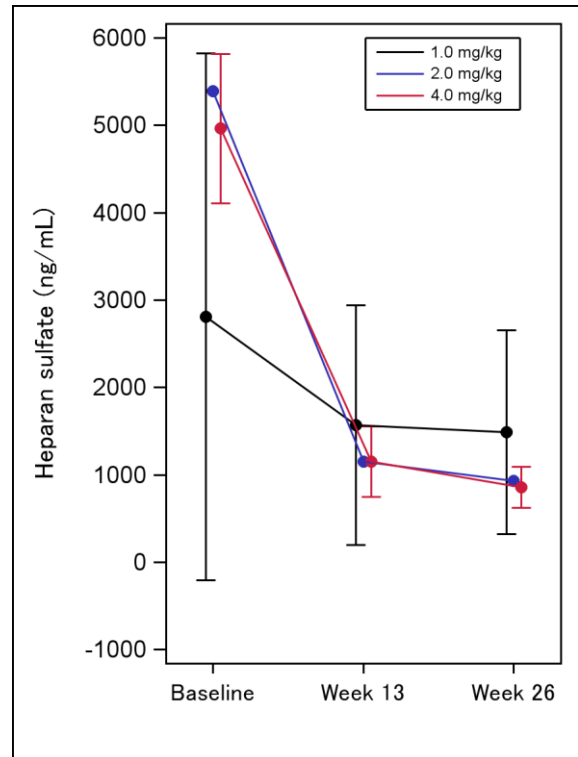
A-1: Urine HS levels in the switched patients



B-1: Urine HS levels in the naïve patients

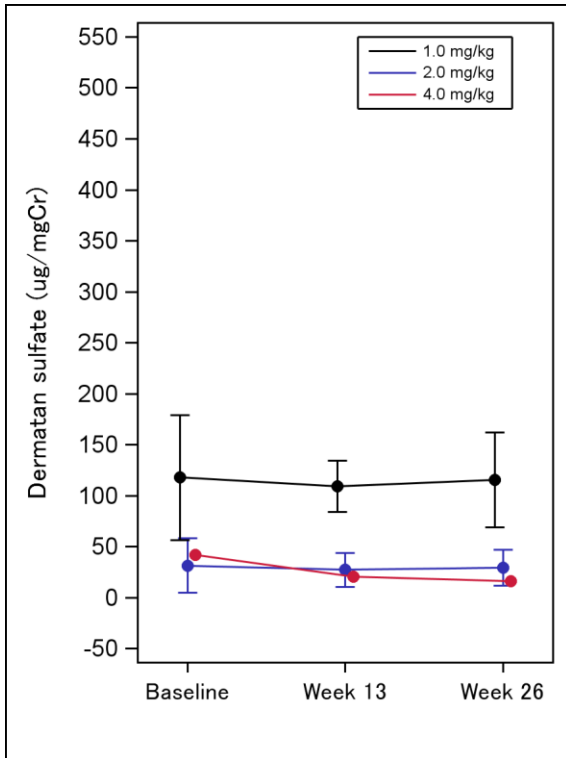


A-2: Serum HS levels in the switched patients

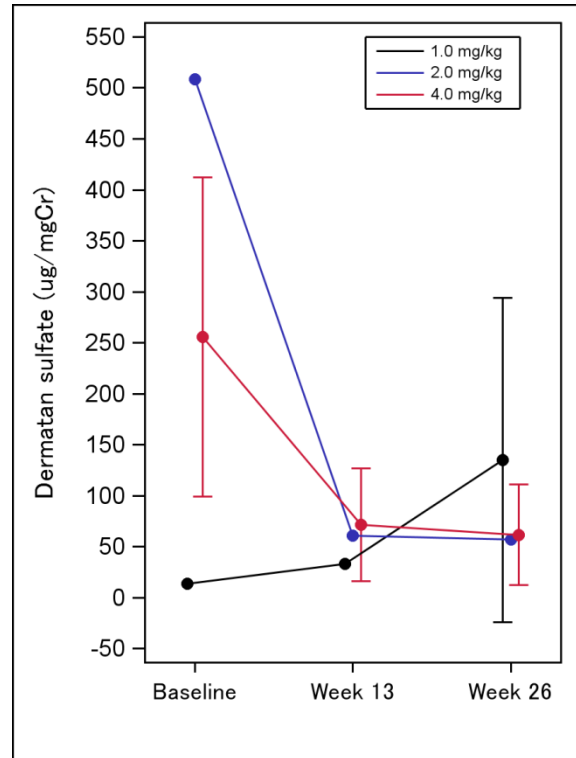


B-2: Serum HS levels in the naïve patients

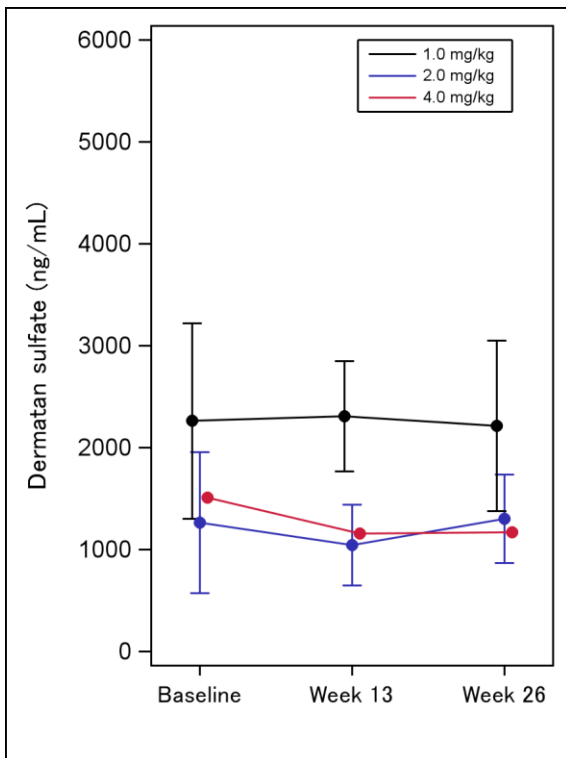
Figure S2. HS concentrations in the urine and serum of the patients previously treated with conventional ERT in the switched and naïve patients. The data represent mean  $\pm$  SD.



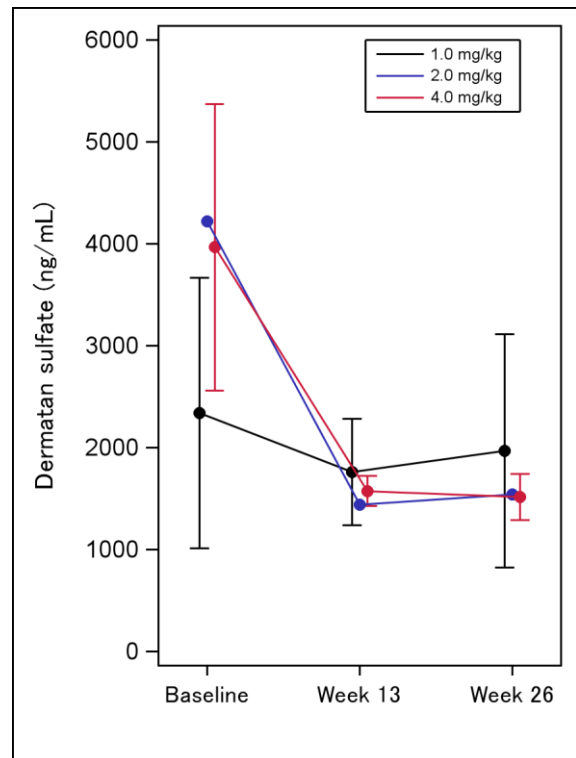
A-1: Urine DS levels in the switched patients



B-1: Urine DS levels in the naïve patients



A-2: Serum DS levels in the switched patients



B-2: Serum DS levels in the naïve patients

Figure S3. DS concentrations in the urine and serum of the patients previously treated with conventional ERT in the switched and naïve patients. The data represent mean  $\pm$  SD.