

Supplementary materials for:
Human neural stem cell-based drug products: clinical and non-clinical characterization

Par. 2.1: Identity - Positionl identity

Table S1: Raw data on real time PCR for the two samples and the total normal human brain (NHB) used as positive control.

Cell Line	Detector	Mean Ct	StdDev Qty	Delta Ct
05/12 B	<i>gapdh</i>	14,930	0,085	
	<i>Emxl</i>	36,655	0,177	21,725
	<i>Foxgl</i>	16,690	0,184	1,760
	<i>Gsh2</i>	19,680	1,202	4,750
	<i>Nkx2.1</i>	30,940	0,891	16,010
	<i>Dlx2</i>	28,045	0,332	13,115
	<i>Emx2</i>	22,765	0,021	7,835
	<i>Irx3</i>	29,660	0,198	14,730
	<i>En2</i>	35,125	0,460	20,195
	<i>Gbx2</i>	22,290	1,075	7,360
	<i>Hoxb1</i>	40,000	0,000	25,070
	<i>Hoxb5</i>	40,000	0,000	25,070

08/12B	<i>gapdh</i>	18,030	0,311	
<i>Emx1</i>	38,750	1,768	20,720	
<i>Foxg1</i>	19,230	0,042	1,200	
<i>Gsh2</i>	23,945	0,262	5,915	
<i>Nkx2.1</i>	40,000	0,000	21,970	
<i>Dlx2</i>	31,780	0,311	13,750	
<i>Emx2</i>	25,015	0,035	6,985	
<i>Irx3</i>	28,965	0,035	10,935	
<i>En2</i>	36,295	0,134	18,265	
<i>Gbx2</i>	26,420	0,057	8,390	
<i>Hoxb1</i>	40,000	0,000	21,970	
<i>Hoxb5</i>	40,000	0,000	21,970	

NHB	<i>gapdh</i>	15,645	0,035	
<i>Emxl</i>	22,835	0,714	7,185	
<i>Foxgl</i>	18,580	0,057	2,930	
<i>Gsh2</i>	28,000	0,014	12,350	
<i>Nkx2.1</i>	28,080	1,004	12,430	
<i>Dlx2</i>	21,775	0,530	6,125	
<i>Emx2</i>	22,340	0,255	6,690	
<i>Irx3</i>	25,645	0,318	9,995	
<i>En2</i>	23,525	0,233	7,875	
<i>Gbx2</i>	28,330	0,368	12,680	
<i>Hoxb1</i>	40,000	0,000	24,350	
<i>Hoxb5</i>	32,105	0,332	16,455	

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Par. 2.1: Identity - In vitro differentiation test

Table S2: For each sample, using a fluorescence microscope and a 20X lens, we counted at least 2000 cells in not less than 5 fields. Then we determined the percentage of each cell type on the total number of counted differentiated cells. Here are reported the calculated percentages of neurons, astrocytes and oligodendrocytes in each batch, the average value for all the batches and the standard deviation.

BATCH #	% Neurons	% Astrocytes	% Oligodendrocytes
1	30	49	21
2	31	52	18
3	31	49	29
4	33	50	18
5	24	54	23
6	29	49	21
7	20	65	15
8	48	41	12
9	30	52	18
10	17	59	24
11	40	47	13
12	28	46	25
13	32	53	15
14	36	46	18
15	43	45	11
16	25	28	36
17	46	26	26
18	17	12	26
19	27	26	19
20	16	40	7
21	13	20	11
22	7	46	36
23	18	48	29
24	16	41	24
25	26	54	18
26	15	55	31
27	22	53	31
28	10	37	11
29	8	40	9
30	10	55	29

Average	24,91	44,57	20,75
ST.DEV.	11,14	11,99	7,98

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Par. 2.3.3 Safety - in vivo test

Table S3: Here we present raw data of the in vivo safety evaluation for the two donors we used in the ALS trial. The hNCSCs lines were unilaterally transplanted into the dorsal striatum (300.000 cells/mice) of Athymic Nude mice and animals were kept for up to 6 months (n=7 animals for B05/12 and n=8 animals for B08/12). See main article for protocol description.

Cell line	Number of cells	Tumors formation	% of survived cells [1]	Ki67 [2]	Stem cells [3]	Astrocytes [4]	Neuronal progenitors [5]	Neurons [6]	Oligodendrocytes [7]	Migration into the brain parenchima (μm)
B05/12	300.000	no	32,98± 4,39	4,83±1,12	20,7±3,37	40,88±5,96	12,39±3,04	11,83±1,83	12,8±0,48	6150 ± 584,05
B08/12	300.000	no	36,78 ± 7,71	3,98±0,72	35,42±2,80	27,29±1,47	6,95±0,72	8,62±0,71	4,44±0,50	4714,29 ± 491,84

[1] % of survived cells respect to the total injected

[2] %Ki67/tot huN

[3] %Nestin+/tot huN

[4] %GFAP+/tot huN

[5] %Dcx+/tot huN

[6] %b-Tub III+/tot huN

[7] %MBP+/tot huN