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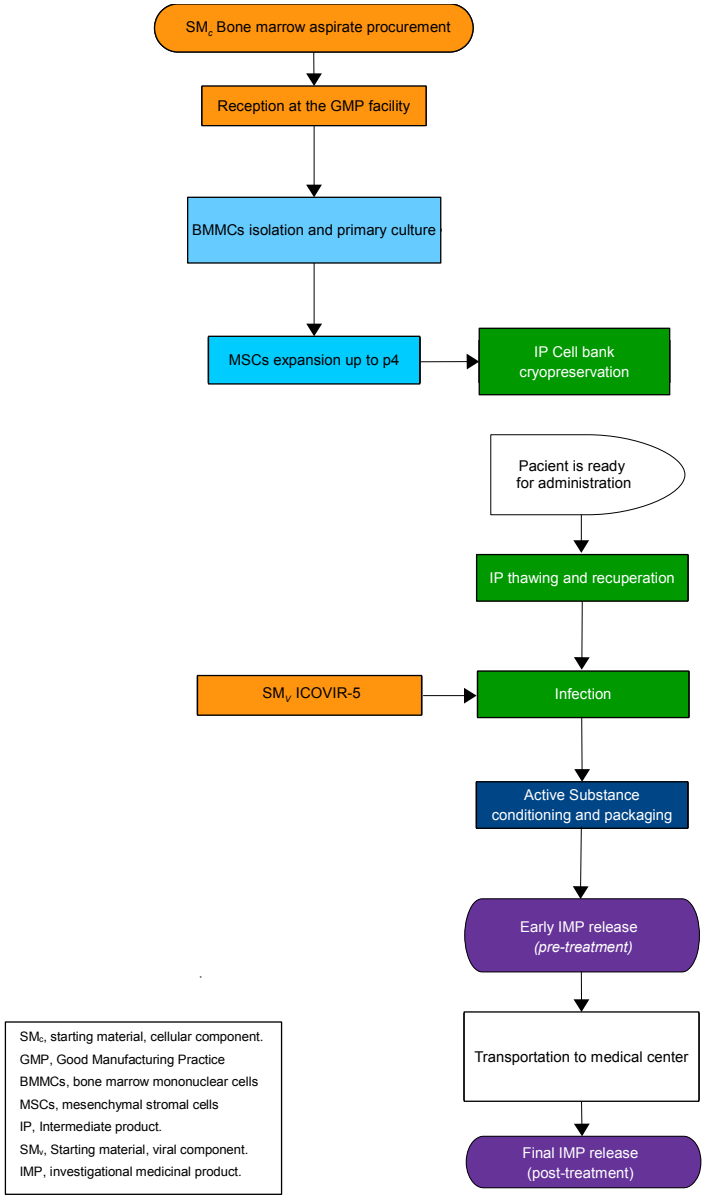
Supplemental Information

First-in-Human, First-in-Child Trial of Autologous MSCs Carrying the Oncolytic Virus Icovir-5 in Patients with Advanced Tumors

David Ruano, José A. López-Martín, Lucas Moreno, Álvaro Lassaletta, Francisco Bautista, Maitane Andión, Carmen Hernández, África González-Murillo, Gustavo Melen, Ramón Alemany, Luis Madero, Javier García-Castro, and Manuel Ramírez



Supplementary Figure. Manufacturing process of Celyvir.

Supplementary Table I. Adverse effects related to therapy with Celyvir.

Cohort	Organs & Systems	Symptoms	GRADE 1 n	1 %	2 n	2 %	3 n	3 %	4 n	4 %
Cohort A	Nervous system disorders	Headache	**1**	**12,5**	0	0	0	0	0	0
	General disorders and administration site conditions	Gait disturbances	1	12,5	0	0	0	0	0	0
		Pyrexia	**3**	**37,5**	0	0	0	0	0	0
	Musculoskeletal and connective tissue disorders	Limb pain	1	12,5	0	0	0	0	0	0
		Bone pain	1	12,5	0	0	0	0	0	0
		Musculoskeletal chest pain	1	12,5	0	0	0	0	0	0
Cohort B	Analytical / bichemical explorations	Increased blood alkaline phosphatase	0	0	1	12,5	0	0	0	0
	Infections	Cellulitis	1	12,5	0	0	0	0	0	0
	Blood and lymphatic system disorders	Anemia	0	0	1	12,5	0	0	0	0
	Metabolism and nutrition disorders	Anorexia	0	0	1	12,5	0	0	0	0
		Hypoalbuminemia	0	0	1	12,5	0	0	0	0
		Hypokalemia	0	0	0	0	1	12,5	0	0
	Gastrointestinal disorders	Diarrhea	0	0	1	12,5	0	0	0	0
		Abdominal distension	1	12,5	0	0	0	0	0	0
		Abdominal pain	1	12,5	0	0	0	0	0	0
		Nausea	2	25	0	0	0	0	0	0
		Vomiting	1	12,5	0	0	0	0	0	0
	General disorders and administration site conditions	Asthenia	**1**	**12,5**	0	0	0	0	0	0
		General deterioration of physical state	0	0	0	0	0	0	1	12,5
		Pain	0	0	0	0	1	12,5	0	0
		Oedema	0	0	1	12,5	0	0	0	0
		Pyrexia	**2**	**25**	0	0	0	0	0	0
	Renal and urinary disorders	Anuria	1	12,5	0	0	0	0	0	0
		Dysuria	1	12,5	0	0	0	0	0	0
		Renal failure	0	0	1	12,5	0	0	0	0
		Oliguria	1	12,5	0	0	0	0	0	0
	Respiratory, thoracic and mediastinal disorders	Epistaxis	1	12,5	0	0	0	0	0	0
	Vascular disorders	Hypotension	1	12,5	0	0	0	0	0	0

In red, adverse effects recorded, not related to therapy. In black, adverse effects related to therapy.

Supplementary Table II. Release criteria

Parameter	Sample	Method	Limits
Early release (pre-treatment)			
Primary container			
Integrity	100 ml-sterile bottle	Visual inspection	Complies
Label			Readable
Content			
Aspect	Cell suspension	Visual inspection	w/o cell aggregates
Volume	Cell suspension	Visual inspection	50 ml
Cell count[1]	Cells	Manual cell count	0,5-1,0x10^6 cells/kg
Cumulative PD[1,2]			≤10
Viability[1]	Cells	Trypan blue exclusion	≥75%
Endotoxinas[2]	Cell suspension	LAL *(Ph. Eur. 2.6.14)*	≤1,0 EU/ml
Immunophenotype[1]			
CD90(+)	Cells	FACS	≥85%
CD73(+)			≥85%
CD29(+)			≥85%
CD14(+)			<5%
CD19(+)			<5%
CD45(+)			<5%
Final release (post-treatment)			
Sterility	Cell suspension	BD BACTEC™ (*Ph. Eur.* 2.6.27)	No growth
Mycoplasma[1]	Supernatant	RT-PCR (*Ph. Eur.* 2.6.7)	Negative
Cytopathic effect[1,3]	Cells	Internal procedure	≥90%
Adenovirus quantification [1,2,4]	Supernatant	qRT-PCR	≥3,5 log

[1]Controls performed on active substance before conditioning and packaging. [2]To be implemented throughout next phase of development. [3]To be replaced by Adenovirus quantification. Cells are seeded following infection and cultured under

permissive conditions during 7 days. Cell confluence is evaluated under optic microscope, confluence percentage is calculated and registered. [4]Cells are seeded following infection and cultured under permissive conditions during 7 days. Supernatant is assessed for adenovirus quantification with a clinical grade-qRT-PCR diagnostic kit.