Getting Cell Line Development off the critical path in Biologics Drug Discovery

Anke Mayer-Bartschmid, Mark Trautwein, Beate Mueller-Tiemann
Cell & Protein Sciences, Global Biologics, Bayer HealthCare

Biologics Congress
February 2nd and 3rd, 2015, Berlin
Agenda

The opportunity: Global Biologics – organizational set-up

The issue: Cell Line Development in Biologics R&D

The solution: Automation to compensate increased resource demands

- Theoretical Screening Considerations
- Workflow
- Automation solution
- Data handling
## Bayer HealthCare Pharmaceuticals

### Top 10 Products

<table>
<thead>
<tr>
<th>Product</th>
<th>2012</th>
<th>2013</th>
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<tr>
<td>Kogenate™</td>
<td>1,182</td>
<td>1,202</td>
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<tr>
<td>Betaferon™ / Betaseron™</td>
<td>1,216</td>
<td>1,038</td>
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<tr>
<td>Xarelto™</td>
<td>322</td>
<td>949</td>
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<tr>
<td>YAZ™ / Yasmin™ / Yasminelle™</td>
<td>1,045</td>
<td>853</td>
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<tr>
<td>Nexavar™</td>
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<td>771</td>
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<td>Mirena™</td>
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<td>Adalat™</td>
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<td>Aspirin™ Cardio</td>
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<td>452</td>
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<td>Avalox™ / Avelox™</td>
<td>486</td>
<td>426</td>
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<tr>
<td>Glucobay™</td>
<td>408</td>
<td>423</td>
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Note: Sales in € million.
### Bayer HealthCare Pipeline by Indication

#### Phase I (20)
- **Cancer** / Anetumab ravantansine (Mesothelin-ADC)
- **Cancer** / PSMA BITE Antibody
- **Cancer** / PI3K Inhibitor
- **Cancer** / FGFR2 Antibody
- **Cancer** / Pan-FGFR Inhibitor
- **Cancer** / FGFR2-ADC
- **Cancer** / Allosteric AKT1/2 Inhibitor
- **Cancer** / PTEFb Inhibitor
- **Cancer** / MPS1 Inhibitor
- **Cancer** / C4.4a-ADC
- **Heart Failure** / Chymase Inhibitor
- **Thrombosis** / FXI antibody
- **Lung disease** / peg Adrenomedullin
- **Wet AMD** / PDGF antibody + aflibercept
- **Endometriosis** / Vilaprisan (S-PR M)
- **Endometriosis** / BAY 98-7196 Intrav. ring
- **Contraception** / Progestin IUS
- **Endometriosis** / AKR1C3 Inhibitor
- **Endometriosis** / PRLR Antagonist
- **Endometriosis** / GnRH Antagonist

#### Phase II (19)
- **SCLC** / Roniciclib (CDK Inhibitor)
- **NHL** / Copanlisib (PI3K Inhibitor)
- **Cancer** / Regorafenib*
- **Cancer** / Refametinib (MEK Inhibitor)
- **Cancer** / Radium-223 Dichloride
- **Additional Indications** / Sorafenib
- **ACS sec prevention** / Rivaroxaban
- **CHF** / Finerenone (MR Antagonist)
- **Diabet. Nephropathy** / Finerenone (MR Antagonist)
- **Cystic Fibrosis** / Riociguat (sGC Stimulator)
- **PH IIP** / Riociguat (sGC Stimulator)
- **Raynaud’s phenom.** / Riociguat (sGC Stimulator)
- **Diff. syst. Sclerosis** / Riociguat (sGC Stimulator)
- **Worsening chronic HF** / Vericiguat (sGC Stimulator)
- **Anemia** / Molidustat
- **Heart Failure** / Partial Adenosine A1 Agonist
- **Bronchiectasis** / Neutr. Elastase Inhibitor
- **wet AMD** / Regorafenib ophthalmology
- **Sympt. Uterine Fibroids** / Vilaprisan (S-PR M)

#### Phase III (18)
- **Prostate Cancer** / ODM-201
- **Adjuvant RCC** / Sorafenib
- **HCC 2nd line** / Regorafenib*
- **Adj. CRC w/ resected liver mets** / Regorafenib*
- **Comb. Study CRPC** / Radium-223 Dichloride
- **Embolic stroke of undetermined source** / Rivaroxaban
- **Peripheral artery disease** / Rivaroxaban
- **Major Adv. Car. Events Prevent.** / Rivaroxaban
- **CHF and CAD** / Rivaroxaban
- **Long-term VTE prevention** / Rivaroxaban
- **Medically ill** / Rivaroxaban
- **Insufficient PDE5 Inhibitor response** / Riociguat
- **Hemophilia** / Damoctocog alfa pegol
- **Contraception** / LCS 16
- **VV Atrophy** / Prasterone (Vaginorm)
- **Non-CF Bronchiectasis** / Cipro DPI
- **Skin and Lung Infections** / Tedizolid
- **Gram-neg. Pneumonia** / Amikacin inhale

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*Regorafenib is a Bayer compound developed solely by Bayer. In 2011, Bayer entered into an agreement with Onyx Pharmaceuticals, Inc. under which Onyx will receive a royalty on any future global net sales of regorafenib in oncology.*
Organizational Set-up at BAYER
Research AND Development within one function

Actively managing the **RtoD Interface** by dedicated organizational set-up ensures competitive timelines and costs for clinical candidate molecules

A. Application of Developability Criteria in lead optimization (biophysical properties)
   - Ensure standardization compatible to mAb platform
   - Ensure overall technical robustness of clinical candidates
   - Prepare for ease in formulation development

B. Expression titers > 2 g/l as starting point for process development
Agenda

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  - Theoretical Screening Considerations
  - Workflow
  - Automation solution
  - Data handling
Cell Line Development: on critical path in Biologics drug discovery and development

- On the critical path after selection of clinical candidate
- Contributes to development timelines!
Cell Line Development in a nutshell

Transfection

DNA + CHO-Cells

Selection and Cloning

Expansion

Stability of Cell Line

1

~8

10-20

~50

Product Quality

Productivity & Growth Rate

Suitability in Bioreactor

- Biologics Congress, 2015, Berlin • B. Mueller-Tiemann

Bayer HealthCare
Reduction of overall project timelines by parallelization in cell line development

<table>
<thead>
<tr>
<th>Yesterday: Start of Cell Line Development at handover to Development</th>
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<tr>
<td><strong>Research</strong></td>
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<tr>
<td>Start Lead Optimization</td>
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<tr>
<td><strong>Development</strong></td>
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<tr>
<td>Start Pre-Clinical Development</td>
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<td>GMP-Lot</td>
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<table>
<thead>
<tr>
<th>Today: Start of Cell Line Development for multiple Candidates in late Research Phase</th>
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<tbody>
<tr>
<td><strong>Research</strong></td>
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<tr>
<td>Start Lead Optimization</td>
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<td><strong>Development</strong></td>
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<tr>
<td>Start Pre-Clinical Development</td>
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<td>GMP-Lot</td>
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Reduction of Development Timeline

6 months
Agenda

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How to screen for clones with top fed-batch performance?

Cell Line Development in a nutshell

Need for predictive Higher Throughput fed-batch format

Transfection

DNA + CHO-Cells

Selection

Cloning

Stability of Cell Line

Suitability in Bioreactor & extended Product Quality

Product Quality

Productivity & Growth Rate in fed-batch

> 48
Screening concept: Predictivity

How to screen >1000 clones in a fed-batch predictive for the later bioreactor?

Comparison of small-scale models with different feeding strategies in parallel

- Deep well plates
- System “Duetz”
- Shake flasks
- TubeSpins®
- ambr™
Fed-batch scale comparison:
one clone, four different feedings, four incubation devices
From dozens to hundreds
Early screening of clones in fed-batch

http://www.tpp.ch

http://www.enzyscreen.com
How many clones to screen?

If it is technologically feasible to screen hundreds of clones –

- What is a reasonable number of clones to screen?
- At which point do we reach saturation?
- How can we dedicate resources in an effective & efficient way?

The answer will also depend on what you aim for –

- One TOP clone is not enough in our view
- A variety of TOP clones is desirable due to
  - Variations in product quality
  - Stability issues
  - Bioreactor suitability & growth profile
  - Metabolic profile
Theoretical Screening Considerations

**System A**
- mAb X frequency at 2 g/l: 5%
- mAb Y frequency at 2 g/l: 1%
- mAb X frequency at 3 g/l: 1%

**System B**
- mAb X frequency at 2 g/l: 1%

**Probability of Success** will depend on:
- Expression System used, comprising:
  - Expression Vector
  - Selection Pressure
  - Host Cell Line
  - Medium & Process
- Aim
- Molecule to be expressed
Theoretical Screening Considerations

How many clones to be screened to find 8 clones for the bioreactor evaluation?

Bernoulli Experiment

\[ P(X \geq a) = \sum_{k=a}^{n} \binom{n}{k} \cdot p^k \cdot (1-p)^{n-k} \]

http://www.brinkmann-du.de/mathe/rbtest/1sonstiges/zufall/binomialvert_01.htm

How many random clones to analyze?

- Probability of obtaining at least 8 clones from TOP 5%

http://www.brinkmann-du.de/mathe/rbtest/1sonstiges/zufall/binomialvert_01.htm

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Theoretical Screening Considerations

Fan et al., Biotechnol Bioeng. 2012

~ 2%
~ 11%

<table>
<thead>
<tr>
<th>Parental</th>
<th>Molecule</th>
<th>Bulk MSX</th>
<th>SCC MSX</th>
<th>Frequency for clones ≥ 2 g/L</th>
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<tbody>
<tr>
<td>CHOK1SV</td>
<td>mAb-B</td>
<td>50</td>
<td>50</td>
<td>6/320</td>
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<tr>
<td>GS-KO</td>
<td>mAb-B</td>
<td>25</td>
<td>50</td>
<td>9/80</td>
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</table>
Theoretical Screening Considerations
Raising the odds?

<table>
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<tr>
<th>Test screening</th>
<th>Molecule</th>
<th>Frequency of clones ≥ 2 g/l</th>
<th>Highest Titer in Shake Flask</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most advanced BAYER expression system</td>
<td>mAb X</td>
<td>3 / 96</td>
<td>4.5 g/l</td>
</tr>
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</table>

⇒ Check at least 400 clones in a fed-batch screen!

Rejection of low performers by terminal batch

Screening strategy for the Bayer CHO EXPRESSion System

- Use a batch screening to eliminate low performers
- Screening of 384 clones in fed-batch should yield ~ 5-8 clones with > 2 g/l
- Enough to carefully analyze these to select the final clone with respect to product quality, stability, bioreactor suitability and metabolic profile

Standard mAb molecules without issues

Difficult mAb or other protein format

- Use a batch screening to eliminate low performers
- The fed-batch has to be increased to max 1,000

⇒ Anything between 384 and 1,000 seems reasonable to be checked in a predictive fed-batch
Cell Line Development in a nutshell

Transfection

DNA

CHO-Cells

Selection

Cloning

Stability of Cell Line

Suitability in Bioreactor & extended Product Quality

Product Quality

Productivity & Growth Rate in fed-batch

1

> 48

> 384
Workflow envisioned

Transfection → Plating → Copy #3 → Passaging → Freeze (backup)

Copy #1

Copy #2

Terminal batch

Rejection of low performers (50% of clones)

Fed-batch screen

Clone Picking ~ 800

DWP fed-batch

Expansion & Cryopreservation

TubeSpin in depth Fed-batch Characterization

384

TOP48

TOP48
Real test case: mAb Y in manual workflow

Devised workflow yields five clones exceeding 2 g/l, three are close

⇒ Excellent starting point for bioreactor characterization
Screening cascade for future cell line development projects

**Project A**
- 1,000 clones in DWP fed-batch
- 2,000 clones in terminal batch
- 50x384 MTP seeded

**Project B**
- 6 x 384 clones in DWP fed-batch
- 6 x 800 clones in terminal batch
- 6 x 20x384 MTP seeded

- One candidate
- Six candidates in parallel

➤ Need for automation solution
Cell Line Development Station from Beckman-Coulter (3D simulation)

- Management of up to 6 candidates in parallel
- Flexible scheduling and staggered workflows
- A well-based hit picking process
- Start DWP fed-batch process with defined cell number
- Full traceability for each clone with data transfer to our in-house Biologics database
Automation Software – Biologics Data Platform

BDP => Bayer’s Large Molecule Workflow Management Platform
- Therapeutic mAbs, incl. ADCs & next-gen. Abs
- non-mAb therapeutic proteins
- Protein reagents & tools for HTS and structural biology

- The core platform for management of molecules (BMOLs, tools)
- Registration interface to Bayer’s overall compound registration system
- The clone and sample (batch) tracking tool
- The central DB for key data from screening and automation projects in GBR
- The data repository for key assay and analytics data

Based on Page 27 • Biologics Congress, 2015, Berlin • B. Mueller-Tiemann
Biologics Data Platform (BDP) – Bayer’s Integrated Biopharma R&D Workflow Platform

Full molecule & sample life cycle traceability throughout the whole process

- Antigen Registration
- Panning, Immunization
- Plate Management
- Assay Results
- Sequencing Run
- Redundancy Analysis
- Hit Selection
- Protein engineering

- Target Protein
- Vectors
- Cell Lines
- Protein Expression
- Protein Purification
- Analytics, Assay Data
- Sample Management
- Final Candidate

Based on Genedata Biologics™

Supporting cell line development campaigns

Automated Cell Line Development
Microbioreactor Evaluation
Bioreactor Evaluation
Production Cell Line

Bayer HealthCare
BDP: Guiding the automated CLD process

- **Complete traceability of clones**
  - From host cell line passaging, transfection, screening, subcloning to final clone
  - Automated & manual processes with flexibility for switching between these modes during the CLD process

- **Decision-making**
  - Evaluation of monoclonality (CSI)
  - Confluence, passaging decisions (CSI)
  - Hit selection based on titer (Octet), confluence (CSI), or both

- **Instruction of automation platform**
  - Creation of work lists (i.e. picking list)

- **Cell banking**

**Culture Processing Reports:**
- Biologics Congress, 2015, Berlin
- B. Mueller-Tiemann

**CLD Workflow:**
- Seeding & selection
- Incubation
- Passaging
- Analyzing
- Cryo-conservation

**Automation System**
## Hit Selection for Cell Line Development Campaign CLD-5

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<tr>
<th>Cell Line Clone</th>
<th>26.03.2014</th>
<th>29.03.2014</th>
<th>31.03.2014</th>
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**Hit Selection table including imaging data (CSI)**
Implementation of Cell Line Development Automation at Bayer
Cell Line Development in a nutshell

Transfection

DNA + CHO-Cells

Selection

Up to 6 candidates

Cloning

Stability of Cell Line

Productivity & Growth Rate in fed-batch

Suitability in Bioreactor & extended Product Quality

> 48

> 384 < 1,000

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Summary

- Start of cell line development in late research phase allows overall timeline reduction by at least 6 months
- This results in an amplification of cell line development efforts
- An efficient & effective workflow has been designed which is amenable to automation
- Automation and Data management system critical for success
- Compact automation solution allows parallel management of up to 6 candidates in early stages of cell line development
An R-to-D Interface to Enable Rapid and Cost-effective CMC Development

Smooth hand-over from Research to Development

R-to-D Interface Leadership Team

Cross-functional leadership team of GB Research and Development to integrate expertise and manage resources globally and flexibly across projects

Team objectives

- Decreased cost and time from start preclinical development to GMP
- Increased cell line productivity
- Increased PoS by prioritizing drug-like properties in Lead Optimization

CHO cell line productivity achieved at development candidate nomination – g/l

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<thead>
<tr>
<th>Year</th>
<th>mAb a (System A)</th>
<th>mAb B (System B)</th>
<th>mAb c</th>
<th>mAb d</th>
<th>mAb e</th>
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<th>mAb g</th>
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Timelines (relative):

start preclinical development -GMP DP

Bayer HealthCare
## Acknowledgements

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<th>Bayer, Global Biologics Development, Wuppertal</th>
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<td>Mark Trautwein</td>
<td>Tobias Thuete</td>
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<tr>
<td>Claudia Goetzberger-Schad</td>
<td>Jennifer Dietrich</td>
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<tr>
<td>Alexander Korseska</td>
<td>Uwe Langer &amp; Teams</td>
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<td>Michael Strerath &amp; Teams</td>
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<th>Bayer, Global Biologics Development, Berkeley</th>
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<th>Beckmann Coulter</th>
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<td>John Thrift</td>
<td>Christoph Freiberg</td>
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<td>Roy Kimura</td>
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Thank you!

Science For A Better Life
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