Biotechnology: Regulatory Requirements on Continuous Manufacturing for (glycosylated) monoclonal Antibodies

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List of Abbreviations

AAPS	American Association of Pharmaceutical Scientists
ADCC	Antibody dependent cell cytotoxicity
AEX	anion exchange chromatography
Asn	Asparagine
BLA	Biologic License Application
BWP	Biologics Working Party
CEX	cation exchange chromatography
CFR	Code of Federal Regulations
CHMP	Committee for medicinal products for human use
CMC	Chemistry, Manufacturing, and Controls
CoA	Certificate of Analysis
CQA	Critical quality attribute
CVMP	Committee for medicinal products for veterinary use
DF	Diafiltration
DSP	Downstream Processing
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EC	European Commission
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
Ig	Immunoglobulin
IgG	Immunoglobulin G
IMPD	Investigational Medicinal Product Dossier
kDa	Kilo Dalton
LOQ	Limit of quantification
MAA	Marketing authorisation application
mAB	Monoclonal antibody
MAH	Marketing authorisation holder
MCB	Master Cell Bank
N	Asparagine
n.a.	Not applicable
NL	Netherlands
PAT	Process Analytical Technologies
Ph. Eur.	European Pharmacopeia

pI	Isoelectric point
PTM	Post-translational modification
QbD	Quality by Design
QTPP	Quality target product profile
RBP	Reference Biotherapeutic Product
RMP	Reference Medicinal Product
RTRT	Real-Time-Release Testing
TFF	Tangential Flow Filtration
TNF	Tumor necrose factor
TSE	Transmissible Spongiform Encephalopathy
UF	Ultrafiltration
US (USA)	United States (of America)
USP	Upstream processing
VEGF-A	Vascular endothelial growth factor A
WCB	Working Cell Bank

1 Introduction

To meet today's and future patient needs pharmaceutical manufacturing must be optimized in order to avoid disruption of drug supply. Drug shortages are a serious challenge for health care systems and patients. They can have various reasons e.g. quality or good manufacturing practice (GMP)-compliance issues, active pharmaceutical ingredient (API) shortages, delays in manufacturing or increase in market demand. To get their products to the market fast enough and to avoid drug shortages pharmaceutical companies must be efficient and flexible. Therefore the goal clearly is not only to avoid quality issues causing drug shortages or drug recalls, but also to be flexible to react fast to any required drug amount changes.

Another goal of the pharmaceutical industry is to increase the productivity and at the same time decrease the costs of the production process without negative effects on product quality or safety and efficacy. In order to meet this objective pharmaceutical manufacturing is modernising. In the last decade continuous manufacturing was further developed and is now an efficient solution for biologics manufacturing.

Beside the fact that the pharmaceutical industry is interested in the advantages of continuous production also national competent authorities promote the development of continuous manufacturing.

2 Aim and structure of the master thesis

The aim of this master thesis is to provide information on the regulatory requirements of different manufacturing procedures for monoclonal antibodies and to examine the advantages and disadvantages of these production forms. The focus is on the pros and cons of batch versus continuous upstream combined with adapted downstream manufacturing procedures for biotechnological drug products in general and in particular for (glycosylated) monoclonal antibodies. Not within the scope of this thesis is the continuous manufacturing of small or chemical molecules.

Glycosylation is the most common and complex post-translational protein modification form. Glycans are critical for different protein functions, e.g. folding, stability, localisation and protein interactions, and promote herewith not only the efficacy of biopharmaceuticals, but also influence drug safety. Glycosylated monoclonal antibodies are used in different therapeutic fields for often serious or life threatening diseases, like cancer or autoimmune disease therapies.

Different manufacturing approaches of pharmaceutical companies will be compared and discussed. Pharmaceutical manufacturing companies can use this thesis as an overview and strategic decision guidance.

In **section 3** I will give an introduction on **(glycosylated) monoclonal antibodies (mABs)**, the therapeutic fields they are used and the standard manufacturing process for mABs.

The following **section 4** focuses on **continuous manufacturing**, how it differs to other manufacturing processes and why there is a need for manufacturing process optimization.

Section 5 discusses the **regulatory basis for continuous manufacturing**. This section compares the different positions of ICH, EMA and FDA on batch/lot definition and batch traceability for a continuous process and the special requirements that applicants for a market authorisation must consider for continuous manufacturing.

Afterwards, **section 6** will discuss the potential possibilities for a **switchover** from batch to continuous manufacturing process. I will compare the ICH, EMA and FDA guidance on manufacturing changes. These changes will be further described using Humira™ and Remicade™ as examples. Two theoretical examples will discuss the best time point to implement continuous manufacturing.

The **Discussion and outlook** section will discuss the possibilities of a continuous mAB manufacturing process and introduce some international initiatives which promote continuous manufacturing.

3 (Glycosylated) monoclonal antibodies

3.1 Why are monoclonal antibodies of interest?

"Monoclonal antibodies (mABs) are immunoglobulins (Ig) with a defined specificity derived from a monoclonal cell line"[1] and are today among the most important pharmaceuticals in various therapeutic fields. But the path to this point was, especially in the first years, quite rocky. Soon after the first mABs were described in 1975, scientists recognized the possibilities that the mAB-specific antigen binding could bring as therapeutic drug. However the first clinical trials with murine mABs were disappointing, due to the fact that the administration of these mABs to humans was limited, and the human immune system reacted against the administered mAB[2]. Therefore the murine mAB was not able "to interact with the human immune in a manner that led to immune destruction of the cancer"[2].

The success story of mABs as therapeutic drugs began with the "development of techniques that allowed for genetic modification of murine mAB to produce chimeric mouse – human, or humanized mAB that behave in most ways like naturally occurring human IgG"[2].

In 1986 the first therapeutic mAB, OKT3, was approved in the United States (US). The first mAB product that is still on the market was approved in the US in 1994. Since then the US Food and Drug Administration (FDA) approved altogether 68 therapeutic monoclonal antibodies till January 2017. In Europe the first therapeutic mABs were approved in 1996. Figure 1 shows a database analysis on the number of therapeutic mAB drugs approved in the US (FDA) in comparison to Europe (EMA).

As Figure 1 shows, the approval of mABs continuously increases reaching a maximum in 2015 with 10 approved mABs in the Europe and the US market.

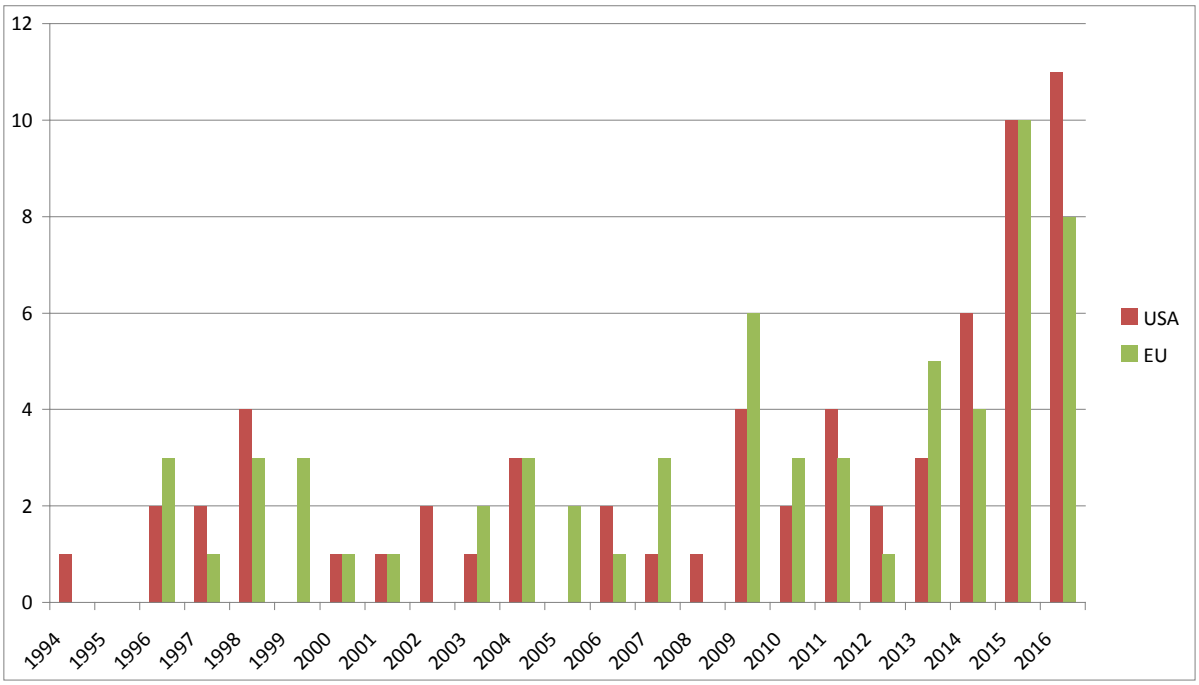


Figure 1: Approved therapeutic mAB drugs still on the US and EU market – A database analysis (Data from Drugs@FDA: FDA Approved Drug Products download date March 8, 2017; Data from EMA download date March 12, 2017).

Monoclonal antibodies are biological drugs, which are defined as medicinal products that contain a biological substance[3].

After market approval the manufacturing process of biologicals often changes. There are several reasons leading to those changes, e.g. when the market demand increases or the mAB gets an approval for a new indication. In these cases the initial manufacturing site can for example be not sufficient for the market demand or the process needs to be transferred to another or an additional manufacturing site. Another possible change in these cases could be the change of the production scale. The scale can be increased in a process called scale-up. In the life cycle of a pharmaceutical drug the manufacturing process can also be further modified to increase process robustness, change raw material suppliers or to introduce new technologies. For each of these changes it must be shown that product quality, safety, efficacy and biological activity is comparable to the product produced with the previous / approved manufacturing process.

The development of a manufacturing process for a biological product always presents unique challenges. Due to their specific characteristics this also applies for mABs. The following chapter will examine these specific mAB characteristics in more detail.

3.2 What are (glycosylated) monoclonal antibodies (mAB)?

Antibodies are the biggest group of approved glycosylated therapeutic products. As source for therapeutic monoclonal antibodies different cell types can be used, e.g. mammalian, plant or bacterial cells. The name convention for mABs is based on the "General policies for monoclonal antibodies" by the World Health Organization[4]. "The common stem for mAbs is -mab, placed as a suffix" that is used for "all products containing an immunoglobulin variable domain which binds to a defined target"[4]. The ending of the mAB contains further a substem A to define the target and a substem B to define the species (**Table 1**).

The WHO defines the chimeric and humanized antibodies as follows[4]:

- "A **chimeric antibody** is one that contains contiguous foreign-derived amino acids comprising the entire variable domain of both heavy and light chains linked to heavy and light constant regions of human origin."
- "A **humanized antibody** has segments of foreign-derived amino acids interspersed among variable domain segments of human-derived amino acid residues and the humanized variable heavy and variable light domains are linked to heavy and light constant regions of human origin."

Table 1: mAB nomenclature (after WHO 2009 "General policies for monoclonal antibodies"[4])

Prefix	Substem A indicates the target		Substem B indicates the species		Suffix
	Substem A	**Target**	**Substem B**	**Species**	
random ***Name-***	***-b(a)-***	bacterial	***-a***	rat	***-mab***
	-c(i)-	cardiovascular	***-axo*** *(pre-sub-stem)*	rat/mouse	
	-f(u)-	fungal	***-e***	hamster	
	-k(i)-	interleukin	***-i***	primate	
	-l(i)-	immunomodu-lating	***-o***	mouse	
	-n(e)- *(under discussion*	neural	***-u***	human	
	-s(o)-	bone	***-xi***	chimeric	
	-tox(a)	toxin	***-xizu-*** *(under discussion)*	chimeric/ humanized	
	-t(u)	tumour	***-zu***	humanized	
	-v(i)-	viral			

Monoclonal antibodies (mABs) are large (approximately 3000 amino acids, 150 kDa) and highly complex but relatively stable proteins. MABs can recognize and bind specifically to other proteins. They consist, like all antibodies, of four polypeptide chains – two heavy (each 50 kDa) and two light chains (each 25 kDa) (**Figure 2**). These chains are connected in a V-like structure by disulfide bridges. Each heavy and light chain contains a variable light (V_L) and a variable heavy (V_H) domain. The other domains are called the constant light (C_L) and constant heavy (C_H1–3) domains.

Human monoclonal antibodies can be divided into five classes depending on their structure: IgG, IgM, IgA, IgE and IgD. Most therapeutic mABs on the market belong to the IgG1 subclass. **Figure 2** shows the structure of an IgG mAB.

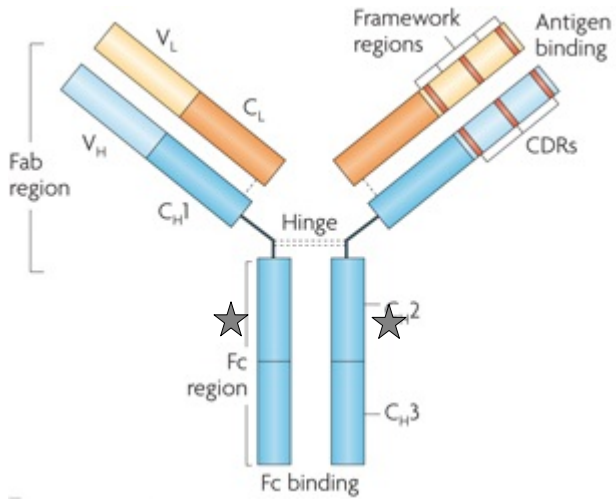


Figure 2: Structure of an Immunoglobulin G (IgG) mAB (from Hansel et al, 2010[5])

In the context of therapeutic drug manufacturing protein glycosylation is "described as the most complex form of all post-translational modifications (PTM)[7]. Studies show that "50% of all native human proteins are glycosylated"[6]. Glycosylation can be defined as the covalent attachment of a oligosaccharide molecule to the polypeptide backbone of a protein[6]. Protein glycosylation can influence "protein folding and assembly", "targeting and trafficking of a newly synthesized protein to its final destination", "increase solubility, influence biological half-life" and increase biological activity"[6] by promoting ligand recognition and binding[6].

Typically, mABs have only one N-Glycosylation site in the F_C heavy chain (C_2H) region (Asn297). At this site N-Acetelyglucosamine is linked to the amino acid Asparagine (Asn/N), e.g. the asparagines at site 297 (Asn297, Figure 2, star) in IgGs. The amino acid consensus

sequence for N-Glycosylation is Asparagine (Asn) – random (X) – Serine (Ser) / Threonine (Thr).

Monoclonal antibodies can act by different mechanism for example on cancer cells. These mechanisms are highly complex and require the involvement of additional cellular mechanisms, e.g. “antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)”[1] or antibody-dependent phagocytosis (ADCP)[7]. **Figure 3** shows a scheme for the mechanism of action for mAB therapeutic drugs.

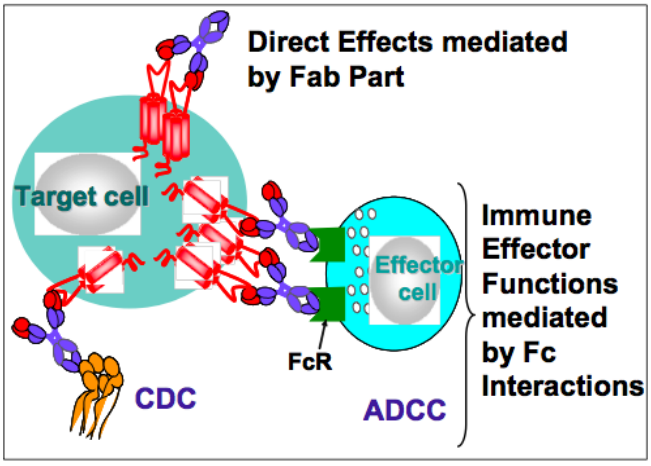


Figure 3: mAB-mode of action, Figure from G.-B. Kresse, CMC, 2009[8]

Human IgGs “bound to an antigen on a target cell surface can interact with Fc receptors for IgG (FcγRs) on effector cells and may support the destruction of target cells by antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP), whereas interaction with the complement component C1q may support killing by complement-dependent cytotoxicity (CDC)“[9]. The interaction of the IgG Fc domain with the receptor FcγR is responsible for the long biological half-life of the mAB[9]. Modifications of the amino acid sequence of the Fc region can lead to “modulation of effector functions (ADCC, ADCP and CDC) and/or half-life”[9] (see **Appendix 1**).

In contrast to other pharmaceutical manufacturing processes the “biosynthesis of glycans is not directly template-driven but, rather, is a result of a complex network of metabolic and enzymatic reactions that are influenced by many factors, including the genetic profile of the cells in which the glycoconjugates are expressed, epigenetics and the extracellular environment”[10]. The biotechnological manufacturing of mABs requires stable conditions.

Already slight changes in the composition of cell media, temperature, cell density or other factors can have tremendous effects on the heterogeneous glycosylation pattern.

N-Glycosylations can be highly heterogeneous[11]. Usually the heavy chain of mABs carries a N-Glycosylation site at Asn297 and the light chain is usually not glycosylated. The heavy chain can carry more than one glycosylation. The core structure of the N-Glycosylation at Asn297 contains N-Acetelyglucosamine and Mannose molecules. These N-Acetelyglucosamine molecules can further be modified by mannosylation, galactosylation, sialylation and fucosylation. These modifications affect the biological function of the mAB.

The natural occurring post-translational modification in cells leads to a heterogeneous mixture of slightly different mAB glycan profiles. Therefore it is always necessary to confirm the presence or absence of all glycosylation site(s) and their modifications in a therapeutic mAB[1]. It is necessary to characterise the glycosylation structure of mABs by analysing the "carbohydrate content (neutral sugars, amino sugars and sialic acids)"[1], the "structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy"[1].

3.3 For which indications are therapeutic (glycosylated) monoclonal antibodies important?

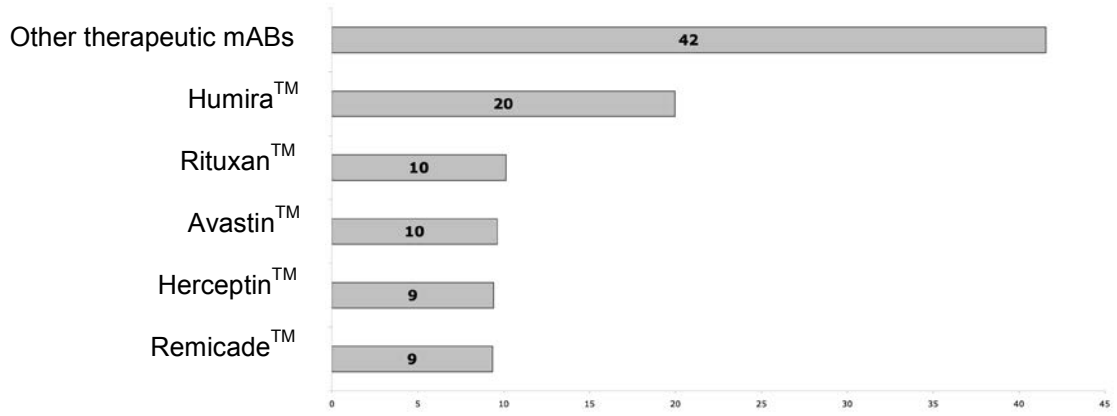
In the therapeutic drug market the specific binding of mABs is used for different indications, e.g. Cancer, autoimmune and cardiovascular diseases.

Some of the mABs ranking in the top 10 of best-selling biologicals are shown in the table below. These mABs also have top rankings in the annual list of pharmaceutical drug sales successes. The market share of these mABs is shown graphically in the bottom of the following table.

Table 2: Best-selling mABs under the top 10 best-selling Biologicals of 2015[12] and their percentage of mAB-market share in 2015[13].

Trade name	Active pharmaceutical ingredient (API)	Producer	Mechanism of action	Target	Revenue in Millions (USD)	Patent expired
Humira™	Adalimumab	AbbVie	By binding to TNFα it reduces inflammation	Rheumatoid arthritis, psoriasis, Crohn's and other autoimmune diseases	$14.01Bn	2016 (US), 2018 (EU)[14]
Rituxan™	Rituximab	Roche	Binds to CD20 on B-cells	Leukemia, lymphomas, lupus	$7.33Bn	2014 (EU), 2017 (US)[15]
Avastin™	Bevacizumab	Roche	Inhibits VEGF-A and thus inhibits angiogenesis	Cancer	$6.95Bn	2019 (US), 2022 (EU)
Herceptin™	Trastuzumab	Roche	Inhibits the HER2/neu receptor	Cancer	$6.80Bn	2014 (EU), 2019 (US)
Remicade™	Infliximab	Janssen	Binding to TNFα	Rheumatoid arthritis, psoriasis, Crohn's and other autoimmune diseases	$6.56Bn	2014 (EU), 2018 (US)

MAB-Market Share in percent in 2015[13] (see Appendix 2)



The biological function of mABs like Adalimumab, Rituximab, Bevacizumab, Trastuzumab, Infliximab depends on their N-glycosylation in the CH2 domain in the heavy chain. Beside these five the regulatory agencies in the EU and the US approved since December 2007 further glycosylated mABs, e.g. **"**Bexxar, Erbitux, **(…)** Humaspect, **(…)** Mabcampath/ Campath-H1, Mabthera/**(…)**, Mylotarg, Neutrospec, Oncoscint, Orthoclone OKT-3, Prostascint, Raptiva, (…) Simulect, Soliris, Synagis, Tysabri, Vectibix, Xolair, Zenapax, Zevalin" (trade names of the products)[6].

The block buster Humira™ (API: Adalimumab) was first approved in the US in 2002 for rheumatoid arthritis[16]; later further targets like psoriasis, Crohn's and other autoimmune diseases followed. Adalimumab carries a N-Glycosylation site in each heavy chain (C_2H). Adalimumab is a recombinant human IgG1 mAB that can specifically bind to the tumor necrose factor (TNF) and carries an N-linked glycosylation site. Since its first market access the manufacturing process for Adalimumab was changed several times, including scale ups, manufacturing site transfers, changes of test methods and specifications. Due to the importance of the N-glycosylation site one of the main tasks for the manufacturer was to show that the changes in the manufacturing process do not affect the glycosylation (chapter 6.1.1).

Rituxan™ (API: Rituximab) is a human-mouse chimeric mAB that binds to CD20 on B-cells. Rituximab was approved in the US in 1997 for the indication "low-grade or follicular non-Hodgkin's lymphoma (LG/F NHL)" and in the EU in 1998 for "Stage III/IV, follicular, chemoresistant or relapsed NHL"[17]. Like Adalimumab, Rituximab is glycosylated at the heavy chain"[11].

Avastin™ (API: Bevacizumab) is a humanized recombinant IgG1 mAB. That was first approved in the US in 2004 and in the EU in 2005 for the treatment of metastatic colorectal cancer. Later further approvals for other cancer indications, e.g. ovarian, breast and kidney cancer followed. Bevacizumab acts through inhibition of VEGF-A and thus inhibits angiogenesis. The heavy chain of Bevacizumab is N-glycosylated.

Herceptin™ (API: Trastuzumab) is a humanized recombinant IgG1 mAB carrying N-glycosylations at the heavy chain. Trastuzumab was first approved for the treatment of breast cancer acting through the inhibition of the HER2/neu receptor in the US in 1998 and in the EU two years later in 2000.

Remicade™ (API: Infliximab) is a chimeric (mouse/human) IgG1 mAB. In the EU and the US Remicade was first approved for the treatment of rheumatoid arthritis in 1999. Infliximab acts

by binding to TNFα and thereby inhibits TNFα. For its proper function the heavy chain of the Infliximab is N-glycosylated at Asn^{300} (chapter 6.1.2).

3.4 What is the standard manufacturing process for mABs?

The traditional approach of pharmaceutical production uses batch processing. Batch processing is also the most common approach for biologicals like mABs. In mAB manufacturing the focus has been on batch processing for decades.

The standard biological manufacturing process for mABs is divided into the following process steps:

- **Cell development**: This describes the process in which the cell clone that contains the desired transgene will be identified and subsequently the research cell bank from this cell clone is established.
- **Cell banking**: During this process step the master cell bank (MCB) and the working cell bank (WCB) are established and fully characterised. The WCB will then be used in the following steps.
- **Upstream processing** (USP): The first step during USP is the scale up of cells usually by using an aliquot of the WCB. Seeding the bioreactor follows this step. Then the fermentation starts followed by harvesting.
- **Downstream processing** (DSP): During DSP the drug product will be first recovered form the harvest, and then purified and polished using several chromatography and filtration steps. In the last step the product will be sterile filtrated and formulated.

3.4.1 Guidelines and Standards that must be considered for the manufacturing of mABs and biologics

All steps during pharmaceutical manufacturing must be done in compliance with the "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients" (ICH Q7)[18].

In Europe the legal basis for the manufacturing of medicinal products and investigational medicinal products (IMP) for human use has been laid down in the Commission Directives 2003/94/EC[19]. The EudraLex – Volume 4 – Good Manufacturing Practice (GMP) guidelines and its annexes describe how the "Commission Directives 2003/94/EC of 8 October 2003 laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use"[19] should be practically interpreted.

In the US the FD&C Act 501(a)(2)(b) requires conformity with the Current Good Manufacturing Practice (CGMP) regulations published[20]. The applicable US regulations for Food and Drug can be found in the Code of Federal Regulations (CFR) in Title 21. For pharmaceutical manufacturing of mABs for human use especially the regulations contained in 21 CFR Parts 200-299 should be considered. These regulations are not exclusively written

for biologics. However 21CFR211 describes the minimal requirements for “current good manufacturing practice for preparation of drug products (…) for administration to humans” (21CFR211.1 (a)).

In general any pharmaceutical manufacturing process should be done on a risk-based approach. The **ICH quality guidelines** on Pharmaceutical Development (ICH Q8), Quality Risk Management (ICH Q9) and Pharmaceutical Quality System (ICH Q10) are the foundation for a flexible GMP-based risk management during process development:

- “Pharmaceutical Development” ICH Q8 from 2005[21]. The scope of this guideline is on the pharmaceutical development of the drug product and how to include this information into the manufacturing application dossier.
- “Quality Risk Management” ICH Q9 from 2005[22]. This guideline describes a systematic approach to manage quality risks for drug substance and drug products during
    - “development, manufacturing, distribution” and
    - “throughout the lifecycle of drug substances, drug (medicinal) products, biological and biotechnological products (including the use of raw materials, solvents, excipients, packaging and labeling materials in drug (medicinal) products, biological and biotechnological products).”[22]
- “Pharmaceutical Quality System” ICH Q10 from 2008[23]. The three main objectives of this guideline are to
    - “establish, implement and maintain a system that allows the delivery of products with the quality attributes appropriate to meet the needs of patients, health care professionals, regulatory authorities”,
    - “develop and use effective monitoring and control systems for process performance and product quality”, and
    - “identify and implement appropriate product quality improvements, process improvements, variability reduction, innovations and pharmaceutical quality system enhancements”[23].
- “Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)” ICH Q11 from 2012[24]. Gives guidance on
    - Manufacturing process development,
    - Process controls and parameters,
    - Starting material selection,
    - Process validation, and
    - Life-Cycle-Management.

In addition to the ICH Q7 / Q8 / Q9 / Q10 guidelines the following ICH guidelines on quality should also be consulted for the pharmaceutical manufacturing of mABs:

- "Viral safety evaluation of Biotechnology products derived from cell lines of human or animal origin" ICH Q5A from 1997[25]. The focus is on "testing and evaluation of the viral safety of biotechnology products derived from characterised cell lines of human or animal origin"[25]. In the application the risk of all possible viral contaminations should be applied. The application should also include:
    - A strategy to avoid viral contaminations,
    - Methods to test viral contaminations and
    - Methods for "virus removal and inactivation" during the manufacturing process.
- "Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products" ICH Q5B from 1997[26]. The application must include the characterisation of the expression construct and expression system including the integration in the host cell genome[26].
- "Stability Testing of Biotechnological/Biological Products" ICH Q5C from 1995[27]. Guidance for marketing applications regarding:
    - Types of stability studies
    - Generation and submission of stability data for mABs
- "Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" ICH Q5D from 1997[28]: The required information for an application need to contain
    - Characterisation of cell line – origin, source, history and generation[28]
    - Generation and characterisation of used cell banks (MCB and WCB)[28]
    - Methods for characterisation and testing of cell lines / cell banks
- "Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process" ICH Q5E from 2004[29].
    - Evaluate „comparability of biotechnological/biological products before and after changes are made in the manufacturing process for the drug substance or drug product"[29].
    - Collection of "relevant technical information which serves as evidence that the manufacturing process changes will not have an adverse impact on the quality, safety and efficacy of the drug product."[29]
- "Validation of Analytical Procedures: Text and Methodology" ICH Q2(R1) from 2005[30].
- "Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products" ICH Q6B from 1999[31]. Requirements for marketing authorisation are:

- Characterisation of the biotechnological/biological drug substance and drug product[31].
- Setting and justifying drug substance and drug product specifications, e.g. appearance, identity, purity, potency[31].

Besides these quality guidelines the following **safety and efficacy ICH guidelines** need to be taken into account by applicants of a marketing authorisation for a therapeutic mAB:

- "Preclinical Safety Evaluation of Biotechnological-Derived Pharmaceuticals" ICH S6(R1) from 1997[32]. Scope of the guideline:
  - Performing preclinical safety testing
  - Investigation of the potential for undesirable pharmacological activity in appropriate animal models, e.g. Single Dose Toxicity Studies, Repeated Dose Toxicity Studies, Genotoxicity Studies, Carcinogenicity Studies
- "Nonclinical Evaluation for Anticancer Pharmaceuticals" ICH S9 from 2009[33]. This guideline specifically applies to the development of anti-cancer pharmaceuticals, which are "intended to treat cancer in patients with serious and life threatening malignancies"[33] with limited or no other treatment option. The application must include „type and timing of nonclinical studies" e.g. safety pharmacology, pharmacokinetics, toxicology, genotoxicity, carcinogenicity[33].
- "Pharmacovigilance Planning" ICH E2E from 2004. The intention of this guideline is to "aid in planning pharmacovigilance activities, especially in preparation for the early postmarketing period of a new drug"[34], e.g. biotechnological-derived pharmaceuticals. The purpose of ICH E2E is on:
  - Safety Specification
  - Pharmacovigilance Plan

The EMA and FDA provide additional guidance to applicants for marketing authorisation in addition to their legal regulations. Some of the most important guidelines for mABs are listed in the following sections.

EMA guidance for the pharmaceutical manufacturing of mABs:

- "Guideline on development, production, characterisation and specification for monoclonal antibodies and related products" from 2016[1]. The focus is on
  - quality requirements for the marketing authorisation of mABs – mABs for clinical trial are not in the scope.
  - "importance of characterisation and control of relevant glycosylation structures and biological activity".

FDA guidance for the pharmaceutical manufacturing of mABs:

- "Guidance for Industry: Monoclonal Antibodies Used as Reagents in Drug Manufacturing" from 2001[35]. The main scope of this guidance is on "chemistry, manufacturing, and control (CMC) issues" that should be addressed during marketing authorisation application (MAA).
- "Guidance for Industry: Q5E Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process" from 2005[36].
- "Guidances (Drugs) - Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products" from 1996[37]. Guidance to implement manufacturing changes.
- In 1997 the FDA published a document on "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human"[38] use. This document provides manufacturers with the information on how to develop and manufacture, but also on the requirements for a new drug application and licensing procedures. The focus of this guidance is on:
    - cell development – characterisation of master vector, generation of a genetically stable expression in the cell line, MCB and WCB, appropriate cell line testing,
    - manufacturing of mABs and purification,
    - characterisation of mABs,
    - quality control of drug substance and drug product,
    - product safety and stability testing, etc.
- "Points to Consider on the Characterization of Cell Lines Used to Produce Biologicals" by CBER from 1993[39]. The points are:
    - "production, identification and characterization of the cell substrate",
    - "validation of the manufacturing process for removal and/or inactivation of adventitious agents", and
    - "testing of the bulk and final product to assure safety"[39] .
- "Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologic & Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability" by CBER from 1992[40]. This document gives guidance on the characterisation of expression constructs and cell banks (MCB, WCB)[40].

3.4.2 Cell development / cell banking

Mammalian cell lines are usually the preferred cell line to express large and complex proteins with post-translational modifications like glycosylated mABs. The most common used cell line for mAB pharmaceutical manufacturing is the Chinese hamster ovary (CHO) cell line. Beside

CHO other mammalian cell lines used for the expression of therapeutic mABs for human use are the murine myeloma cell lines NS0 and Sp2/0[41]. Thus, for example four of the five mAB blockbusters Adalimumab, Rituximab, Bevacizumab and Trastuzumab are expressed in CHO cell lines[41]. Only Infliximab is expressed in Sp2/0 cell lines[41]. These mammalian (not human) cells express additional post-translational modification enzymes, which are able to modify mABs with non-human glycosylations (galactose-α1,3-galactose (α-gal) and N-glycolylneuraminic acid)[41]. To avoid adverse immune reactions in patients the glycan expression pattern of the cell clones must be analysed during cell line development. Only cell clones with acceptable glycan expression profile are used.

Human cell lines that could be used for the expression of therapeutic antibodies are human embryonic kidney cells 293 (HEK293) and human fibrosarcoma cells (HT-1080). The great advantage of these human cell lines is the human post-translational modification (e.g. glycan) pattern. Recently some therapeutic biologics were approved by EMA and FDA which are produced in HEK293 or HT-1080 cells[41], e.g. the Fc fusion protein (rFVIIIFc) from Biogen is expressed in HEK293 cells[42]. Beside the absence of non-human glycosylations these human cell lines have further advantages, e.g. they are able to grow in "suspension serum-free culture", rapidly reproduce and there are many transfection methods available[41]. On the downside, compared to other mammalian cell lines like CHO the clinical experience is still far less extensive and another disadvantage is the unknown risk-potential of human viral contaminations[41].

Beside the cell line and the efficacy of protein expression, mAB glycosylation further depends on the cell culture medium, the viability and status of the cells and therefore the fermentation process.

3.4.3 Upstream processing: Fermentation and Harvesting

Batch manufacturing is the traditional approach of pharmaceutical production. Today most active pharmaceutical ingredients (APIs) of approved pharmaceuticals are still manufactured by a batch process in which the drugs are processed step by step. Other common fermentation and harvesting methods are fed-batch and perfusion:

- In a batch process the "Initial medium charge provides all nutrition for the entire run"[43] and is completely discharged at the end of the run.
- Fed-batch (or semi-batch) "refers to an approach in which a concentrated solution of nutrients is added at particular intervals, with no product harvested until the end of the run"[43].

- In a perfusion process nutrients and medium are continually filled into the bioreactor while the product is also continually harvested from the bioreactor.

Today, fed batch and perfusion mammalian cell culture are becoming the most commonly used upstream manufacturing techniques used for the production of large amounts of glycosylated proteins[44].

Critical parameters for the upstream process are "(i) the time until a desired cell density is reached, determined by the specific growth rate (μ) of the cells; (ii) the duration of the production phase enabling an accumulation of recombinant protein from a high-density and viable culture; and (iii) the obtainable product titer determined by the specific production rate (qP) and the overall process duration"[45]. These critical parameters depend on process parameters like temperature, cell medium composition; ph, osmolarity, oxygen and CO_2 value, etc. and influence not only the product titer but also post-translational protein modifications and therefore the biological functionality of the product. Thus it is essential to understand the influence of each critical parameter and to control them.

Perfusion is known since the 1980s and was initially used for products (e.g. antibodies) that were not stable when left in the fermenter (batch / fed batch) for a longer time. The cells secrete the glycosylated mAB into the medium. In a perfusion bioreactor the spent medium with the mAB is removed continuously while the cells are retained. The perfusion rates can be less than the reactor volume or up to several times of the reactor volume per day. The biggest difference between a batch or fed-batch and a perfusion reactor is the need for an additional cell-retention system. These cell retention systems can work for example by centrifugation or filtration. Due to the constant addition of new medium and removal of spent medium the cells are kept at optimal process conditions. Typically the cell density in a perfusion reactor is significantly higher than in batch or fed-batch reactor and thereby performs a higher product yield.

At the end of the batch or fed-batch run, or continuously from the perfusion reactor the mAB is usually harvested by centrifugation. After harvesting the mAB purification process can be directly started.

3.4.4 Downstream processing: Purification

The downstream process is a multi step process starting with the cell harvest from the USP and ending with the purified mAB. Figure 4 gives a schematic overview over the downstream process.

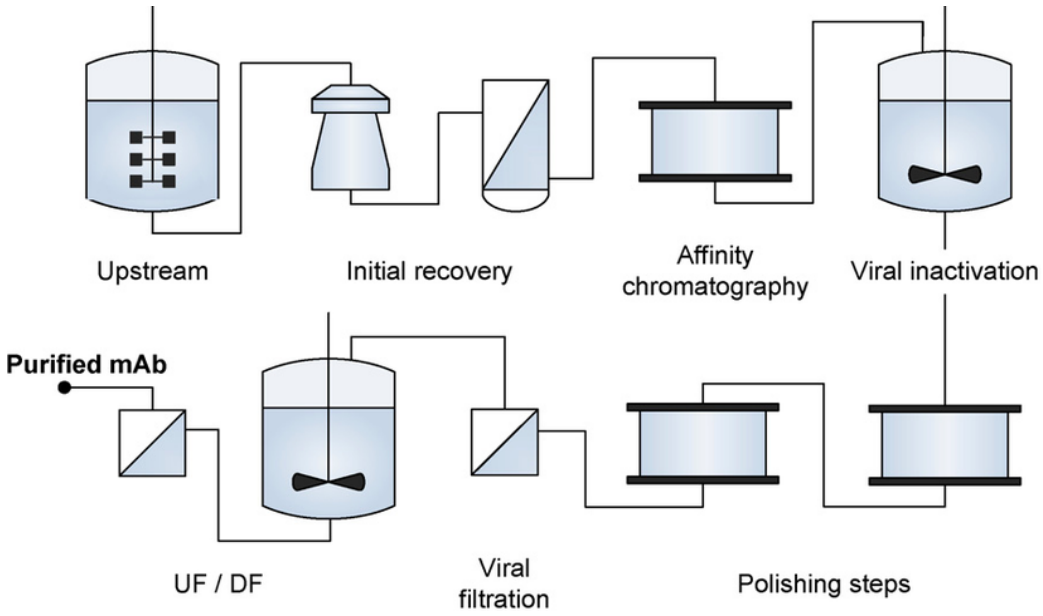


Figure 4: Standard downstream platform for mABs from Rosa et al., 2010[46].

The following table lists some of these different processes during a mAB purification downstream process. MAB purification processes highly differ from product to product. The essential steps are discussed subsequent to the table.

Table 3: Downstream process. Depending on the product the process can differ not only in the number of process steps but also in the sequences of the individual steps[44,47,48].

Process step	Function	Remarks
Upstream process: Harvesting (Centrifugation and/or filtration)	Cells and cell residues are removed.	This clarification step is essential, to prevent column agglutination in the following downstream process.
Capture (Protein A chromatography)	Product purification.	The majority of mAB purification processes uses Protein A chromatography as the main purification step. During this step host cell-related proteins and other impurities are removed. Risk: The low pH of the elution buffer can increase product aggregation.
Viral inactivation (Low pH treatment)	Inactivation of endogenous and adventitious viruses[47].	To improve viral safety two independent mechanisms (viral inactivation and filtration) are included in the downstream processing. Risk: The low pH treatment can increase product aggregation.
Polishing chromatography	Product and process impurities and viruses are sequentially removed.	Using ion exchange chromatography for reduction of "high molecular weight aggregate, charge-variants, residual DNA and host cell protein, leached Protein A and viral particles"[47]
Viral filtration	Endogenous and adventitious viruses are removed.	Removes viral contaminations depending on the pore size of the membrane.
Ultrafiltration / Diafiltration (UF/DF)	Final sterile filtration and concentration step.	The product is sterile filtered and concentrated into the final formulation buffer[47].

After harvesting the antibody purification process starts with the capture step using Protein A chromatography. Depending on the protein titer in the harvest an additional ultrafiltration / diafiltration step can be appropriate to increase mAB concentration prior to the Protein A chromatography step. Protein A, a protein of microbial origin, is the most common used option for mAB affinity chromatography. During this step the mAB and other proteins bind to the Protein A matrix while other components in the supernatant flow through the chromatography column. After several washing steps the purified mAB will be eluted from the column. A problem with Protein A chromatography is that although it can specifically bind proteins, beside the wanted mAB it also binds unspecifically to impurities in the supernatant, e.g. process impurities, host cell DNA or proteins[47]. These unwanted impurities are process dependent. To improve the Protein A chromatography it is essential to define the binding

capacity of the wanted mAB precisely. Thereby the elution conditions can be more specific for the wanted mAB. The elution buffer has a low pH. One common problem with low pH buffers is that they can lead to unwanted protein aggregations.

Following Protein A chromatography one or more ion exchange chromatography steps are carried out. Ion exchange chromatography is highly selective and compared to the Protein A chromatography relatively inexpensive[47]. One can distinguish between two types of ion exchange chromatography resigns, anion or cation exchange chromatography (AEX or CEX). Depending on the isoelectric point (pI) of the mAB either the anion or cation exchange chromatography will be used as a polishing step[47]. Depending on the amount of impurities in the supernatant the loading volume needs to be adjusted[47].

To further purify the wanted mAB a number of different filtration methods can be performed during downstream processing, e.g. ultrafiltration / diafiltration (UF/DF) and viral filtration. The last filtration step is a sterile filtration that reduces the bioburden before final storage of the product.

The main challenges for downstream processing are to avoid the drug product aggregation, loss of wanted product isoforms and any degradation of the wanted product. To prevent this from occurring the conditions of downstream processing must be optimised. Particularly during early stages of pharmaceutical drug development the downstream process is often not optimal when it comes to “process economics, yield, pool volumes”[47], etc. Once a new drug has successfully passed the early clinical trials the development of downstream process often concentrates on the simplification and reduction of chromatography and polishing process steps[47].

Recently, several initiatives have been started throughout the industry to answer these challenges to downstream process, one of the most innovative being the so-called continuous downstream process. To understand the possible benefits and challenges of such technologies the next chapter will concentrate on continuous manufacturing.

4 Continuous manufacturing

In the annual meeting of the American Association of Pharmaceutical Scientists (AAPS) in October 2011 Dr. Janet Woodcock, director of the FDA's Center for Drug Evaluation and Research (CDER) stated

“Right now, manufacturing experts from the 1950s would easily recognize the pharmaceutical manufacturing processes of today. It is predicted that manufacturing will change in the next 25 years as current manufacturing practices are abandoned in favour of cleaner, flexible, more efficient continuous manufacturing.”

Dr. Woodcock used the term continuous manufacturing. Next to that term also continuous production or continuous process are often used in the literature, and sometimes they are used synonymously[49]. To clarify how these terms are used in this thesis they will be defined in the next session. Afterwards I will focus on matters like what is continuous manufacturing, how does it differ from other manufacturing processes, how can new approaches improve manufacturing and why is a change of the manufacturing practices required.

4.1 What is continuous manufacturing?

To clarify the use of the term continuous manufacturing I will differentiate it from continuous production or continuous process.

Continuous production is a general term used for a manufacturer that continuously produces. This can be completely independent of the used manufacturing processes, which can include continuous but also non-continuous processes like a batch process.

The term **continuous process** or **continuous process step / unit** describes a discrete process / unit in which input materials are continuously added at the start and product is continuously removed at the end of the process and this process runs continuously over a considerable period of time. In biopharmaceutical manufacturing a continuous process runs over a longer period, e.g. a perfusion upstream process can run up to 60 sometimes 90 days. In other industries the perfusion upstream process of biopharmaceuticals would be rather defined as a semi-continuous process.

Continuous manufacturing is often used to describe a manufacturing that consists of continuous processes that are interlinked to continuously convert raw materials without interruption into final product that is continuously removed from the process. In this thesis I will use the term continuous manufacturing for connected continuous parts of the manufacturing process, e.g. for the upstream or downstream process of mAB manufacturing.

Since in present-days continuous processes are often combined with non-continuous processes in manufacturing I will use the term **adapted continuous manufacturing** for these hybrid-manufacturing processes that are “composed of both batch and continuous unit operations”[50].

4.2 How does continuous manufacturing differ from other manufacturing processes?

Batch manufacturing was the traditional approach of pharmaceutical manufacturing and most approved biologicals are still manufactured by a batch process, e.g. Humira (section 3.3). In the last decades more and more other manufacturing process techniques became available including also continuous processes like perfusion mammalian cell culture techniques that became more and more popular for the production of large amounts of glycosylated proteins. The reasons for this development vary. Since it was initially used in the 1980s one of the central success factors for the perfusion process is the possibility to manufacture proteins that become unstable when left in the fermenter for a longer time, e.g. glycosylated mABs. Another reason for the success of this continuous process is more practical. To increase the production volume with a non-continuous batch or fed-batch process the process must be restarted over and over again or the reactor size must be increased, whereas the perfusion process could run for a longer period of time.

Compared to non-continuous pharmaceutical manufacturing the advantages of continuous manufacturing are fewer steps, “no manual handling, increased safety, shorter processing times” and “increased efficiency”[51], smaller equipment footprint, and therefore “More flexible operation, Reduced inventory, Lower capital costs, less work-in-progress materials” and “Smaller ecological footprint”[51], and easier scale changes[52].

In the perspective of the FDA further mayor advantages of continuous manufacturing are the possibilities of “On-line monitoring and control for increased product quality assurance in real-time”[51] by using Real-Time-Release testing (RTRT). The FDA believes that on-line monitoring and testing would significantly increase the consistency of drug quality. Due to its impact on manufacturing and continuous manufacturing in particular these on-line monitoring and testing methods will be explained in the next session in association with the ICH quality by design (QbD) and design space approach.

4.3 How can Quality by design (QbD) and Process Analytical Technology (PAT) move continuous manufacturing forward?

A central point of biopharmaceutical process development is to collect information and knowledge, and to use them to increase drug quality and safety. Quality is defined as the "suitability of either the drug substance or drug product for its intended use" including "such attributes as the identity, strength, and purity"[53]. Critical quality attributes include also specifications, which are defined as "a list of tests, references to analytical procedures, and appropriate acceptance criteria, which are numerical limits, ranges, or other criteria for the tests described"[53].

Today the development of biopharmaceuticals and their manufacturing strategies, e.g. continuous manufacturing, should be done on a risk-based approach in accordance with the ICH quality guidelines on Pharmaceutical Development (ICH Q8(R2)), Quality Risk Management (ICH Q9) and Pharmaceutical Quality System (ICH Q10) (section 3.4). These ICH guidelines are the foundation of the Quality by Design (QbD) concept "that entails building quality into the process and product in a systematic, science- and risk-based manner"[54]. This means that quality cannot be tested into the product but must be developed into the process and product. ICH Q8(R2) defines QbD as a "systematic approach to development that begins with predefined objectives and emphasises product and process understanding and process control, based on sound science and quality risk management"[21]. According to ICH Q8(R2) pharmaceutical drug development should at least include the definition or identification of the quality target product profile (QTPP), the "potential critical quality attributes (CQAs) of the drug product", the "critical quality attributes of the drug substance, excipients etc.", the "control strategy" and the suitable manufacturing process[21].

The drug design space is a multidimensional overall combination of all drug quality-influencing factors during pharmaceutical processing[21]. To monitor pharmaceutical manufacturing Process Analytical Technology (PAT)[55] can be used as a "system for designing, analysing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality" [21]. The establishment of the design space requires the enhanced "knowledge gained from pharmaceutical development studies and manufacturing experience"[21]. Once established for a drug product the design space can be used "to facilitate:

- risk-based regulatory decisions (reviews and inspections);
- manufacturing process improvements, within the approved design space described in the dossier, without further regulatory review;

- reduction of post-approval submissions;
- real-time quality control, leading to a reduction of end-product release testing"[21].

ICH Q8(R2) distinguishes two different approaches for the development of pharmaceuticals. The comparison of the minimal and enhanced QbD approach is shown in Table 4[21].

Table 4: Different Approaches to Pharmaceutical Development adopted from ICH Q8(R2), Annex 1[21].

Aspect	Minimal Approaches	Enhanced, Quality by Design Approaches
Overall Pharmaceutical Development	• Mainly empirical • Developmental research often conducted one variable at a time	• Systematic, relating mechanistic understanding of material attributes and process parameters to drug product CQAs • Multivariate experiments to understand product and process • Establishment of design space • PAT tools utilized
Manufacturing Process	• Fixed • Validation primarily based on initial full-scale batches • Focus on optimisation and reproducibility	• Adjustable within design space • Lifecycle approach to validation and, ideally, continuous process verification • Focus on control strategy and robustness • Use of statistical process control methods
Process Controls	• In-process tests primarily for go/no go decisions • Off-line analysis	• PAT tools utilised with appropriate feed forward and feedback controls • Process operations tracked and trended to support continual improvement efforts post- approval
Product Specifications	• Primary means of control • Based on batch data available at time of registration	• Part of the overall quality control strategy • Based on desired product performance with relevant supportive data
Control Strategy	• Drug product quality controlled primarily by intermediates (in-process materials) and end product testing	• Drug product quality ensured by risk-based control strategy for well understood product and process • Quality controls shifted upstream, with the possibility of real-time release testing or reduced end-product testing
Lifecycle Management	• Reactive (i.e., problem solving and corrective action)	• Preventive action • Continual improvement facilitated

The enhanced QbD approach is already a very successful approach for small molecules, however for biologics it is still developing. Today most pharmaceutical manufacturer develop a process that lies in between of these two approaches[21].

The EMA founded in 2003 the Process Analytical Technology (PAT) Team to support PAT and QbD approaches in the European Union. With the FDA pilot in 2008 the QbD approach was first introduced into pharmaceutical development of biologics, e.g. during tech transfer or other post-marketing changes. But it took some time for the first biologics using the QbD approach for marketing authorizations.

With Gazyva (2013, API: Obinutuzumab) and Tecentriq (2016, API: Atezolizumab) Roche/Genentech obtained the FDA approval for two therapeutic mABs with applications based on QbD with a defined design space[56,57]. Due to the approved design space the FDA "does not except any regulatory notification for movements within the design space, any other changes in the manufacturing, testing, packaging, or labeling or manufacturing facilities for GAZYVA (obinutuzumab) will require the submission of information to your biologics license application for our review and written approval, consistent with 21 CFR 601.12"[57].

In the last years a couple of FDA projects focused on continuous manufacturing and QbD/PAT approaches for biologics. These are included in FDAs Regulatory Science Progress Report (FY 2015-2016)[58]: One topic is to optimize PAT for bioreactors for the production of biologics by improving process control and real-time monitoring[58]. Another named study in the FDA report uses the QbD approach to analyze the impact of process changes on the heterogonous glycosylation pattern of mABs[58,59].

After having conducted projects independent from each other, EMA and FDA started the "EMA-FDA pilot program for parallel assessment of Quality-by-Design applications" in 2011[60]. The scope of this international pilot program was to "ensure consistent implementation between EU and US of ICH Q8, 9, 10, 11 guidelines in the assessment process and to facilitate sharing of regulatory decisions on new regulatory concepts"[60]. In this EMA-FDA pilot program one case was the review of a marketing application that uses continuous manufacturing. Based on the EMA-FDA review of this application the "following areas related to CM were harmonized: batch definition; control of excipients; material traceability; strategy for segregation of non-conforming material; real-time release testing (RTRT) methods and prediction models; and good manufacturing practice (GMP) considerations for RTRT, validation strategy, models, and control strategy"[61].

Today although many manufacturers are still reluctant to introduce QbD into their pharmaceutical drug development, the concept by itself already influences the development

of drugs. During the last years several guidelines were released focusing especially on better process development. To be compliant with current EMA and FDA process validation guidelines manufacturers already gather more process information. Both EMA and FDA introduced the QbD approach into their current validation guidelines, although they do not call it QbD or design space in their guidance, like the FDA with the Guidance for Industry on "Process Validation: General Principles and Practices"[62]. The FDA distinguishes in this guideline three phases of process validation "process design", "process qualification" and "continued process verification". The EMA on the other side also distinguishes three phases of the drug product life cycle: development, validation and ongoing process validation[63]. The three guidelines applying to these stages are:

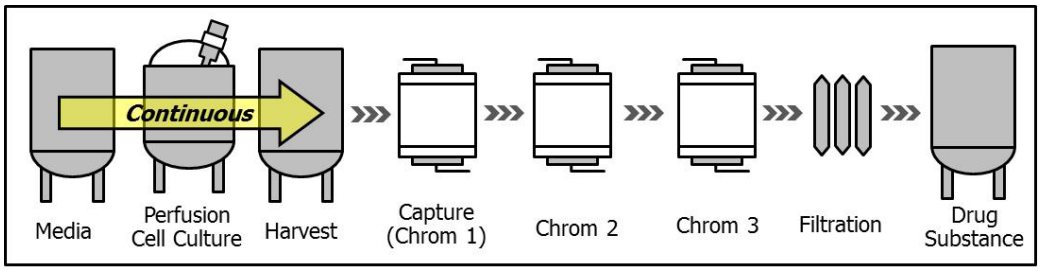
- Process development: "Pharmaceutical development" (ICH Q8(R2)[21]
- Validation: "Guideline on process validation for finished products - information and data to be provided in regulatory submissions" (CHMP, 2014)[63]
- Ongoing process validation: EudraLex volume 4, Annex 15 on "Qualification and Validation"[64]

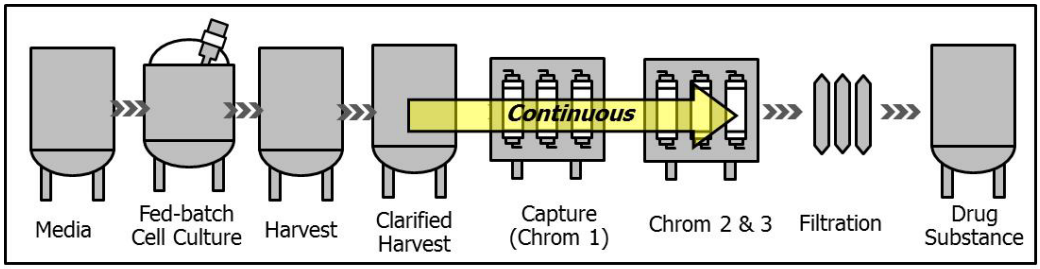
Additionally for biopharmaceuticals the EMA "Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission" that came into effect in November 2016 mentions the possibility to use the design space also for biopharmaceutical drugs[65].

4.4 Comparison of different continuous manufacturing approaches

The continuous manufacturing approach is a relatively new approach for biopharmaceuticals. **Table 5** summarizes the existing and potential continuous manufacturing platforms.

Table 5: Examples for pharmaceutical protein manufacturing systems using mammalian cell culture[50]. The content of the table summarizes the examples from Konstantinov and Cooney, 2014[50].

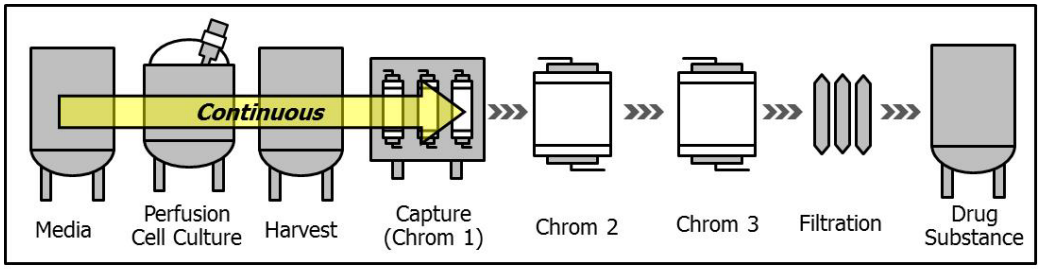
<table>
<tr><td colspan="2">Hybrid system: Continuous upstream with batch downstream</td></tr>
<tr><td colspan="2"> 

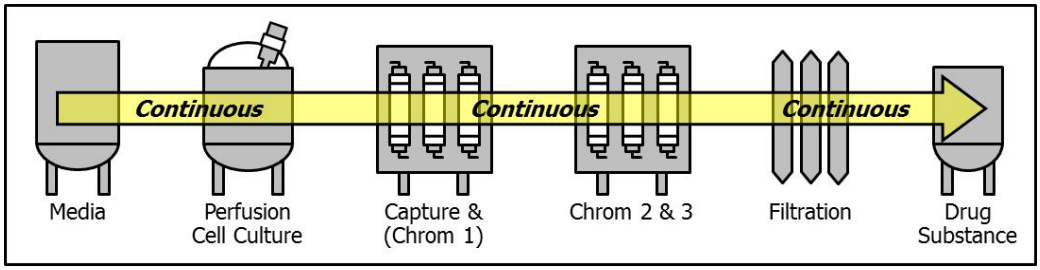
- Upstream perfusion bioreactor is often used for complex / unstable proteins like mABs because of the short period of retention in the bioreactor.
- Downstream process is done in a traditional batch approach.</td><td rowspan="3">Companies*)
Genzyme, Bayer, Janssen, BioMarin, Shire, Merck-Serono, Novartis and Pfizer</td></tr>
<tr><td>Advantages</td><td>Disadvantages</td></tr>
<tr><td>- Widely used for commercial manufacturing for over 25 years
- High cell density
- Small bioreactor footprint
- Production of unstable proteins
- High productivity</td><td>- High downstream process footprint</td></tr>
<tr><td colspan="2">Hybrid system: Batch upstream with continuous downstream</td></tr>
<tr><td colspan="2"> 

- Upstream fed-batch reactor
- Downstream process contains one or more continuous process steps</td><td rowspan="3">Companies*)
none</td></tr>
<tr><td>Advantages</td><td>Disadvantages</td></tr>
<tr><td>- Smaller volume of continuous chromatography columns</td><td>- Continuous downstream process steps are still in development</td></tr>
</table>

*) Commercially manufactured proteins

Table 5 continued:

<table>
<tr><td colspan="2">Hybrid system: Continuous bioreactor and capture followed by batch (post-capture) downstream</td></tr>
<tr><td colspan="2"> 

- Upstream process contains a continuous perfusion bioreactor that is directly linked to the continuous capture step.
- Downstream process is a traditional batch approach.</td><td rowspan="3">Companies*)
none</td></tr>
<tr><td>Advantages</td><td>Disadvantages</td></tr>
<tr><td>- No storage between perfusion bioreactor and capture
- Reduction in column size and amount of buffer
- High cell density
- Small bioreactor footprint
- Production of unstable proteins
- High productivity</td><td>- First continuous capture steps are commercially available, but little or no experience</td></tr>
<tr><td colspan="2">Fully integrated continuous process</td></tr>
<tr><td colspan="2"> 

- Upstream and downstream process consists exclusively of continuous process steps</td><td rowspan="3">Companies*)
none</td></tr>
<tr><td>Advantages</td><td>Disadvantages</td></tr>
<tr><td>- Small equipment footprint
- Low residence and cycle times
- Low cost
- High cell density
- Small bioreactor footprint
- Production of unstable proteins
- High productivity</td><td>- Still in development
- Not all process steps are commercially available</td></tr>
</table>

*) Commercially manufactured proteins

In the last decades the focus of the biopharmaceutical industry was on the optimisation of the upstream process. Continuous perfusion is already established since decades and has many advantages that lead to an increase in production flexibility, reduced investment and operational costs, and increased production speed due to faster readiness and therefore shorter downtimes. In the last decade the use of single-use equipment (disposables) further improved biopharmaceutical manufacturing and also the perfusion process.

Today the biggest and most complex bottleneck on the way to realize continuous manufacturing (also named as a fully integrated continuous process, **Table 5**) for therapeutic mABs is the downstream process. Initially the downstream process was developed for the purification of mABs from an upstream batch process with low titer[66]. However the development of new cell lines, expression constructs, cell media and perfusion bioreactors led to a significant increase in cell density and product expression, and therefore protein titer and yield.

For the downstream process steps the development of a continuous platforms is still in its infancy. Until today no continuous downstream platform has been reported for the manufacturing of an approved biopharmaceuticals. In 2014, the Genzyme Cooperation filed the patent “Integrated Continuous Manufacturing of Therapeutic Protein Drug Substances” (US 20140255994 A1)[67] that integrates a continuous upstream and downstream process for the manufacturing of therapeutic monoclonal antibodies[67] (for further information see **Appendix 3**).

The second big bottleneck for continuous manufacturing is the development of suitable on-line analytical test methods as they are already established for continuous manufacturing of small molecules.

5 Regulatory basis for Continuous manufacturing

The restraint of companies to develop and use continuous manufacturing is often accompanied with the uncertainty of current regulatory requirements and the unknown future demands of competent authorities. The development of legal requirements and controls over the past decades has shown that guidelines will be developed further or new guidelines will be reissued in order to respond adequately to face the upcoming challenges.

In 2012 Sharmista Chatterjee, at that time a CMC Lead for QbD department at the FDA, gave a presentation on the topic "FDA Perspective on Continuous Manufacturing"[51]. Chatterjee stated that in the perspective of the FDA there are no special requirements for continuous drug manufacturing except one and that is the requirement to precisely define "batch" and "lot" in continuous drug manufacturing[51]. The FDA takes the view that with assistance of the Quality by Design (QbD) approach, quality can directly be built into the process design and therefore improve the "assurance of quality and consistency of drugs"[51].

In the US the application for a therapeutic mAB would be as a Biologics License Application (BLA), which is regulated under CFR section 21, 600 – 680. Examples are the blockbusters Humira, Rituxan, Avastin, Herceptin and Remicade. In the EU biotechnological-derived mABs belong to those medicinal products that need to be authorised by the European Community under the so-called "centralised procedure" that is laid down in Regulation (EC) No 726/2004 on "Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency"[68].

Next to that there are no limitations or special regulations for continuous manufacturing, neither in the US nor in the EU.

In the next sections I will first compare the batch/lot definition of ICH, EMA and FDA and subsequently discuss how batch traceability can be handled in continuous manufacturing.

5.1 How can a batch/lot be defined in a continuous process?

The batch/lot definition of ICH, EMA and FDA will be assessed with regard to the requirements for continuous manufacturing.

5.1.1 International Conference on Harmonization (ICH)

ICH Q7 defines a Batch (or Lot) as "A specific quantity of material produced in a process or series of processes so that it is expected to be homogeneous within specified limits." The guideline continues, that "In the case of **continuous production**, a batch may correspond to a **defined fraction of the production**. The batch size can be defined either by a fixed quantity or by the amount produced in a fixed time interval."

5.1.2 European Medicines Agency (EMA)

The Eudralex, volume 4 Good Manufacturing Practice (GMP) guidelines defines **batch** or **lot** as “A defined quantity of starting material, packaging material or product processed in one process or series of processes so that it could be expected to be homogeneous.”[69] It is noted that depending on the “stages of manufacture, it may be necessary to divide a batch into a number of sub batches, which are later brought together to form a final homogeneous batch.”[69]

For **continuous manufacturing** “the batch must correspond to a defined fraction of the production, characterised by its intended homogeneity.”[69]

“For control of the finished product, the following definition has been given in Annex 1 of Directive 2001/83/EC as amended by Directive 2003/63/EC: ‘For the control of the finished product, a **batch** of a proprietary medicinal product comprises **all the units of a pharmaceutical form which are made from the same initial mass of material and have undergone a single series of manufacturing operations or a single sterilisation operation** or, in the case of a **continuous production process**, **all the units manufactured in a given period of time’.”**[69]

The Eudralex, volume 4 Good Manufacturing Practice (GMP) guidelines defines the **batch/lot number** as “A distinctive combination of numbers and/or letters which specifically identifies a batch.” [69]

5.1.3 Food and Drug Administration (FDA)

A **batch** can be defined as ‘‘a specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture” (21CFR 210.3)[70]. This batch definition in the CFR focuses only on the **quantity of the produced drug** regardless of the manufacturing process. A **lot** is then further defined as “a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a drug product produced by **continuous process**, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having uniform character and quality within specified limits” (21 CFR 210.3)[70]. The lot definition focuses clearly also on continuous processing. Both definitions for “batch” and “lot” in the Code of Federal Regulations (CFR) can be applied to continuous manufacturing.

The CFR defines that the exact **batch** or **lot designation number** can be “any distinctive combination of letters, numbers, or symbols, or any combination of them, from which the

complete history of the manufacture, processing, packing, holding, and distribution of a batch or lot of drug product or other material can be determined."[70]

5.1.4 Why do batch/lot definition matters anyway?

After the current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals (21 CFR 211) in the CFR[71] the definition for batch/lot is required for

- Drug testing and release – "For each batch of drug product, there shall be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product, including the identity and strength of each active ingredient, prior to release" (21 CFR 211.165 (a)).
- Manufacturing records – § 211.186 "Master production and control records", § 211.188 "Batch production and control records", § 211.105 "Equipment identification", § 211.110 "Sampling and testing of in-process materials and drug products", § 211.115 "Reprocessing", § 211.130 "Packaging and labelling operations", etc.
- Traceability in case of complaints (§ 211.198), or recalls (§ 211.192) or any other necessary investigations (211.192).

These requirements are comparable to the requirements in the European Union (EU) as laid down in Directive 2003/94/EC[19] and Directive 2001/83/EC[3] for medicinal products for human use. Annex 16 of the EudraLex Volume 4 GMP guideline on "Certification by a Qualified Person and Batch Release" provides further guidance on batch release in the EU[72].

5.2 How can batch traceability be handled in continuous manufacturing?

Drug traceability is a particular challenge in the global drug-manufacturing world of today, e.g. the risk of falsified medicines getting to patients (Falsified Medicines Directive 2011/62/EU)[73]. The whole life cycle of a drug product from manufacturing until patient application must be comprehensible. Therefore batches/lots require being traceable forward and backward. This means for example that in case of any adverse drug reaction in a patient the batch of the drug substance needs to be identified quick, easy and reliable and if necessary for all products containing this drug substance a recall procedure can be started. Due to their complex nature mABs represent a particular challenge for traceability e.g. in case of serious adverse immune reactions.

As introduced in the previous paragraph 5.1, different approaches can be used to specify a batch or lot like production period, amount of material, equipment or changes in production. Continuous manufacturers should consider a few characteristics regarding batch or lot definition. The CFR defines a continuous batch as a certain unit of **time** or **quantity.** The Eudralex, volume 4 GMP-guideline uses a more restricted definition for batch in continuous

manufacturing. Here the continuous manufactured batch is defined as "**all the units manufactured in a given period of time"**[69]**.** Therefore to be compliant with EMA and FDA regulations on the global pharmaceutical market the manufacturer should define batch/lot as a unit of time. Furthermore a batch/lot in a continuous process needs to be of "uniform character" (21 CFR 210.3)[70]. To ensure this uniformity each batch needs to be monitored on a detailed level, including excipients, raw material traceability, etc. Any changes in quality parameters that exceed defined thresholds could lead to the termination of the respective batch.

During drug development and post-marketing manufacturers continuously try to improve their products or have to make changes to meet the current market demands. The following chapter gives an overview on mAB manufacturing process changes and the regulatory guidelines and guidance that should be consulted.

6 Manufacturing process changes to glycosylated monoclonal antibodies

In this chapter I will first present briefly the regulatory requirements for changes to approved biological drug applications and later discuss when it would be the best time point to implement a continuous manufacturing process.

6.1 Regulatory basis

MABs are highly complex proteins manufactured in a multi-complex process. Due to their complex structure they are more sensitive to manufacturing process changes than small molecules. Even small changes in the manufacturing process can dramatically impact quality, safety or efficacy, e.g. “changes in antibody glycosylation are a major cause of batch-to-batch variability during production”[74]. Appropriate glycosylation is among others one of the critical quality attributes for manufacturing of mABs and therefore must be demonstrated to obtain the market approval of competent authorities. In accordance with ICH and WHO guidelines the glycosylation of mABs should be appropriately characterised and tested and their impact on drug safety and efficacy thoroughly analysed[31,36,75,76]. According to ICH Q6B appropriate specifications with acceptable limits must be set and justified[31]. These specifications can be used to assess comparability of mABs “before and after changes are made in the manufacturing process for the drug substance or drug product” (ICH Q5E)[29] with an appropriate number of batches[76].

Due to the high variability of the glycan structure in proteins, manufacturers of therapeutic mABs always try to have a “humanized” mAB meaning a mAB with the human glycan profile. However the glycan profile of mABs is often so heterogeneous that it is sometimes unclear what the human structure really is. Therefore for each type of mAB the glycan structure must be evaluated on a case-by-case basis.

Another serious problem with complex mABs is that it is impossible to fully characterise mABs with the available analytical methods. There are also still gaps in the knowledge of the functional consequences of the process change on the therapeutic mABs and on the mode of action. Therefore it is absolute essential to demonstrate for any change the comparability in accordance with the ICH Q5E guideline on “Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process” from 2004[29]. **Table 6** summarizes the legal requirements for manufacturing changes for biologicals or biotechnological-derived products on the US and European market.

Table 6: The table provides an overview of the most important guidelines and guidance for post-marketing changes for biologicals or biotechnological-derived products in the US and EU.

Guidance	Remarks
Food and Drug Administration (FDA)	
21CFR601.12 "Changes to an approved application"[77]	21CFR601.12 (2) prescribes that "Before distributing a product made using a change, an applicant must assess the effects of the change and demonstrate through appropriate validation and/or other clinical and/or nonclinical laboratory studies the lack of adverse effect of the change on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product". Changes, e.g. - **major changes** - require "supplement submission and approval prior to distribution of the product made using the change" (21CFR601.12b) - „Supplement - Changes Being Effected in 30 Days" (**CBE-30 supplements**) - require „supplement submission at least 30 days prior to distribution of the product made using the change" (21CFR601.12c) - **minor changes** – „Changes to be described in an annual report" (21CFR601.12d)
"Guidances (Drugs) - **Demonstration of Comparability** of Human Biological Products, Including Therapeutic Biotechnology-derived Products" from 1996[37]	Describes the FDA concept of product comparability.
"Guidance for Industry: **Changes to an Approved Application** for Specified Biotechnology and Specified Synthetic Biological Products" from 1997[78].	Describes changes under 21CFR601.12.
"**Comparability Protocols** for Human Drugs and Biologics: Chemistry, Manufacturing, and Controls Information Guidance for Industry" DRAFT from April 2016[79]:	This guidance will replace the DRAFT guidance on "Comparability Protocols: Chemistry, Manufacturing, and Controls Information" from 2003. The aim of this guidance is to improve manufacturing continuously by - "Effective use of knowledge and understanding of the product and manufacturing process - A robust control strategy - Risk management activities over a product's life cycle - An effective pharmaceutical quality system"
"**Established Conditions**: Reportable CMC Changes for Approved Drug and Biologic Products Guidance for Industry" Draft from May 2015[80].	The Draft guidance defines the established conditions and how an applicant should prepare the application.

Table 6 continued:

Guidance	Remarks
European Medicines Agency (EMA)	
COMMISSION REGULATION **(EC) No 1234/2008** of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products[81]	§2 of the **variation regulation** defines the different types of variations depending on there impact on quality, safety or efficacy of the medicinal product concerned: - **Minor variation of type IA** (...) has only a minimal impact, or no impact at all, - **Major variation of type II** (…) may have a significant impact, - **Minor variation of type IB** means a variation which is neither a minor variation of type IA nor a major variation of type II Further variations are e.g. extensions.
COMMISSION REGULATION **(EU) No 712/2012** of 3 August 2012 amending Regulation (EC) No 1234/2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products[82]	Amendment to the variation regulation.
"Guideline on the details of the various categories of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products"[83] from 2013 (2013/C 223/01)	The variation guideline gives a list on the details of different variations.

Before implementing a process change the manufacturer should assess the consequences of this change using a risk-based approach (ICH Q9). Depending on the process knowledge or experience with these kind of change the risks could be assessed differently. However it is the responsibility of the manufacturer to demonstrate that product quality, safety and efficacy is not affected.

The first developed mAB manufacturing processes with recombinant cell lines had low protein expression levels leading to titers at or below 1 g/L, e.g. Humira™, Rituxan™, Herceptin™ and Remicade™[48]. The combination of low titers, high clinical dose requirements and the high market demand especially for these later block blusters led marketing application holders to increase there manufacturing capacity by building more plants and / or increasing the bioreactor size[48].

Section 6.1.1 and 6.1.2.outlines two examples for such manufacturing process changes.

6.1.1 Humira – an example for post-marketing manufacturing process changes

Humira (API: Adalimumab, section 3.3), one of the mAB blockbusters, was initially produced in a 3000 L fed batch bioreactor. Chinese Hamster Ovary (CHO) cells were used to express the recombinant human Adalimumab. During product life cycle the manufacturing process for Adalimumab needed to be changed several times dramatically. The scale of the fed batch bioreactor changed from 3000 L in 2003 to 6000 L in 2004, further manufacturing sites were added, one site added in 2007 with a scale of 12000 L, another one in 2013 with a scale of 20000 L. Taken together Abbvie manufactured Adalimumab at five different production scales at four different sites for over a decade[16]. Therefore the task for Abbvie was to align the different manufacturing sites, process control procedures and specification to comply with the requirements of product comparability[16].

To analyse the product quality of Adalimumab it was necessary to understand the product quality and heterogeneity of the glycosylations, and therefore be able to measure the effects of any manufacturing process changes on the biological functionality. Adalimumab carries a N-glycosylation site at the heavy chain. Therefore the N-glycosylation sites of Adalimumab were characterised in detail by using normal phase high performance liquid chromatography[16]. Beside the structural analysis also the biological activity, this means in the case of Adalimumab the binding to TNF, was analysed using an anti-TNF enzyme-linked immunosorbent assay (ELISA)[16]. These studies demonstrated that the oligosaccharide profile and the biological activity were rather comparable throughout the different scales and years.

Taken into account, that the process design is a crucial factor for the manufacturing of therapeutic mABs in the next example not only the post-marketing changes but also the changes during drug development will be considered.

6.1.2 Blockbuster Remicade – an example for manufacturing process changes

The drug substance of Remicade, Infliximab, is manufactured in murine myeloma cells[84] (section 3.3). Infliximab can occur in “five IgG glycoforms containing 0 to 4 galactose residues distributed between the 2 N-linked biantennary oligosaccharides structures located on Asn-300 of each heavy chain[84].

During drug development the focus changes. Clinical trial phase 1 is the proof of concept. In this phase the production process is not designed for commercial manufacturing. As clinical trials are progressing the manufacturing process was permanently improved during these later trial phases (see **Table 7**).

Table 7: Manufacturing process changes. The content of the table summarizes a selection of the manufacturing process changes during Remicade development. Data from Wojciechowski and colleagues[84].

Manufacturing process changes during Remicade drug development:		
Milestones	**Process changes**	**Remarks**
Toxicological studies and Phase 1 clinical trial	None (initial process)	At every stage of clinical development **comparability** for Infliximab was tested. **Analytical tests** to analyze protein, aggregates, carbohydrates, activity, purity, etc. were selected to "demonstrate comparability of identity, purity, primary structure, and bioactivity". Drug product from the initial manufacturing process was used for proof of concept. **Manufacturing process**: **perfusion bioreactor**, cell clone produced <9 µg/mL, perfusion bioreactor duration 45 to 50 days, 2 lots manufactured
Phase 2 clinical trial	**New cell line** – ten fold higher productivity **Serum free media** for bioreactor **Fewer purification steps** **Improved liquid formulation**	Comparability test revealed no significant changes in oligosaccharide structure. Manufactured lots: 5
Phase 3 clinical trial	Bioreactor **media composition** and **run time** - two fold increase of productivity. **4X scale up of the purification** process including high performance resins. Formulation change from liquid to **lyophilisate**.	To test comparability lots from previous manufacturing runs were tested side-by-side. Bioassays to test the inhibition of TNF-α were done. Manufactured lots: 3
BLA preparation	Bioreactor time **8X scale up of the purification** process	To further expand the scale of the purification process additional comparability lots were verified for BLA approval. Manufactured lots: 3
BLA approval by FDA; MAA approval by EMA	**Manufacturing site:** Leiden, NL approved	Show comparability to drug product used in clinical trials. Process validation was done with 5 consistency batches.

Although the manufacturing process of Remicade was developed continuously during the clinical trial phases the final marketing process was not sufficient to cover the current market demand. Therefore further adjustments were necessary to adjust the approved manufacturing process accordingly, e.g. by

- additional manufacturing site – “five drug product manufacturing facilities were filed to the BLA and MAA”,
- taken together dozens of major changes were submitted to FDA, and
- comparability protocols were submitted to apply for downgrades of reporting categories[84].

6.2 Manufacturing process change – from fed-batch to perfusion bioreactor

In this thesis I presented the advantages of a continuous manufacturing process and how the quality by design approach could improve the manufacturing of glycosylated monoclonal antibodies. Combining continuous manufacturing and QbD would bring the following benefits for the manufacturing of therapeutic mABs (the list is not exhaustive):

- Improvement of drug development and manufacturing, due to better process understanding
- Knowledge of the impact of critical materials, process parameters and quality attributes
- Continuous process monitoring of critical process parameters
- Reduction of risks and an increase of safety, due to less manual handling, less process steps and use of PAT and RTRT
- Reduced batch release tests
- Higher efficiency
- More flexibility
- Reduction of environmental food print
- Reduction of post-approval change applications

Today a fully integrated continuous process (as described in section 4.4) for glycosylated mABs cannot yet be realized due to the lack of commercially available equipment for the downstream process for a market production. But an available continuous manufacturing

process would be a continuous upstream manufacturing process that could be combined with a batch downstream process.

In the next sections I will compare two different approaches to change the upstream fermentation process from a fed-batch to a perfusion bioreactor.

6.2.1 Is this the time for a post-marketing change?

A manufacturing process change like the change from a fed-batch to a perfusion bioreactor would be in both the US and the EU a major change and would require extensive comparability studies. But in this scenario the changed bioreactor would not be the only change, because a cost-effective perfusion process requires a cell line that is compatible with the process. Many cell expression platforms are not suitable for the conditions in a perfusion reactor. Therefore it could be necessary to change not only the bioreactor but also the cell line. To make the whole process more cost-efficient one would not only have to change the cell line but would also try increase the productivity of the cell line. So the changes would require further changes, e.g. the composition of the cell media and other critical quality attributes. Until now this scenario focused only on the upstream process but these changes would also have a major impact on the downstream process, which must therefore also be taken into account.

The Draft Guidance for Industry on "Comparability Protocols"[79] suggests for recombinant DNA-derived proteins that have a complex and difficult to characterize structure like mABs to contact the FDA for any changes that could possibly affect the protein structure or post-translational modifications[79]. Such changes would require "appropriate comparative structural (e.g. primary and higher order structure, carbohydrate and attachment site analysis) and functional characterization (e.g., biological activity, binding assay), analytical procedures to be used, and criteria to demonstrate that the products before and after the change are analytically comparable"[79].

It is essential to demonstrate the comparability of mAB glycosylation after changes during drug development and also during post-approval manufacturing. Depending on the seriousness of the change comparability studies often require additional non-clinical and clinical data. Only in cases when the application is based on the QbD approach with an applied design space the manufacturing changes within the design space do not require any notification of authorities like FDA[57].

6.2.2 Biosimilars – Taking the chance to change?

Biosimilars for mABs are recently drawing more and more attention due to patent expiration of the mAB blockbusters (section 3.2, **Table 2**). After patent expiration of the originator the so-called biosimilars or "similar biotherapeutic products (SBPs)"[76] can enter the market. A biosimilar is defined as "a biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product, RMP) in the European Economic Area (EEA)"[85]. To apply for a biosimilar the applicant must show that the product is comparable to the originator.

As already discussed for manufacturing process changes in section 6.1 it is impossible to reproduce exactly the same glycosylated mAB with a slightly changed manufacturing process. Furthermore, the manufacturing process itself is another challenge for the applicant / manufacturer of a biosimilar product. The reason for this is that the originator keeps the manufacturing process confidential. Therefore it is always necessary to perform appropriate comparative pre-clinical test and clinical trials for biosimilar mAbs, to demonstrate, that the product is similar to the reference product in terms of "quality characteristics, biological activity, safety and efficacy"[85].

Today the first biosimilar mABs already entered the biopharmaceutical market.

The following table lists the guidelines and guidance that the applicant should take into account (in addition to the guidelines for biologics, section 3.4.1) then applying biosimilar.

Table 8: Important guidelines and guidance for biosimilar application.

Guidance	Remarks
WHO "Guidelines on Evaluation of Similar Biotherapeutic Products (SBPs)" from 2009[76].	The WHO guideline recommends for the development of a biosimilar to use - a "state-of-the art science and technology" manufacturing process; - the same host cell type for the expression of glycosylated protein, - the same formulation and - the same container closure system as used for the reference medicinal product. The applicant / manufacturer must demonstrate that the "structure of the molecule is not affected or that the clinical profile of the product will not change." The application must include all necessary information on the manufacturing process, e.g. development of - expression platform (expression vectors, cell line) - fermentation, - harvest, - purification, - formulation, etc.
EMA "Guideline on Similar Biological Medicinal Products Containing Biotechnology-derived Proteins as Active Substance: Non-clinical and Clinical Issues" from 2013[85].	According to the EMA guideline "the applicant "shall provide a full quality dossier together with data demonstrating comparability with the reference medicinal product by using appropriate physico-chemical and in vitro biological tests, non-clinical studies and clinical studies."
FDA "Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product." from 2015[86]	The FDA guidance points out that it is important to understand "heterogeneity of the proposed product and the reference product (e.g., the nature, location, and levels of glycosylation) and the ranges of variability of different isoforms, including those that result from posttranslational modifications."

To apply for a biosimilar mAB the applicant must demonstrate that the glycosylation pattern is similar compared to the reference medicinal product[76,85,86]. Already small differences in the heterogeneous glycosylation pattern that could possibly "alter the biodistribution of the product and thereby change the dosing scheme" can be evaluated as non-similar[76] (section 3.2). Such differences in the glycosylation pattern require additional non-clinical and clinical investigations.

In 2013 the EMA approved with Remsima™ and Inflectra™, both containing Infliximab as active substance, the first two biosimilar mABs[87,88]. Both biosimilars were approved for the same therapeutic indications as Remicade (section 3.3), e.g. rheumatoid arthritis, adult and

paediatric Crohn's disease, ulcerative colitis, psoriasis[87,88]. The active substance Infliximab is in all three drug products N-glycosylated at Asn[300]. In case of Remsima™ the heterogeneous N-glycan structure slightly differs to the originator and thus may lead to the decreased biological activity detected by the FcγRII-binding or ADCC assay[89]. Albeit these differences Remsima was approved by the EMA to be similar enough, because the clinical data showed comparable results to the reference product. The difference can be explained by a different manufacturing process, compared to the reference product, because the comparator does not know the exact manufacturing process of the originator.

In 2016 the FDA approved Inflectra[90]. Since 2013 four more mAB biosimilars received a marketing authorisation by the EMA (**Table 9**).

Table 9: A database search at ema.europa.eu "European public assessment reports" for authorised biosimilars on 22.05.2017 leads to the following results[91].

Originator			**Biosimilar**		
Reference medicinal product	**API**	**Marketing Authorisation Holder**	**Biosimilar**	**Marketing Authorisation Holder**	**Date of authorisation**
Humira	Adalimumab	AbbVie	Amgevita	Amgen Europe B.V.	22.03.2017
			Solymbic	Amgen Europe B.V.	22.03.2017
Rituxan	Rituximab	Roche	Truxima	Celltrion Healthcare Hungary Kft.	17.02.2017
Remicade	Infliximab	Janssen	Flixabi	Samsung Bioepis UK Limited (SBUK)	26.05.2016
			Inflectra	Hospira UK Limited	10.09.2013
			Remsima	Celltrion Healthcare Hungary Kft.	10.09.2013
Avastin	Bevacizumab	Roche	none		
Herceptin	Trastuzumab	Roche	none		

The MAA for the Adalimumab biosimilars, Amgevita and Solymbic were "based on a comprehensive data package supporting biosimilarity to adalimumab based on analytical, pharmacokinetic and clinical data, including results from two phase III"[92]. These clinical trial phase III studies "met their primary endpoint showing no clinically meaningful differences to

adalimumab"[92]. Parts of the application were also safety studies that demonstrated comparability to the reference medicinal product, Adalimumab[92].

The patents for Humira (API: Adalimumab) are expected to expire in Europe in 2018 (section 3.3, **Table 2**). Therefore the market entry for the biosimilars can only start afterwards. In the US Amgen received the approval for Amjevita (Adalimumab-atto) by the FDA in 2016[93].

Beside these already approved biosimilars a database search revealed the huge number of ongoing clinical trials for the active pharmaceutical ingredients of these blockbusters. A database search on ClinicalTrial.gov for the term "monoclonal antibodies" and the search parameter recruitment "open studies" on 19.04.2017 showed a total of 1326 studies. Most of these studies are in clinical trial phase 1 (number of clinical trials: 559[94]) and 2 (number of clinical trials: 701[95]). A closer examination of these studies revealed that a significant number of these studies are down for the known APIs of the mAB blockbusters Adalimumab, Rituximab, Bevacizumab, Trastuzumab, and Infliximab (**Table 10**).

Table 10: A database search at ClinicalTrial.gov for the term "*API*" for recruitment "open studies" on 19.04.2017 leads to the following results.

API	Total number of clinical trials
Adalimumab	126
Rituximab	349
Bevacizumab	355
Trastuzumab	187
Infliximab	96

Therefore we can expect that a number of biosimilars will enter market in the next few years, which could implement the continuous manufacturing. This development will further increase the pressure on all biotechnology companies and could thereby promote the development of more flexible and cost-effective manufacturing processes in the near future.

6.3 Theoretical examples for a switch to a fully integrated continuous manufacturing (CM) process

After understanding the regulatory basis and further background for manufacturing process changes the following chapter will outline two theoretical case studies how switch to a fully integrated continuous manufacturing process could be implemented.

For those exemplary case studies the following assumptions were made:

- The procurement of equipment to enable a manufacturer for continuous manufacturing is a significant investment not to be decided upon easily.
- The validation of continuous manufacturing processes causes more effort and is hence more expensive than standard batch processes.
- The implementation of QbD is more expensive than standard batch processes.
- In general there is little experience with continuous manufacturing in the industry. It is difficult to acquire proper personnel.
- Due to permanent technology and process innovations the manufacturing process for each product is constantly further improving.

The following case studies deal with the implementation of a fully integrated continuous manufacturing process for a) a biological and b) a biosimilar in the context of drug development.

6.3.1 Case study 1: Develop an Original Biological with a fully integrated CM

The development of a completely new biopharmaceutical drug product (Biological) is a long term and cost intense process. **Figure 5** depicts the general development steps and shows each of their development time frame, the overall success rate, the effort for developing according Design Space / QbD approach and the gathered process knowledge. At the beginning of the drug discovery for a new potential drug molecule the prospect of success is very limited. Only 5 out of 5000 tested molecules will ever reach the clinical trials. Hence from the commercial point of view it is crucial to define the best point in time to switch to continuous manufacturing.

As the complete development of a new Biological will span over a decade during this time the manufacturing design options will be constantly reviewed and updated with the newest knowledge of the development team, regulatory requirements, expected market share and the overall cost-benefit ratio. Depending on the detailed circumstances that decision might be quite diverse comparing different companies and / or products.

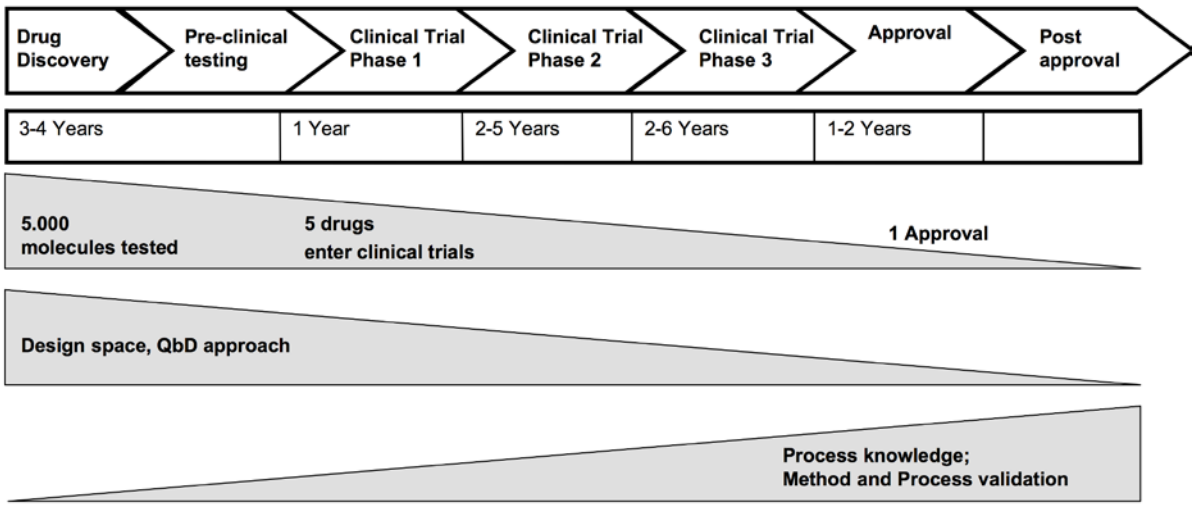


Figure 5: Biological – From Drug discovery to Approval.

The effort for comparability studies required for process changes is less the earlier continuous manufacturing is introduced in the development (see chapters 6.1.1 and 6.1.2 for Humira and Remicade). The disadvantage of an early introduction of CM is the early high invest in equipment, personnel etc. that might not at all be in relationship to the possible success rate of the respective product.

Considering those factors the author's opinion is to wait for the successful completion of the Clinical Trial Phase 1 before implementing CM. This makes comparability studies necessary – but those can be combined with further manufacturing process changes that are to be expected anyhow, e.g. formulation change or change of packaging size. That decision could also be taken after the first clinical trial of phase 2 to have more certainty about the possible success. But at this point at the latest the window of a good trade-off is increasingly diminishing.

6.3.2 Case study 2: Develop an Biosimilar with a fully integrated CM

The development of Biosimilars is faster and less cost intensive compared to the originator / the referenced medicinal product (RMP). As several factors that are unclear during the originator developments are known, also the overall approach is completely different.

The development of the Biosimilar is based on the comparability to the RMP. **Figure 6** depicts the general development steps. The drug discovery is obviously not necessary and also the preclinical and clinical studies are strongly shortened or can be cancelled totally if comparability could be proven sufficiently.

For a Biopharmaceutical the manufacturing process is one of the main influencing factors of product quality and is also crucially influencing the comparability. So it is necessary to start early with the implementation of the CM process for Biosimilars.

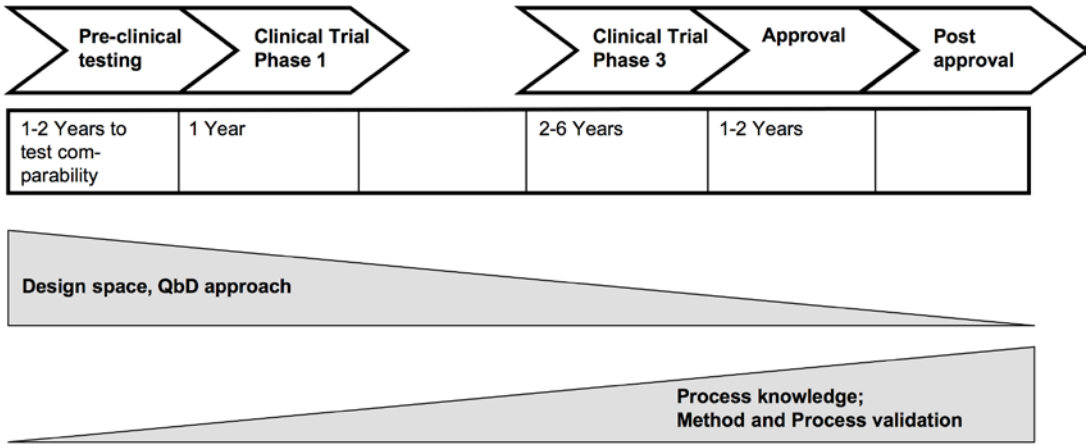


Figure 6: Biosimilar – From Drug discovery to Approval.

As with Biologicals the efforts, regulatory requirements as well as expected market share and cost and benefits will drive the decision. This consideration will be very different for each company / product and cannot be defined in general. The author proposes to decide upon the integrated manufacturing process at the very beginning of the development. If this would be tried later in the Biosimilar development process this very process would need to be started completely anew!

7 Discussion and Outlook

The chapters 3 to 6 portrayed the current discussion to introduce continuous processing to replace batch processing for biopharmaceutical substances on the example of glycosylated monoclonal antibodies (mABs). Special focus was given to the regulatory aspects in general as well as guidelines to consider during switchover from batch to continuous processing in particular. It became clear that although pharmaceutical companies producing small and chemical molecules increasingly use continuous processes combined with PAT for their production, still this trend cannot be observed in biopharmaceutical manufacturing where batch production is the standard for production of therapeutics for decades. Although it is common understanding that batch processing is inefficient and more time and resource consuming than continuous processing, the introduction of continuous manufacturing still lacks solutions to several obstacles:

- Absence of commercially available continuous downstream process units for large-scale production of mABs (see section 4.4).
- The manufacturers' uncertainty of the current regulatory requirements. As of today, the authorities did not release special requirements for biologic drug applications using continuous manufacturing beside the appropriate batch/lot definition (see chapter 5).
- The experiences with continuous manufacturing for biopharmaceuticals are still very limited.
- Difficulties with integrating the QbD approach into biopharmaceutical manufacturing itself and especially for the manufacturing of post-translational modified mABs.
- Challenges on the way to define an appropriate design space for mAB manufacturing.
- Without an approved design space process change are more complex and difficult.
- High process variability. To run a continuous manufacturing process better monitoring tools are required to collect more process data.
- Absence of suitable on-line PAT methods. The methods that are commercially available today are not able to analyse biologics to the same extant as small molecules.

- Absence of appropriate production planning and analytic software to adequately analyse the obtained process data, which could then be used for automatic adjustments in the following process steps.

- Furthermore there are some specific conditions regarding product quality, which should be considered when introducing continuous manufacturing, e.g. material stress, product contamination and batch uniformity. These specific risks for continuous manufacturing processes and possible risk mitigation and control strategies are listed in **Appendix 4**.

This list of obstacles is long and needs to be resolved in the upcoming years. But as the promise of higher efficiency, process stability and flexibility at the same time is still valid and unanswered, this leads to increasing efforts in the biopharmaceuticals community.

Recently continuous manufacturing of biologicals received a new push by the first approval for a switchover from an approved batch manufacturing process to a continuous manufacturing process by the FDA in 2016. Prezista (API: Darunavir) is a protease inhibitor that "prevents human immunodeficiency virus (HIV) cells from multiplying in the body"[96]. It is manufactured by Johnson & Johnson and Janssen as an "orally administered solid dose-form biologics"[96].

On the other hand, not only the biopharmaceutical companies make efforts, but also a number of international competent authorities have started different initiatives and / or published different guidance to support the implementation of continuous manufacturing in the development of new biologics, e.g.

- The **Emerging Technology Program** features the Emerging Technology Team – In December 2015 the FDA published a draft guidance on "Advancement of Emerging Technology Applications to Modernize the Pharmaceutical Manufacturing Base"[97]. With it the FDA "intends to develop guidance and standards, as necessary, on emerging technologies and approaches to enable the modernization of the pharmaceutical manufacturing base"[97]. To be able to participate in this CDER program the new technology must have "the potential to modernize the pharmaceutical manufacturing body of knowledge to support more robust, predictable, or cost-effective processes"[97].

- The European Commission launched the **Horizon 2020** initiative for "INDUSTRIAL LEADERSHIP - Leadership in enabling and industrial technologies – Biotechnology" as part of the Community Research and Development Service (Cordis) program. One of the programs funded is the project the "Next-generation biopharmaceutical

downstream process“[98]. The main task of this particular project is to optimize downstream processing by developing continuous downstream process steps (see **Appendix 5**).

To sum up this chapter as well as the thesis it can be stated, that continuous manufacturing of glycosylated monoclonal antibodies promises several major advantages in terms of efficiency, stability and flexibility at the same time. There are several obstacles that are not yet overcome. But industries as well as authorities do not stop to strive for a change from batch to continuous manufacturing. Having this in mind manufacturers do well in observing the next few years of development to not lose track with rather significant changes in manufacturing paradigms.

8 Glossary

Term	Definition
Acceptance Criteria	"Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet."[31] (ICH Q6B)
Biological Activity	"The specific ability or capacity of the product to achieve a defined biological effect. Potency is the quantitative measure of the biological activity."[31] (ICH Q6B)
Biosimilar	A biosimilar is a similar biological medicinal product.
Cell bank	"A cell bank is a collection of appropriate containers, whose contents are of uniform composition, stored under defined conditions. Each container represents an aliquot of a single pool of cells." (ICH Q5D)
Cell line	"Type of cell population which originates by serial subculture of a primary cell population, which can be banked." (ICH Q5D)
Critical Process Parameter (CPP)	A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality. (ICH Q8 (R2))
Critical Quality Attribute (CQA)	A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. (ICH Q8 (R2))
Critical Material Attribute (CMA)	"A physical, chemical, biological or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material."[99]
Design Space	"The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval." (ICH Q8)
Desired Product	"(1) The protein which has the expected structure, or (2) the protein which is expected from the DNA sequence and anticipated post-translational modification (including glycoforms), and from the intended downstream modification to produce an active biological molecule."[31]
Drug Product (Finished Product)	"A pharmaceutical product type that contains a drug substance, generally, in association with excipients." (ICH Q6B)
Drug Substance (Bulk Material)	"The material which is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients including other components such as buffers." (ICH Q6B)
Design Space	"The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval." (ICH Q8)
Expression construct	The expression construct is defined as "the expression vector containing the coding sequence of the recombinant protein"[26]

Term	Definition
First generation biopharmaceuticals	First generation biopharmaceuticals are unengineered native proteins.
MCB (Master Cell Bank)	“An aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a new MCB (from a previous initial cell clone, MCB or WCB) should be the same as for the MCB unless justified.” (ICH Q5D)
Potency	The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties.
Process Analytical Technology (PAT):	“A system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.” (ICH Q8)
Quality	The suitability of either the drug substance or drug product for its intended use. This term includes such attributes as the identity, strength, and purity. (ICH Q6A)
Quality by Design (QbD)	“A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.” (ICH Q8 (R2))
Quality Target Product Profile (QTPP)	“A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product.” (ICH Q8 (R2))
Reference biotherapeutic product (RBP) or RMP	“A reference biotherapeutic product is used as the comparator for head-to-head comparability studies with the similar biotherapeutic product in order to show similarity in terms of quality, safety and efficacy. Only an originator product that was licensed on the basis of a full registration dossier can serve as an RBP. The term does not refer to measurement standards such as international, pharmacopoeial or national standards or reference standards.”[76] (WHO)
Reference Medicinal Product (RMP) or RBP	Medicinal product that has been granted with a marketing authorization in the European Economic Area (EEA).
Real Time Release Testing (RTRT)	“The ability to evaluate and ensure the quality of in-process and/or final product based on process data, which typically include a valid combination of measured material attributes and process controls.” (ICH Q8 (R2))
Second generation biopharmaceuticals	“Are engineered proteins, which can contain changed amino-acid sequences or post-translational modifications, e.g. glycosylations.” (ICH Q6B)
Similarity	“Absence of a relevant difference in the parameter of interest.”[76] (WHO)
Specification	“A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. ““Conformance to specification” ” means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval.” (ICH Q6B)
Variation	“A variation is a change to the terms of a marketing authorisation.”[100]

Term	Definition
WCB (Working Cell Bank)	“The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.” (ICH Q5D)

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10 Appendixes

Appendix 1: “Glycosylation strategies for modifying FcγR and complement interactions”[9].

Appendix 2: mAB sales in 2015 adapted from “2015 Product Sales Data From Annual Reports Of Major Pharmaceutical Companies”[13].

Appendix 3: Integrated Continuous Manufacturing of Therapeutic Protein Drug Substances (US 20140255994 A1) [67].

Appendix 4: Specific Risks for Continuous manufacturing processes.

Appendix 5: Next Generation Biopharmaceutical Downstream Process[98].

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Appendix 1 – "Glycosylation strategies for modifying FcγR and complement interactions"[9]

The "variable region is responsible for the antigen binding properties of IgG antibodies, it can also influence their pharmacokinetics, pharmaceutical properties and immunogenicity"[101]. "The Fc region is responsible for the effector functions and the pharmacokinetics"[101]. Igawa et al. are discussing a set of possibilities to engineer the variable region of therapeutic IgG antibodies[101]. The table shows glycosylation strategies and their potential impact on biologic activity.

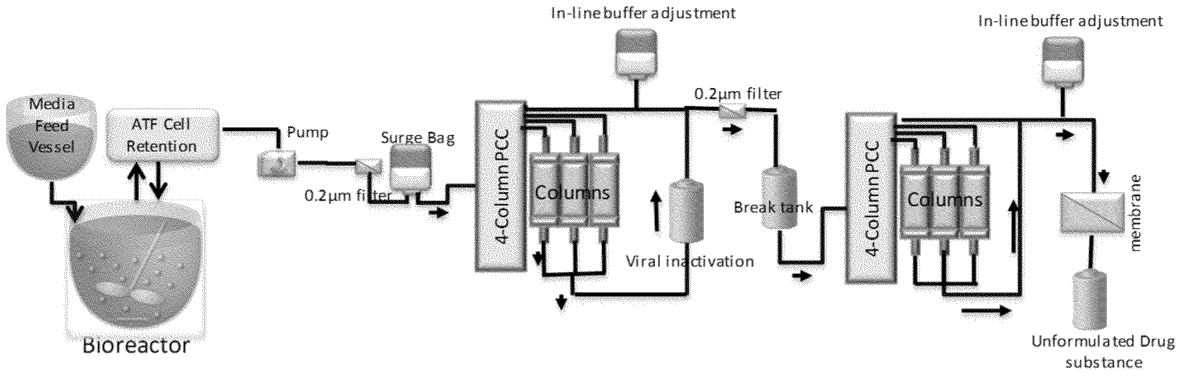
Strategies for modifying interactions	**Potential impact of modification**	**Comment**
Aglycosylation	⇓ ADCC, ⇓ ADCP and ⇓ CDC	„mutating the conserved aspargine, Asn297, prevents glycosylation to generate aglycosylated antibodies lacking effector functions"[9]
Bisecting N-acetylglucosamine	⇑ ADCC	„ADCC can be enhanced by increasing the bisecting N-acetylglucosamine in the Fc carbohydrate"[9]
Non-fucosylation	⇑ ADCC	„ADCC can be enhanced (...) by eliminating fucose"[9]

Appendix 2 – mAB sales in 2015 adapted from "2015 Product Sales Data From Annual Reports Of Major Pharmaceutical Companies"[13]

Product Name	Company Name	Active Ingredient	Main Therapeutic Indication	Currency	2014 Revenue in Millions	2015 Revenue in Millions	2014 Revenue in Millions (USD)	2015 Revenue in Millions (USD)	Sales Difference in Millions (USD)	Growth (%)
Humira	AbbVie	Adalimumab	Immunology (Organ Transplant, Arthritis etc.)	USD	12.543	14.012	12.543	14.012	1.469	12%
Avastin	Roche	Bevacizumab	Oncology	CHF	6.417	6.684	6.481	6.751	270	4%
Herceptin	Roche	Trastuzumab	Oncology	CHF	6.275	6.538	6.338	6.603	265	4%
Remicade	Johnson & Johnson	Infliximab	Immunology (Organ Transplant, Arthritis etc.)	USD	6.868	6.561	6.868	6.561	-307	-4%
MabThera/Rituxan	Roche	Rituximab	Oncology	CHF	5.603	5.640	5.659	5.696	37	1%
Soliris	Alexion Pharmaceuticals	Eculizumab	Blood Related Disorders	USD	2.234	2.590	2.234	2.590	356	16%
Stelara	Johnson & Johnson	Ustekinumab	Immunology	USD	2.072	2.474	2.072	2.474	402	19%
Lucentis	Novartis	Ranibizumab	Opthamology	USD	2.441	2.060	2.441	2.060	-381	-16%
Tysabri	Biogen	Natalizumab	Neuroscience and Mental Health	USD	1.960	1.886	1.960	1.886	-74	-4%
Remicade	Merck & Co	Infliximab	Immunology (Organ Transplant, Arthritis etc.)	USD	2.372	1.794	2.372	1.794	-578	-24%
Lucentis	Roche	Ranibizumab	Opthamology	CHF	1.701	1.520	1.718	1.535	-183	-11%
Perjeta	Roche	Pertuzumab	Oncology	CHF	918	1.445	927	1.459	532	57%
Actemra/RoActemra	Roche	Tocilizumab	Immunology (Organ Transplant, Arthritis etc.)	CHF	1.224	1.432	1.236	1.446	210	17%
MabThera/Rituxan	Roche	Rituximab	Oncology	CHF	1.297	1.405	1.310	1.419	109	8%
Xgeva	Amgen	Denosumab	Oncology	USD	1.221	1.405	1.221	1.405	184	15%
Simponi/Simponi Aria	Johnson & Johnson	Golimumab	Immunology (Organ Transplant, Arthritis etc.)	USD	1.187	1.328	1.187	1.328	141	12%
Prolia	Amgen	Denosumab	Bone Health	USD	1.030	1.312	1.030	1.312	282	27%
Xolair	Roche	Omalizumab	Respiratory Disorders	CHF	975	1.277	985	1.290	305	31%
Yervoy	Bristol-Myers Squibb	Ipilimumab	Oncology	USD	1.308	1.126	1.308	1.126	-182	-14%

Product Name	Company Name	Active Ingredient	Main Therapeutic Indication	Currency	2014 Revenue in Millions	2015 Revenue in Millions	2014 Revenue in Millions (USD)	2015 Revenue in Millions (USD)	Sales Difference in Millions (USD)	Growth (%)
Opdivo	Bristol-Myers Squibb	Nivolumab	Oncology	USD	6	942	6	942	936	15600%
Xolair	Novartis	Omalizumab	Respiratory Disorders	USD	777	755	777	755	-22	-3%
Synagis	AbbVie	Palivizumab	Respiratory Disorders	USD	835	740	835	740	-95	-11%
Simponi	Merck & Co	Golimumab	Immunology (Organ Transplant, Arthritis etc.)	USD	689	690	689	690	1	0%
Synagis	AstraZeneca	Palivizumab	Respiratory Disorders	USD	900	662	900	662	-238	-26%
Keytruda	Merck & Co	Pembrolizumab	Oncology	USD	55	566	55	566	511	929%
Vectibix	Amgen	Panitumumab	Oncology	USD	505	549	505	549	44	9%
Erbitux	Bristol-Myers Squibb	Cetuximab	Oncology	USD	723	501	723	501	-222	-31%
Erbitux	Eli Lilly	Cetuximab	Oncology	USD	373	485	373	485	112	30%
Cyramza	Eli Lilly	Ramucirumab	Oncology	USD	76	384	76	384	308	405%
Benlysta	GlaxoSmithKline	Belimumab	Rare Disease	GBP	173	230	246	327	81	33%
Lemtrada	Sanofi	Alemtuzumab	Neuroscience and Mental Health	EURO	34	243	37	267	230	622%
Cosentyx	Novartis	Secukinumab	Immunology (Organ Transplant, Arthritis etc.)	USD	0	261	0	261	261	New Launch
Ilaris	Novartis	Canakinumab	Rare Disease	USD	199	236	199	236	37	19%
Reopro	Eli Lilly	Abciximab	Cardiovascular	USD	111	98	111	98	-13	-12%
Praluent	Sanofi	Alirocumab	Hypercholesterolaemia	EURO	0	9	0	10	10	New Launch
Empliciti	Bristol-Myers Squibb	Elotuzumab	Oncology	USD	0	3	0	3	3	New Launch

Appendix 3 – Integrated Continuous Manufacturing of Therapeutic Protein Drug Substances (US 20140255994 A1)[67]



*) Figure from US 20140255994 A1[67]

The perfusion upstream bioreactor is connected to a downstream manufacturing process containing two Periodic Counter-Current Chromatographic System (PCCS)[67].

The advantages of the PCC system compared to a standard batch downstream process include "an increase in the volumetric productivity, an increase in the chromatography media capacity utilization, a decrease in the volume of buffer usage, and a decrease in the volume of the individual column size"[67]. These are summarized in the following two tables[67]:

TABLE 3

Exemplary Advantages of Two PCC System

Parameters		Units	Batch	Continous Processing: Protein A column	Continous Processing: Capto-S column
Resin capacity		Normalized (%)	100	120	110
Buffer Usage		Normalized (%)	100	75	80
Column	Volume	Normalized (%)	100	3.9 (X 3)	4.7 (X3)
	Diameter	Normalized (%)	100	16	40
	Height	Normalized (%)	100	47	30

TABLE 4

Exemplary Advantages of Two PCC System

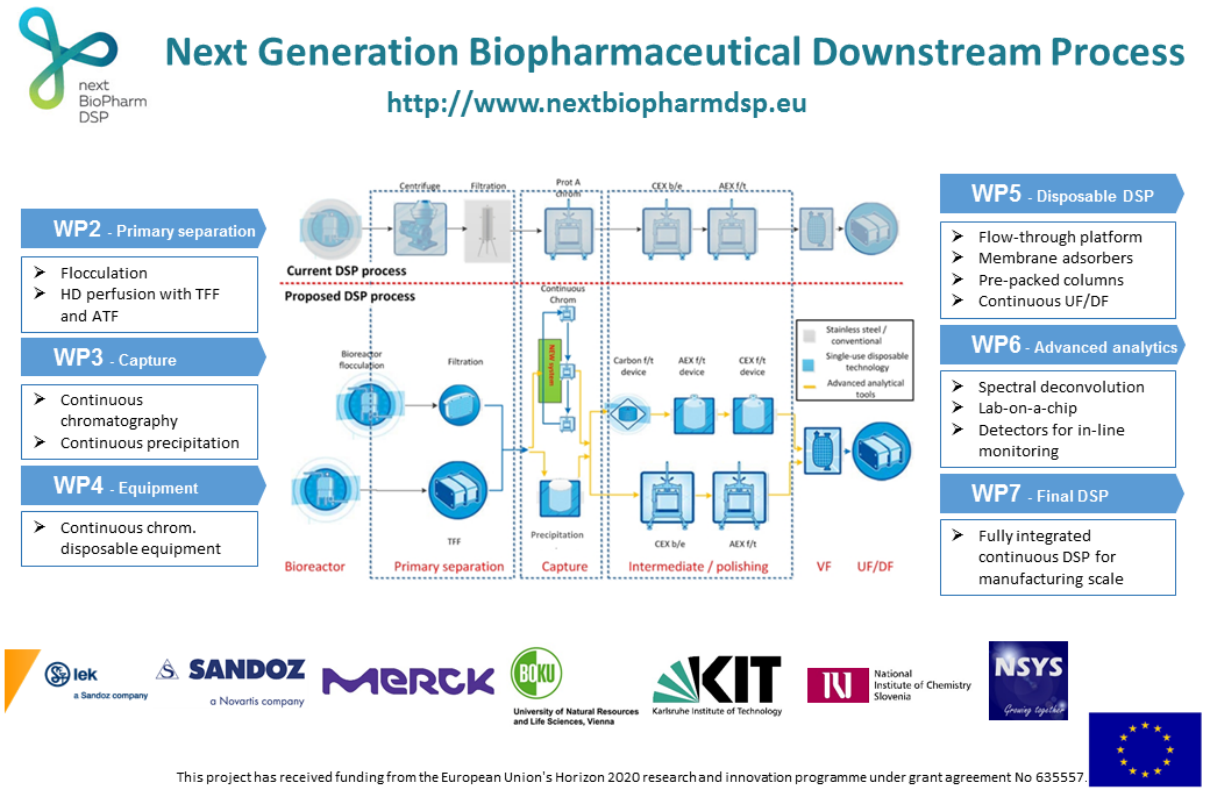
Parameters	Current Batch	Continuous Platform Process
Upstream Cycle Time	1 BRX volume/ 14 days	1 BRX volume/ 12 hours
Downstream Cycle Time	Days-Months	~10 hours
Volumetric Productivity (g/L-Day)	~0.1-0.2	~1.2
Automation	Batch mode, discrete unit ops	Fully automatic
Total Number of downstream unit Operations	9 (harvest, Centrifugation, depth filtration, Pro A, VI, Capto S, Capto Q, viral filtration, sterile filtration)	3 (PCC1, PCC2 and Viral filtration)
Hold Steps	9	None

*) Tables from US 20140255994 A1[67]

Appendix 4 – Specific Risks for Continuous manufacturing processes

Risk Assessment	Risk mitigation	Risk control	Remarks
Material stress, e.g. material fatigue	Use of suitable materials, validated processes	Suitable in-process tests	Materials (e.g. single use or stainless steel bioreactors) are longer in contact with the medium.
Product contamination, e.g. extractable and leachable	Use of suitable qualified materials	Acceptance criteria	Single use equipment has increased product contact times.
Batch uniformity depending on process conditions, e.g. pH, O_2, medium, cell line, buffers	Use of qualified equipment, processes and parameter monitoring	Suitable in-process and / or RTR testing	Process understanding and knowledge is essential to keep the process in acceptable ranges over time.
Batch uniformity depending on raw material	Qualified suppliers, suitable materials	Raw material control, specifications, traceability of raw material lots	Different lots of raw materials can be used.

Appendix 5 – Next Generation Biopharmaceutical Downstream Process[98]



*) Figure from http://www.nextbiopharmdsp.eu

The project has a term of 3 years and started in March 2015. The Figure presents a scheme of the work packages (WP). WP1 (not shown) is the Management work packages and is responsible for the project coordination and communication with the European commission[98]. The task of WP2 "Primary separation" is to develop a „cell separations based on flocculation, depth filtration, tangential flow filtration (TFF) and alternate flow filtration (ATF) rather than disc stack centrifugation"[98]. Both flocculation and filtration will be developed to run continuously[98]. In WP3 the project team will evaluate the continuous multi-column chromatography (CMC) and a continuous precipitation[98]. In WP4 the "Continuous chromatography equipment" the project team will develop a "fully automated disposable equipment (including Hardware, Single-use flowpath and automation platform)"[98]. In WP5 the "Disposable DSP" one task is the development of a continuous UF/DF process[98]. "WP6 aims to develop analytical techniques for in-line/at-line use to ensure that product quality attributes are monitored during continuous processing"[98]. In WP7 the "Final DSP" the project team "will implement a fully integrated manufacturing platform based on continuous chromatography in combination with single-use disposable techniques for all unit operations of DSP on small production scale together with incorporation of advanced analytical tools"[98].

Erklärung

Hiermit erkläre ich an Eides statt, die Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Frankfurt am Main,

Danksagung

Ich danke meinem Mann für die Unterstützung während der gesamten Zeit des Studiengangs und insbesondere auf den letzten Metern.