Monoclonal antibodies and antibody fragments process development:

Laying the foundations of a manufacturing process at an early stage

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Summary of presentation

1. Objectives and challenges of early phase process development

2. Monoclonal antibodies & antibody fragment: product quality & regulatory focus

3. Reducing the process development critical path while building the foundations for a manufacturing process
Objectives and challenges of early phase process development
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- Cost containment is key
- Candidate selection following FIH studies
- Having product to test is a necessary means to an end
- Time to start pre-clinical activities critical
  - GMP manufacturing happens in parallel to these activities
- Process development under pressure
- Challenge to avoid fundamental process design flaws
Process development: cost of getting it wrong

Cost of redesigning unit operations that impact product quality:
R&D, comparability studies, re-validation, time to market, post approval changes

Importance of trying to get it right first time!!
Objectives of early phase processes: the foundations

- Adequate product yield and quality in the shortest possible development time
- Early understanding of impact of the processes on the product (ICHQ8)
- Expectation that product quality can remain “comparable” through drug development
- GMP compliant and scaleable manufacture e.g.
  - Pre-clinical & Phase I-II: 100L - 1000L scale fermentation
  - Phase III and beyond: > 1000L
- Early integration of “cost of goods” [e.g. PEGylation, resin]
Monoclonal antibodies & antibody fragment manufacture: product quality & regulatory focus
Monoclonal and antibody fragment therapeutics

Monoclonal antibodies come of age

- 1990 – 2008 (Janice Reichert; Tufts university; 2009)
  - 440 candidates
  - 240 clinical studies
- 22 approved in US and other countries as of 2009
- Big 5: Avastin®, Herceptin®, Rituxan®, Remicade®, Humira®
  - Mammalian cell derived

Antibody fragments

- 3 FDA / EMEA approved Fab in 2009
  - ReoPro® [enzymatic digestion of full length IgG]
  - Lucentis® [E. coli]
  - Cimzia® [E. coli]
Human immunoglobulin gamma (IgG): structure overview

“Traditional “ mAbs are successful drugs:

- IgG class
- Humanized or fully human
- High specificity
- Flexibility of mode of action
- Bivalent
- Long serum persistence
- Mostly mammalian cell derived
- Volumetric yields > 5g/L
- Glycoengineered yeast (*P. pastoris*) i.e. Glycofi

MW ~ 150KDa
Monoclonal IgG DS manufacture: general process overview

All steps have the potential to impact product quality and purity:

- Process related impurities
- Product-related impurities
- Product-related substances
Monoclonal IgG DS: impurities and product-related variants

- **Process related impurities**
  - HCP, residual protein A
  - Residual DNA
  - Endotoxin, bioburden, adventitious agents

- **Product-related impurities**
  - Degradation / truncation products
  - Aggregates

- **Product-related substances**
  - Glycosylation variants
  - Disulphide isoforms
  - Charge (acidic) variants: deamidation, pyroglutamate, sialylated species
  - Oxidation
  - C-terminal lysine variants
Monoclonal IgG DS: post translational modifications

- Conversion of N-term glutamine to pyroglutamate
- C-terminal lysine removal by carboxypeptidase
- Fragmentation (proteolysis)
- Asparagine deamidation
- Methionine & Cysteine oxidation
- Disulphide isoform / shuffling
- Glycation (e.g. lysine): non-enzymatic
- Glycosylation
  - High Mannose
  - Sialylation
  - Variation of site occupancy
- 9600^2 ~ 10^8 combinations (S. Kozlowski)
Antibody fragments and domains: structural designs

V_{HH} domain (~15 kDa)
V-NAR domain (~15 kDa)
V_{L} domain (~15 kDa)

Fab (~55 kDa)

Fab_{2} (bispecific) (~110 kDa)

Fab_{3} (trispecific) (~165 kDa)

Fv (~25 kDa)

Bis-scFv (bispecific) (~55 kDa)

Triabody (trivalent) (~75 kDa)

Diabody (bispecific) (~50 kDa)

Minibody (bivalent) (~75 kDa)

Tetraabody (tetravalent) (~100 kDa)

P. Holliger et al.; Nature Biotechnology, 2005
Antibody fragments and domains: key advantages

Key advantages of Fab and domains
- Small size
  - Target access, tissue penetration, improved bio distribution
- Microbial and yeast derived i.e. no viral clearance requirements
- Fab / domains produced in *E. coli* or *P. pastoris* are aglycosylated
- Absence of Fc: potential safety advantage
- Easier conjugation: PEG, toxins

Expression strategies
- Periplasmic secretion of Fab with *E. coli*
- Few inclusions bodies / refold strategies from bacteria
- Exclusively extra cellular secretion with *P. pastoris*
- Guided by complexity of disulphide pairing & glycosylation
- Volumetric yield > 1-2 g/L
- Improved cell line generation & time to FIH
Antibody fragments and domains: quality attributes

Key differences
- Pronounced impact of CDR sequence
- Greater variability in size, pI, hydrophobicity
- Less generic, more bespoke processes
- Gluconoylation in *E. coli* (+178 / 256Da species)
- Process specific residuals e.g. ITPG, antibiotics

High degree of commonality of other impurities and product-related variants
- Deamidation, oxidation, pyroglutamate, disulphide isoforms, truncation, aggregation
- Residuals: DNA, HCP, endotoxin, bioburden
Regulatory challenges for process development: emerging needs

- “Process is product” paradigm
- ICH Q8: Pharmaceutical Development
- ICH Q9: Quality risk management
- Process Analytical Technologies (PAT)
- Quality by Design (QbD)
- Design Space

Product and process performance characteristics should be scientifically designed to meet specific objectives and not empirically derived from the performance of test batches.
Reducing the process development critical path while building the foundations for a manufacturing process
→ Choose the right product
→ Standardise processes
→ Integrate a multidisciplinary approach & reduce experimental cycle time
→ Make pre-clinical material earlier
Choose the Right Product!

- Design the final molecule to make manufacture easier
  - Avoid sequences known to create heterogeneity problems
  - Opportunity for rational protein engineering
  - Quicker simpler processes
  - Higher yields
  - Reduced cost of manufacture

- Antibody fragments / domains in *E. coli* or *P. pastoris*
  - Replace AsnGly sequences to prevent deamidation
  - Remove glycosylation sites
  - Use *Pichia* leader that doesn’t require Ste13
  - Design N+1 residue for Met removal / non-removal

- Warning…
  - Try not to retrofit – e.g. PEGylation
  - Will likely give better yielding and more robust process
  - BUT….time saving difficult to quantify
Standardize Processes

- When the same process can be used for all products it does save development time!
- .. and brings consistency to product quality attributes
- Confidence in regulatory aspects at early stage e.g. viral clearance / inactivation steps
- Better & earlier mechanistic understanding of impact on product quality
- Preliminary design space prior to process characterization
- More accurate and earlier CoG estimation
Fast track upstream process development

- Standardization of elements in pAVEway™ system leads to rapid development of high titer fermentations in *E. coli*
  - Vectors, strains, fermentation conditions
- Modulation of expression based on level of inducer added
  - Maximise secretion of soluble biologically active protein in the periplasm
  - Manipulate partitioning of secreted proteins: periplasm vs. growth medium
- Typical development time for first evaluation ~ 6 weeks

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<th>Protein</th>
<th>Cellular location</th>
<th>Host</th>
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<td>Fab fragments (various)</td>
<td>Secretion/soluble</td>
<td><em>E. coli</em></td>
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<td>Antibody domain: Avecia pAVEway™ ‘platform’ process</td>
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<td>Antibody domain: Pichia ‘platform’ process</td>
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<td><em>E. coli</em></td>
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<td>Antibody domain: <em>Pichia</em> ‘platform’ process</td>
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<td><em>P. pastoris</em></td>
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Antibody Fragment Purification – Alternative Schemes

- Variety of non-mAbs require bespoke processes
- But … despite all molecules being different, most processes are remarkably similar
- Some parts of processes can be standardized and integrated into “platforms”
  - Purification for some groups of molecules e.g. Fab, ScFv, domains

![Diagram of purification processes](image)
Purification schemes

- Small Molecule Affinity Ligand + CIEX
- Specific for ‘FAB’ structures
- Selective FAB removal from \textit{E coli} HCP in single step
- DBC > 20g/L
- Flexible
- Scaleable
- NaOH CIP
- Potential to reduce/streamline DSP

\textit{Purification of antibody fragment obtained in single step by Avecia affinity approach.}
Integration of a multidisciplinary approach

- Upstream process sciences
- Downstream process sciences
- Manufacturing sciences:
  - Consideration of manufacturing environment: process control, large scale equipment limitations & specification, operational practices
- Analytical sciences:
  - Not just DS / DP analysis & quality control
  - Essential role in process creation, understanding, monitoring and implementation of “quality by design”
- Knowledge management and experimental design
  - Higher throughput: gain maximum information from minimum experiments
  - Improved process understanding and clarity

Process development is a multidisciplinary activity which requires the parallel integration of all relevant scientific fields
Reduce Experimental Cycle Time

- Resin Screening
  - Can be minaturized
  - Adapted to high throughput mode
  - Sample requirements reduced
  - Small and very small systems becoming more readily available from resin manufacturers

- UPLC v HPLC
  - From 40mn per cycle to less than 20mn
  - RP & IEX resins
  - Peptide mapping: deamidation, oxidation, glycation etc..
  - Decrease cycle time for iterative development

- Chromo Cycle
  - SDS-PAGE
Make Pre-Clinical Product Earlier

- Material for pre-clinical work to support IMPD is the critical path
- Material needs to be representative of clinical product but not cGMP
- Often supplied from initial Engineering run in GMP plant
  - Requires scale up work completed
  - Equipment qualification activities
  - cGMP facilities require batch records, raw material assurance etc to protect facility and other products
- Avecia supply from pre-GMP demonstration run within R&D
  - Duplicate plant equipment
  - Flexible and adaptable to last minute changes
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Layer 1. Choose the right product
Layer 2. Standardize development activities
Layer 3. Multidisciplinary approach & high throughput
Layer 4. Provide non-GMP material early
Multiple approaches can be successfully used to reduce process development critical path

- **BUT ..... It is still the critical path**

- Successful antibody fragment process design requires integration of elements
  - Experience to understand causes and types of heterogeneity
  - Multidisciplinary teams
  - Investment in platform technologies
  - Willingness during development to try a new way

- Future targets is to develop antibody fragment / domain bespoke processes as fast as applying generic processes
A world leader in the development and manufacture of biopharmaceuticals