



cevec 

Human Cells
for Human Pharmaceuticals

CEVEC Pharmaceuticals GmbH

3rd Science to Market Conference

Posttranslational Modifications of Therapeutic Proteins
and Implications for Development

Vienna, February 24 – 25, 2010

Management and Brief Corporate History

Management: Rainer Lichtenberger, PhD, MBA - CEO
Wolfgang Kintzel, MSc - Chief Commercial Officer
Gudrun Schiedner, PhD - CSO
Gary Boch - Director BD North America

Technology: In development for 5 years, global IP protection

Location: Cologne, Germany

Biopharmaceuticals are a success story – but with room for improvement

Requirements for production of complex human proteins:

- Safe agents with high specific activity
- Non-immunogenic profile
- Patient-friendly mean residence time
- Produced in ethically acceptable systems

Existing biopharmaceutical production systems often do not satisfy the requirements

- Lack of glycosylation (yeast, E.coli)
- Non-human carbohydrate residues that cause immune reactions (CHO, murine myeloma cells)
- Lack of authentic sialylation (human HEK293 cells)
- Failure to produce complex proteins (e.g., Surfactant, AAT)

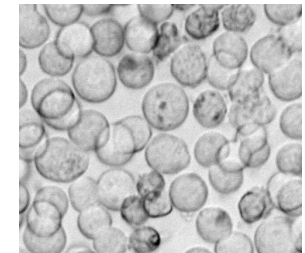
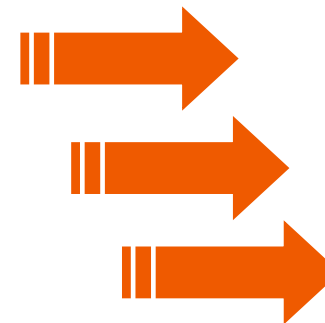
Summary Cell Source:

CEVEC's Amniocyte Production (CAP) Technology



Human native primary cells

- Ethically obtained
- Non tumor origin
- Non-viral components
- Robust and scaleable

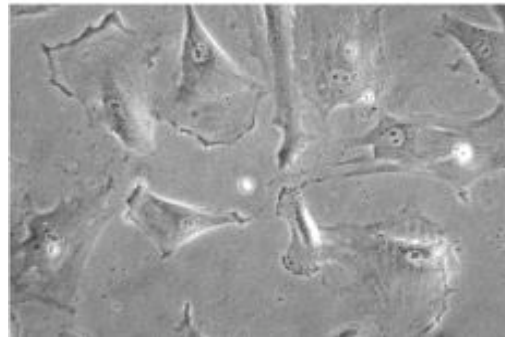
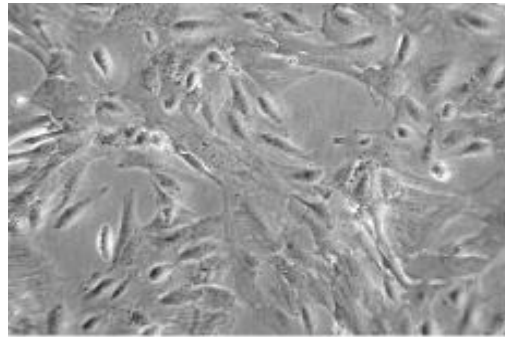


Suspension culture from immortalized single cell colony

Amniocytes are the only human cell type that is easily accessible and can be reproducibly immortalized by gene functions not oncogenic in humans.

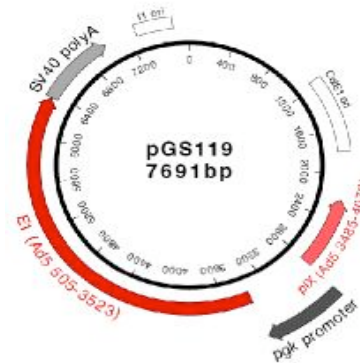
CAP Technology Development – Immortalization of Primary Human Amniocytes

Primary Amniocytes

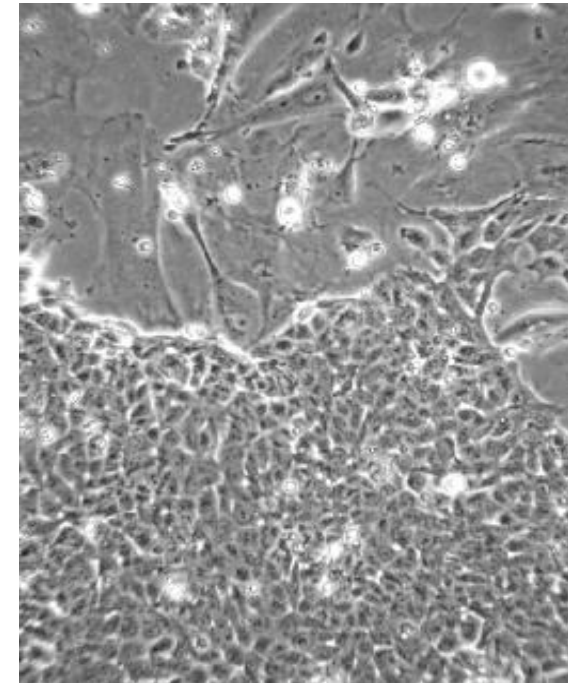


Transfection

adenoviral
E1/pIX functions



Transformed Foci



CAP Technology Development – **Cell Line Documentation & Process Development**

Master Cell Bank (MCB) established, tested & certified:

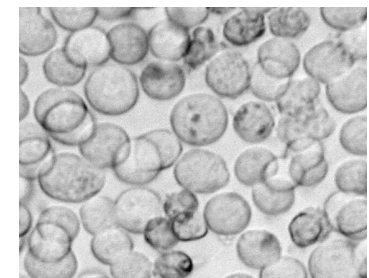
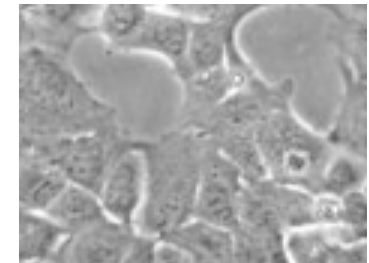
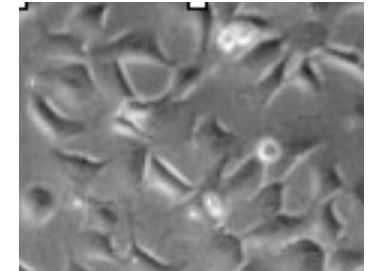
- ICH guidelines
- European Pharmacopoeia 2005
- tested for 18 different human viruses

Full documentation according to current guidelines:

- Cell donor fully documented incl. informed consent
- All materials and process steps fully documented according to SOPs

Process Development:

- Serum-free growth in suspension culture
- Lab scale: shaker, spinner
- Bioreactors: stirred tank (up 10L), perfusion, wave
- Process optimization with commercial media and feeding
- Development of medium optimized for CAP cells started

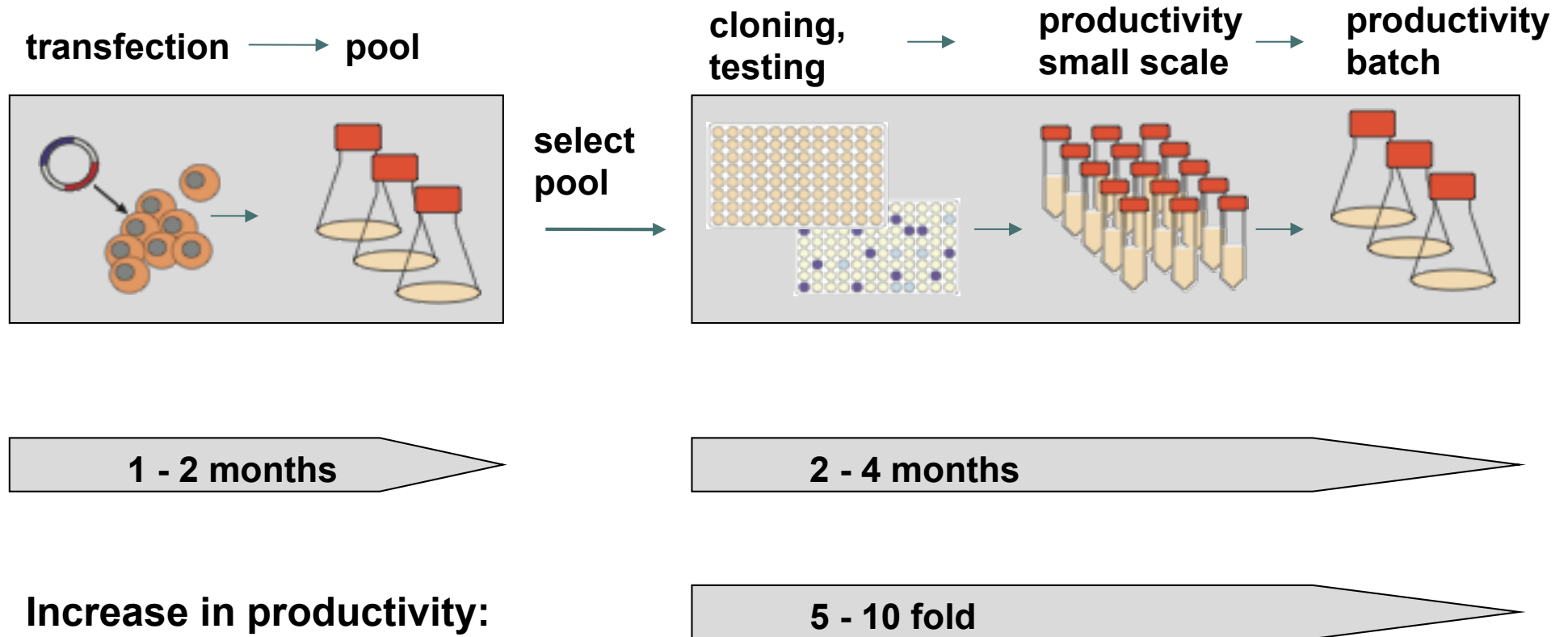


CAP Cells:

human cell line for *stable* expression of proteins

Evaluation of CAP Cells:

Procedure and Time Lines For Stable Expression



Evaluation of CAP cells:

Expression of Human Alpha-1 Antitrypsin (hAAT)

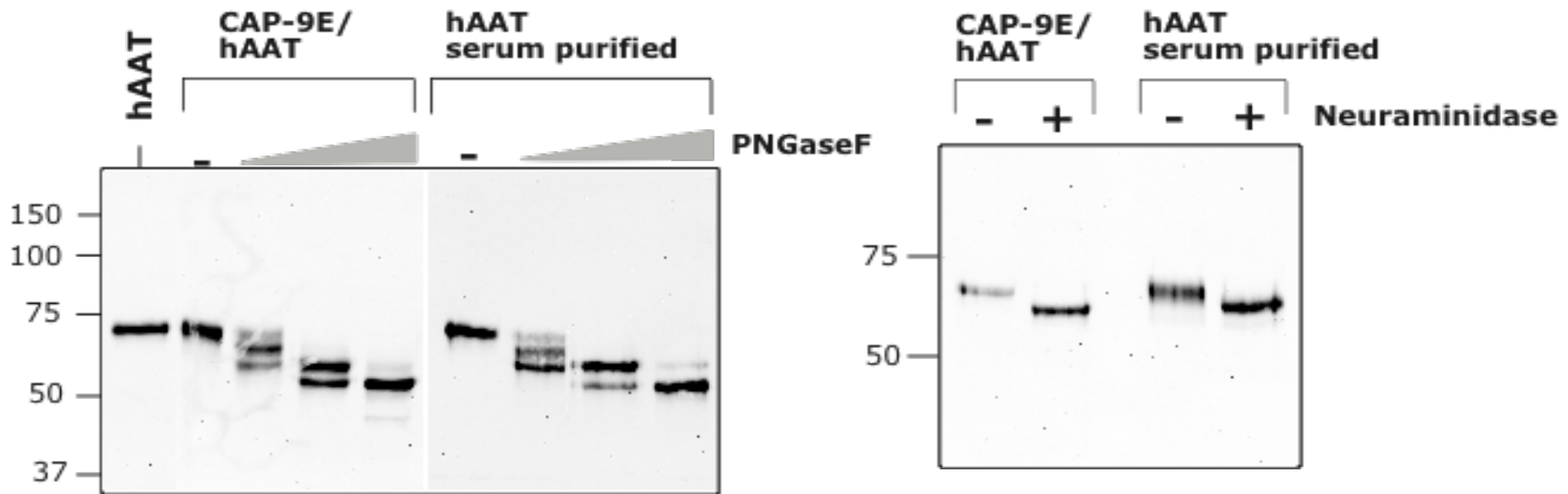
Alpha-1 Antitrypsin (hAAT)

- ⇒ highly polymorphic glycoprotein: 3 N-glycosylation sites
- ⇒ serine protease inhibitor; primary target: neutrophil elastase
- ⇒ hAAT deficiency: alveolar destruction by neutrophil elastase, emphysema,

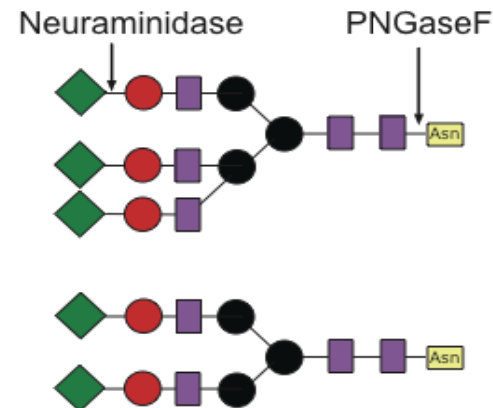


hAAT	4 mg/L	10 mg/L	70 mg/L	700 mg/L
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CAP Cells Generate Fully Human Glycosylated hAAT



Western Blot (anti-hAAT mAb) of supernatants from CAP-hAAT cells and hAAT purified from human serum, digested with increasing amounts of PNGase F or Neuraminidase, respectively



Evaluation of CAP Cells:

Growth Parameters

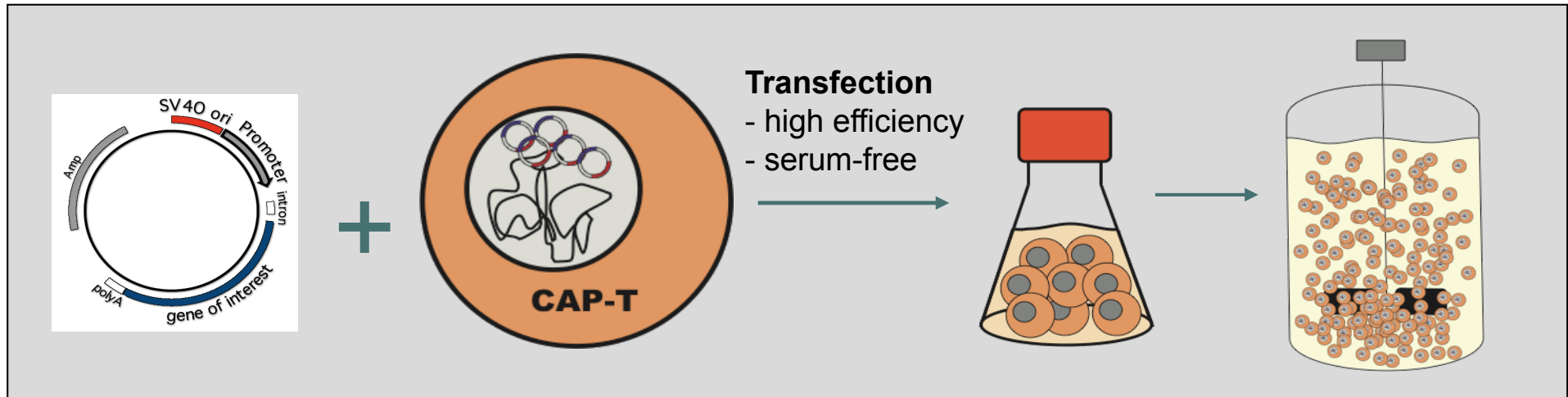
- Parental CAP cell: stable growth for > 100 passages
- Protein expressing CAP clone: stable protein expression for > 75 passages
- Growth in suspension culture :
spinner, shaker, fermenter up to 10 L,, Perfusion, WAVE
- Growth densities up to 1×10^7 cells/ml
- Splitting rate 1:20
- High transfection efficiency (>90%)
- Fast cell line development

CAP-T Cells:

human cell line for *transient* expression of proteins

CAP-T Cells:

Rapid High Yield Transient Expression Tool



Optimized plasmid:

- SV40 ori
- gene of interest

CAP-T cell line:

- human cell line
- expresses SV40 T-Antigen

Cultivation

- serum-free
- scalable

transient protein expression

- fast
- high yields
- authentic human glycosylation

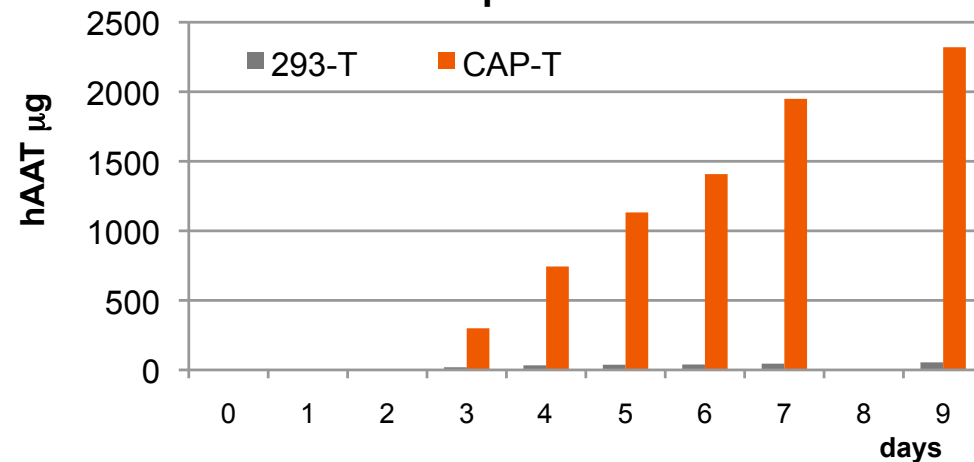
2 weeks

CAP-T and HEK293-T Cells: Transient Expression of hAAT

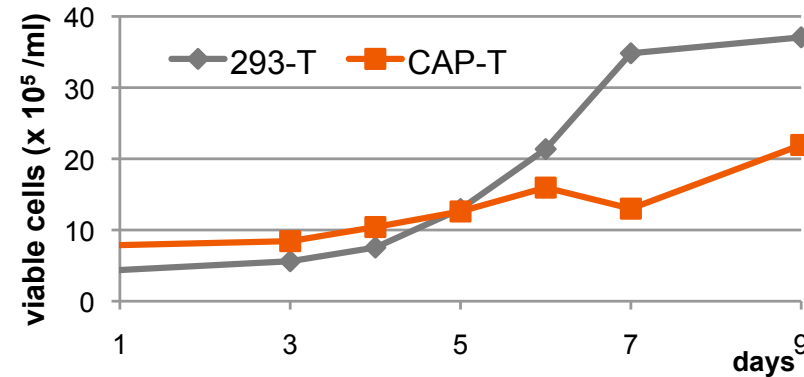
transfection: 1×10^7 cells
 volume: 40 ml
 medium: serum-free
 cultivation: shaker flask
 DNA: 5 μ g

CAP-T: 2.5 mg
 HEK293-T: 50 μ g

transient hAAT expression in CAP-T / 293-T

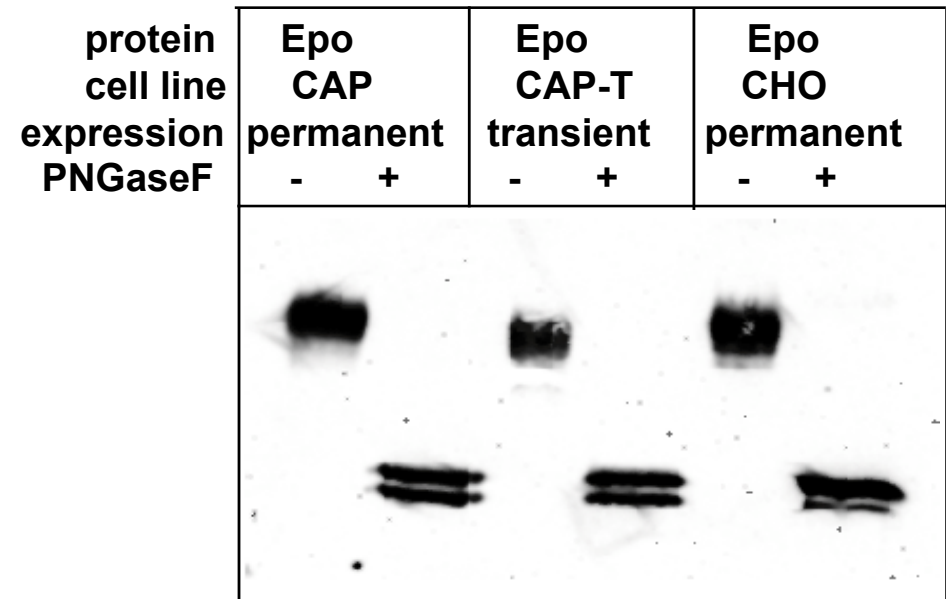
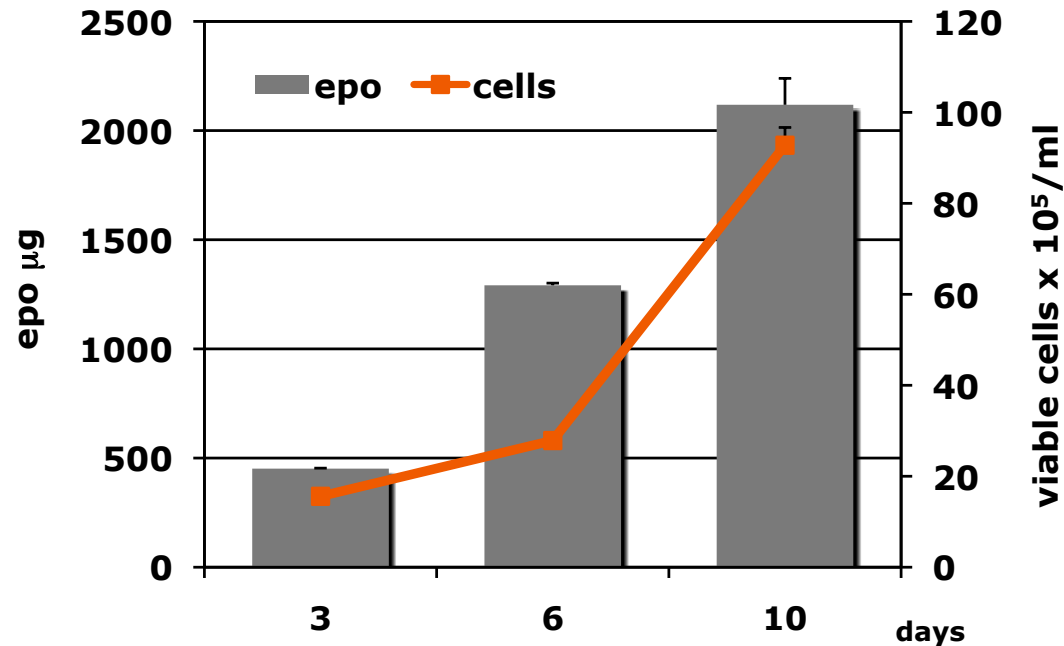


viable cell densities CAP-T / 293-T



CAP-T Cells:

Transient Expression of N-/O-Glycosylated Epo



transfection: 1x10⁷ cells
volume: 40 ml
medium: serum-free
cultivation: shaker flask
DNA: 5 µg

CAP-T Cells:

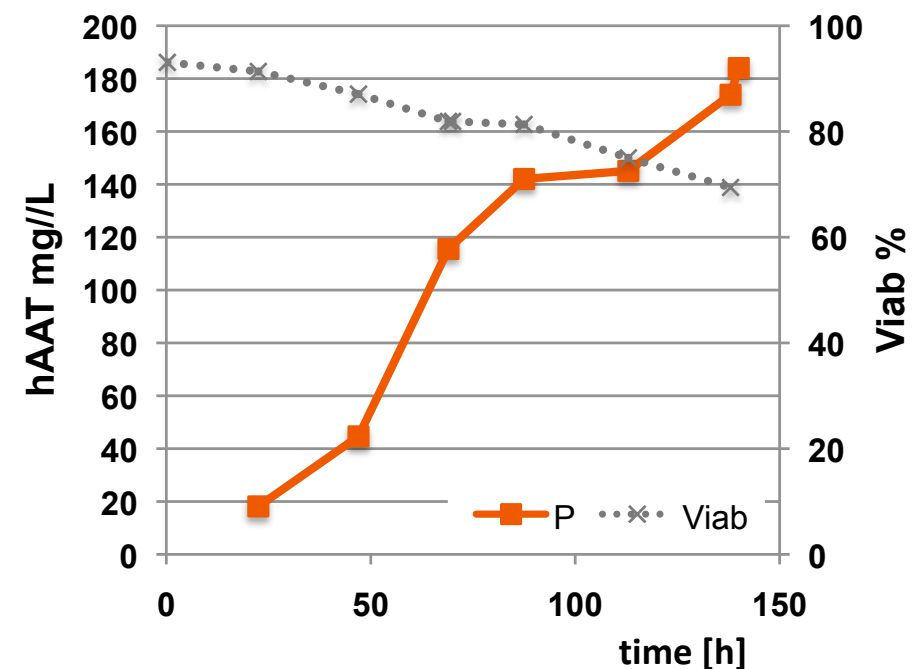
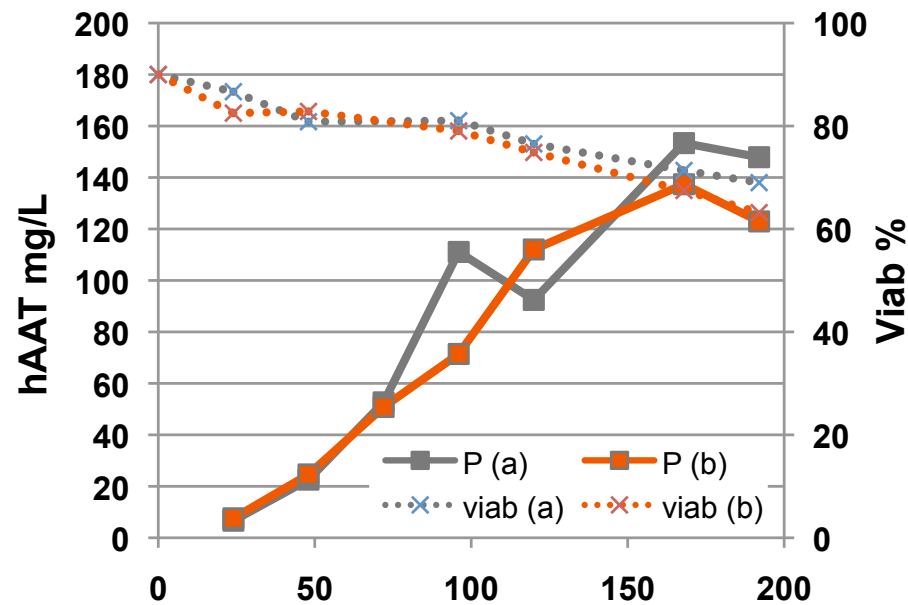
Upscaling of transient hAAT Expression

Shake flask, vol 300 ml:

transfection: 5×10^8 cells
 medium: Na-butyrate
 DNA: 500 μ g
 reagent: PEI
 time: 8 days

Bioreactor, vol 1L:

transfection: 1.7×10^9 cells
 medium: Na-butyrate
 DNA: 1.5 mg
 reagent: PEI
 time: 6 days



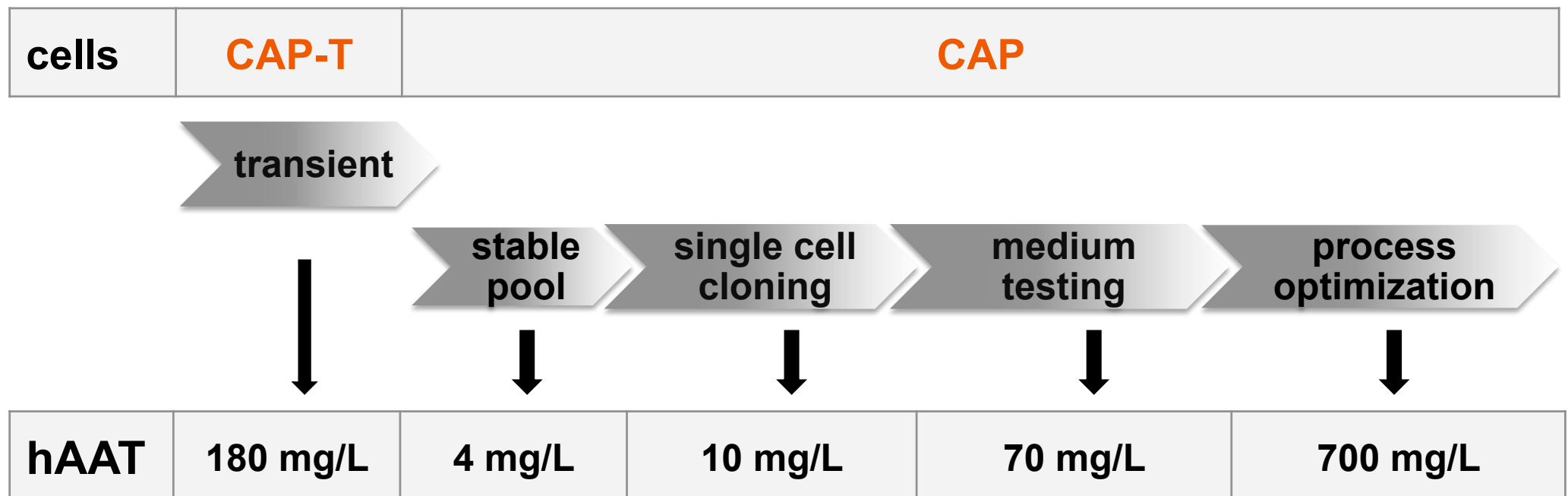
High Yield Transient Expression of Glycosylated Proteins in CAP-T - Summary

	hAAT		EPO	C1-INH
cell transfected	1×10^7	1.7×10^9	1×10^7	1×10^7
culture volume (ml)	60	1000	60	30
culture time (days)	9	6	10	12
viability at harvest (%)	85	70	80	87
total amount of protein (mg)	3.8	180	2.3	2.9
volumetric productivity (mg/L)	64	180	38	35

Evaluation of CAP Platform:

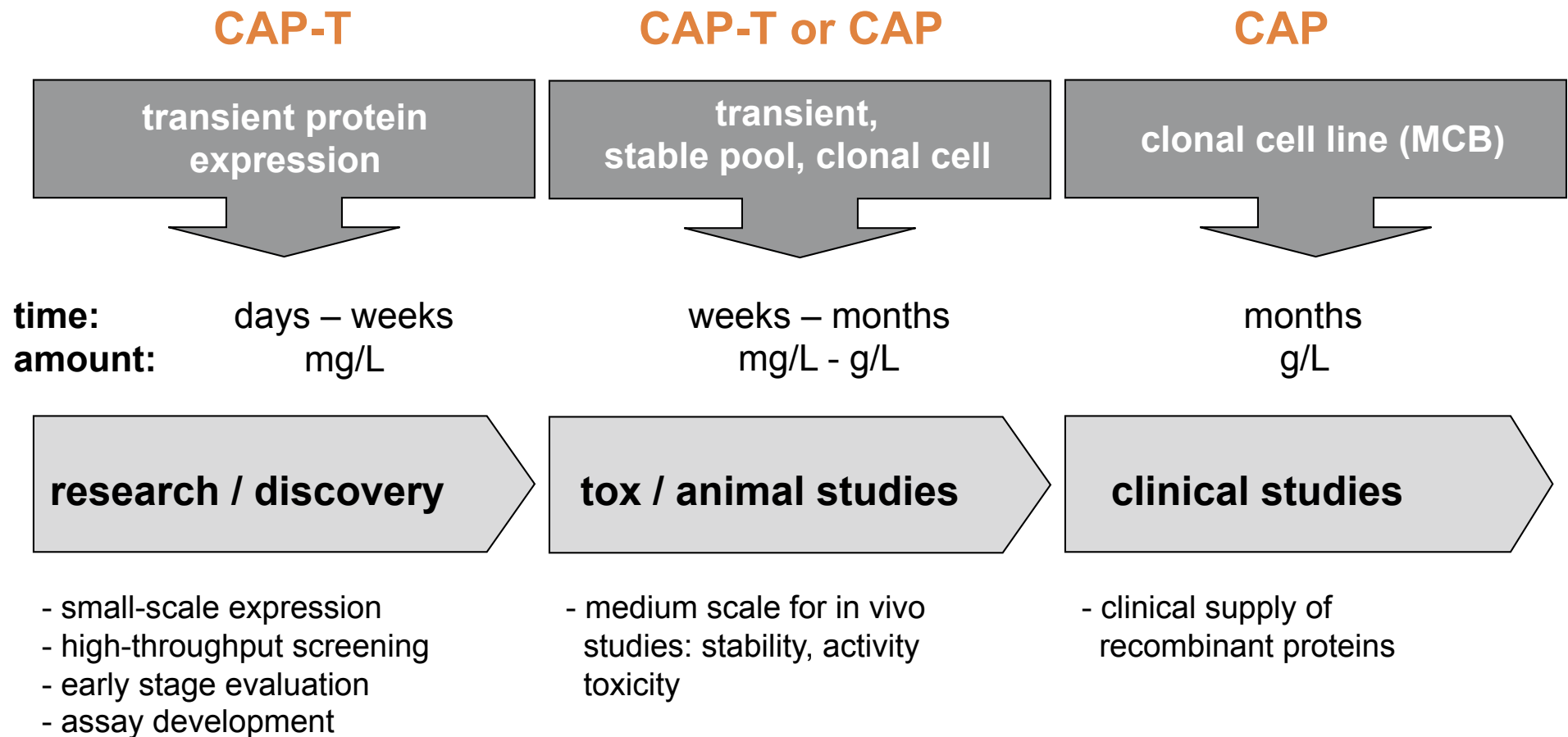
Transient Expression in CAP-T Reduces Timeline

Transient and Stable expression of hAAT



One Platform for All Your Needs:

A Continuum for Rapid and Versatile Product Development



Thank you for your attention!



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