The EB66 cell line for the industrial production of high potency antibodies and analytical methods for low-fucosylated clones screening

PEGS, Boston
May 3rd 2012
Cell substrates for vaccines production

A stem cell alternative to the embryonated chicken eggs

IDEAL CELL LINE

- Broad virus susceptibility
- Indefinite self-renewal
- Genetically stable
- Scalable
- Growth in suspension
- Chemically-defined media
- High yielding
- Cost effective

BUT BESET BY ISSUES

- Cumbersome manufacturing process
- Slow reactivity in pandemic crisis
- Exposure to risks of outbreak of bird diseases & eggs penury
- Egg-component allergies
- Quality concern
- Susceptible to contaminations
- (e.g. Shortage of Influenza vaccines in the US in 2004)
Derivation of Duck Embryonic Stem Cells

A new established cell line meeting industry and regulatory requirements

- IMMORTAL
- GENETICALLY STABLE

No genetic modifications and no viral nor chemical modifications

Fully documented from animal substrate to isolated EB66® Cells
The duck EB66® cell line

Maintenance of ES cells unique properties

ULTRASTRUCTURE SIMILAR TO EMBRYONIC STEM CELLS

- Abundant mitochondria & ribosomes
- Large nucleus & nuclear bodies
- Small size (~8-10 µm)

EXPRESSION OF « STEMNESS » GENES

- NANOG
- OCT-4
- GAPDH

EXPRESSION OF ES CELL SURFACE MARKERS

- EB66
  - SSEA-1
  - EMA-1
- CHO
  - SSEA-1
  - Control

From cells to therapeutics
The duck EB66® cell line

Maintenance of ES cells unique properties

STRONG EXPRESSION OF TELOMEREASE, INVOLVED IN MAINTENANCE OF IMMORTALITY & GENETIC STABILITY

![Graph showing absorbance (A450-A655) of different cell lines: ES Cells, Duck EB66, Chick Fibroblasts, DF1 cells, MDCK cells. The graph indicates a significantly higher absorbance for ES Cells and Duck EB66 compared to the other cell lines.]

- **KARYOTYPING**
  - **MCB** p134
  - **EPC** p154
  - **STABLE** (modal chromos. n°: 78)
  - **STABLE** (modal chromos. n°: 78)
Cell growth in stirred tank bioreactors

Cell culture characteristics; currently up to 1000L

Custom serum-free medium:
- Liquid & powder
- R&D grade & GMP grade
- Devoid of components of primary & secondary animal origin
- Cost-effective
Cell growth in stirred tank bioreactors

Cell culture characteristics; currently up to 1000L

No accumulation of lactate or ammonium, & limited consumption of glutamine

Short Population Doubling Time
(~12 hours at 39°C, ~15 hours at 37°C)
The EB66® cell line

A new standard for the production of vaccines

- 18 Commercial licenses + ~12 research licenses
- 2 Phase I clinical trials completed for flu vaccines in the USA & Japan
- A first veterinary EB66 vaccine marketed in 2013
Antibody-Dependent Cytotoxicity Activity (ADCC)

IgG with low fucose display high affinity towards FcγRIIIa receptors

LOW FUCOSE-IgG DISPLAY HIGHER AFFINITY TO FcγRIIIa RECEPTOR

ELIMINATION OF TUMOR, INFLAMMATORY OR VIRUS-INFECTED CELLS
Biomanufacturing of Antibodies with enhanced ADCC

Development of a novel cell substrate for antibody production
Cell Culture Media in fed-batch Culture

mAb production and upscaling

FEDBATCH PRODUCTION PROCESS IN SHAKE FLASK

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>IgG conc. (g/L)</th>
<th>Cell density (x10^6 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>1.3 gr/L</td>
<td>40</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>D2</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>D3</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>D4</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>D5</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>D6</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D7</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>D8</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

FEDBATCH PRODUCTION PROCESS IN 20L DISPOSABLE BIOREACTOR

<table>
<thead>
<tr>
<th>Days in Culture</th>
<th>IgG conc. (g/L)</th>
<th>Cell density (x10^6 cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>1 gr/L</td>
<td>35</td>
</tr>
<tr>
<td>D1</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>D2</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>D3</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>D4</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>D5</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D6</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>D7</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>D8</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
Glycosylation profile of EB66-IgGs

Increased percentage of G0/G1/G2 vs GOF/G1F/G2F populations (MALDI-TOF analysis)
LOW FUCOSE ANTIBODIES PRODUCED BY EB66 SHOW IMPROVED ADCC ACTIVITIES (Similar results with 5 independent antibodies)
Selection of low fucosylated mAb producers

Analytical methods

- MALDI TOF MS
- NP HPLC
- RP HPLC

mAb PURIFICATION

- CLONES
  30 to 80% a-fuc

GLYCOPROFILING

- chromium
- No radioactive assay (Calcein)

CD16a BINDING ASSAY

- CISBIO
TagLite technology

*FcγRIIIαV158 competition assay*

**PRINCIPLE**

- **FRET signal**
- **Human IgG-d2 low a-fuc**
- **Unlabeled IgGs with various a-fuc degree**
- **FCγRIIIA and γ-chain co-expression labeled with SNAP-Tb**

**HEK**

**PROTOCOL**

1. 10μL labeled cells
2. 5μL sample (antibody or Fc protein)
3. 5μL IgG-d2

Incubate 2h at RT

Read on an HTRF compatible reader
TagLite technology

*FcγRIIIaV158 competition assay - Results*

**BINDING OF ANTIBODY Fc PORTIONS TO FcγRIIIA ON CELLS CORRELATE WITH GLYCOPROFILING**
**TagLite technology**

*FcγRIIIaV158 competition assay - Results*

---

**IgG-X**

![Graph showing EC50 IgG (M) vs % a-fuc for IgG-X.](image)

- **EC50 IgG (M):**
  - 3.30x10^-8
  - 1.53x10^-8
  - 50% of non-fucosylated oligosaccharides is sufficient to have the best CD16a affinity and thereby to confer maximum ADCC activity.

- **ADCC Activity (% of specific cell lysis):**
  - IgG-X: 82% of non-fucosylated glycans
  - IgG-X: 48% of non-fucosylated glycans
  - IgG-X: 29% of non-fucosylated glycans

*Olivier et al., Mabs 2010*
Selection of low fucosylated mAb producers

Analytical methods

- MALDI TOF MS
- NP HPLC
- RP HPLC
- Chromium
- No radioactive assay (Calcein)

CD16a BINDING ASSAY

GLYCOPROFILING

mAb PURIFICATION

ADCC

Cell supernatant
TagLite technology

*FcγRIIIaV158 competition assay - perspective*

USE THE TagLite ASSAY FOR CLONE SCREENING FROM CELL SUPERNANT?
The EB66® Platform

A unique technology in Biologics manufacturing

EB66® cells display unique technical, industrial & regulatory features

- **A safe substrate.**
  - Derivation from duck ES cells with no genetic, viral or chemical modifications
  - Absence of endogenous retroviral particles
  - cGMP Master Cell Bank available & Full process documentation & traceability
  - Biological Master File (BMF) filed with the U.S. FDA.

- **Unique industrial properties.**
  - Long term genetic stability, short PDT, high cell densities (>40 million cells/mL) in suspension
  - Broad susceptibility to human and veterinary vaccines
  - mAbs produced in EB66 cells display low fucose content & enhanced ADCC activity
  - Potential platform for difficult to express therapeutic proteins

- **Substrate for the production mAbs**
  - Promising production yields: >1.2gr/L in basic fedbatch process
  - Low fucose content: 30 to 80% α-fucosylated IgG
  - Optimization of screening process to selected best candidates based on glycosylation profile and mAb productivity
  - Implementation of several analytical methods including TagLite CD16a binding assay which is a robust and easy to use tool to study binding of antibody Fc portions to FcγRIIIα
Acknowledgements

Fabrice LeGall
Fabienne Guehenneux
Goeffrey Blanc
Marine Jacoby
Majid Mehtali

From Cisbio
Delphine Jaga
Stephane Martinez
We invite you to visit our booth, #18 and CISBIO’s booth, #64