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CASE STUDY

Maximize Working Cell Bank (WCB) Manufacturing Quantity while controlling batch costs

PROBLEM STATEMENT

Client Overview & Challenge

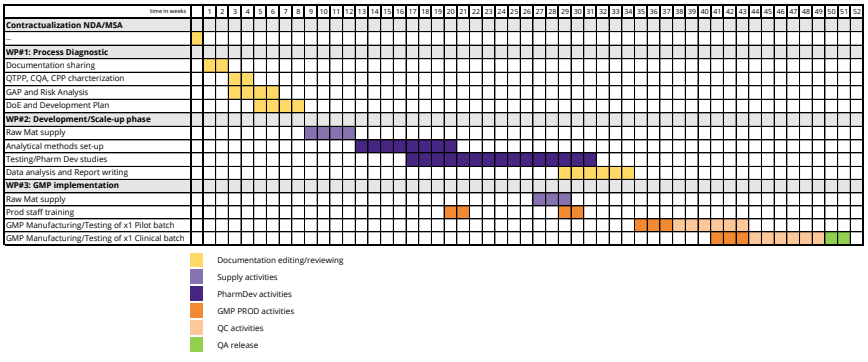
To develop a cell therapy product based on NK cells for a Phase 1 clinical study, a European biotech company partnered with Cell-Easy to scale up and manufacture a GMP-compliant Working Cell Bank (WCB) from an immortalized feeder cell line. While the biotech had already established the 2 weeks process at lab scale, the challenge was to scale it up and manufacture within a tight timeframe: **12 months to produce the first GMP batch of 50 billion cells.**

Our Role

As a CDMO specializing in Cell Therapy, we were tasked with optimizing the manufacturing process for clinical-stage production while ensuring GMP compliance. At Cell-Easy, every project starts by defining the customer's key needs and expectations. In this case, scaling up the manufacturing process, including the WCB production, had to be completed within a strict 12-month deadline. Additionally, limiting the cost per billion cells produced was an important add-on requested.

OUR APPROACH

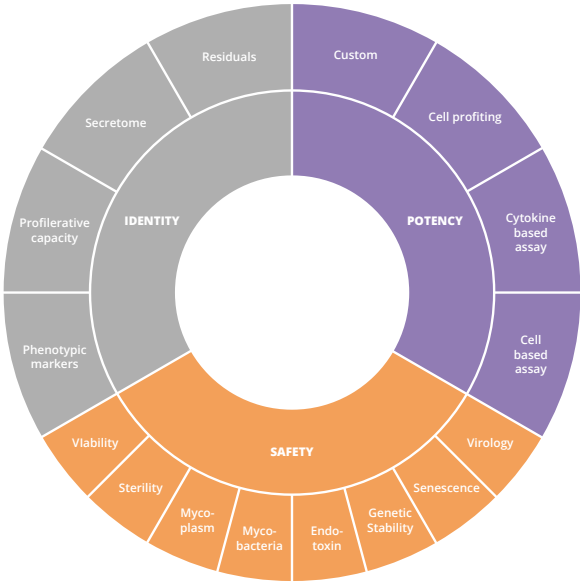
Starting with a **process diagnostic**, the following roadmap was agreed.



1 Development phase: Implementation of analytical methods

Beyond safety analytics, mastering identity and potency analytics is crucial for assessing the quality of the generated cells during the scale-up phase, making this the first development activity we initiated. By leveraging our **in-house analytic platform**, we could rapidly analyze the various conditions evaluated during scale-up. Had we externalized our analytics, we would not have been able to implement such an aggressive development plan.

This platform also facilitated faster batch release in subsequent stages.



In this case, the following **methods** were deployed :

	Parameter	Phase 1: Regulatory expectation	Status	Equipment	Action performed
Safety	Mycoplasmas PhEur_2.6.7.	Full validation	Method internalized	Biorad PCR	Matrix suitability
	Sterility assay PhEur_2.6.27	Full validation	Method internalized	bacTec	Matrix suitability
	Bacterial endotoxins PhEur_2.6.14	Full validation	Method internalized	Endosafe	Matrix suitability
	Human virus detection PhEur_2.6.21.	Full validation	Method sub-contracted	PCR	Matrix suitability
Identity	Numeration, Viability, Yield (Flow cytometer) PhEur_2.7.29	No validation required	Method internalized	MACSquant	Gating strategy and IS (index staining) set-up
	Immunophenotyping (Flow Cytometry) PhEur_2.7.29	No validation required	Method internalized	MACSquant	Gating strategy and IS (index staining) set-up
Potency	Measurement of NK-cell growth support (read-out by Flow Cytometry)	No validation required	Method internalized	MACSquant	Ab pannel, Gating strategy and IS (index staining) set-up

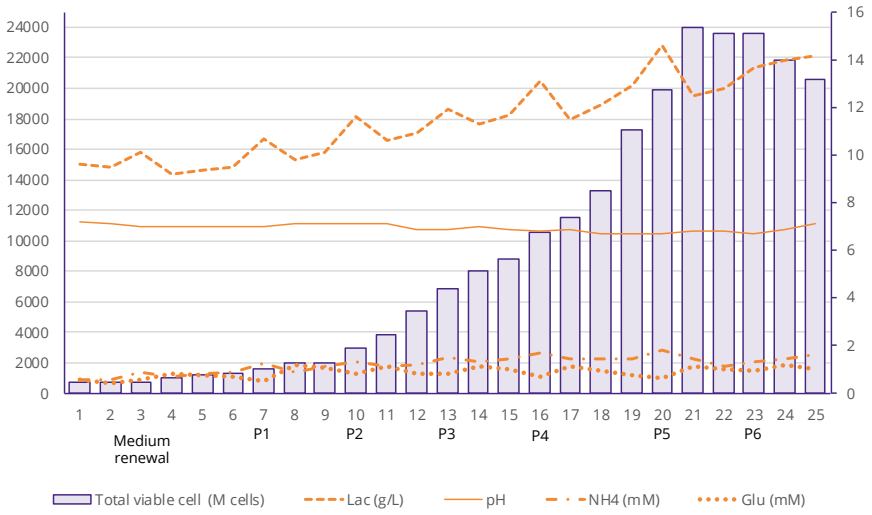
2 Scale up phase: Evaluation and testing of culture supports and conditions

When scaling up, it's essential to minimize the negative impact on cell quality while maximizing quantity. In this case, due to the large number of cells required and the very tight development timeline, closed automated systems were excluded from the development plan. The biotech's consistent results using T-flasks led us to favor similar culture systems. We evaluated both Cell Stack-type factories and wave bioreactors. Each system had its pros and cons, but we chose to continue with Cell Stack® for several reasons:

- Faster and more **reliable procurement**,
- **Cell growth kinetics** closely matching those achieved at lab scale,
- No new Aseptic Process Simulation runs were required, as operators were already trained and validated to handle **up to x12 CellStack10 units**, avoiding extra budget and time.

With the culture system established, we focused on optimizing the culture conditions by working primarily on the Critical Process Parameters (CPPs). Our DEV team employed a **Quality by Design (QbD)** approach (ICHQ8) and systematic live monitoring of metabolic parameters to evaluate various cell culture conditions, including the composition of the culture media, seeding densities, and media passage/change durations.

Live monitoring of cell growth parameters



By the end of the development studies, we were able to establish the **optimal manufacturing process** for Cell Stack, as outlined in the flowchart below.

			Volume	Support			Cells count	Concentration	
	Thawing Yielding at 70,00%		2 mL				10,0 Cells/vial	5,0 M Cells/mL	Tuesday-17-Sep
			2 mL				7,0 M Cells	3,5 M Cells/mL	Tuesday-17-Sep
				Nb of vial to thaw		2 vials			Tuesday-17-Sep
P. 0	Cell-culture passage Duration #72 hrs Douling time 50 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		5 mL	1 units	T25	25 cm2	12,5 M Cells	500 000 Cells/cm2	Tuesday-17-Sep
			5 mL	1 units	T25	25 cm2	27,1 M Cells	5 426 417 Cells/mL	Friday-20-Sep
P. 1	Cell-culture passage Duration #96 hrs Douling time 35 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		10 mL	2 units	T25	50 cm2	25,0 M Cells	500 000 Cells/mL	Friday-20-Sep
			10 mL	2 units	T25	50 cm2	133,9 M Cells	13 387 894 Cells/mL	Tuesday-24-Sep
P. 2	Cell-culture passage Duration #72 hrs Douling time 30 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		50 mL	1 units	T225	225 cm2	112,5 M Cells	500 000 Cells/mL	Tuesday-24-Sep
			50 mL	1 units	T225	225 cm2	475,0 M Cells	9 500 457 Cells/mL	Friday-27-Sep
P. 3	Cell-culture passage Duration #72 hrs Douling time 30 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		200 mL	1 units	CS1	636 cm2	318,0 M Cells	500 000 Cells/mL	Friday-27-Sep
			200 mL	1 units	CS1	636 cm2	1 342,7 M Cells	6 713 656 Cells/mL	Monday-30-Sep
P. 4	Cell-culture passage Duration #72 hrs Douling time 30 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		800 mL	2 units	CS2	2544 cm2	1 272,0 M Cells	500 000 Cells/mL	Monday-30-Sep
			800 mL	2 units	CS2	2544 cm2	5 370,9 M Cells	6 713 656 Cells/mL	Thursday-3-Oct
P. 5	Cell-culture passage Duration #96 hrs Douling time 30 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		2000 mL	1 units	CS10	6360 cm2	3 180,0 M Cells	500 000 Cells/mL	Thursday-3-Oct
			2000 mL	1 units	CS10	6360 cm2	23 378,3 M Cells	11 689 154 Cells/mL	Monday-7-Oct
P. 6	Cell-culture passage Duration #72 hrs Douling time 30 PDT hrs Seeding 500 000,0 Cells/cm2 Yielding at 80,00%		12000 mL	6 units	CS10	38160 cm2	19 080,0 M Cells	500 000 Cells/mL	Monday-7-Oct
			12000 mL	6 units	CS10	38160 cm2	80 563,9 M Cells	6 713 656 Cells/mL	Thursday-10-Oct
IRR	Irradiation x3 35 Gray Yielding at 70,00% Cell concentration 300 000 000,0 Cells/cm2		269 mL				80 563,9 M Cells	300,0 M Cells	Thursday-10-Oct
						Viable Cell Nb before thawing	56 394,7 M Cells		Thursday-10-Oct
	Downstream & filling Yielding at 70,00% Final C : 50,0 M Cells/mL Vial volume : 5,0mL		1128 mL				56 395 M Cells	50,0 M Cells/mL	Thursday-10-Oct
						Nb of vials to fill	226 vials		Thursday-10-Oct

Finally, to ensure a smooth transfer of the process to the GMP environment, **two lock-down runs were executed at scale**. This allowed us to secure all critical process steps, ensuring a seamless clinical batch production while strenghtening the production staff training and documentation efficiency.

3 GMP implementation

The integration and close collaboration between the development and production teams enabled a quick transfer of the process to our state of the art cGMP facility. At Cell-Easy, all developments are defined and executed in close partnership with a GMP referent, while all GMP production is supported by a development engineer.

Thanks to this, the following batches were manufactured successfully, with the expected quantity and requested quality :

- **x1 GMP Pilot batch,**
- **x1 GMP Clinical batch**.

Batches were manufactured in accordance with release specifications and user requirement specifications, then frozen at -170°C with a Control Rate Freezer using pre-established freezing parameters. Ultimately, the clinical batch was released in a record timeframe of just 8 weeks, largely due to the internalization of most QC methods at Cell-Easy.
Vials are stored in gaseous nitrogen tanks at Cell-Easy until they are used to generate NK cell-based Drug Product.
In the meantime, a WCB stability study has begun and will last for 3 years, depending on customer demand.

RESULTS

Thanks to our Quality by Design approach and the Cell Biology expertise of our multi-disciplinary teams, the WCB manufacturing process was successfully scaled up — achieving a sixfold increase in cell production compared to the initial process — while maintaining the cell line's quality attributes, adhering to the overall project timelines. In addition, the control of scale-up, batch-to-batch reproducibility and the increase in cell viability post-thawing have mechanically led to a 3-fold reduction in cost per billion cells manufactured.

CONCLUSION AND LESSONS LEARNED

The success of this project is founded on three main pillars that Cell-Easy consistently emphasizes as a CDMO expert in Cell Therapy:

ATMP Expertise (Advanced Therapy Medicinal Products): We understand the scientific challenges associated with cell-based products, whether engineered or not, having produced 50+ GMP batches across various cell types.

State-of-the-Art DEV and GMP Facilities: We master technologies and aseptic operations to ensure the qualitative, quantitative, and timely production of your cell products.

Alliance: We provide ongoing CMC and regulatory support to secure your IMPD/IND.

ABOUT US

Cell-Easy is a science-centric CDMO specializing in advanced cell therapies. We offer a comprehensive range of services, including process and analytical development, GMP cell banking, GMP manufacturing, and CMC/regulatory support for biotech and pharmaceutical companies.

Our team of scientists and Quality by Design (QbD) approach facilitate seamless technology transfer and development for cell-based therapies in oncology, auto-immunity, and regenerative medicine, covering T cells, NK cells, MSCs, macrophages, iPS cells, stem cells, and other immortalized cell lines.

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