



Leading the way in Cell & Gene therapy

Process Development and Large-Scale GMP Production for Lentiviral (LV) vectors

*Luca Alberici,
CBO*

*Margherita Neri,
USP DEV Manager*

*Francesca Bellintani
DSP DEV Manager*



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Agenda

1. **Company Presentation**
2. *LV Upstream production in CFs and bioreactor*
3. *LV Downstream processing*



MolMed is a pure player in the Cell&Gene arena



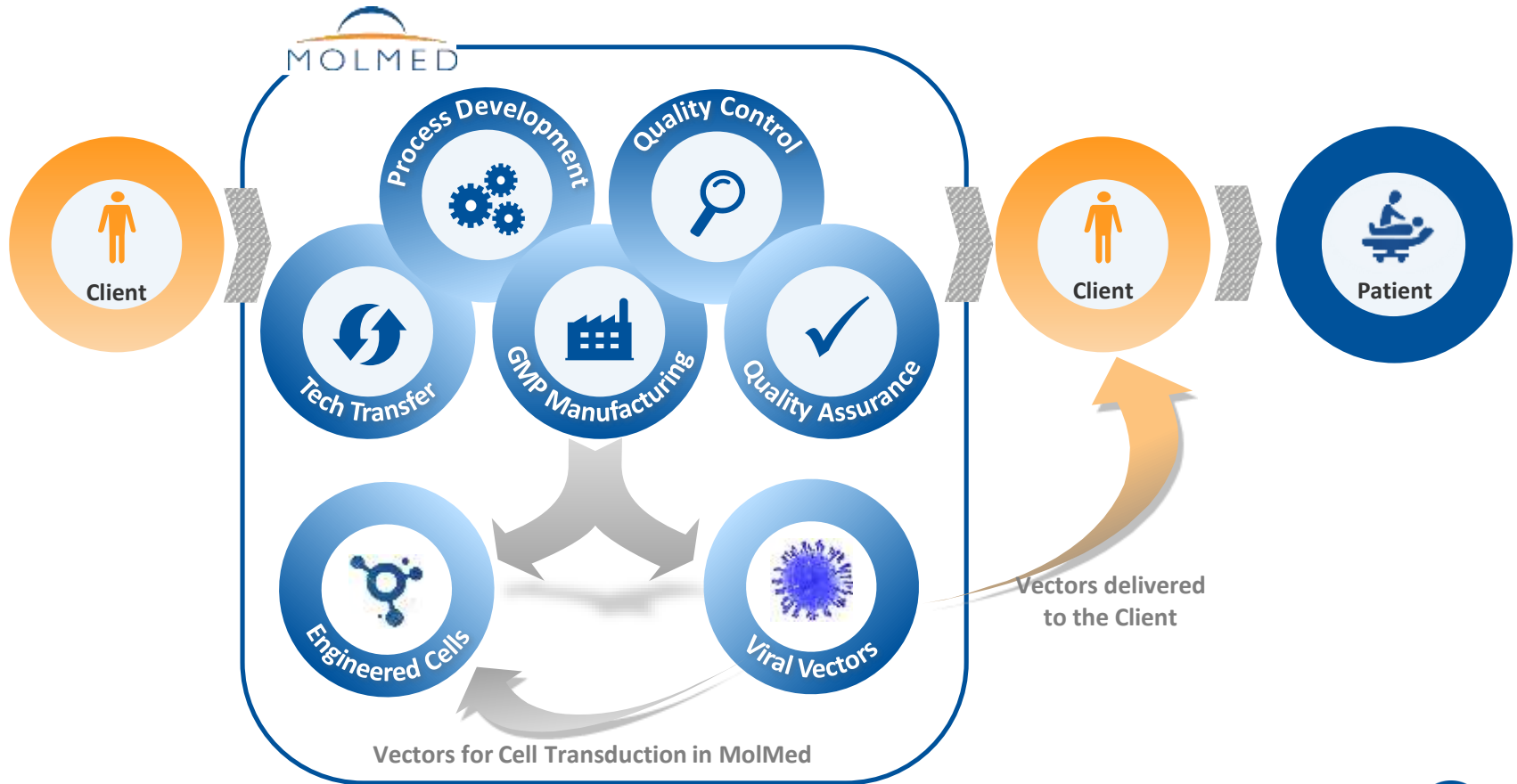
Focusing on **innovative cell and gene therapies** that can meet the therapeutic needs in the treatment of **tumors and rare diseases**, with a clear and solid industrial project based on **research, development** and **production excellence**



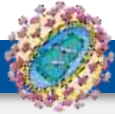
CDMO Business, with 35+ Programs developed with our Partners

R&D Business on our Autologous Product CAR-T CD44v6

CDMO business experience in manufacturing of vector and modified cells



Current manufacturing platforms



Viral Vector Manufacturing

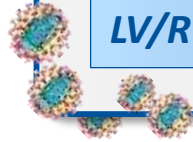
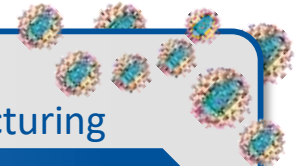
Development/Feasibility

Tech transfer/Engineering

GMP manufacturing

LV/RV 48L Cell Factories – adhesion

LV/RV 200L bioreactor - adhesion



Cell Engineering

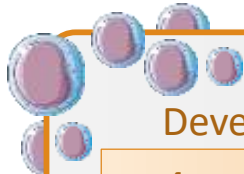
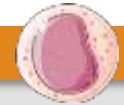
Development/Feasibility

Tech transfer/Engineering

GMP manufacturing

LV/RV T-Cell Transduction

LV CD34+ Cell Transduction



Excellent GMP capacity with more than 230 scientists and support staff

Milan Site (San Raffaele)

- **1,500 SQM** (16,000 SQF) and **6 grade B/C suites**
- **2003:** Authorized GMP manufacturing facility for **Clinical programs**
- **2015:** Authorized GMP manufacturing facility for **Commercial products**

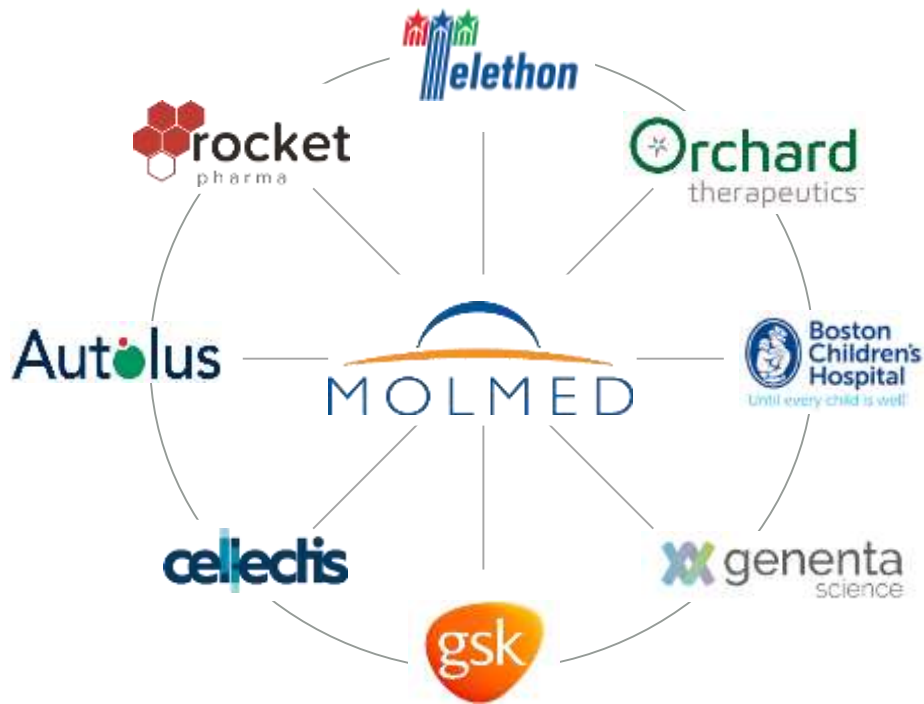


Bresso Site



- **3,300 SQM** (36,000 SQF) and **>20 Grade B/C suites**
- Authorized for **GMP manufacturing** and **QC** for the production of **clinical** and **commercial** products
- **Recently authorized Stream#2**, for further services and new collaborations

Development and manufacturing partners in EU and US geographies



35+ Programs currently in Development and GMP

2 Commercial Cell-Engineered Products in EU

2 Commercial Viral Vectors in EU

15+ Cell-Engineering programs for EU&US

20+ Viral Vectors programs for EU&US

Track Record

300+ Treated Patients (autologous)

220+ Manufactured GMP Vectors

30+ C&G Clinical Trials Supplied in EU&US

8+ International Service Partners

Strengths of MolMed CDMO

High **GMP manufacturing Capacity** thanks to new facility in Milan area

25yrs **Experience** in proprietary projects now available for CDMO collaborations

Recognized **Flexibility** in accommodating Partners' requests



160 QC tests internalized, ensuring reduction in time and cost

Ready **Proprietary Processes** for vectors and cells engineering

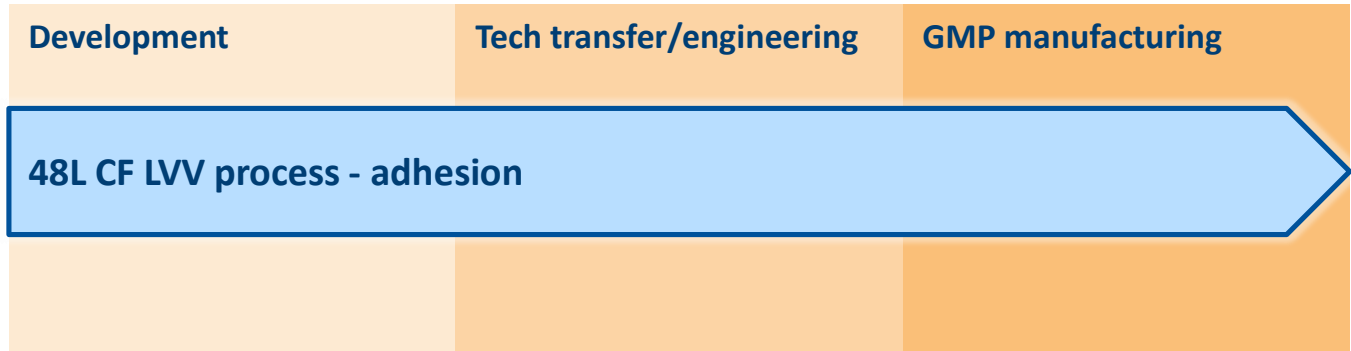
1st Approved Facility for C&G therapies

Agenda

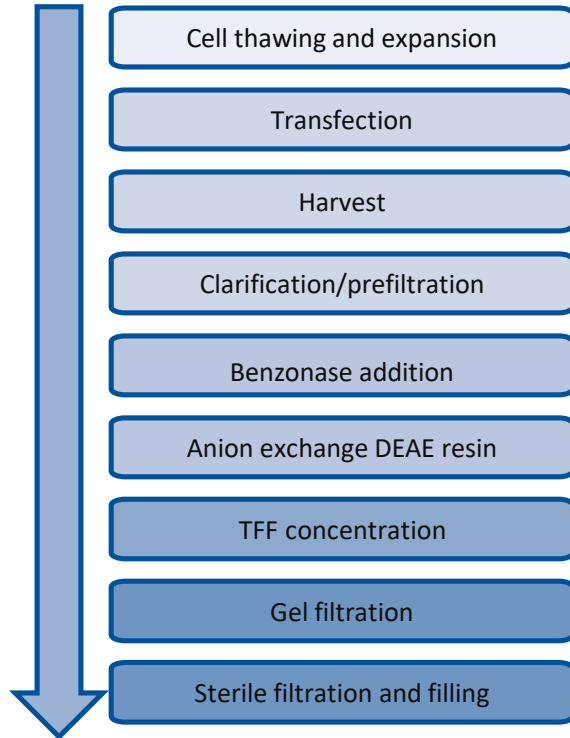
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Current vector manufacturing processes – Cell factories 48L



LV production process – 48L Cell Factories



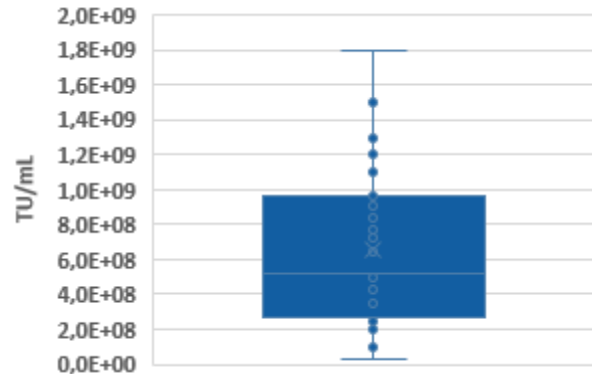
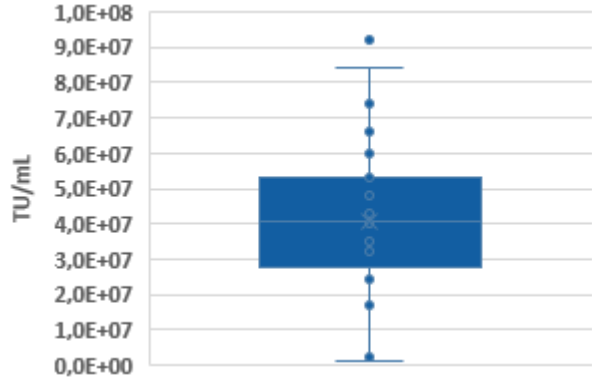
- **Cell line** adherent HEK293T
- **Culture Area** 24CF (15.2m²)
- **Harvest** 48L
- **DSP** Purification and concentration

- **Process currently in GMP for clinical and commercial application**
- **More than 100 batches manufactured in GMP**

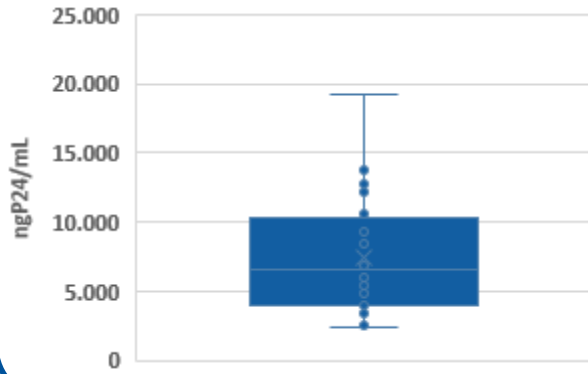
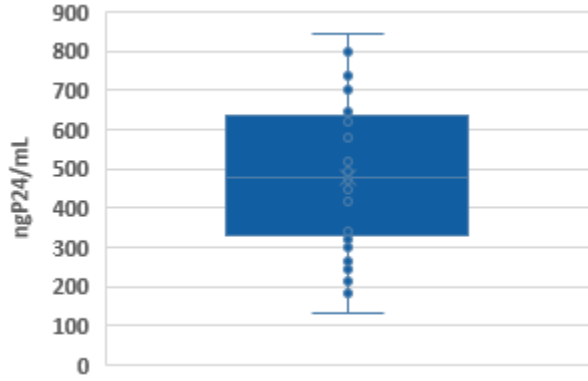
LV production process – 48L Cell Factories - Productivity



Infectious viral titer



Physical Viral Titer

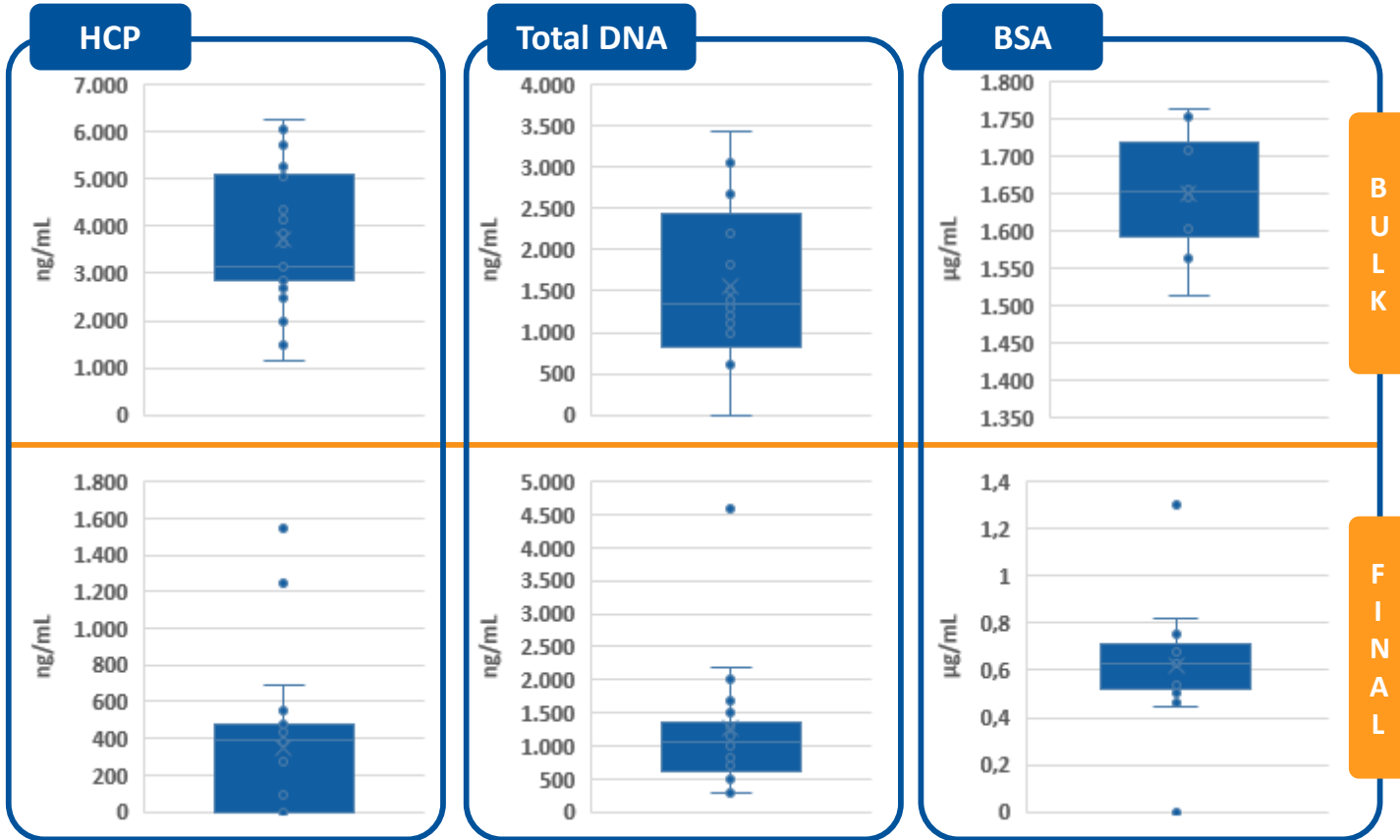


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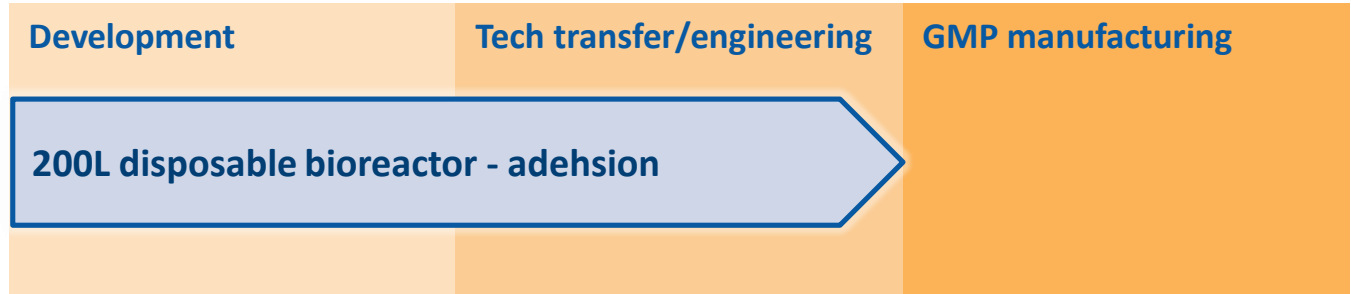
	Bulk	Final
TU/mL	4.1×10^7 $\pm 2.2 \times 10^7$	6.5×10^8 $\pm 4.6 \times 10^8$
ngP24/mL	481 ± 185	7428 ± 4109

LV production process – 48L Cell Factories - Residuals



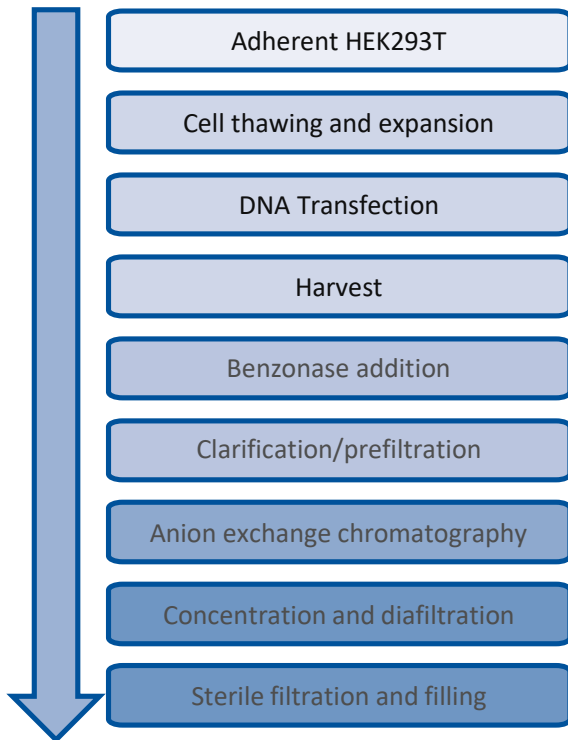
	Removal Contaminants
HCP	>99%
Total DNA	>99%
BSA	>99%

Current vector manufacturing processes – Bioreactor 200L



- iCellis system allows the development of fully **cGMP** compliant manufacturing processes for LV vectors compliant with **commercial vector application**
- Vectors produced in iCellis systems:
 - ✓ are **qualitative** comparable to CF clinical batches
 - ✓ batch size is **cost-effective** in terms of number of patients treated versus cost of production and QC
 - ✓ high **reproducibility** of genetically modified cells batches

LVV production in iCELLis 500: process flow chart



Full Scale



iCellis 500



ÄKTA Ready and
pre-packed column



ÄKTA Ready Flux
System

Scale down



iCellis Nano



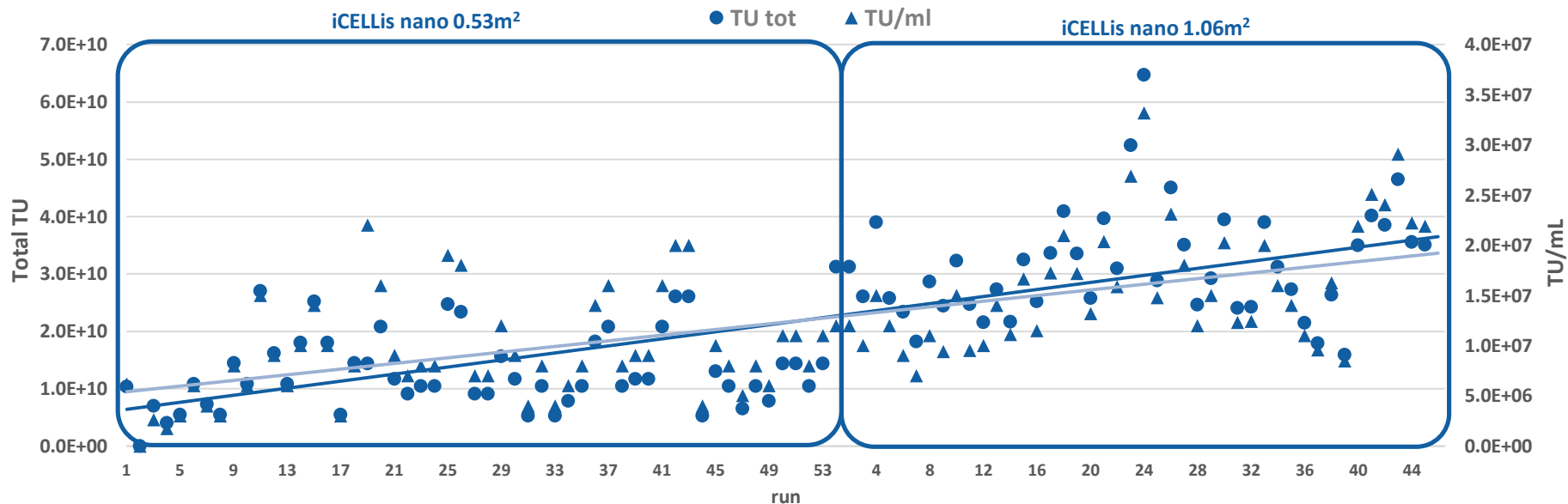
ÄKTA Pilot and
Axichrom column



KrosFlo System

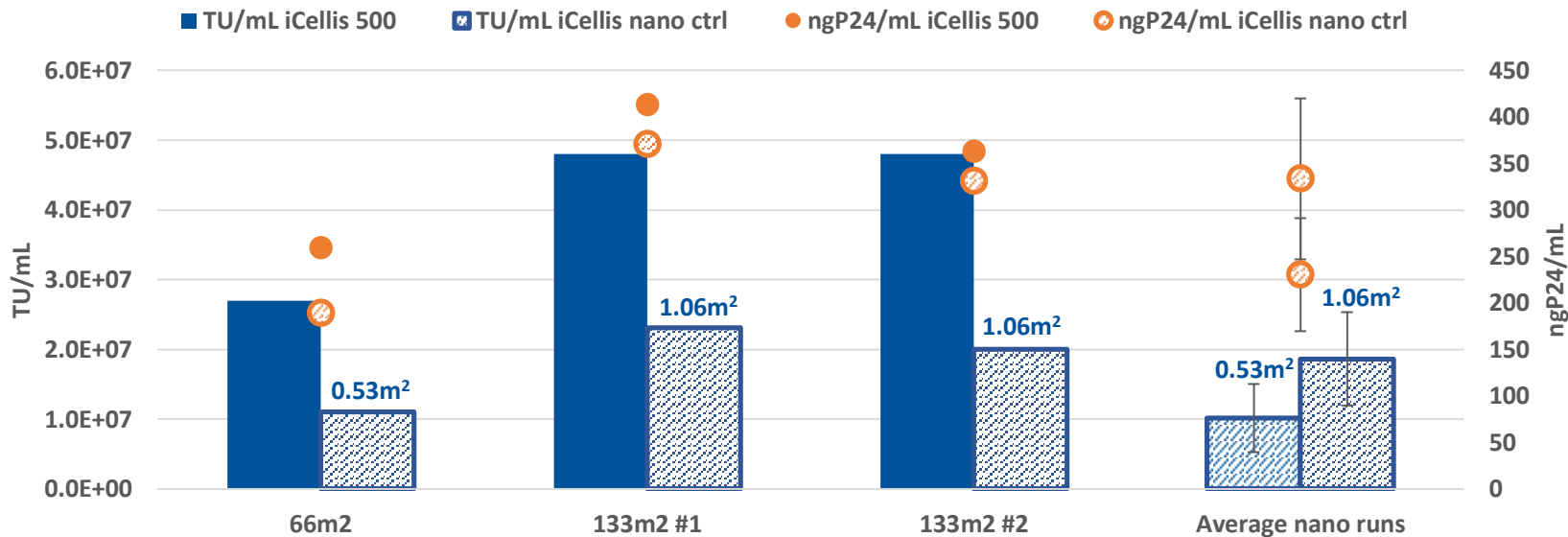
LVV production in iCELLisnano: development data USP

Productivity on bulk supernatant



Conditions optimization permitted to increase system productivity of 3-4 fold

LVV production in iCELLis 500: development data USP



Total TU

4.1x10¹²

1.1x10¹³

1.0x10¹³

Scale up in iCellis500 runs confirmed system scalability
Linearity in production using different packed-bed surface areas

From Upstream to Downstream

Culture Conditions

Adherent vs Suspension cell culture

Transient transfection vs stable producer

Serum containing vs serum free culture medium

Addition of USP additives

Residual Levels

Cell supernatant vs cell lysate

Residual HCPs and DNA levels

Residual BSA presence

Additives removal

Downstream Strategies

Clarification strategy:
Centrifugation / TFF / NFF

Chromatography:

Ion exchange

Size exclusion

Affinity

Other ligands

TFF:

Ultrafiltration

Diafiltration

Upstream process parameters can impact the desing of Downstream processing

LVV purification from clarified bulk

Process designed to remove main contaminants and to have the same level of quality of CF vector



Anion exchange chromatography

- Captures and concentrates LV
- Removes HCP
- Removes DNA contaminants
- Removes BSA

67% vector recovery
98% HCP removal
>88% DNA removal



Concentration and diafiltration (TFF)

- Concentrates LV
- Reduces HCP
- Reduces small DNA contaminants
- Reduces BSA

57% vector step recovery
>99% HCP removal
44% DNA removal

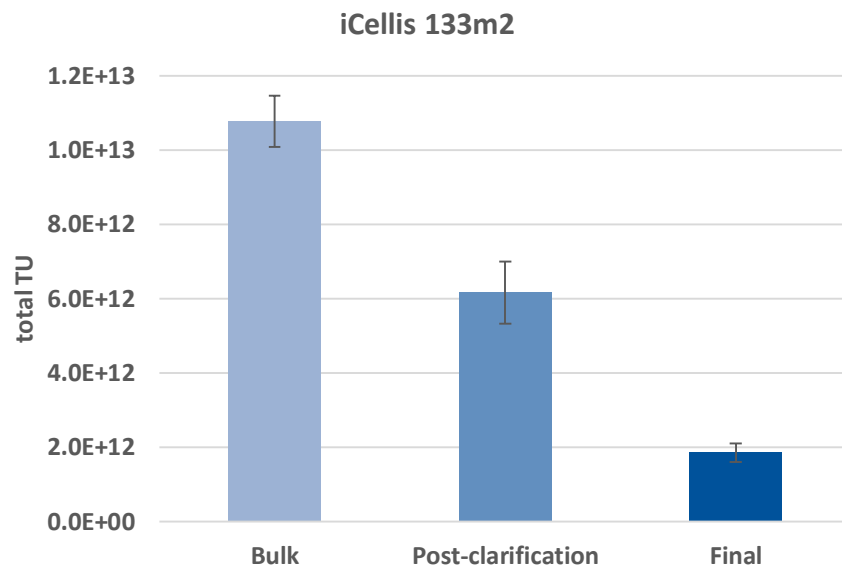
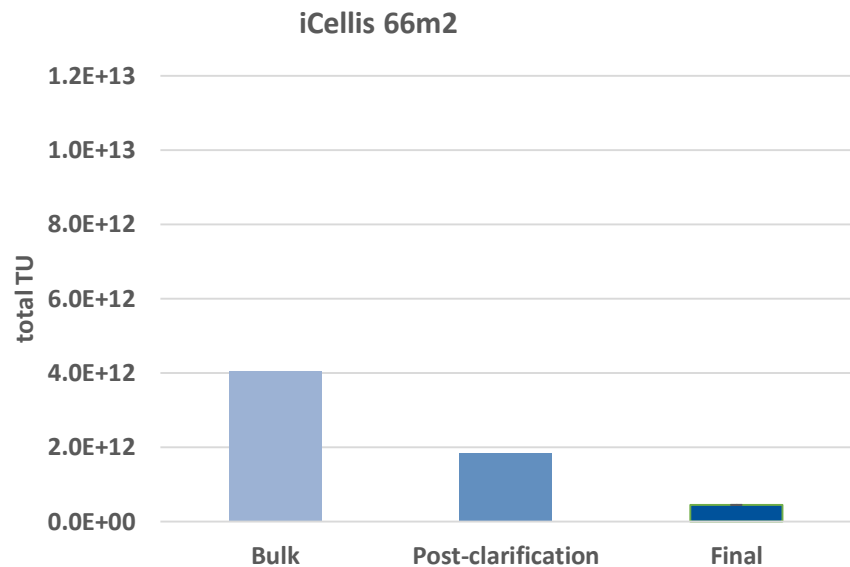


Sterile filtration and filling

- Bioburden reduction and sterile filtration
- Filling in target vials/bottles/bags

75% vector step recovery
>99% HCP removal
74% DNA removal

LVV purification from clarified bulk



Yield

45%

24%

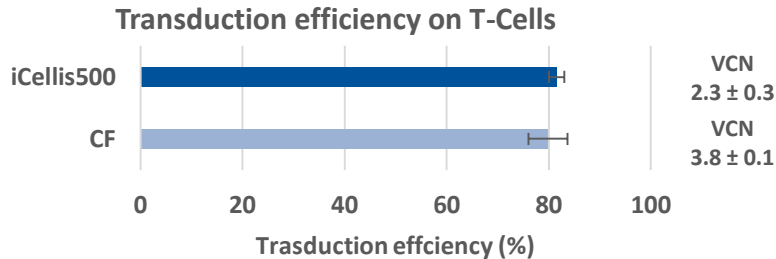
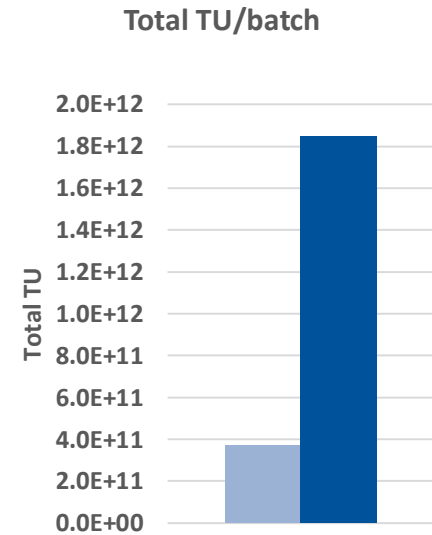
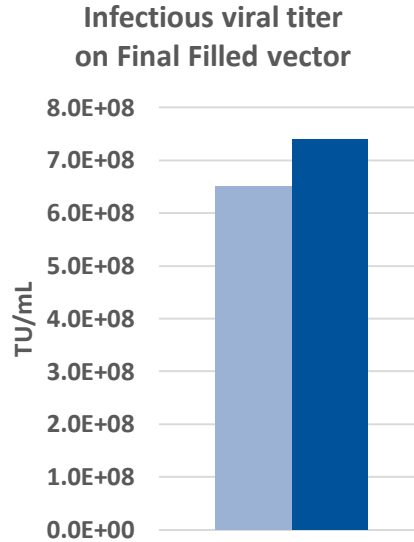
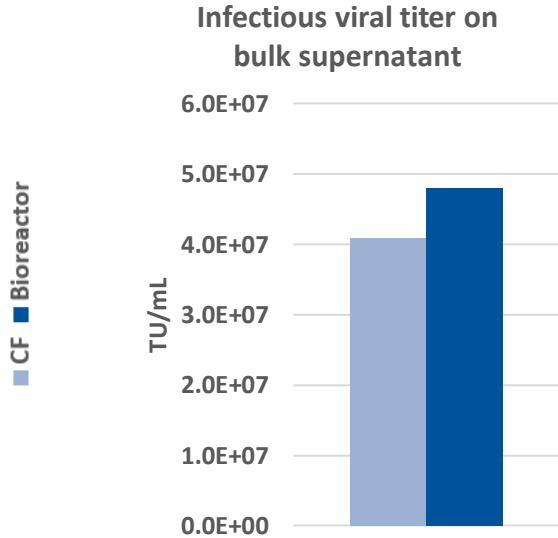
58%

30%*

**Process yield calculated applied downstream scale down model*

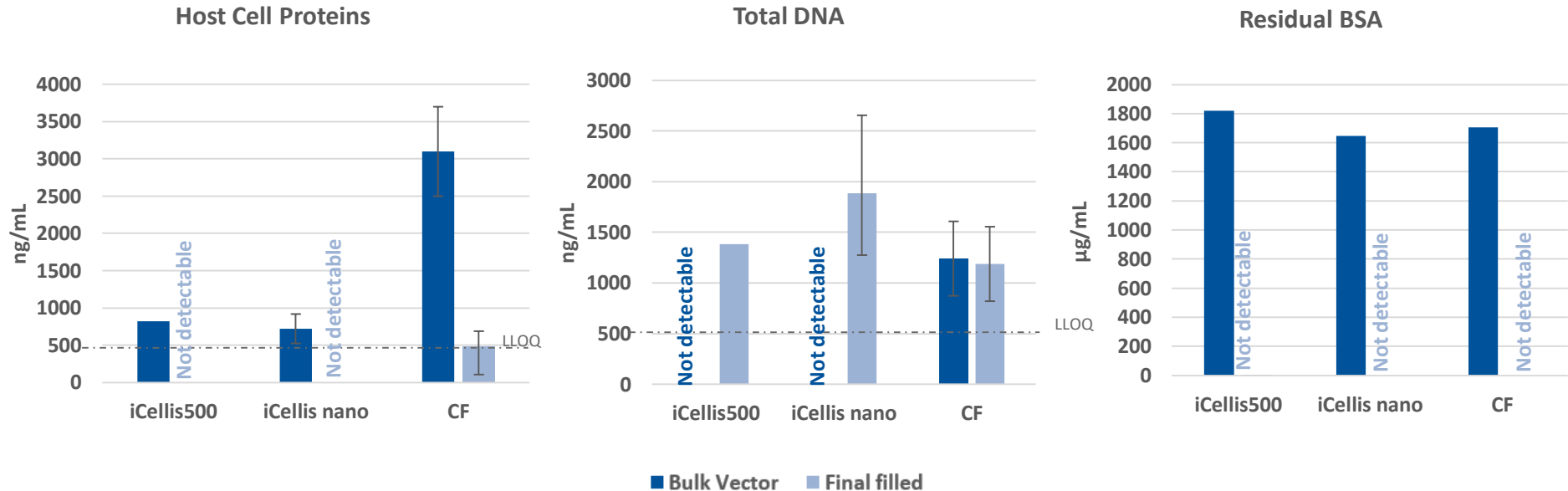
Total Process yield is very high and consistent

Comparison LVV production in iCELLis500 vs standard CF process



5 Fold increase in total vector TU and same potency on primary cells

Characterization of LVV produced in iCellis – Impurities profile



Improved impurities profile for vector produced in bioreactor

From vector to patients

Patients treated with 1 batch of manufactured vector:

	CF	Bioreactor
T-cells transduction at MOI 4	~ 90 patients	~ 400 patients
CD34 cells transduction at MOI 50	~ 10 patients	~ 50 patients



Considering to transduce about 1×10^9 T cells/pt and 0.7×10^9 CD34+ cells/pt



Thank you for your attention!

Luca Alberici

CBO

e-mail:

luca.alberici@molmed.com

Margherita Neri

USP DEV Manager

e-mail:

margherita.neri@molmed.com

Francesca Bellintani

DSP DEV Manager

e-mail:

francesca.bellintani@molmed.com