

Table S1. ABCB5⁺ MSC product release data

Date of production	Patient	Responder	Lot	Vitality ¹	Viability ^{2,3}	Potency ³				
						Immunomodulation		Angiogenesis:	Endothelial trans-differentiation	
						IL-1RA secretion ⁴		VEGF secretion ⁵	Tube formation ⁶	
dd/mm/yyyy	#	Y/N	#	% of cells	% of cells	pg/ml	Ratio _{stim/unstim}	pg/ml	Well 1	Well 2
11/01/2018	01-003	Y	C-AL04000-01	98.3	99.4	4,838	1.9	> 49.6	B	B
19/04/2018	01-005	N	C-AL05000-03	98.2	99.8	4,318	1.8	> 49.6	B	C
26/06/2018	03-001	Y	C-AL05000-04	98.9	96.6	3,859	2.3	> 49.6	B	B
26/07/2018	01-007	Y	C-AL05000-07	98.4	99.3	8,550	3.0	> 49.6	A	B
14/08/2018	01-008	Y	C-AL05000-08	97.6	99.3	8,550	3.0	> 49.6	A	B
06/09/2018	01-009	N	C-AL05000-10	96.8	99.5	7,730	2.7	> 49.6	A	B
06/09/2018	01-007	Y	C-AL05000-11	98.6	99.0	3,835	2.0	> 49.6	B	B
12/09/2018	05-001	N	C-AL05000-12	96.4	99.5	7,730	2.7	> 49.6	A	B
24/09/2018	07-001	Y	C-AL05000-13	95.9	99.3	8,550	3.0	> 49.6	A	B
26/09/2018	01-008	Y	C-AL05000-14	96.6	99.3	8,550	3.0	> 49.6	A	B
23/10/2018	01-009	N	C-AL04000-04	95.0	95.6	4,802	3.3	> 49.6	A	A
23/10/2018	08-001	Y	C-AL04000-05	98.5	99.7	5,535	2.2	> 49.6	B	B
24/10/2018	05-001	N	C-AL04000-06	97.5	95.6	4,802	3.3	> 49.6	A	A
28/11/2018	05-002	Y	C-AL05000-15	97.0	99.6	15,450	5.7	> 49.6	B	B
05/12/2018	05-004	Y	C-AL05000-17	97.0	99.0	3,835	2.0	> 49.6	B	B

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						IL-1RA secretion ⁴	Ratio _{Ostim/unstim}	VEGF secretion ⁵	Tube formation ⁶	
dd/mm/yyyy	#	Y/N	#	% of cells	% of cells	pg/ml	Ratio _{Ostim/unstim}	pg/ml	Well 1	Well 2
11/12/2018	08-001	Y	C-AL05000-18	95.5	99.0	3,835	2.0	> 46.9	B	B
10/01/2019	05-002	Y	C-AL05000-19	98.0	99.6	15,450	5.7	> 46.9	B	B
16/01/2019	05-004	Y	C-AL04000-09	95.5	99.5	10,991	3.1	> 46.9	B	B
14/05/2019	04-002	N	C-AL05000-24	96.5	99.0	3,835	2.0	> 46.9	B	B
23/05/2019	05-005	N	C-AL04000-14	96.5	99.4	6,126	2.3	> 46.9	B	B
29/05/2019	01-011	Y	C-AL04000-16	97.0	99.1	10,130	2.9	> 46.9	B	B
03/07/2019	05-005	N	C-AL08000-08	96.5	99.7	7,450	2.5	> 46.9	B	C
03/07/2019	04-002	N	C-AL05000-31	96.0	98.2	3,268	2.3	> 46.9	A	A
10/07/2019	01-011	Y	C-AL05000-32	96.5	99.3	16,180	6.0	> 46.9	B	B
31/07/2019	02-003	Y	C-AL05000-33	97.0	99.9	3,931	2.4	> 46.9	A	B
08/08/2019	05-006	Y	C-AL05000-34	96.0	99.3	16,180	6.0	> 46.9	B	B
22/08/2019	01-012	Y	C-AL07000-08	97.0	99.8	5,370	3.0	> 46.9	C	C
12/09/2019	02-003	Y	C-AL07000-10	95.5	99.5	7,340	3.3	> 46.9	B	B
12/09/2019	05-007	Y	C-AL07000-11	92.0	99.4	7,820	3.4	> 46.9	B	B
12/09/2019	05-009	N	C-AL07000-12	93.0	99.4	7,820	3.4	> 46.9	B	B
19/09/2019	05-006	Y	C-AL07000-14	94.5	99.4	7,820	3.4	> 46.9	B	B
30/09/2019	06-002	Y	C-AL05000-35	95.0	99.3	16,180	6.0	> 46.9	B	B

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						IL-1RA secretion ⁴		VEGF secretion ⁵	Tube formation ⁶	
dd/mm/yyyy	#	Y/N	#	% of cells	% of cells	pg/ml	Ratio _{Ostim/unstim}	pg/ml	Well 1	Well 2
02/10/2019	01-012	Y	C-AL05000-36	93.5	99.3	16,180	6.0	> 46.9	B	B
24/10/2019	05-009	N	C-AL05000-38	96.0	99.3	16,180	6.0	> 46.9	B	B
24/10/2019	05-007	Y	C-AL05000-39	96.5	99.3	16,180	6.0	> 46.9	B	B
30/10/2019	02-005	N	C-AL05000-41	95.5	98.6	16,430	6.5	> 46.9	B	A
14/11/2019	06-002	Y	C-AL07000-19	97.5	99.7	7,250	4.0	> 46.9	B	C
27/11/2019	09-004	Y	C-AL07000-21	96.5	99.7	7,250	4.0	> 46.9	B	C
12/12/2019	02-005	N	C-AL08000-14	97.4	99.7	7,450	2.5	> 46.9	B	C
08/01/2020	04-004	Y	C-AL07000-23	96.3	99.6	6,970	3.1	> 46.9	B	C
10/01/2020	04-006	N	C-AL07000-24	96.4	99.7	7,250	4.0	> 46.9	B	C
14/01/2020	08-002	Y	C-AL08000-16	97.8	99.1	2,880	2.4	> 46.9	C	B
15/01/2020	04-005	Y	C-AL07000-27	95.5	99.7	3,789	2.8	> 46.9	B/C	C/B
21/01/2020	02-009	Y	C-AL05000-42	94.9	99.3	16,180	6.0	> 46.9	B	B
21/01/2020	02-008	n.a. ⁷	C-AL05000-43	94.4	99.3	16,180	6.0	> 46.9	B	B
30/01/2020	02-010	Y	C-AL07000-30	93.3	99.5	7,340	3.3	> 46.9	B	B
17/02/2020	06-003	Y	C-AL05000-44	91.2	98.6	16,430	6.5	> 46.9	B	A
19/02/2020	04-004	Y	C-AL05000-45	97.0	98.6	16,430	6.5	> 46.9	B	A
25/02/2020	04-006	N	C-AL05000-46	94.9	98.6	16,430	6.5	> 46.9	B	A

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dd/mm/yyyy	#	Y/N	#	% of cells	% of cells	pg/ml	Ratio _{Ostim/unstim}	pg/ml	Well 1	Well 2
27/02/2020	02-011	N	C-AL07000-32	93.1	> 46.9	7,820	3.4	> 49.6	B	B
03/03/2020	04-005	Y	C-AL05000-47	95.2	> 46.9	16,430	6.5	> 49.6	B	A
04/03/2020	02-009	Y	C-AL08000-19	98.1	> 46.9	2,880	2.4	> 49.6	C	B
12/03/2020	02-010	Y	C-AL10000-12	93.7	> 46.9	10,740	2.0	> 49.6	B	C
Specification				≥ 75%	≥ 90%	> 125	> 1	> 46.9	A, B or C	
% of products fulfilling the specification				100%	100%	100%	100%	100%	100%	
Mean (SD) all products				96.1 (1.7)	99.1 (0.9)	9,157.2 (4,830.8)	3.7 (1.6)			
Mean (SD) products applicated to responders				96.2 (1.8)	99.3 (0.6)	9,436.3 (4,944.3)	3.8 (1.6)			
Mean (SD) products applicated to non-responders				96.0 (1.4)	98.8 (1.3)	8,090.1 (4,378.4)	3.5 (1.5)			

For more detailed description of the release testing algorithm and the methods see Ballikaya et al. (2020).

¹Defined as percentage of live cells determined as propidium iodide-excluding cells.

²Defined as percentage of metabolically active cells determined as cells converting calcein acetoxymethylester to calcein.

³Values were adopted from drug substance release testing; transferability of the result from drug substance release testing onto the final drug product was demonstrated in stability studies and method validation (Ballikaya et al., 2020).

⁴Determined after cocultivation with M1-polarized macrophages to evaluate the immunomodulatory potency of the ABCB5⁺ MSCs.

⁵Determined after 48 h culture under hypoxic conditions to evaluate the angiogenic potency of the ABCB5⁺ MSCs.

⁶Determined after 19-22 h incubation on extracellular matrix gel to evaluate the endothelial trans-differentiation capacity of the ABCB5⁺ MSCs.

A: tubular branches of several cells forming a defined network-like structure; B: tubular branches of several cells clustering together forming broad strands, formation of syncytia, areas of high cellular density lacking formation of tubular branches; C: cells clustering together, building nodes and forming tubular branches that connect the nodes with each other; D: only sporadic cells form tubular branches, partial node formation, but no or nearly no connections between nodes, no or only sporadic apoptotic cells; E: largely apoptotic cells, no or only sporadic tubular branches; F: no tubular branches.

⁷Subject dropped out before week 12.

Reference:

Ballikaya S, Sadeghi S, Niebergall-Roth E, Nimitz L, Frindert J, Norrick A et al. Process data of allogeneic ex-vivo expanded highly pure ABCB5⁺ mesenchymal stromal cells for human use: Off-the-shelf GMP-manufactured standardized and donor-independent ATMP. *Stem Cell Res Ther* 2020; 11:482.