EDITION
2022

BIOPROCESSWATCH
MAB PRODUCTION

mabdesign
THE BIOTHERAPY EXPERT

ABOUT **MABDESIGN**

MabDesign, the French Association of the Biotherapy Industry

MabDesign, the French biotherapy industrial association, aims to support, federate and increase the visibility of the biopharmaceutical industry, foster exchanges, promote the development and competitiveness of companies, and stimulate innovation by encouraging the emergence of start-ups from academic research.

In order to carry out its development strategy and to adapt to changes in the industrial ecosystem, MabDesign's governance has evolved to meet the specific needs of the various companies working in the biotherapy industrial sector. Therefore, the Board of Directors of MabDesign already composed of DBV Technologies, Lyonbiopole, Pierre Fabre and Sanofi, has been strengthened with the arrival of ABL Europe, bioMérieux, Institut Pasteur, Thermo Fisher Scientific and TreeFrog Therapeutics as well as four Qualified Persons with Nicola Beltramineli (Innate Pharma), Hervé Broly (Merck), Philippe Germanaud (SANOFI), and Stéphane Legastelois (33 California). Their arrival to the Board of Directors reinforces MabDesign global vision of the current challenges and opportunities of the biopharmaceutical industry.

Moreover, to achieve its goals MabDesign sets up a coherent set of actions promoting exchanges, collaborations and skills development. In this dynamic MabDesign has developed a **national directory** that brings together industrial and academic players in biotherapy and allows to identify online the know-how available in France. MabDesign organizes high-level **international scientific events**, in collaboration with key ecosystem players, to highlight innovation and stimulate exchanges between companies in the sector. With the help of its Scientific Committee (**COSSF**), MabDesign writes summary reports (**ImmunoWatch and BioprocessWatch**) for the biotherapy industry. MabDesign offers specialized and **innovative continuous professional training** solutions to enable companies to adapt their skills to the market evolution and maintain their competitiveness. Finally, MabDesign offers its members a **wide range of services** to help companies of all sizes to optimize their positioning, protect and enhance their innovations, conquer new markets and raise public funds.

Operational since September 2015, MabDesign currently has over **270 member companies** and its diversity is its strength. MabDesign's dynamic network includes pharmaceutical and biotech companies, service providers (eg. CROs, CDMOs, etc), professional training actors, high-tech equipment suppliers and specialized consultants.

Bât. L'Initial
17, rue Crépet
69007 Lyon
Tel. 04 78 02 39 88
contact@mabdesign.fr
www.mabdesign.fr

mabdesign
THE BIOTHERAPY EXPERT

TABLE **OF CONTENTS**

INTRODUCTION

For several years now, MabDesign has been actively participating in national and regional programmes and organising scientific events and gatherings focusing on bioprocessing. In parallel, we have also been providing strategic consultancy services together with various training opportunities to key actors of this field, including academia, public bodies, SMEs and biotech and pharmaceutical companies, that are involved in the shaping of the bioprocessing industry in France through their R&D, innovation, technologies, services and products. In line with these past and current actions and to further our commitment and support to the French bioprocessing industry, MabDesign has launched in 2021 a second information-monitoring letter, the BioprocessWatch series.

Each edition of BioprocessWatch will focus on current challenges, a critical step or a recent innovation linked to the manufacturing of a specific biopharmaceutical product or affecting the whole field. BioprocessWatch will feature invited scientific contributions from academia and/or the industry, the most recent pipeline, economic and financial data (where applicable), insights into the intellectual property related to the theme and opinion articles and interviews from one or two experts working in the field.

Finally, we would like to acknowledge the continued support from the Centre-Val-de-Loire (CVL) region, through their Ambition Recherche et Developpement (ARD) CVL Biomédicaments programme, in making the launching of the BioprocessWatch series possible.

Editorial

Alain Beck

Senior Director Biologics CMC & Developability

Pierre Fabre

Hervé Broly

Executive CMC Consultant for Biologics

Merck-Serono SA

Monoclonal antibodies-based products (mAbs) remain dominant in biopharmaceuticals approvals (54 % of all approvals in the past four years in the United States and/ or in Europe, Walsh G and Walsh E, 2022). Products approved over these four years include 97 mAbs, 19 hormones, 16 nucleic acid/gene-therapy-based products and 16 vaccines (5 of the COVID-19 vaccines are nucleic acid based). Additional approval categories include colony-stimulating factors (CSFs biosimilars), cell-based products, enzymes, fusion products, and clotting factors.

In terms of sales, originator mAbs represented 80% of total protein-based global biopharmaceutical sales last year (218 USD billions, La Merie Publishing, 2022); Non-mAb recombinant proteins (54 USD billions), Covid vaccines (Comirnaty and Spikevax, 54 USD billions), biosimilars (11 USD billions) and nucleic acid and cell therapies (7 USD billions).

On the production side, Chinese Hamster Ovary (CHO) cells are the most frequently used mammalian system (95 products approved in the last four years) thanks in great part to the ability to produce antibodies at titers of 3–8 g/liter at production scale (www.actip.org). Other mammalian systems used NS0 mouse myeloma (7 products), baby hamster kidney (BHK), human embryonic kidney (HEK), SP2/0 mouse myeloma, PER.C6 immortalized primary human embryonic retinal and rat YB2/0 cells (1 product each). A single new product (Sevenfact) is produced via transgenic means (milk of transgenic rabbits). For non-mammalian production platforms, Escherichia coli continues to dominate (36 products approved since 2018) versus Pichia pastoris (5) and Saccharomyces cerevisiae (4).

In 2022, 13 antibody therapeutics had been granted first approvals by the Food and Drug Administration (FDA, www.fda.gov) and/ or the European Medicine Agency (EMA, www.ema.europa.eu) : tebentafusp (Kimmtrak), faricimab (Vabysmo), sutimlimab (Enjaymo), relatlimab (Opdualag), tixagevimab/cilgavimab (Evusheld), mosunetuzumab (Lunsumio), teclistamab (Tecvayli), spesolimab (Spevigo), tremelimumab (Imjudo; combo with durvalumab), nirsevimab (Beyfortus), mirvetuximab soravtansine (Elahere), teplizumab (Tzield) and ublituximab (Briumvi) including 4 bispecific antibodies (BsAbs), 1 ADC and multiple Fc-engineered mAbs (Kaplon H et al, 2022; www.antibodysociety.org). In addition, Biologic License Applications (BLAs) or MAAs (Marketing Authorization Application) for 23 antibody therapeutics are undergoing review by either FDA or EMA, respectively. Based on PhRMA estimations, 7800 biopharmaceuticals are in clinical development including 2533 mAbs, 546 nucleic acid and gene-based therapies and 348 gene-modified cell therapies (www.phrma.org).

From the start of the COVID-19 pandemic in January 2020 through December 2022, at least 7 mAbs or cocktails (casirivimab + imdevimab, bamlanivimab + etesevimab, sotrovimab, regdanvimab, tixagevimab + cilgavimab, bebtelovimab, amubarvimab + romlusevimab) were authorized in the USA, Europe, South-Korea, or China. These treatments reached the patients within 1–2 years of the project start thus showing reduced time to clinical trials by 75% or more without creating unacceptable patient or product-safety risks (Kelley B et al, 2022). The product Critical Quality Attributes (CQAs), process development, and manufacturing risks associated with recombinant mAbs are now very well-understood. Similar pathways to pre-clinical toxicology studies, accelerate pharmaceutical development and first-in-human studies may also be used in the future for oncology, inflammation, and rare diseases.

BIOASSAYS QUALIFICATION FOR QC BATCH RELEASE

Building a reliable, reproducible cell-based potency bioassay that will be suitable as a current good manufacturing practice (#cGMP) release test for Biodrugs and Biosimilars characterization:

The better controlled the cells are, the more precise the bioassays are.

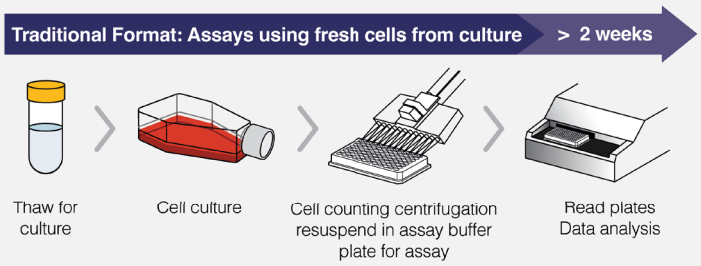
Need for ready-for-use cells?

40+ years CUSTOM EXPERIENCE

INNOVATIVE PARTNER

Thaw and Use (TnU) and Cell Propagation Model (CPM)

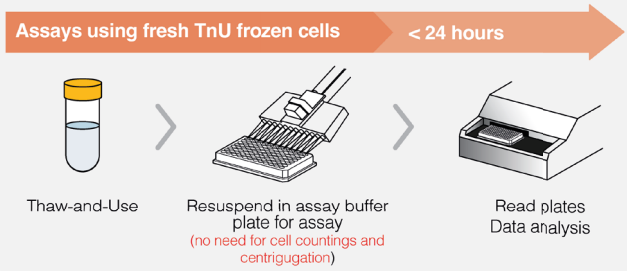
Cell Propagation Model



Challenges:

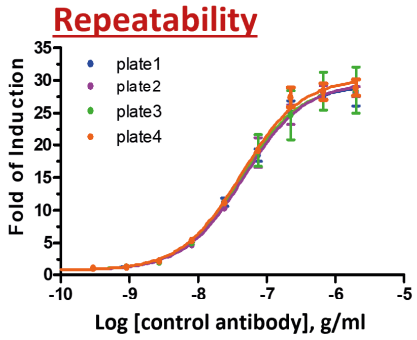
- Cell culture is labor intensive
- Cell maintenance has high cost
- Daily cell culture causes high run-to-run variation
- Slow and variable
- Robustness

"Cells As Reagents" Method



Benefits:

- Simple and convenient
- Fast (time savings)
- Cost savings
- Controlled run-to-run consistency
- Improved reproducibility
- Easy lab-to-lab transfer
- Compatible with automation



ICH Q6B guidelines

Validation of Analytical Procedures

- Accuracy
- Precision:
  - ▶ Repeatability (intra-assay precision)
  - ▶ Intermediate precision (day to day, analyst-to analyst)
  - ▶ Reproducibility (lab to lab)
- Specificity
- Linearity
- Range
- Robustness

Discover more Promega Bioassays as PBMC ADCC Assays, Lumit Immunoassays, Gene Reporter Bioassays…

www.promega.com

GLOBAL ANTIBODY market

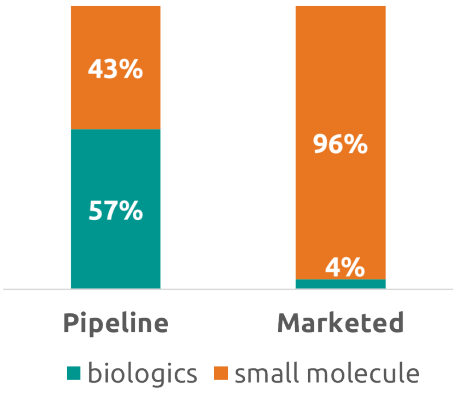
Discover the marketed products, pipeline drug candidates, major deals and biopharmaceutical companies

Monoclonal antibody market

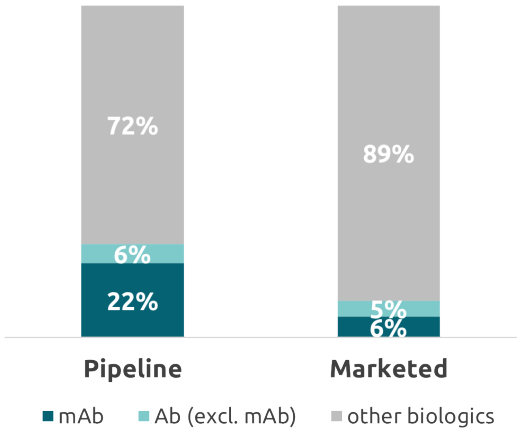
170 Marketed Antibodies

- **139** Monoclonal Antibodies
- **12** Biosimilars
- **19** ADCs
- **13** Antibody therapeutics FDA or EMA approved in 2022

Small molecules vs biopharmaceuticals

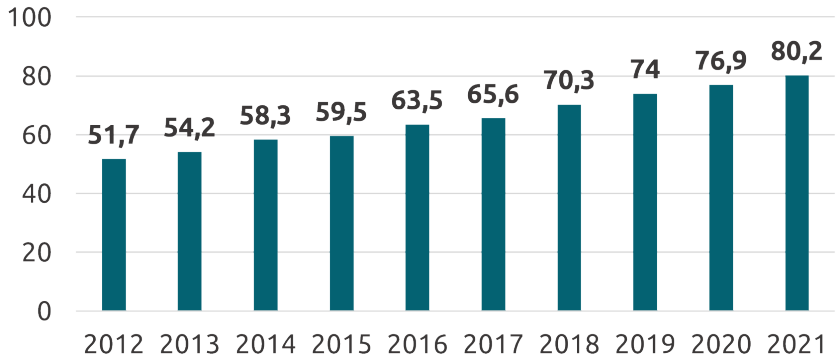


Antibodies vs other biopharmaceuticals



Currently, biopharmaceuticals represent the main driver of R&D innovation. More than half of the products in development are biologics. The most mature market is the mAbs market, which accounts for nearly 1/4 of the biologics under development. Antibodies, of which mAbs represent the majority of products on the market, account alone for over 80% of biologics sales in 2021.

Percentage antibody sales (of total biologics sales)

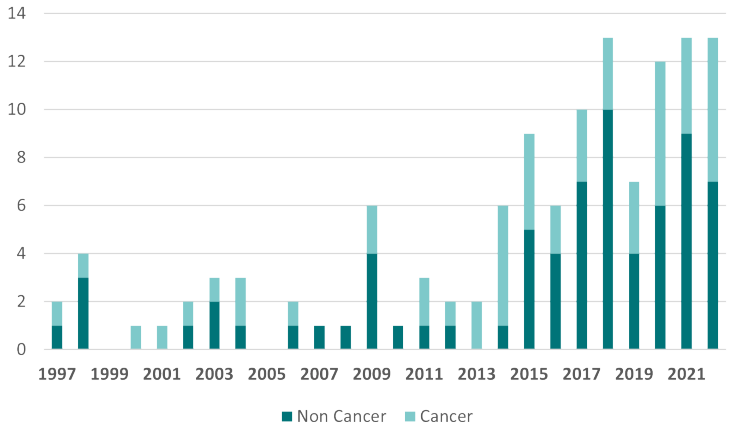


Sources: GlobalData, Antibody Society, LaMerie Business Intelligence

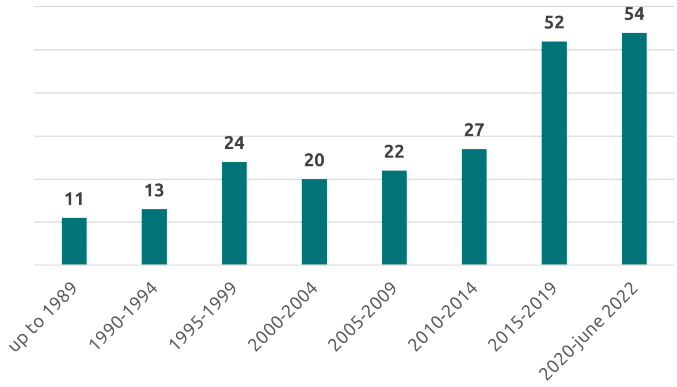
Monoclonal antibody market

The market for mAbs is dynamic and growing, with more mAbs being approved each year. Since 2015, more than half of marketed biopharmaceuticals are mAbs.

Number of antibody therapeutics granted a first approval in US or EU each year, 1997-2022

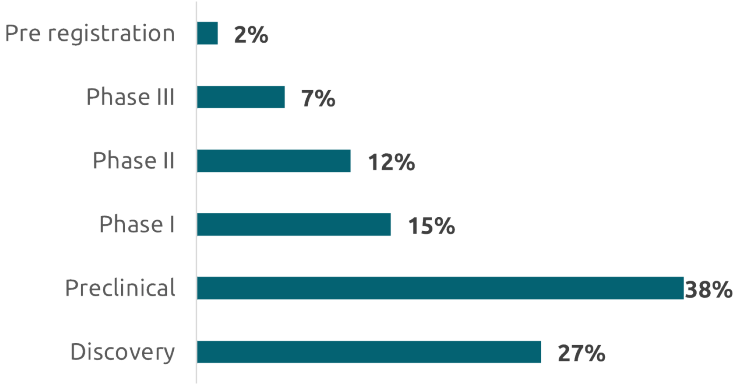


mAbs approval as percentage of total biopharmaceuticals approvals

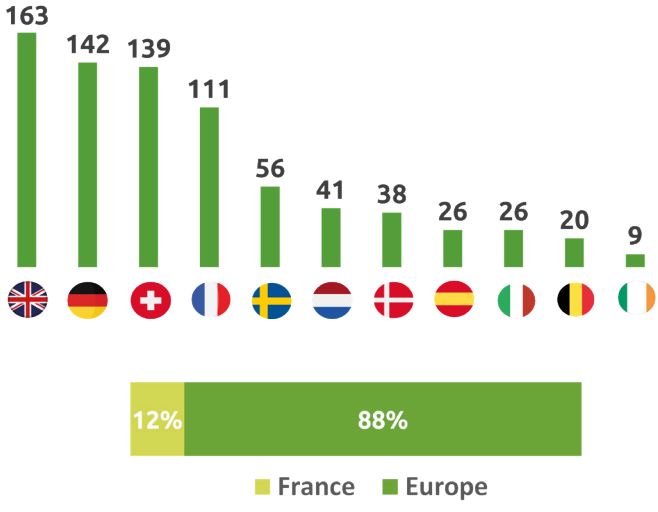


This mature market relies on a robust pipeline and dynamic research. In this specific market, France are in top 5 European countries for mAbs development with French companies currently developing 12% of the products

Monoclonal Antibody Pipeline



Number of mAbs in development by company location



Sources: GlobalData, Antibody Society, LaMerie Business Intelligence

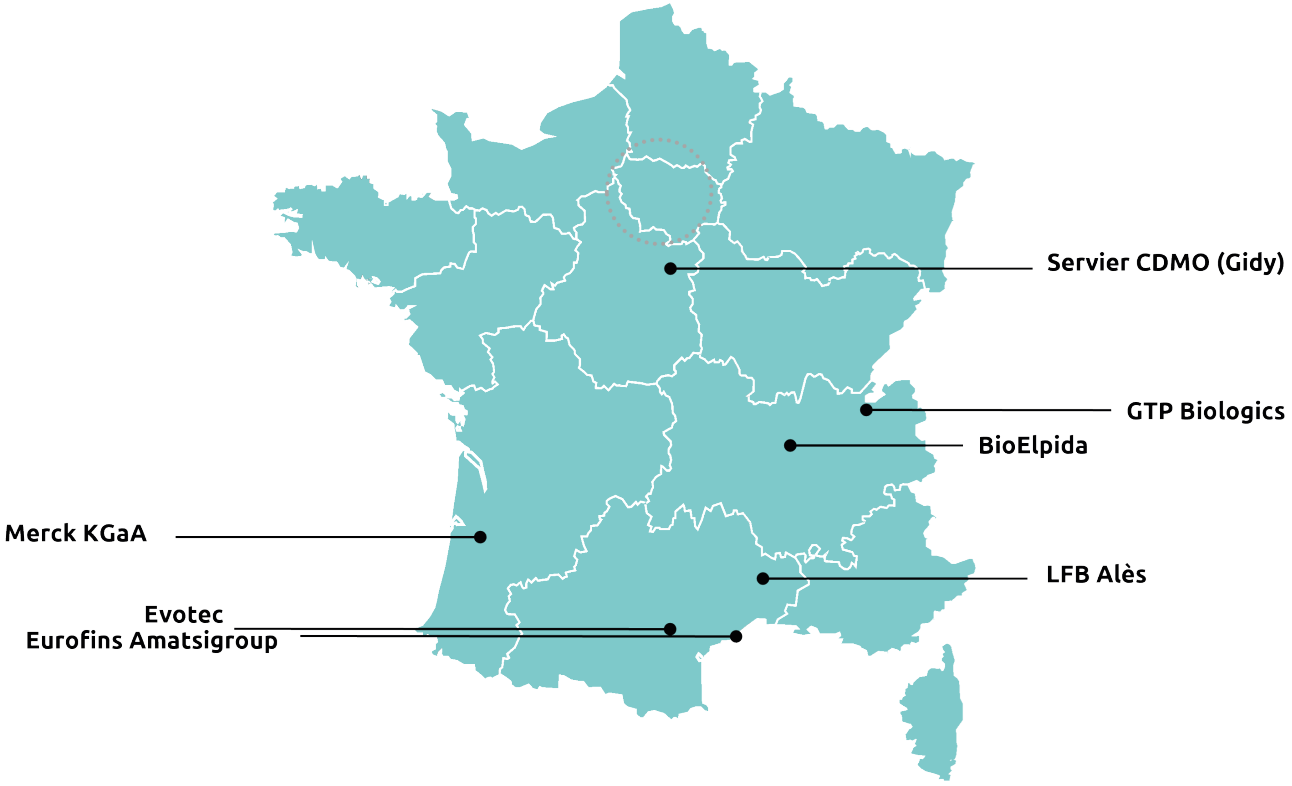
Monoclonal antibody CMO/CDMO

Distribution by location of Headquarters

North America

Europe

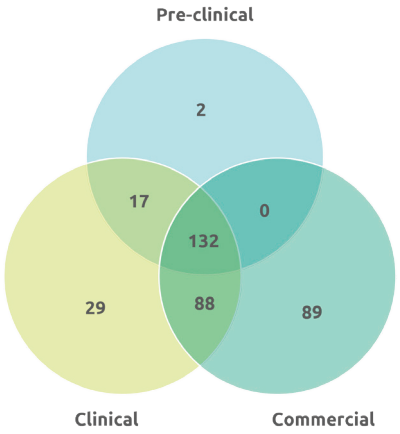
France



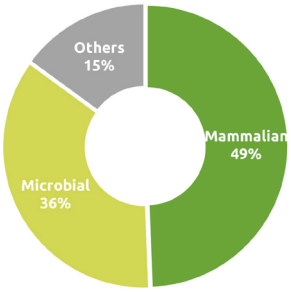
Source: GlobalData

Capabilities and Deals of CMOs

Distribution by Scale Of Operation

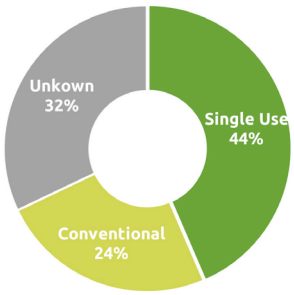


Distribution by expressions system



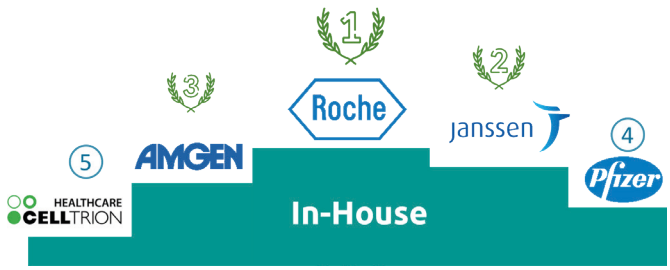
For mAbs production, Mammalian cells remains the most frequently used system

Distribution by Types of Bioreactors



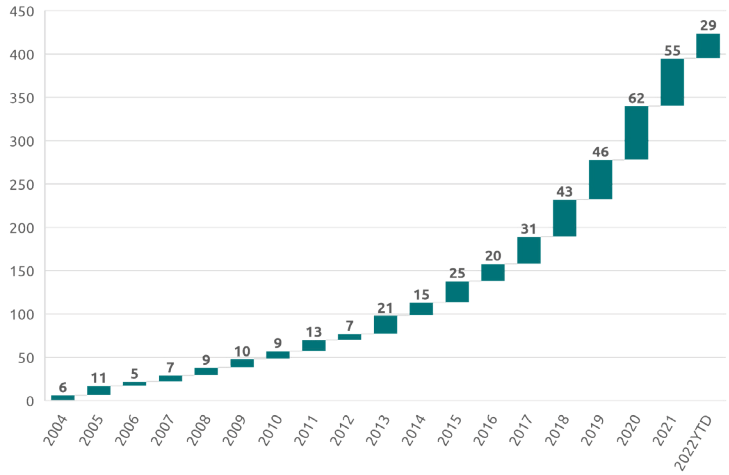
Single-use products have been developed and implemented by industry for the manufacturing of biologics. This is the most common technology used by the CDMOs analyzed.

Companies manufacturing most marketed mAbs in-house & Outsourced



Major antibody developers have in-house manufacturing capabilities, but if not, manufacturing is out-sourced to major CDMOs with significant capabilities.

Antibody Contract Service Agreement distribution by year



Since the early 2000's we have seen an increase in the number of Contract Service Agreements per year, which reflect a still growing dynamism in therapeutic antibodies development

Source: Biopharma Contract Manufacturing Market (3rd Edition), 2019-2030; Roots Analysis

interview

Expert Insights from the industry

OSE IMMUNO THERAPEUTICS

Jean-Pascal Conduzorgues
DIRECTEUR INDUSTRIEL ET PHARMACIEN RESPONSABLE

Jean-Pascal Conduzorgues bénéficie d'une très large expérience de pharmacien responsable, responsabilité pharmaceutique requise pour les médicaments en Europe. Fondateur, dirigeant et pharmacien responsable de CRID Pharma à Montpellier, devenu Amatsi puis récemment Eurofins, qu'il a dirigé pendant 20 ans (Etablissement pharmaceutique prestataire de services spécialisé dans le développement pharmaceutique). Jean-Pascal a fusionné CRID Pharma en 2011 avec Avogadro pour créer Amatsi, un groupe spécialisé dans le développement et la fabrication de médicaments, établi en France et aux Etats-Unis. En 2013, après avoir créé sa propre société de conseils Ibero, il a rejoint OSE Immunotherapeutics pour apporter son expertise dans la stratégie et les actions de développement pharmaceutique de médicaments pour essais cliniques et commercialisation.

Claire-Marine Albertus
SENIOR CMC PROJECT MANAGER

Claire-Marine Albertus est docteur en pharmacie spécialisé par un Master 2 en biotechnologies pharmaceutiques et thérapies innovantes. Après une première expérience sur des sites de production de médicaments biologiques (CellForCure puis LFB), Caire-Marine a rejoint les équipes d'OSE Immunotherapeutics en 2019 tout d'abord sur des fonctions qualités avant d'apporter son expertise dans le développement pharmaceutique des différents produits du portefeuille d'OSE Immunotherpaeutics.

À PROPOS D'OSE IMMUNOTHERAPEUTICS

OSE Immunotherapeutics est une société de biotechnologie qui développe des produits first-in-class en immuno-oncologie et immuno-inflammation. Son portefeuille clinique first-in-class comprend :

• Tedopi® (immunothérapie d'activation des lymphocytes T spécifiques contre les cellules cancéreuses, « off-the-shelf » à base de néo-épitopes) : le produit le plus avancé de la Société ; résultats positifs de l'essai de Phase 3 (Atalante 1) dans le cancer du poumon non à petites cellules (CPNPC) chez les patients en résistance secondaire après échec d'un inhibiteur de point de contrôle. D'autres essais, promus par des groupes cliniques en oncologie, de Tedopi® en combinaison sont en cours dans des tumeurs solides.

• OSE-279 (anti-PD1) : au stade préclinique avancé.

• OSE-127/S95011 (anticorps monoclonal humanisé antagoniste du récepteur IL-7) : développé en partenariat avec Servier ; Phase 2 en cours dans la rectocolite hémorragique (promoteur OSE Immunotherapeutics) et une autre Phase 2 en cours dans le syndrome de Sjögren (promoteur Servier) ; des travaux de recherche préclinique sont en cours dans les leucémies.

• VEL-101/FR104 (anticorps monoclonal anti-CD28) : développé en partenariat avec Veloxis Pharmaceuticals, Inc. dans la transplantation ; Phase 1/2 en cours dans la transplantation rénale (sous la promotion du Centre Hospitalier Universitaire de Nantes) ; Phase 1 en cours aux Etats-Unis (promoteur Veloxis Pharmaceuticals, Inc.).
• BI 765063 (anticorps monoclonal anti-SIRPα sur l'axe SIRPα/CD-47) : développé en partenariat avec Boehringer Ingelheim (BI) dans les tumeurs solides avancées ; résultats positifs de la Phase 1 d'escalade de dose en monothérapie et en association, en particulier avec l'anticorps anti-PD1 ezabenlimab ; Phase 1b internationale promue par BI en cours en association avec ezabenlimab seul ou avec d'autres médicaments dans le cancer de la tête et du cou en rechute ou métastatique et dans le carcinome hépatocellulaire.

1. Comment se déroule le processus de développement et le transfert en bioproduction au sein d'OSE Immunotherapeutics ?

Les laboratoires de recherche et développement d'OSE Immunotherapeutics sont situés à Nantes. Dans ces laboratoires, les équipes découvrent, développent et réalisent la preuve de concept d'anticorps innovants. Une fois ces premières étapes réalisées (qui prennent plusieurs années), les équipes en charge du développement pharmaceutique prennent le relais afin de développer un procédé de production conforme aux exigences réglementaires ; l'objectif est de produire le premier matériel nécessaire aux études pré-cliniques et cliniques. Un plan de développement vers les phases cliniques doit être mis en place au sein duquel sont entre autres définis la lignée cellulaire utilisée ou les procédés de culture et de purification. OSE Immunotherapeutics ne possède pas sa propre unité de fabrication donc l'ensemble de ces étapes sera sous-traité à différents façonniers. Une des étapes primordiales dans le développement pharmaceutique, en dehors des étapes de production, est la rédaction du Dossier d'Investigation Médical du Produit (IMPD) pour déposer une demande d'étude clinique. Ce dossier est à construire par les équipes pour répondre aux exigences réglementaires de la phase clinique dans laquelle se trouve le produit. La réalisation de ces différentes étapes doit répondre à des contraintes de temps et de budgets bien définies.

2. Quelles sont les grandes étapes clés de ce développement vers la clinique ?

La première grande étape dans le développement d'anticorps monoclonaux innovants est de définir la lignée cellulaire à partir de laquelle l'anticorps va être produit. Cette étape est critique dans la définition de l'anticorps (structure de l'anticorps) et dans l'obtention d'un titre (rendement de production) acceptable.

Ensuite, les conditions de culture doivent être déterminées et diffèrent selon la lignée cellulaire choisie. L'objectif de cette étape est d'obtenir la meilleure productivité possible de l'anticorps et permettre une montée en échelle pour produire des lots à échelle clinique (50L, 200L voir 1000L) selon les besoins du projet.

Enfin, l'optimisation des étapes de purification et d'inactivation virale sont un vrai défi pour obtenir le produit le plus pur possible en répondant aux exigences réglementaires des études cliniques.

3. Quels sont les verrous technologiques et non technologiques que vous pouvez rencontrer lors de ces étapes ?

Depuis la crise liée à la pandémie du COVID19, l'approvisionnement en matières et en matériel est devenu l'un des principaux verrous, sans qu'aucun retour à la normale ne se profile, les délais pour le plastique (poche de production, filtre…) sont de plusieurs dizaines de semaines pour certaines références. Cela combiné avec le peu de créneaux de production disponibles, implique aujourd'hui un délai minimum de 6 à 12 mois entre la réservation du créneau et la production du lot. Ces difficultés d'approvisionnement et de réservation de créneaux impliquent un retard important quand de nouvelles productions non prévues initialement doivent être intégrées au planning, suite par exemple à des écarts en cours de production impliquant une non-conformité du lot. Les problèmes peuvent vite arriver et une production sans écart est rare. L'anticipation n'est pas toujours évidente car la connaissance des opérateurs sur les anticorps est restreinte (production ponctuelle) et prévoir un surplus de stock demande beaucoup de moyens et de ressources.

Il existe des obstacles plus économiques, par exemple concernant le choix de la lignée cellulaire. Ici, il est important de prévoir son indépendance vis-à-vis de la lignée sélectionnée. Choisir une lignée avec laquelle le façonnier fonctionne de manière exclusive implique des contraintes, notamment si on souhaite ensuite changer de sous-traitant (coût ou changement de lignée).

Il est important de savoir rester flexible avec son sous-traitant afin d'éviter un schéma trop rigide.

En conclusion, il reste primordial de garder un œil critique sur les choix de sous-traitant et de bien suivre et maîtriser les étapes de la bioproduction en tenant compte du fait que certaines étapes, comme celle de la purification, sont plus complexes que d'autres. Disposer des ressources et des compétences en interne permet par exemple de suivre les projets avec un œil critique et de prendre les meilleures décisions possibles.

4. Quels sont vos critères prioritaires pour le choix des CDMO partenaires ?

Nous essayons de privilégier des partenaires français mais ils sont parfois limités en capacités de production pour accueillir tous nos projets. Nous sommes donc obligés d'aller prospecter ailleurs, notamment aux Etats-Unis et en Europe.

Le critère financier est important lors du choix, particulièrement pour une société de biotechnologie. En Europe les prix commencent à augmenter mais il y a un réel écart de prix entre les prestataires européens et américains. En effet, chez certains acteurs aux Etats-Unis les prestations restent hors de prix pour nous.

La spécificité de certains anticorps monoclonaux, comme les anticorps bispécifiques, nous pousse aussi à sélectionner des entreprises qui ont une expertise sur ce type de produit.

Nous essayons au maximum de créer des partenariats privilégiés avec nos sous-traitants et d'utiliser toujours les mêmes pour la production de nos anticorps ; cela permet d'avoir une relation de confiance et également de réduire les coûts de gestion de projet (contrat et accords qualité déjà en place, qualification réalisée…). Malheureusement cela n'est pas toujours possible pour des contraintes de planning et de coût.

5. Quels délais peut-on prévoir pour les projets de développement jusqu'à la production ?

Les délais vont de 2 à 3 ans entre le démarrage du projet et la production d'un premier lot clinique. Ils peuvent être raccourcis lors d'un transfert de technologie ou si le procédé est déjà existant.

6. Quels sont les aléas que vous avez pu rencontrer et que vous n'aviez pas forcément anticipé ?

Une des difficultés que nous avons récemment rencontrées sur nos différentes productions est de réussir à estimer la bonne taille de lot afin d'avoir des quantités suffisantes de produit pour nos études pré-cliniques et cliniques. En effet, lors de la montée en échelle le titre de production de l'anticorps ainsi que les rendements obtenus sur les différentes étapes de purification peuvent être en deçà de l'attendu. De plus, il faut prendre en compte les nombreux prélèvements nécessaires aux différentes études réglementaires (stabilité, inactivation virale…). C'est un point de vigilance à avoir, bien qu'anticiper ces valeurs n'est pas évident.

7. Quels sont les conseils et points d'alerte que vous pouvez transmettre à des jeunes sociétés biotech qui se lancent dans des phases cliniques ?

La gestion de projet avec le sous-traitant n'est pas toujours simple. Au départ il faut traiter avec les commerciaux puis une fois dans le projet, les équipes techniques prennent le relais et le chef de projet est clé notamment en termes de réactivité et de connaissance sur le projet et le produit.

Il y a aussi un travail à bien réaliser en amont sur les termes et les conditions de contractualisation avec les sous-traitants. Cela peut avoir beaucoup d'impact par la suite en cas de retard, d'abandon de lots, etc. Les moyens d'une biotech exigent d'être vigilants sur ces aspects. Ce sont des points qui peuvent être négligés pour aller vite mais qui peuvent causer plus de tort que le temps passé en amont.

Le choix du partenaire doit prendre en compte l'échelle sur laquelle on se trouve. La collaboration avec des gros industriels mondiaux implique un rapport de force plus complexe et donc des discussions et une flexibilité difficile. De la même manière, ces gros acteurs appliquent des étapes et une réglementation identique pour toutes les étapes cliniques alors que la réglementation n'impose pas les mêmes exigences pour des produits commerciaux ou cliniques. Il faut donc éviter un gros décalage d'échelle entre la biotech et le façonnier et procéder étape par étape. Prévoir un plan de développement jusqu'à l'AMM quand on se lance en préclinique et en phase I n'est pas toujours judicieux.

scientific articles

Read the different inputs from the scientific community on various aspects of antibodies

ACTIP

Connecting Industry Expertise

The Animal Cell Technology Industrial Platform (ACTIP)

Our vision

The vision of ACTIP is to be the leading non-profit industry association for the promotion of information exchange, networking and cooperation between European biotech companies, academia and other stakeholders involved in advanced cell technologies.

Our mission

The mission of ACTIP is to connect experts in the field of advanced cell technology enabling intensive networking among member companies, academia, and regulatory agencies to increase European competitiveness.

ACTIP

Connecting Industry Expertise

objectives

Intensive networking

Enable intensive networking among member companies, together with experts in academia, regulatory agencies and other associations involved in advanced cell technologies.

Representing industry

Represent advanced cell technology-based industry practitioners and being a source of industrial expertise.

Directing attention

Direct attention on the initiation and execution of advanced cell technology research and related projects sponsored by the European Commission.

Focusing on challenges

Bring together experts to identify and overcome challenges for the industrial use of advanced cell technologies.

Supporting early career scientists

Improve the recognition of early career scientists in the field of advanced cell technology research and introduce them to the ACTIP community through the ACTIP fellowship award program.

Staying up to date

Follow new aspects in advanced cell technology research, development, manufacturing and regulatory affairs.

background

The Animal Cell Technology Industrial Platform (ACTIP) is an independent non-profit association of European companies and institutions engaged in the industrial use of advanced cell technology for research, development and/or production of biotherapeutics (e.g. antibodies, proteins, vaccines, cell & gene therapies) and other areas. ACTIP was established in 1990 at the request of the European Commission to improve the transition of fundamental animal cell technology research to commercial applications. Composed of European industry members, the platform set itself the task to initiate and enabling intensive networking among cell technology experts in industry, academia, regulatory agencies and other stakeholders to increase European competitiveness. Over the years the scope of ACTIP has expanded from being not only focused on animal cell technologies, but to include other types of advanced cell technology applications. Through numerous scientific meetings, networking activities, position papers and active support to early career scientists, ACTIP has become a respected European and worldwide recognised association connecting industry expertise.

ACTIP's members meet twice a year at a plenary scientific meeting. These informal meetings feature high-level presentations on selected topics of mutual interest, with invited speakers from industry, academia, own member companies, small and medium-sized enterprises and regulatory agencies. As well, early career scientists are invited to present their research through the ACTIP Fellowship award. The meeting program also includes an informative site visit at the member company hosting the meeting and social activities to facilitate networking and cooperation.

ACTIP
Connecting Industry Expertise

organisation

ACTIP Steering Committee

ACTIP is led by a Steering Committee, which is elected every two years amongst its members. The Steering Committee makes decisions on ACTIP business and activities. The current Steering Committee members are:

Dr. Matthieu Stettler
Cytiva – ACTIP Chairman

Dr. Holger Lübben
GSK Vaccines

Dr. Jochen Sieck
Merck Life Science

Dr. Adrian Haines
Swedish Orphan Biovitrum

Dr. Nienke Vriezen
Byondis

Dr. Jonathan Bones
NIBRT

Dr. Ralf Ostendorp
MorphoSys AG

ACTIP Office

The ACTIP office takes care of the day-to-day management, i.e., memberships, meetings, fellowships, newsletters, communications and is the first point of contact.

Dr. Erwin van Vliet
ACTIP Executive Secretary
exec.secr@actip.org

Els van den Berg
ACTIP Secretariat
secretariat@actip.org

For more information, please visit the ACTIP website (www.actip.org) or contact the ACTIP secretariat via secretariat@actip.org

ANTIBODY-BASED PRODUCTS FDA OR EMA APPROVED IN 2022: TARGETS, FORMATS, PIVOTAL CLINICAL DATA, AND MANUFACTURING.

Alain Beck[1] and Hervé Broly[2]

[1]Biologics CMC and Developability, Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France. alain.beck@pierre-fabre.com, Alain Beck (0000-0002-4725-1777) (orcid.org)

[2]Biotech Process Sciences, Merck-Serono, Corsier-sur-Vevey, Switzerland. herve.broly@merckgroup.com.

1- Introduction

2022 was again a very successful year for therapeutic antibody-based products with 13 FDA or EMA first approvals equalizing the annual records of 2018 and 2021 (Kaplon H et al, 2023). The indications covered are oncology (6; relatlimab + nivolumab, tremelimumab + durvalumab, tebentafusp, mosunetuzumab, teclistamab and mirvetuximab soravtansine), immunology (3; sutimlimab, spesolimab and ublituximab), ophthalmology (1; faricimab), virology (2; faricimab and nirsevimab) and diabetes (1; teplizumab). These biologics are based on different antibody formats (2 combination therapies, 4 bi-specific antibodies, 1 antibody drug conjugate), with specific engineering Fc-engineering ("Fc-silent", "Fc-enhanced", increased FcRn affibity). To reach the market, multiple developability, production and formulation challenges have been solved and may be used as benchmarks.

In the first part of this paper, we will review these 13 antibody-based products (format, targets, indications, pivotal clinical trial summaries). In a second part, we will discuss the developability and Chemistry Manufacturing and Control (CMC) challenges and solutions as well FDA and EMA accelerated registration pathways use for most of them. Forecasts events that might occur in 2023 for late-stage antibody-based products will also be briefly discussed as an outlook.

2- Clinical success in 2022: 13 FDA or EMA first approvals (Kaplon H et al, 2023).

Six drugs have been approved in oncology (melanoma, hepatocellular carcinoma, lymphoma, myeloma, and ovarian cancer). The 7 others in immunology (cold agglutinin disease, psoriasis, multiple sclerosis), in ophthalmology (age macular disease), in virology (Covid-19 and RSV) and in diabetes.

2.1- Clinical successes in Oncology: combination Immuno-Oncology antibodies (2), T-Cell engagers (3) and ADC (1).

• Opdualag® (BMS); relatlimab (anti-LAG-3) + nivolumab (anti-PD1) combination IgG4 antibodies (Melanoma); "Fc-silent" (1)

Opdualag is a fixed dose combination of relatlimab and of nivolumab immune-oncology antibodies, administered as a single intravenous infusion. The drug was approved by FDA on March 18, 2022 for the treatment of adult and pediatric patients twelve years of age or older with unresectable or metastatic melanoma. Like nivolumab, relatlimab (BMS 986016, ONO4482; Bristol Myers Squibb / Ono) is a hIgG4k antibody stabilized in the hinge domain by a Ser228Pro mutation. FDA's approval was based on data from the Phase 2/3 RELATIVITY-047 trial (NCT03470922), which evaluated the effects of relatlimab combined with nivolumab versus nivolumab in a total of 714 patients with previously untreated metastatic or unresectable melanoma. Patients were randomized 1:1 and administered a fixed-dose combination of 160 mg relatlimab and 480 mg nivolumab or 480 mg nivolumab by intravenous infusion every 4 weeks until disease recurrence, unacceptable toxicity or withdrawal of consent. The study's primary endpoint, progression-free survival (PFS) by blinded independent central review, was met. The median PFS in the group that received both relatlimab and nivolumab (n=355) was significantly longer, 10.1 months as compared with 4.6 months with nivolumab alone (n=359) (Tawbi HA et al, 2022).

• Imjudo® (Astra Zeneca) tremelimumab (anti-CTLA-4) IgG2 antibody + Imfinzi® / durvalumab (anti-PD-L1) IgG1κ (L238F, L239E, P335S) antibody combination (Hepatocellular carcinoma); "Fc-silent" (2)

On October 24, 2022, AstraZeneca announced that Imjudo (tremelimumab) in combination with Imfinzi (durvalumab) has been approved in the US for the treatment of adult patients with unresectable hepatocellular carcinoma (HCC), the most common type of liver cancer.

Tremelimumab (CP-675,206), originally developed by Pfizer using Abgenix's XenoMouse technology, is a human IgG2κ antibody targeting CTLA-4. In 2011, MedImmune (now AstraZeneca) gained tremelimumab's global development rights, while Pfizer retained the rights for use in certain combination therapies. Tremelimumab blocks the activity of the immune checkpoint CTLA-4, contributing to T-cell activation, fostering antitumor immune responses and cancer cell death. Tremelimumab and durvalumab were granted **Orphan Drug designation in the US** for the treatment of hepatocellular carcinoma (HCC), and tremelimumab was also granted **Orphan Drug designation** for HCC in the EU. On October 24, 2022[JR1], FDA approved the combination of tremelimumab durvalumab for unresectable advanced liver cancer based on the results of the Phase 3 HIMALAYA trial. Marketing applications for this combination for liver cancer is under review by regulatory authorities in other countries and regions. Moreover, based on the results of the POSEIDON trial, marketing applications for the combination of tremelimumab durvalumab and chemotherapy for first-line metastatic NSCLC are also under review.

HIMALAYA (NCT03298451) is a randomized, open-label, global Phase 3 trial evaluating the safety and efficacy of durvalumab monotherapy and the combination of durvalumab and tremelimumab versus sorafenib, a standard-of-care multi-kinase inhibitor, as first-line treatment in patients with unresectable HCC who had not received prior systemic therapy and were not eligible for localized treatment. The combination of durvalumab and tremelimumab, called the STRIDE regimen (Single Tremelimumab Regular Interval Durvalumab), comprises a single priming dose of 300 mg of tremelimumab added to 1500 mg of durvalumab followed by durvalumab every four weeks. Patients were randomized to STRIDE (n=393), durvalumab (n=389), or sorafenib (n=389). The primary outcome measure was overall survival. Results of the HIMALAYA trial were presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium held January 20-22, 2022 in San Francisco. At data cutoff, the primary objective was met: median OS was significantly improved for STRIDE (16.4 months) and durvalumab (16.6 months) vs sorafenib (13.8 months). The ORRs were higher for STRIDE and durvalumab (20.1% and 17.0%, respectively) than for sorafenib (5.1%) whereas the median duration of response was longer for STRIDE (22.3 months) as compared with 16.8 months for durvalumab and 18.4 months for sorafenib.

• Kimmtrak® (Immunocore): tebentafusp, a bispecific gp100 peptide-HLA-directed CD3 T cell engager (Melanoma); No Fc-domain (3)

On January 26, 2022, Immunocore announced the approval by the FDA of KIMMTRAK® (tebentafusp-tebn) for the treatment of HLA-A*02:01-positive adult patients with unresectable or metastatic uveal melanoma (mUM). Tebentafusp (IMCgp100) is a bispecific fusion protein composed of 1) a T cell receptor (TCR) recognizing a human leukocyte antigen (HLA)-A*02:01 complexed with a peptide derived from gp100 antigen expressed by melanoma cells, and 2) an antibody single-chain variable fragment that binds CD3 present on T cells. This molecule creates a bridge between tumor cells and immune cells, and thus facilitates tumor-cell killing by T cells. As the TCR domain recognizes a peptide presented on HLA-A*02:01, tebentafusp can only be used to treat patients expressing this HLA type. Tebentafusp has been granted **Breakthrough Therapy, Fast Track, and Orphan Drug** designations by the FDA.

The marketing application is based on a late-stage clinical trial (NCT03070392) that enrolled 378 patients with advanced uveal melanoma who were HLA-A*0201–positive. In the study, patients were randomized

2:1 to receive tebentafusp or investigator's choice of therapy (either pembrolizumab, ipilimumab, or dacarbazine). Tebentafusp was administered at a dose of 20 □g on cycle 1 Day 1, then 30 □g on cycle 1 Day 8, then 68 μg on cycle 1 Day 15 and weekly thereafter by IV infusion until confirmed disease progression or unacceptable toxicity. The primary outcome measure is overall survival. As reported in September 2021, the median OS for KIMMTRAK was 21.7 months as compared with 16.0 months for investigator's choice (82% pembrolizumab; 13% ipilimumab; 6% dacarbazine). Moreover, the 1-year survival rate was 73% for patients in the experimental arm vs. 59% in the investigator's choice arm.

• Lunsumio® (Roche): mosunetuzumab (CD20 x CD3) bispecific knobs-into-holes antibody (Lymphoma); "Fc-silent" (4)

On June 8, 2022, Roche announced that the European Commission granted conditional marketing authorization for Lunsumio® (mosunetuzumab), a T-cell engaging bispecific antibody that redirects CD3+ T cells to eliminate malignant CD20+ B cells, for the treatment of adult patients with relapsed or refractory follicular lymphoma (FL) who have received at least two prior systemic therapies. Mosunetuzumab (RG7828, BTCT4465A) is an aglycosylated (N297G) humanized IgG1k bispecific antibody constructed using knobs-into-holes technology.

The approval is based on positive results from the Phase I/II GO29781 study (NCT02500407) in which Lunsumio demonstrated high complete response rates, with most complete responders maintaining responses for at least 18 months, and favorable tolerability in people with heavily pre-treated FL. After a median follow-up of 18.3 months, the median duration of response among responders was 22.8 months, the complete response rate was 60% (n=54/90), significantly higher than the historical control complete response rate with copanlisib of 14%, the objective response rate was 80% (n=72/90). Lunsumio is currently being evaluated in two Phase 3 studies: CELESTIMO, investigating Lunsumio plus lenalidomide in second line plus (2L+) FL, and SUNMO, investigating Lunsumio plus Polivy® (polatuzumab vedotin) in 2L+ diffuse large B-cell lymphoma.

• Teclayli® (Jansen): teclistamab (BCMA x CD3) bispecific antibody IgG4 duobody (Multiple Myeloma); "Fc-silent" (5)

On October 25, 2022, the **FDA granted accelerated approval** to teclistamab-cqyv (Tecvayli, Janssen Biotech, Inc.) for adult patients with relapsed or refractory multiple myeloma (MM) who have received at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody. Teclistamab (TECVAYLI) is a T-cell redirecting IgG4λ bispecific antibody recognizing B cell maturation antigen (BCMA) expressed on the surface if myeloma cells and CD3 expressed on T cells. Generated from Ligand's transgenic mouse (OmniAb) and Genmab's DuoBody technology, the Fc was engineered with the stabilizing S228P mutation and L234A/L235A mutations to minimize its effector functions. Teclistamab was granted Orphan Drug designations for the treatment of MM in both the US and EU, and received the **Breakthrough Therapy designation** for the treatment of relapsed or refractory MM (RRMM) by the FDA, and a **PRIority MEdicines (PRIME)** designation by the EMA for treatment of adult patients with RRMM who previously received ≥3 prior lines of therapy. TECVAYLI (teclistamab) was granted **conditional marketing authorization** in the EU as monotherapy for the treatment of adult RRMM patients who previously received ≥3 prior lines of therapy in August 2022.

The authorizations for marketing were based on the results from the multicohort, open-label, Phase 1 and Phase 2 MajesTEC-1 studies (NCT03145181, NCT04557098, respectively), evaluating the safety and efficacy of teclistamab in adults with RRMM. The ongoing first-in-human dose escalation and dose expansion clinical study (NCT03145181) is assessing the efficacy of teclistamab in patients with RRMM, with the antibody administered IV (range: 0.3–19.2 μg/kg [once every 2 weeks] or 19.2–720 μg/kg [once per week]) or subcutaneously (range: 80–3000 μg/kg [once per week]) in different cohorts, with step-up

dosing for 38.4 µg/kg or higher doses. Based on the dose escalation data, in the Phase 2 portion of the study patients received a weekly subcutaneous dose of teclistamab (1.5 mg/kg), after receiving step-up doses of 0.06 mg/kg and 0.3 mg/kg. Results of the MajesTEC-1 study (n=165) showed that teclistamab induced durable responses that deepened over time in patients with triple-class refractory disease (n=128) with an overall response rate of 63%, including a complete response in 39.4% of the patients. The median duration of response and duration of progression-free survival were 18.4 months and 11.3 months respectively.

• Elahere® (Immunogen): mirvetuximab soravtansine (Folate receptor) ADC (Ovarian cancer); Fc competent (6)

The FDA granted an **accelerated approval** for mirvetuximab soravtansine-gynx (ELAHERE™) for the treatment of adult patients with FRα-positive, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer, who have received one to three prior systemic treatment regimens, on November 14, 2022. FDA also approved a companion diagnostic, VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, developed by Roche.

Mirvetuximab soravtansine, developed by ImmunoGen as a treatment for epithelial malignancies such as ovarian adenocarcinoma, is an antibody-drug conjugate (ADC) targeting folate receptor alpha (FRα). The cytotoxic warhead, the tubulin-targeting maytansinoid drug DM4, is conjugated to the humanized IgG1κ antibody via a cleavable disulfide linker. The ADC has been granted **Orphan Drug designations for ovarian cancer in the US and EU, and FDA's Fast Track** designation for a specific subset of ovarian cancer patients with medium to high FRα-positive platinum-resistant lesions who received between one and three prior systemic treatments, and for whom single-agent chemotherapy is appropriate as the next line of therapy.

FDA's approval was based on positive results of the Phase 3 SORAYA study (NCT04296890), which evaluated the efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant advanced high-grade epithelial ovarian, primary peritoneal or fallopian tube cancer, whose tumors express a high-level of FRα. A total of 106 platinum-resistant ovarian cancer patients with high FRα expression previously treated with at least one, but less than three prior systemic treatments, at least one of which included bevacizumab, received mirvetuximab soravtansine (6 mg/kg adjusted ideal body weight) administered on day 1 of every 3-week cycle. Results from the SORAYA trial were presented at the Society of Gynecologic Oncology (SGO) annual meeting held in March 2022. Additional efficacy analyses based on a 120-day cut-off date showing tumor reduction in 71.4% of patients, an objective response rate of 32.4% as assessed by the investigator, and a preliminary median OS of 13.8 months were presented at the American Society of Clinical Oncology (ASCO) Annual Meeting held June 3-7, 2022. A retrospective safety analysis based on 464 patients with FRα positive, recurrent ovarian cancer pooled across three studies (a Phase 1 first-in-human trial and the Phase 3 FORWARD I and SORAYA trials) demonstrating a differentiated and consistent safety profile was also presented at the 2022 ASCO meeting.

Mirvetuximab soravtansine was also evaluated in the randomized Phase 3 FORWARD I trial (NCT02631876), which enrolled 366 patients with platinum-resistant ovarian cancer, randomized 2:1 to receive either the ADC or the physician's choice of pegylated liposomal doxorubicin, topotecan, or weekly paclitaxel. Improved patient-reported outcomes associated with mirvetuximab compared with chemotherapy were presented at the European Society for Medical Oncology (ESMO) held in Paris, France in September 2022. In addition, ImmunoGen continues to enroll patients in the randomized, open-label Phase 3 MIRASOL study (NCT04209855), which is evaluating mirvetuximab soravtansine vs. investigator's choice of chemotherapy in platinum-resistant, advanced high-grade epithelial ovarian, primary peritoneal, or

fallopian tube cancers with high folate receptor-alpha expression. Top-line data from the confirmatory MIRASOL study are expected to be announced in early 2023. If positive, the results may support a full approval by FDA.

2.2- Clinical successes in Immunology: "Fc-silenced", "Fc-enhanced" and canonical antibodies.

• Enjaymo® (Sanofi): sutimlimab (C1s) IgG4-L235E antibody (Cold agglutinin disease); "Fc-silenced" (7)

On February 4, 2022, the FDA approved Enjaymo (sutimlimab-jome) infusion to decrease the need for red blood cell transfusion due to hemolysis in adults with cold agglutinin disease (CAD). This rare autoimmune disorder is characterized by hemolysis caused by activation of the classic complement pathway. Sponsored by Sanofi, sutimlimab is a hinge stabilized, humanized IgG4k antibody that targets and inhibits complement component 1s (C1s). A mutation in the Fc region (L235E) reduces the effector functions of the antibody. Sutimlimab contains a serine-to-proline mutation (S241P), based on the Kabat numbering system, which stabilizes the core-hinge region of the molecule. In addition, sutimlimab contains a leucine-to-glutamic acid mutation (L248E) that abrogates Fcγ receptor binding. Sutimlimab received **FDA's Breakthrough Therapy and Orphan Drug designations** for CAD, and **Orphan Drug designation in the EU** for this indication.

The BLA was based on data from the CARDINAL open-label, single-arm study (NCT03347396), which enrolled 24 adult patients with CAD who received a recent blood transfusion. All participants received Enjaymo for up to six months and could choose to continue therapy in a second part of the trial. Based on body weight, participants received either a 6.5 g or 7.5 g infusion of Enjaymo on day 0, day 7, and every 14 days through week 25. In total, 54% of participants responded to Enjaymo. In this study, sutimlimab administration rapidly halted hemolysis, increased hemoglobin levels, and reduced fatigue.

• Spevigo® (BI): spesolimab (anti-IL-36) IgG1 antibody (Psoriasis); Fc-competent (8)

Spesolimab (SPEVIGO®), a humanized anti-IL-36 IgG1k antibody developed by Boehringer Ingelheim, was approved by the FDA as a treatment option for generalized pustular psoriasis (GPP) flares in adults, as announced by BI on September 1, 2022. GPP is a rare and potentially life-threatening neutrophilic skin disease characterized by episodes of widespread eruptions of painful, sterile pustules. The FDA had previously granted spesolimab **Breakthrough Therapy and Orphan Drug designations** for the treatment of GPP, and the BLA for spesolimab received a Priority review.

The approval by FDA was based in part on results from the 12-week pivotal Phase 2 Effisayil™ 1 clinical trial (NCT03782792), which evaluated the efficacy, safety, and tolerability of a single 900 mg dose of IV administered spesolimab, with the option of a second dose if symptoms persisted on Day 8, vs placebo in 53 patients (35 active, 18 placebo) experiencing a GPP flare. After one week, 19 (54%) of patients treated with SPEVIGO showed no visible pustules compared to 1 (6%) patient who received placebo. A 3-arm, 5-year Phase 2 study (NCT03886246) to evaluate spesolimab in GPP patients who took part in previous studies with spesolimab is currently recruiting an estimated 155 participants. Patients will be administered SPEVIGO® at 4-, 6- or 12-week intervals. The primary outcome measure of the study is the occurrence of treatment emergent adverse events (TEAEs) up to week 252 of maintenance treatment; secondary outcome measures relate to the efficacy of the drug.

• BriumviTM (TG Therapeutics, licenced form LFB): ublituximab (anti-CD20) IgG1 antibody (Multiple sclerosis); "Fc-enhanced" (9)

On December 28, 2022, the FDA approved BRIUMVI™ (ublituximab-xiiy), for the treatment of relapsing forms of multiple sclerosis (RMS), to include clinically isolated syndrome, relapsing-remitting disease, and active secondary progressive disease, in adults. Ublituximab (TG-1101) is a chimeric anti-CD20 IgG1k antibody glycoengineered for enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) that was originally developed by LFB Biotechnology (de Romeuf C et al, 2008) and licensed to TG Therapeutics as a treatment for CLL and multiple sclerosis (MS).

The approval was based on results of the randomized, double-blinded, active-controlled Phase 3 trials ULTIMATE I (NCT03277261) and ULTIMATE II (NCT03277248) evaluating ublituximab (450 mg dose IV every 6 months, following a Day 1 infusion of 150 mg over four hours and a Day 15 infusion over one hour) compared to teriflunomide (14 mg oral tablets taken once daily) in a total of 1,094 patients with relapsing MS for both studies. The primary endpoint was the annualized relapse rate (ARR). Results from the ULTIMATE I and II Phase 3 trials showed that the primary endpoint was met, with a significant reduction of the ARR for ublituximab vs. teriflunomide over a period of 96 weeks (Steinman L et al, 2022).

2.3- Clinical success in Ophtalmology: bispecific antibody.

• Vabysmo™ (Roche): faricimab (VEGFA x Ang-2) bispecific crossmab antibody (AMD); "Fc-silenced" (10)

On January 28, 2022, Genentech announced that the FDA has approved Vabysmo™ (faricimab-svoa) for the treatment of wet, or neovascular, age-related macular degeneration (AMD) and diabetic macular edema (DME). Faricimab (RO6867461, RG7716) is an anti-vascular endothelial growth factor-A (VEGF-A) and anti-angiopoietin-2 (Ang-2) bispecific antibody derived from Roche's CrossMab technology.

Faricimab (RG7716) is a heterodimeric 1 + 1 VEGF/ Ang-2 CrossMabCH1–CL optimized for intraocular use and high concentration formulation by the introduction of P329G LALA and Triple A mutations in the knobs- into-holes technology (KiH) containing IgG1 Fc portion to abolish FcγR-mediated effector functions and FcRn recycling for low systemic exposure (Surowka M et al, 2021).

The approval was based in part on results from four Phase 3 studies in wet AMD and DME. The randomized, double-masked, and active comparator-controlled TENAYA (NCT03823287) and LUCERNE (NCT03823300) studies evaluated the effects of faricimab (6.0 mg administered at fixed intervals of every two, three, or four months) and aflibercept (Eylea®) (2.0 mg administered at fixed two-month intervals) in wet AMD patients. The primary endpoint of the studies, average change in best-corrected visual acuity (BCVA) from baseline through week 48, was met in both studies. The average vision gains from baseline in the faricimab arms were +5.8 and +6.6 letters, compared to +5.1 and +6.6 letters in the aflibercept arms, in the TENAYA and LUCERNE studies, respectively, demonstrating the non-inferiority of faricimab compared to aflibercept. The study also showed that faricimab's treatment interval could be longer than that of aflibercept – nearly 80% of patients treated with faricimab were able to go three months or longer between treatments during the first year.

The 3-arm, randomized, double-masked, active comparator-controlled YOSEMITE (NCT03622580) and RHINE studies (NCT03622593) compared the effects of faricimab (6.0 mg administered at personalized treatment intervals (PTI) of up to four months or 6.0 mg administered at fixed two-month intervals) to those of aflibercept (2.0 mg administered at fixed two-month intervals) in DME patients. The primary endpoint, average change in BCVA score from baseline at one year, was met, with faricimab again showing non-inferiority in visual acuity gains compared to aflibercept. In the YOSEMITE study, the average vision gains from baseline were +11.6, +10.7, and +10.9 letters in the faricimab PTI, faricimab two-month, and

aflibercept arms, respectively. The average vision gains from baseline were +10.8, +11.8, and +10.3 letters in the faricimab PTI, faricimab two-month, and aflibercept arms, respectively, in the RHINE study.

2.4- Clinical successes in Virology: ultrafast development of canonical antibody (Covid-19) and Fc-engineered antibody to double half-life (RSV)

• Bebtelovimab (Covid 19, omicron variant); Fc-competent (Emergency Use Authorization USA) (11).

On February 11, 2022, the FDA has issued an **Emergency Use Authorization (EUA)** for bebtelovimab (LY-CoV1404), an anti-SARS-CoV-2 hIgG1 monoclonal antibody that demonstrates neutralization against the Omicron variant. Bebtelovimab targets the SARS-CoV-2 spike glycoprotein receptor binding domain. The EUA was issued to Eli Lilly and Co.

The EUA for bebtelovimab is supported by clinical and nonclinical data. The clinical data are from a Phase 2, randomized, single-dose clinical trial (NCT04634409) evaluating the efficacy of bebtelovimab alone and bebtelovimab combined with other mAbs for treating mild to moderate COVID-19.

Bebtelovimab is authorized for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (12 years of age and older weighing at least 40 kg) with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19, including hospitalization or death, and for whom alternative COVID-19 treatment options approved or authorized by FDA are not accessible or clinically appropriate. The authorized dose of bebtelovimab is 175 mg given as an intravenous injection over at least 30 seconds.

On November 30, the FDA announced that bebtelovimab is no longer authorized for emergency use in the U.S. because it is not expected to neutralize Omicron current dominant subvariants BQ.1 and BQ.1.1.

• Beyfortus® (AstraZeneca and Sanofi): nirsevimab (RSV); Fc-engineered IgG1YTE antibody (12).

The European Commission (EC) has approved AstraZeneca and Sanofi's Beyfortus® (nirsevimab) for the prevention of respiratory syncytial virus (RSV) lower respiratory tract disease in newborns and infants during their first RSV season. Nirsevimab is human IgG1κ antibody targeting RSV. The Fc domain was engineered using AstraZeneca's proprietary YTE half-life extension technology. Developed by AstraZeneca and Sanofi, nirsevimab is designed to offer newborns and infants direct protection against RSV and help prevent RSV-related lower respiratory tract infections.

Nirsevimab received regulatory designations to facilitate development, including **Breakthrough Therapy designation from FDA; and PRIME designation from EMA**. The approval by the EC was based on results of the Phase 3 MELODY (NCT03979313), Phase 2/3 MEDLEY (NCT03959488), and Phase 2b (NCT02878330) clinical trials.

The Phase 2b trial is a randomized, placebo-controlled trial designed to measure the efficacy of nirsevimab in preventing medically attended RSV-related lower respiratory tract infections through 150 days post-dose. The study was conducted on healthy preterm infants (29–35 weeks' gestation) who were randomized (2:1) to receive a single intramuscular injection of nirsevimab (50 mg) or placebo. The primary endpoint of the study was met, with a reduction of the incidence of medically attended RSV-related lower respiratory tract infections by 70.1% compared to placebo.

MELODY is a randomized, placebo-controlled Phase 3 trial evaluating the safety and efficacy of nirsevimab for the prevention of medically attended lower respiratory tract infections in healthy late preterm and term infants (i.e., born at 35 weeks' gestation or later). Participants (n=1490) up to 1 year of age were randomized (2:1) to receive a single intramuscular injection of nirsevimab (50 mg if <5 kg or 100 mg

if >5 kg body weight) or placebo. The primary endpoint of this study was met, with a reduction in the incidence of medically attended lower respiratory tract infections caused by RSV of 74.5% compared to placebo.

2.5- Clinical success in Diabetes

• Tzield™ (Provention Bio and Sanofi: teplizumab IgG1 L234A L235A mutated antibody (Type 1 diabetes); Fc-silent (13)

On November 17, 2022, the FDA approved TZIELD™ (teplizumab-mzwv) to delay the onset of Stage 3 type 1 diabetes (T1D) in adult and pediatric patients aged 8 years and older with Stage 2 T1D. The approval was based in part on a clinical trial in Stage 2 T1D patients in which TZIELD delayed the median onset of Stage 3 T1D by 25 months, or approximately 2 years, compared to placebo.

Teplizumab is a humanized, anti-CD3e IgG1k antibody originally developed at Tolerance Therapeutics, Inc. and the University of California. The antibody Fc region was mutated (L234A; L235A) to reduce effector functions. Teplizumab binds CD3 expressed on mature T cells and may induce expansion and/or regulatory function in T cell subsets. In 2005, teplizumab was licensed to MacroGenics. In 2018, Provention Bio acquired all rights to teplizumab and subsequently continued its development for the prevention and treatment on T1D. The FDA granted teplizumab **Orphan Drug designation** for the treatment of recent-onset T1D. Teplizumab was also granted **FDA's Breakthrough Therapy designation** for the prevention or delay of clinical T1D in at-risk individuals and **EMA's PRIority MEdicines (PRIME)** designation for the same indication. As of October 2022, Provention Bio and Sanofi had entered into a co-promotion agreement for teplizumab.

Provention Bio is currently evaluating teplizumab in patients with newly diagnosed insulin-dependent T1D in the global PROTECT (PROvention T1D trial Evaluating C-peptide with Teplizumab) Phase 3 study (NCT03875729). This randomized, double-blind, placebo-controlled, multicenter trial will enroll 300 patients with recent onset T1D who will be randomized 2:1 to either two 12-day cycles of teplizumab (IV) or placebo. The primary efficacy endpoint is C-peptide change. Secondary endpoints include insulin use, HbA1c, hypoglycemic episodes, and safety. The company expects top line data from PROTECT Phase 3 study in 2H 2023.

3- Chemistry Manufacturing Controls considerations for antibody-based products approved in 2022.

12 out of 13 antibodies are produced in CHO cells that dominate the bioprocessing market for glycoprotein production (www.actip.org; Walsh G & Walsh E, 2022; Broly H & Beck, 2023). Ublituximab is produced YB2/0 rat hybridoma cells (Joubert S et al, 2019).

3.1- Fc-competent (IgG1), "Fc-enhanced" (IgG1, low fucose) or "Fc-silenced" formats (IgG4, IgG2, IgG1-Fc mutated).

Therapeutic antibodies (hIgG1, "Fc-competent") rely on two types of functionalities to achieve clinical efficacy: target-specific binding by the Fab (antigen-binding fragment) domain and immune-mediated effector functions—such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP) and complement dependent cytotoxicity (CDC) via interaction of the Fc domain with receptors on various cell types.

Cytotoxic enhancement for Fc domains N-glyco-engineered mAbs with a bisecting GlcNAc or low Fucose content are clinically validated in engineered CHO cells (mogamulizumab, benralizumab, benlatamab

vedotin) or in YB2/0 rat cells (ublituximab) (Beck A and Reichert M, 2012; Wagner-Rousset E et al, 2021).

The Fc-mediated immune effector activities are an important part of an antibody's natural function, but in many therapeutic antibodies these interactions are not desirable and can lead to deleterious side effects. hIgG4 or hgG4 ("Fc-silenced") may be select as well as many other approaches to eliminate (or enhance) effector function (Wilkinson I et al, 2021). Many Fc-mutation are used such as double mutation (L234A/L235A) of isotype IgG1 or IgG4 (S239D/I332E) to reduce ADCC.

The Fc N-glycosylated moiety is easily eliminated by mutation of N297, resulting in a reduction in binding to Fc receptors and C1q (e.g. atezolizumab, clazakizumab and otelixizumab). Alternatively, one of the most widely used IgG1 variants is L234A/L235A (LALA). These substitutions reduce binding to the IgG Fc receptors FcγRI, FcγRII and FcγRIII as well as to complement component C1q. Such antibodies are useful where binding and activation of Fc receptors is undesirable, for example when the product is being used as an antagonist of a cytokine or similar or when ADCC is undesirable because, for example, the cells bearing the checkpoint inhibitor motif are essential to the mechanism of action (e.g. for anti-PD1 penpulimab; Huang et al, 2022). Numerous therapeutic antibodies using the LALA mutations have entered clinical trials (e.g. bimagrumab, cemiplimab, galcanezumab, progolimab, risankizumab, spesolimab and teplizumab) (Wilkinson I & Geoff Hale G, 2022).

Among the 13 FDA or EMA 2022 approved antibodies:

- 7 Fc are "silenced (relatlimab (IgG4)/nivomulab (hIgG4), tremelimumab (IgG2)/ durvalumab (IgG1-L238F, L239E, P335S), mosunetuzumab (BsIgG1 aglycosylated (N297G)), sutimlimab IgG4-L235E, faricimab (bsIgG1, crossmab), teplizumab (IgG1- L234A, L235A), tebentafusp (no Fc-domain)),
- 5 Fc-competent: mirvetuximab soravtansine, spesolimab (hIgG1), bebtelovimab (hIgG1), nirsevimab (hIgG1),
- 1 Fc-enhanced (cIgG1, low fucose).

Binding of the Fc domain to the FcRn receptor is responsible for the comparatively long half-life of IgG. Nirsevimab is engineered to extend the half-life (YTE mutations).

3.2- Combination products

Use of combination of mAbs is another trend in the field as illustrated by the approval of Opdualag® (relatlimab (LAG3) + nivolumab (PD1) in melanoma) and Imjudo® (tremelimumab (CTLA4) + Imfinzi®/ durvalumab (PD-L1) in Hepatocellular carcinoma).

These combination therapies can be administrated separately or as co-formulated drugs. Characterization of co-formulated antibodies can be challenging due to similarities in physicochemical properties, especially in combinations in which the concentrations of the component mAbs are significantly different (Akbarian M et al, 2022). Systematic characterization is key to identify CQAs to be monitored in co-formulated mAbs and to justify the use of platform methods (Kim J et al, 2020).

3.3- Bispecific antibodies: knob-into-holes, crossmabs, duobodies and scFv-TCR fusion protein.

Bispecific antibodies (BsAbs) can exist in many different formats including, for example, tandem monovalent binding fragments as well as immunoglobulin G (IgG)-based antibodies where each arm binds a different antigen or onto which multiple additional antigen-binding domains are attached. These diverse formats allow BsAbs to be designed to match the proposed mechanisms of action and the

intended clinical application (Spiess D et al, 2015). Unique development considerations may be relevant for each of the formats, such as quality, stability, and production yields, but in general the products should be characterized, and the manufacturing processes should be developed in accordance with standard monoclonal antibody development practices. Quality attributes that may affect pharmacology should be studied, including antigen specificity; affinity and on- and off-rates; avidity (for bispecific antibodies that target two molecules on the same cell); potency; product-related impurities such as aggregates, fragments, homodimers, and other mispaired species; stability; and half-life. For example, in vitro and in vivo pharmacology studies may provide information on the relative binding activity and on- and off-rates for each target. Design of the potency assay(s) will depend on the target product attributes. Early in vitro studies may inform selection of an expression construct with optimal affinity and stability properties. The relative amounts of homodimers should be assessed. This evaluation is particularly important for effector cell engaging constructs where homodimers of the anti-CD3 or anti-Fc engaging arm may lead to cytokine release. Also, novel structures could potentially lead to increased immunogenicity.

Compared to the development of canonical mAbs, the development of BsAbs can present many challenges in product expression, purification, product stability, and scale up of the manufacturing process. Therefore, in addition to efforts made with molecular design, CMC strategies are critical for developing and marketing bispecific drugs.

Over a third or more of many big pharma clinical stage pipelines are now composed of bispecific antibodies (Gera N, 2022). These pipelines address various disease areas and span several different formats. As described earlier, most of these molecules are T cell-redirecting bsAbs; however, molecules such as NK cell and macrophage engagers, are rapidly making their way into the clinic. BsAbs, in general, do not fit the existing platform monospecific antibody production processes, and therefore, manufacturability for this complex modality must be assessed. Discovery and development of successful bispecific molecules requires multiparametric optimization of numerous properties to address the biological problem and clinical application at hand. For example, emicizumab was selected from 40,000 bsAbs optimized for FVIII mimetic activity, pharmacokinetics improvement, solubility improvement, deamidation, and deimmunization. This required generation of many additional variants in the process to identify the final candidate molecule hBS910, which became emicizumab and was FDA/ EMA approved in 2017 (Duivelshof BL et al, 2022). Emicizumab is a bispecific IgG (type 2) as defined by Wilkinson I & Hale G, 2022. Bispecific or biparatopic molecule with a classical IgG structure and mutations in the CH3 domain of the heavy chain to favor heterodimerization of the heavy chain.

- **Mosunetuzumab (CD20 x CD3) bispecific knobs-into-holes antibody, Bispecific IgG (type 1)):** Bispecific molecule with a classical IgG structure and mutations in the CH3 domain of the heavy chain to favor heterodimerization of the heavy chains with different VH domains. This molecule contains no light chain modifications to encourage correct light chain pairing.

- **Faricimab (VEGFA x Ang-2) bispecific crossmab antibody, Bispecific IgG (type 5):** Bispecific molecule with a classical IgG structure consisting of mutations in the CH3 domain of the heavy chain to favour heterodimerization of the heavy chains with different VH domains and with the CH1 and CL domains in one Fab arm crossed-over to favour light chain pairing (Surowka M et al, 2021).

- **Teclistamab (BCMA3 x CD3) bispecific IgG4 duobody, Bispecific IgG (type 6):** The final molecule is a bispecific molecule with a classical IgG structure and mutations in the CH3 domain of the heavy chain to favor heterodimerization of the heavy chains with different VH domains. This is obtained by first producing the two monoclonal antibodies independently and then mixing under specific

conditions that favor the recombination of the different heavy chains to form a heterodimeric species.

• **Tebentafusp (GP100 x CD3) bispecific scFv-TCR fusion protein:** A scFv fused at its C-terminus to a T-cell receptor beta chain which heterodimerizes with a separate alpha chain.

3.4- Antibody Drug Conjugates (Dumontet C, Beck A et al, 2023, submitted).

After a slow start, ADCs have entered global markets at an increased pace, with 8 first approvals granted since 2019. Additionally, currently approved antibodies are being explored in a growing number of indications, including alternative tumor types, combination regimens and the adjuvant or neoadjuvant setting. Given their improved therapeutic index over conventional cytotoxic chemotherapy, ADCs have demonstrated their potential to replace such agents in certain indications. Recently approved ADCs are remarkable both in terms of target antigen diversification and the nature of their payloads. The success of this class of drugs has attracted numerous companies that are now developing novel ADCs using a wide array of components. Collectively, the commercial clinical pipeline of ADCs is robust, with 130 agents in clinical trials, including 11 in late-stage studies.

In the case of mirvetuximab soravtansine, the cytotoxic warhead (tubulin-targeting maytansinoid drug DM4), is stochastically conjugated to a chimeric IgG1κ antibody via a cleavable disulfide linker on lysine residues (Joubert N et al, 2020) sur as for trastuzumab emtansine with a complex charge variants profile (Beck A et al, 2016; Beck A et al, 2017; Wagh A et al 2018; Beck A et al, 2019; Beck A et al, 2022).

3.5- State-of-the art analytical methods.

As illustrated above, the biopharmaceutical landscape continues to evolve rapidly, and associated modality complexity and the need to improve molecular understanding require concomitant advances in analytical approaches used to characterize and release the product (Blue L et al, 2022). Multiple progresses have been reported in 2022 including top-down and middledown electron transfer dissociation mass spectrometry (MS) (Fornelli L et al, 2022), de novo sequencing by MS (Suckau D et al, 2022), multiple chromatography-native MS setting (Duivelshof BL et al, 2022) and capillary electrophoresis – hyphenated to MS (Reinert T et al, 2022).

3.6- Risk-based control strategies for recombinant antibodies (Beck A et al, 2022).

Acidic and basic species have drawn substantial attention during the discovery, the lead selection and optimization, the pharmaceutical development, and the commercialization of therapeutic antibodies (mAbs) due to their sensitivity to manufacturing process changes.

Commonly, acidic species are detected as several small peaks when analyzed by Ion Exchange Chromatography (IEX) or by capillary Iso-Electric Focusing (cIEF) based techniques, formed due to modifications such as deamidation, glycation, and sialyation. In contrast, basic species are usually detected as fewer peaks, easier to identified, and formed by modifications such as clipping of C-terminal Lysine or of C-terminal amidation (Goyon A et al, 2017).

It may be challenging to maintain the levels of acidic and basic species within the reasonable ranges defined in specification and comparability acceptance criteria when process changes are introduced during optimization steps, manufacturing scale-up or transfer to different facilities. Candidate selection through developability assessment, early phase process and formulation development are critical steps toward successful late phase development and commercialization (Wagner E et al, 2017). The objective

of developability assessment is to select candidates with inherent properties of generating low and consistent levels of acidic and basic species. The inclination towards advancing a program through Investigational New Drug (IND)-enabling toxicology, and early-phase development quickly should be balanced with the need to understand the degree of controls over charge variants. Since characterization of the acidic and basis species at early development stage is not necessary nor is it a common practice, basic understanding of process parameters and their effects on charge profiles is essential to support process optimization, transfer and scale up. Process parameters are further studied, qualified and tightly controlled during Process Performance Qualification (PPQ) to be commercialization ready. Overall, parameters during cell culture have the most substantial effect on charge variants. To a much lesser degree, downstream process, formulation, and storage can be explored to control charge variants. Qualitative difference, such as the appearance of new species, is more concerning compared to quantitative difference. It is more manageable in early phase compared to late-phase development and commercialization. Nevertheless, maintaining acidic and basic species within a controlled range throughout development including animal toxicology, early phase and late phase development and commercialization can ensure product safety, efficacy and overall Regulatory Authority acceptance.

Critical Quality Attributes (CQAs) evaluation evolves along with the program development process. For early-stage programs, with no or limited information on the chemical nature of acidic and basic species, lack of structure-function relationship and clinical experience, acidic and basic species are most likely categorized as CQAs. Although based on the published information, if no difference in antigen binding, potency and pK is observed between acidic, basic and the main species, it may not be justifiable to classify acidic and basic species as non-CQA due to the lack of extended characterization. If not carried out earlier, peak isolation and extended characterization of acidic and basic species must be included in a Biologics License Application (BLA) submission. If necessary, CQA assessment can be re-evaluated. Whether or not classified as an CQA, it is prudent to maintain acidic and basic species at consistent levels. When differences arise, additional peak isolation and characterization are most likely required to demonstrate the presence of the same species and lack of adverse effect on safety and efficacy. Sometimes, in vitro data alone may not be sufficient to justify lack of impact especially for safety.

3.7- Covid-19 antibodies: accelerated development timelines (Broly H & Beck A, in preparation).

From the start of the COVID-19 pandemic in January 2020 through December 2021, multiple companies livered significant quantities of mAb therapies to COVID-19 patients, reducing hospitalization rates and saving lives (Eli Lilly's bamlanivimab and etesevimab, Regeneron's mixture of imdevimab and casirivimab, Vir's sotrovimab, Celltrion's regdanvimab, and Lilly's bebtelovimab to name a few; Emergency Use Authorization in the USA (EUA)). The quality, safety, and stability of these products reflect the maturation of the biopharma industry to develop and commercialize mAbs since the licensure of the first recombinant mAb in 1997 (rituximab). The companies that brought these COVID therapies to market accepted business risk and early investments but did not compromise product quality or accept safety risks. Now, this class of products has been made available at unprecedented speed, and scales leveraging-platform processes and global manufacturing capacity at existing CDMOs and innovator companies (Popkin ME et al, 2022).

The race against time is illustrated by the accelerated CMC development timelines, and the emergency Use Authorization date for bebtelovimab (Feb 11, 2022) and withdrawn date (Nov 30, 2022) due to epitope escape (not effective to neutralize Omicron subvariants BQ.1 and BQ.1.1).

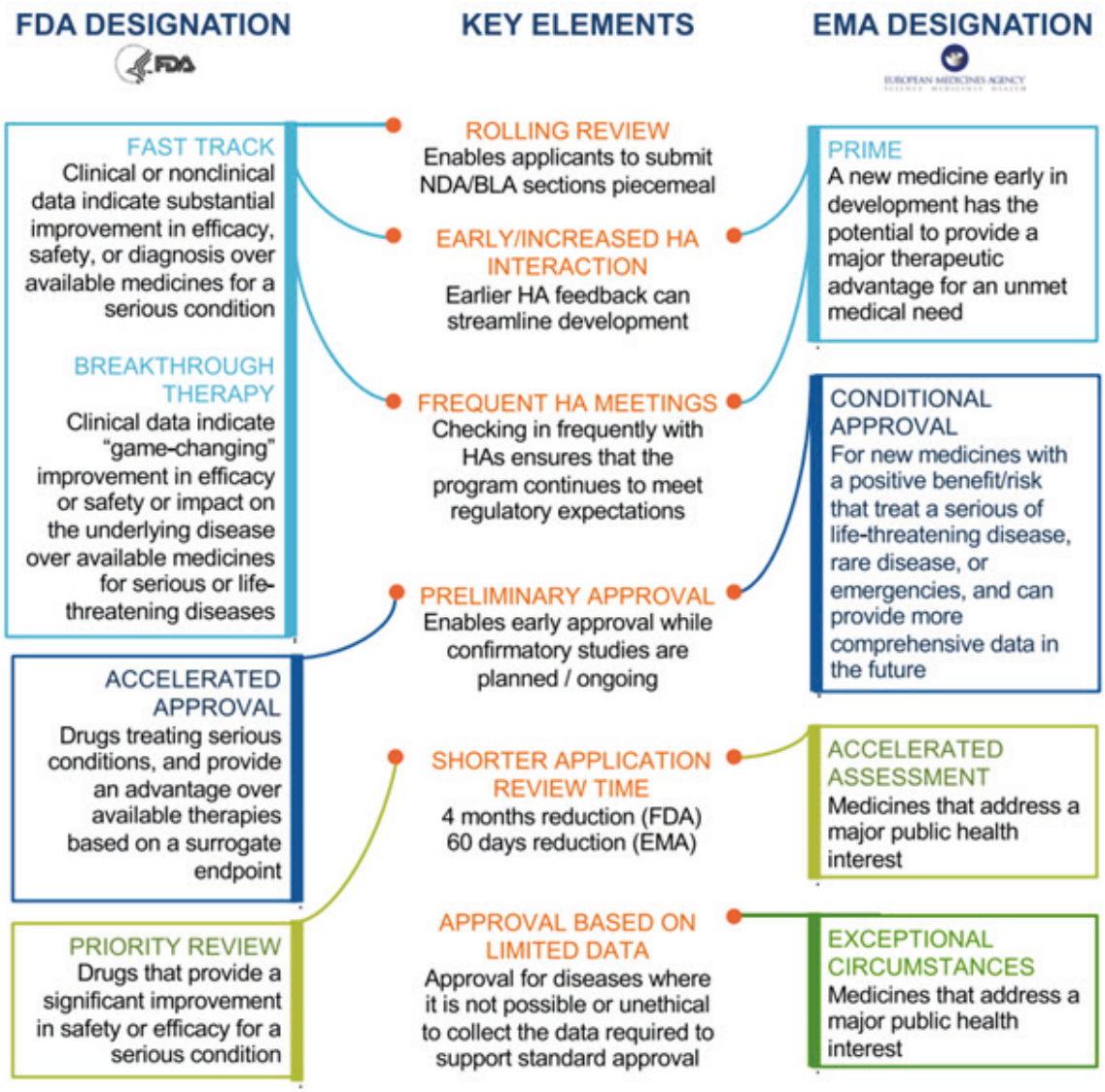
Because the Omicron variant has become the dominant variant in the United States, and because emerging variants of Omicron have become resistant to the currently available monoclonals, all of these EUAs have been revoked, and there is no monoclonal antibody currently recommended for use to treat

COVID-19 (www.idsociety.org). Tixagevimab/cilgavimab (Evusheld) is emergency-authorized as pre-exposure prophylaxis against COVID-19 for immunocompromised individuals or those who cannot be vaccinated or mount post-vaccination immune response.

3.8- EMA and US FDA accelerated approval pathways.

As highlighted in part 1 of this article, 9 out of the 13 antibody products were approved based on accelerated regulatory pathways. The definitions of these US and EU pathways can be found on EMA (https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/conditional-marketing-authorisation) and FDA (www.fda.gov) websites and summarized in Figure 1 (Cox E et al, 2020).

Figure 1: US Food and Drug Administration (FDA) and European Medicines Agency (EMA) Expedited Programs



4- Outlook for 2023

By the end of 2022, at least 24 investigational antibody therapeutics are undergoing review by regulatory agencies. With antibodies for COVID-19 excluded, the late-stage commercial clinical pipeline grew by

~20% in the past year to include nearly 140 investigational antibody therapeutics that were designed using a wide variety of formats and engineering techniques. Of those in late-stage development, marketing application submissions for at least 23 may occur by the end of 2023, of which 5 are bispecific (odronextamab, erfonrilimab, linvoseltamab, zanidatamab, and talquetamab) and 2 are ADCs (datopotamab deruxtecan, and tusamitamab ravtansine) (Kaplon H et al, 2023).

The biopharmaceutical sector's impressive response to the global COVID-19 pandemic is likely to inform and accelerate broader innovation in the field, particularly within the vaccine space but also with antibody-based therapeutics. Regulatory experience accrued in the last survey period should accelerate the speed of the drug development and approval processes for future biologics (Walsh G & Walsh E, 2022; Broly H & Beck A, in prep.).

Acknowledgements

The authors would like to acknowledge Dr. Janice M Reichert, the Antibody Society (www.antibodysociety.org), and The Animal Cell Technology Industrial Platform (ACTIP, www.actip.org).

List of Abbreviations

ADC, antibody–drug conjugate; ADCC, antibody dependent cell-mediated cytotoxicity; ADP, adenosine diphosphate; ALK, anaplastic large-cell lymphoma kinase; AMD, age-related macular degeneration; AML, acute myeloid leukemia; Ang-2, angiopoietin-2; ASCO, American Society of Clinical Oncology; BCMA, B cell maturation antigen; BCVA, Best-corrected visual acuity; BLA, biologics license application; BMS, Bristol Myers Squibb; BMT, bone marrow transplant; ; CAD, cold agglutinin disease; CAPOX, capecitabine/oxaliplatin; CDC, complement- dependent cytotoxicity; ; CHMP, Committee for Medicinal Products for Human Use; CLL, chronic lymphocytic leukemia; CMC, Chemistry, Manufacturing and Controls; CNS, central nervous system; COVID-19, coronavirus disease 2019; CR, complete response; CRS, cytokine release syndrome, CSCC, cutaneous squamous cell carcinoma; CSU, chronic spontaneous urticaria; CTLA-4, cytotoxic T lymphocyte antigen-4; DCR, disease control rate; DLBCL, diffuse large B-cell lymphoma; DM4, N2'-deacetyl-N2'-(4-mercapto- 4-methyl-1-oxopentyl) maytansine; DME, diabetic macular edema; dMMR, deficient mismatch repair; EC, European Commission; ECMO, extracorporeal membrane oxygenation; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; ESCC, esophageal squamous cell carcinoma; ESMO, European Society for Medical Oncology; EU, European Union; EUA, Emergency use authorization; Fab, antigen-binding fragment; Fc, crystallizable fragment; FcγR, Receptors for IgG Fc; FcRn, neonatal Fc receptor; FDA, US Food and Drug Administration; FL, follicular lymphoma; FRα, folate receptor alpha; GEA, gastroesophageal adenocarcinoma; GEJ, gastroesophageal junction; GM-CSF, granulocyte-macrophage colony stimulating factor; GPP, generalized pustular psoriasis; GPRC5D, G Protein-Coupled Receptor Class C Group 5 Member D; GvHD, graft-vs-host disease; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; HLA, human leukocyte antigen; HR, hazard ratio; HSCT, hematopoietic stem cell transplant; hTfR, human transferrin receptor; iADRS, Integrated AD Rating Scale; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; IM, intramuscular; INN, International Nonproprietary Names;; IV, intravenous; LAG- 3, lymphocyte-activation gene 3; MAA, marketing authorization application; mAb, monoclonal antibody; MHLW, Ministry of Health, Labor and Welfare; MM, multiple myeloma; MMR, mismatch repair; MTX, methotrexate; NDA, new drug application; NHL, non-Hodgkin's lymphoma; NIH, National Institutes of Health; NK, natural killer cells; NPDR, non-proliferative diabetic retinopathy; NMPA, National Medical Products Administration; NSCLC, non-small cell lung cancer; OR, overall response; OS, overall survival; PD, pharmacodynamics; PD-1, programmed

cell death protein 1; PD-L1, programmed cell death protein ligand 1; PD-L2, programmed death ligand 2; PDUFA, Prescription Drug User Fee Act; PFS, progression-free survival; PHN, paroxysmal nocturnal hemoglobinuria; PK, pharmacokinetics; PMDA, Pharmaceuticals and Medical Devices Agency; PR, partial response; PRIME, Priority Medicines; PTCL, peripheral T cell lymphoma; PTI, personalized treatment intervals; RA, rheumatoid arthritis; RECIST, Response Evaluation Criteria in Solid Tumors; RSV, respiratory syncytial virus, RT-qPCR, Quantitative reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SC, subcutaneous; SCAC, squamous cell carcinoma of the anal canal; scFv, single-chain variable fragment; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; TCR, T cell receptor; US, United States; VEGF, human vascular endothelial growth factor.

References

• Akbarian M, Chen SH. Instability Challenges and Stabilization Strategies of Pharmaceutical Proteins. Pharmaceutics 2022, 14, 2533. https://doi.org/10.3390/ pharmaceutics14112533.

• Beck A and Reichert JM. Marketing approval of mogamulizumab: a triumph for glyco-engineering. mAbs 4:4, 1-7; July/August 2012.

• Beck A, Terral G, Debaene F, Wagner-Rousset E, Marcoux J, Janin-Bussat MC, Colas O, Van Dorsselaer A and Cianferani S. Cutting-Edge Mass Spectrometry methods for multi-level ADC structural characterization. Expert Review of Proteomics (invited review), 2016, 13, 157-183

• Beck A, Goetsch L, Dumontet C, Corvaïa. Strategies and challenges for the next generation of antibody-drug conjugates. Nature Reviews Drug Discovery, 2017, May;16(5):315-337.

• Beck A, D'Atri V, Ehkirch A, Fekete S, Hernandez-Alba O, Gahoual R, Leize-Wagner E, François Y, Guillarme D, Cianférani S. Cutting-edge multi-level analytical and structural characterization of antibody-drug conjugates: present and future. Expert Review of Proteomics, 2019, Feb 1:1-26.

• Beck A. Opening keynote presentation. Festival of Biologics. Terrapin. Nov 2, 2022. Basel.

• Beck A, Nowak C, Meshulam D, Reynolds K, Chen D, Pacardo DB, Nicholls SB, Carven GJ, Gu Z, Fang J, Wang D, Katiyar A, Xiang T, Liu H. Risk-Based Control Strategies of Recombinant Monoclonal Antibody Charge Variants. Antibodies (Basel). 2022 Nov 20;11(4):73. doi: 10.3390/antib11040073.

• Blue L, Xiaoyan Guan, Marisa K Joubert, Scott T Kuhns, Stephanie Moore, David J Semin, Mats Wikström, Jette Wypych and Chetan T Goudar. State-of-the-art and emerging trends in analytical approaches to pharmaceutical-product commercialization. Current Opinion in Biotechnology 2022, 78:102800.

• De Romeuf C. et al, Chronic lymphocytic leukaemia cells are efficiently killed by an anti-CD20 monoclonal antibody selected for improved engagement of FccRIIIA/CD16. British Journal of Haematology, 140, 635–643 doi:10.1111/j.1365-2141.2007.06974.x.

• Duivelshof BL, Beck A, Guillarme D, D'Atri V. Bispecific antibody characterization by a combination of intact and site-specific/chain-specific LC/MS techniques. Talanta. 2022 Jan 1;236:122836. doi: 10.1016/j.talanta.2021.122836. Epub 2021 Sep 3.

• Cox E, Edmund A, Kratz E, Lockwood S and Shankar A. Regulatory Affairs 101: Introduction to Expedited Regulatory Pathways. Clin Transl Sci (2020) 13, 451–461; doi:10.1111/cts.12745.

• Fornelli L, D Ayoub, K Srzentic, K Nagornov, A Kozhinov, N Gasilova, A. Beck et al. Structural Analysis of Monoclonal Antibodies with Top-down and Middledown Electron Transfer Dissociation Mass Spectrometry: The First Decade. Chimia 76 (1-2), 114-114.

• Huang Z, Pang X, Zhong T, Qu T, Chen N, Ma S, He X, Xia D, Wang M, Xia M and Li B (2022) Penpulimab, an Fc-Engineered IgG1 Anti-PD-1 Antibody, With Improved Efficacy and Low Incidence of Immune-Related Adverse Events. Front. Immunol. 13:924542. doi: 10.3389/fimmu.2022.924542

• Joubert N, Beck A, Dumontet C, Denevault-Sabourin C. Antibody-Drug Conjugates: the last ten years. Pharmaceuticals, 2020, Sep 14;13(9):245. doi: 10.3390/ph13090245.

• Joubert S, Dodelet V, Béliard R, Durocher Y. Biomanufacturing of monoclonal antibodies. Med Sci (Paris). 2019 Dec;35(12):1153-1159. doi: 10.1051/medsci/2019219. Epub 2020 Jan 6.

• Gera N (2022). The evolution of bispecific antibodies, Expert Opinion on Biological Therapy, 22:8, 945-949, DOI: 10.1080/14712598.2022.2040987.

• Goyon A, Beck A et al. Determination of isoelectric points and relative charge variants of 23 therapeutic monoclonal antibodies. J Chrom B, 2017, 1065-1066, 119-128.

• Kaplon H, Silvia Crescioli, Alicia Chenoweth, Jyothsna Visweswaraiah & Janice M. Reichert (2023). Antibodies to watch in 2023, mAbs, 15:1, 2153410, DOI:10.1080/19420862.2022.2153410.

• Kim J et al, 2020. Analytical characterization of co-formulated antibodies as combination therapy. mAbs 2020, e1738691. https://doi.org/10.1080/19420862.2020.1738691.

• Popkin ME et al. Chemistry Manufacturing and Controls Development, Industry Reflections on Manufacture, and Supply of Pandemic Therapies and Vaccines. The AAPS Journal (2022) 24:101. https://doi.org/10.1208/s12248-022-00751-9.

• Reinert T, Beck A, Houzé P, Gahoual R, Francois Y. CE–MS Methods for the Characterization of Monoclonal Antibodies. Capillary Electrophoresis Mass Spectrometry for Proteomics and Metabolomics: Principles and Applications. John Wiley & Sons. 2022.

• Shivji R et al. Considerations for the chemistry, manufacturing and Controls (CMC) - quality package for COVID-19 vaccines- interim lessons learnt by the European medicines Agency (EMA). Vaccine 40 (2022) 5539–5541.

• Sreedhar A et al. End-to-End Approach to Surfactant Selection, Risk Mitigation, and Control Strategies for Protein-Based Therapeutics. The AAPS Journal (2023) 25:6. https://doi.org/10.1208/s12248-022-00773-3.

• Steinman L, Fox E, Hartung HP, Alvarez E, Qian P, Wray S, Robertson D, Huang D, Selmaj K, Wynn D, et al. Ublituximab versus teriflunomide in relapsing multiple sclerosis. N Engl J Med. 2022;387(8):704-714. doi:10.1056/NEJMoa2201904

• Strohl WR et al. Passive Immunotherapy Against SARS-CoV-2: From Plasma-Based Therapy to Single Potent Antibodies in the Race to Stay Ahead of the Variants. BioDrugs (2022) 36:231–323. https://doi.org/10.1007/s40259-022-00529-7

• Suckau D, Evers W, Belau E, Pengelley S, Resemann A, Tang W, Sen KI, Wagner E, Colas O, Beck A. Use of PASEF for Accelerated Protein Sequence Confirmation and De Novo Sequencing with High Data Quality. Methods Mol Biol. 2022;2313:207-217. doi: 10.1007/978-1-0716-1450-1_12.

• Surowka M et al, Ten years in the making: application of CrossMab technology for the development of therapeutic bispecific antibodies and antibody fusion proteins. mAbs 2021, VOL. 13, NO. 1, e1967714. https://doi.org/10.1080/19420862.2021.1967714.

• Tawbi HA, Schadendorf D, Lipson EJ, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. N Engl J Med. 2022;386:24-34

• Wagh A, Hangtian Song, Ming Zeng, Li Tao & Tapan K. Das (2018). Challenges and new frontiers in analytical characterization of antibody-drug conjugates, mAbs, 10:2, 222-243, DOI: 10.1080/19420862.2017.1412025

• Wagner-Rousset E, Feteke S, Morel-Chevillet L, Colas O, Corvaïa N, Cianférani S, Guillarme D, Beck A. Development of a fast workflow to screen the charge variants of therapeutic antibodies. J Chrom A, 2017, 1498, 147–154.

• Wagner-Rousset E, Colas O, Chenu S, François Y, Guillarme D, Cianferani S, Tsybin YO, Sjögren J, Delobel A, Beck A. Fast afucosylation profilling of wild type and glyco-engineered mAb subunits by middle up Mass Spectrometry. Methods Mol Biol. Vol 2271. Mass spectrometry of Glycoproteins: Methods and Protocols. Arnaud Delobel, Ed. Springer 2021.

• Walsh G & Walsh E. Biopharmaceutical benchmarks 2022. Nature Biotechnology 2022.

• Wilkinson I, Anderson S, Fry J, Julien LA, Neville D, Qureshi O, et al. (2021) Fc-engineered antibodies with immune effector functions completely abolished. PLoS ONE 16(12): e0260954. https://doi.org/10.1371/journal.pone.0260954.

• Wilkinson I & Hale G (2022). Systematic analysis of the varied designs of 819 therapeutic antibodies and Fc fusion proteins assigned international nonproprietary names, mAbs, 14:1, 2123299, DOI: 10.1080/19420862.2022.2123299.

Websites

• The Antibody Society, Inc. Antibody therapeutics approved or in regulatory review in the EU or US. https:// www.antibodysociety.org/resources/approved-antibodies/.

• The Animal Cell Technology Industrial Platform (ACTIP). www.actip.org.

• EMA (https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/conditional-marketing-authorisation)

• U.S. Food and Drug Administration. Emergency Use Authorization (https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization)

• COVID-19 Real-Time Learning Network Anti-SARS-CoV-2 Monoclonal Antibodies (www.idsociety.org).

COULD NON-CLONAL CELLS BE CONSIDERED TO SUPPORT EARLY-PHASE PRODUCT DEVELOPMENT?

Hervé Broly[1] & Alain Beck[2]

[1] Biotech Process Sciences, Merck-Serono, Corsier-sur-Vevey, Switzerland

[2] Biologics CMC and Developability, Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France

By end of November 2022, the Johns Hopkins coronavirus resource center reported 643 million total cases and 6.6 million dead since the initial COVID-19 outbreak in late 2019 due to infections with SARS-COV-2 despite huge resources were deployed to identify and develop therapeutics in a timely manner for fighting against the infection and disease sequelae in patients. These data show that new viruses can emerge and may affect dramatically the human population in a short period of time.

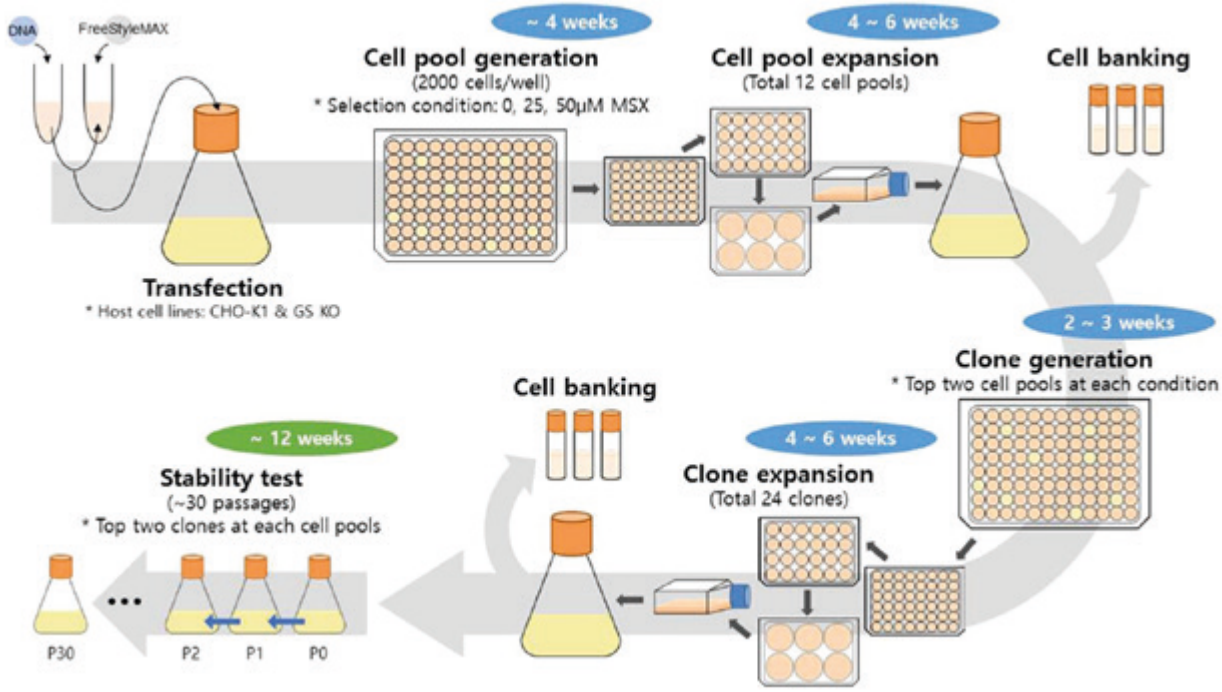
The COVID-19 pandemic highlighted the urgency to make life-saving treatments in a timely manner. One approach was to repurpose approved drugs to treat COVID-19 relatively quickly [1]. The alternative approach was to develop targeted drugs such as vaccines based on mRNA [2, 3] or adenoviral viral vector [4], and monoclonal antibodies (mAbs) directed against SARS-COV-2 motifs [5, 6, 7, 8]. Whereas new targeted drugs may show higher efficacy in principle, classical development pathways would not have been adequate when considering the speed at which the virus spread over a large part of the world. Thus, novel ways to preclinical development [9], clinical development [10] and Chemical, Manufacturing and Control (CMC) [11, 12, 13, 14] have been considered for developing those new drugs at unprecedented speed. As Kelley et al., reported, "*pandemic urgency led to novel development approaches that reduced the time to clinical trials by 75% or more without creating unacceptable patient or product-safety risks*" [15]. The purpose of this paper is to discuss the use of non-clonal cells in generating material to accelerate access to first-in-man trials for recombinant biotherapeutics, such as monoclonal antibodies, Ig-fusion proteins and other protein-based moieties, intended to treat established diseases, especially in areas with critical unmet medical needs, or possible new emerging infectious diseases [16].

Classically, the process for generating manufacturing mammalian cell lines for the expression of recombinant proteins, including mAbs, consists in successive activities once the sequence of the product has been identified:

- preparing or sourcing codon-optimized vectors incorporating the gene of interest and a selection system,
- transfecting a parental cell line,
- screening surviving pool of cells based on the level of expression and the activity of the protein,
- cloning through one round of single cell deposition followed by a cell imaging check [17, 18] to ensure the clonality of the cell line [19], screening and selecting lead clone candidates based on level of expression and major quality attributes, and
- establishing research cell banks (RCB) of the lead candidates and selecting the final manufacturing cell line to generate the master cell bank (MCB) as a compromise between the level of expression, the stability of expression and the quality profile of the recombinant protein expressed using a scale-down model mimicking the large-scale expected manufacturing process.

Based on this traditional linear scenario shown in Figure 1, generating a clonal manufacturing cell line takes about six-seven months [12, 20, 21, 22].

Figure 1: Standard Workflow from Gene to Cell Bank System Qualification (from [23])



One way to save time during development is to parallelize activities by using materials derived from one stage of development which has not yet been fully completed, to perform another independent sequence of activity. This strategy could be considered provided that the use of a preliminary material may not bias the outcome of studies performed with that material.

Multiple regulatory guidelines discuss the topic of the clonal derivation of mammalian production cell lines in the production of recombinant products for human use. ICH Q5D on derivation and characterization of cell substrates stipulates "*For recombinant products, the cell substrate is the transfected cell containing the desired sequences which has been cloned from a single cell progenitor*" [24]. Similarly, FDA's points to consider in the manufacture and testing of monoclonal antibody products for human use notes that "*The MCB is defined as a collection of cells of uniform composition derived from a single tissue or cell*" [25] and the EMA guideline on development, production, characterization and specification for monoclonal antibodies mentions "*The cell substrate to be used for the production of the monoclonal antibodies should be a stable and continuous monoclonal cell line that has been developed by means of recombinant DNA and/or other suitable technologies*" [26]. These guidelines result in the requirement to use a clonally derived cell line for establishing the MCB, which is the starting point of good manufacturing practices (GMP) and subsequent working cell banks (WCB) for preparing manufacturing seeds [27]. However, the manufacture of material for preclinical studies does not fall within the scope of the regulatory guidelines cited above that apply to the manufacture and control of products for human use.

ICH S6(R1) on preclinical safety evaluation of biotechnology-derived pharmaceuticals notes "*The product that is used in the definitive pharmacology and toxicology studies should be comparable to the product proposed for the initial clinical studies*" [28]. But, neither ICH S-guidelines nor ICH Q-guidelines indicate that the material for supplying the preclinical studies should derive from a clonal manufacturing cell line. This opens the door to using pool of cells instead of clonal cells for generating preclinical materials. Indeed, multiple biopharmaceutical companies have published their works supporting that materials issued from pool of cells are not significantly different in quality from materials issued from the clones

subsequently derived from cell pools.

In 2017, several papers have been published on that topic in a specific issue of Biotechnological Progress (volume 33, issue 6):

• Biogen (Wright *et al.*) described an upstream platform capable of delivering equivalent quality material throughout the cell line generation process [21]. The starting point was to subclone parental engineered CHO cells with particular characteristics such as generating highly afucosylated or sialylated recombinant proteins to generate more genotypically and phenotypically homogeneous starting host cell lines. Then, using a standard transfection approach for generating recombinant cell lines, they compare the quality of three different molecules, an IgG1, an IgG4 and an aglycosylated IgG1/IgG4 hybrid at different stages of cell line generation: uncloned pools, pools of clones and lead clones. Using productivity (titer) and quality metrics (% high molecular weight impurities (HMW), impurities, charge variants and glycan distribution), they confirmed the comparability of materials produced at various time points of the cell line generation process.

• At Elli Lily, Rajendra and coworkers used the strategy of transposon (piggy-BAC) technology for ensuring a high level of homogeneity of pools to produce preclinical material [29]. They compared four mAbs (three IgG1 and one IgG4) expressed in CHO cells for stability of expression, genetic stability, titer in bioreactors operated in fed-batch and product quality (HMW, charge variants, purity, glycan distribution, oxidized species and peptide mapping by mass spectrometry) over 55 PDLs. The quality of materials produced at various culture ages was comparable one with the other and comparable to the control derived from a single cell progenitor. They concluded that the genetic stability of transposon-derived cell pools is suitable for generating material for preclinical studies.

• With the same goal, Hu *et al.*, (Genentech) compared titers and product quality (HMW, low molecular weight impurities (LMW) and charge variants) in shake flasks and bioreactors operated in fed-batch of pool of cells and their corresponding top 8 manufacturing cell lines cultured up to 60 days for two mAbs [30]. They concluded that (1) pool and clones have comparable product expression stability and quality profiles for both mAbs, and (2) parallelization of activity and use of cell pools instead of clonal cell lines could be applicable for the manufacture of preclinical material. That strategy allows saving four months from gene to investigational new drug application (IND).

• Fan *et al.* (Bristol-Myers Squibb) propose to use mini-pools to supply preclinical studies and even the first-in-man clinical trials [31]. Their proposal is supported by the comparability of cell growth, productivity and product quality (HMW, charge variants, glycan distribution and sequence) of two monoclonal antibodies produced from mini-pools and corresponding clonal cell lines associated to a more stringent selection from a GSKO CHO host cell line and site-specific integration technology.
At Pfizer, as reported by Scarcelli *et al.*, the strategy for generating manufacturing cell line rests upon the needs of a particular project: a random integration (RI) for high-demand projects where the level of expression is critical, and a site-specific integration (SSI) when speed to clinic is the main driver [32]. Whereas the RI approach associates a CHO K1SV knocked out for GS, the SSI relies on a negative selection by addition of ganciclovir acting through the removal of the thymidine kinase gene of the landing pad and positive selection by addition of hygromycin and the presence of a hygromycin-resistant gene in the landing pad [33]. They compare the performance and product quality of pools of cells and clones for three mAbs developed with the RI approach and six different mAbs issued from the SSI process. The SSI strategy generates highly stable expression across generational age as compared to the RI strategy and enables accelerated development strategies for programs where speed is critical. Whatever the strategy, similar process performance and product quality observed between process and materials based on pools or clones justify the use of pools of cells instead of clones to support preclinical studies and early product development.

• Similarly, Munro *et al.* (Amgen) have compared stability of expression, fed-batch process performance and product quality (charge variants, HMW, LMW, glycan distribution and other quality attributes by multi-attribute mass spectrometry, potency and process-related impurities) of one glycosylated mAb and two aglycosylated mAbs produced from transfected stable pools of cells or from clones. Stable pools exhibited comparable expression stability, process performance and product quality to clonally derived cell lines. They concluded that "*The successful implementation of this approach relies on an in-depth understanding of the expression system being used as well as the bioprocess and analytical platforms to generate and characterize the material irrespective of the cell substrate source*".

Since this series of papers, additional data accumulated to confirm the appropriateness of using non-clonal cells for accelerating process development and decoupling the production of material for preclinical studies from the final clone selection. In 2020, Bolisetty et al. reported that at Bristol-Myers Squibb they parallelize routinely the evaluation of the stability of expression and the final clone selection and the manufacture of preclinical material based on comprehensive observations that platform-derived pools of six top-producing clones mixed at the N-1 stage (one passage before production bioreactor) produce drug substance with very similar product quality profile to the final clone selected based on performance and stability of expression [34].

Even further away, Stuible et al. proposed to generate material for preclinical studies through transient expression in CHO cells [22] thanks to great improvement in yield observed these last years [35, 36]. The authors reported time saving from 8 months to generate material from stable clones to 4 months for material issued from stable pools, or to 3 weeks when considering transient expression. For developing antibodies against SARS-COV-2 in a timely manner, transient expression was used to support process development and even contemplated for supplying preclinical studies [14, 37, 38].

Effectively, in the specific context of FDA's Coronavirus Treatment Acceleration Program [10], companies have used transient expression for selecting antibodies with appropriate characteristics, developing a suitable formulation, checking the suitability of analytical methods and designing a manufacturing process [14, 15]. Producing for preclinical and Phase I clinical studies started with RCBs established under GMP conditions and made of stable pools generated using a site-specific integration (SSI) strategy or transduction to ensure high genetic homogeneity of cells and stability of expression [11, 13, 14, 15]. The RCBs were tested in accordance with ICHQ5A and ICHQ5D to fulfill the safety requirements [24, 39]. In addition, product comparability between Phase I material and later-stage clinical material derived from clonal cell lines that were specifically screened and selected for ensuring product comparability, was demonstrated retrospectively [14, 15]. Xu et al., reported a global approach consisting in transient expression in CHO cells to support preclinical, investigational new drug-enabling toxicology research, and early CMC development, mini-pool materials to supply Phase 1 clinical trials and a single-clone working cell bank for late-stage and pivotal clinical trials [40]. Whereas there were clear differences in process performance between the transient expression and stable expression (mini-pools and single-cell clones), the quality of three batches produced per type of cells was shown comparable using a large set of analytical methods covering the primary structure, higher-order structure, product- and process-related impurities, charge variants, glycan distribution, biological activity, Fc-driven biological functions and 20-day forced degradation profiling at 40°C.

The uncommon practice to use non-clonal cells and corresponding RCBs as starting materials for producing batches of drug substance to supply first-in-man studies have been accepted by regulatory bodies in the specific context of an emergency response to an urgent unmet medical need. However, the absence of demonstration of high level of probability or assurance of clonality of the cells used for producing material intended for human use was counterbalanced by a check of the genetic stability of end-of-production cells and/or an augmented control strategy, e.g., monitoring of sequence variants

by peptide mapping mass spectrometry in drug substance, glycosylation despite not impacting the mechanism of action, and/or tighter limit of *in vitro* cell age in accordance with regulatory considerations on clonality [11, 41].

As an immortalized cell line, the CHO cell line is inherently unstable, and the history of development of that cell line resulted in a genetically and phenotypically diverse family (e.g., CHO K1, K1SV, DXB11, S, DG44) [42]. This genome plasticity led some authors to describe CHO parental cells as a "*quasi species*" [43] and to make questionable the need for cloning manufacturing cell lines with adequate proof of clonality prior to cell bank establishment [44]. On one hand, this may come from a confusion between the semantic sense and the practical sense of clonality. Semantically, clonality means a population of cells that are genetically identical one with the other. Because of the high propensity of CHO cells to chromosomal rearrangements, i.e., about one major chromosomal rearrangement every ten doublings [45, 46, 47, 48], the semantic sense does not apply. However, the practical sense refers to a population of cells with a high probability of being clonally-derived from a single cell progenitor through a manipulation termed "cloning". This is the regulatory sense to clonality [41]. On the other hand, the industry view is that the clonal state of a manufacturing cell line is only one factor that could affect product quality consistency, whereas emphasis should be placed on an augmented control strategy for confirming product consistency [44]. This aspect was supported by the observation that single-cell-derived subclones of clonal manufacturing cell lines generated by random integration in CHO-K1 displayed a range of variation in titer, cell-specific productivity, expression stability, growth and product quality attributes thus showing that cell heterogeneity exists in a cell population even when derived from a single cell progenitor [49]. Therefore, through public communication and publishing, the FDA reemphasized their position on proof of clonality of the population of cells that compose a MCB [19, 41]:

- a non-clonally-derived cell bank may induce more variability in process performance and cellular phenotype-dependent quality attributes (e.g., glycans),
- it may result in drift, shift, or unforeseen selective pressure following a process change,
- the industry view to augment the control strategy is not aligned with the aim of health authorities to promote the quality-by-design concepts [50],
- it may be difficult to differentiate non-clonality from other possible root cause in case of a drift identified by the quality system, including the continued process verification [51], and
- a non-clonally-derived cell bank system may generate variability in product quality due to a lack of consistency in replenishing a working cell bank or during the life cycle of a product.

Finally, Welch & Arden concluded "*Nevertheless, a demonstration that even clonally derived cell lines possess tremendous heterogeneity (or clonal variation) and that nonclonally derived pools can in some cases produce drug substance with CQAs matching those of drug substance produced by a clonally derived line fails to address key unresolved questions*" [19].

In conclusion, as of today, the use of a non-clonally-derived cell bank to produce materials for human studies could be acceptable solely in a specific emergency context where a program exhibits a high benefit to risk ratio and provided that (1) the cell bank has been established in compliance with GMP and tested for adventitious agents in accordance with appropriate ICH guidelines, and (2) the residual uncertainty of the impact of non-clonality is counterbalanced by testing for genetic stability of aged cells and implementing an augmented control strategy such as batch-to-batch amino acid sequence variant analysis.

There is now accumulated evidence on the limited risk of genetic instability, non-reproducibility, and

non-comparability between non-clonally-derived preclinical and clonally-derived Phase I materials. However, there are some precautions to take before implementing a non-clonal strategy, i.e., starting from pools of cells for producing preclinical material. One could consider cell engineering methods such as transduction [52, 53, 54, 55], DNA transposon integration [56, 57, 58], or SSI [59, 60, 61] to deliver stable pools with a high level of homogeneity of cells and a lower risk of significant genetic diversity and instability of expression. Alternatively, transfecting parental CHO cells double-knocked out for glutamine synthetase allows higher stability of expression of pools of cells than wild-type CHO cells [23, 31]. In addition, selecting the final manufacturing cell line by screening clones derived from the pool of cells which has been used for producing preclinical material may further minimize the risk of non-comparability between preclinical and Phase I materials [30].

Recent new technologies for transient expression make that approach an appropriate way to generate very quickly sufficient amounts of materials for developing a manufacturing process, analytics and a suitable formulation. However, there are still limited published reports to really appreciate the probability of occurrence of the materialization of a risk of non-comparability when using transient expression for generating preclinical material and stable clonal cells for producing Phase I material. It may be prudent to further accumulate data prior to considering transient expression as a valuable strategy for the manufacture of preclinical material.

References

[1] J. T. Gordy, K. Mazumdar and N. K. Dutta, «Accelerating drug development through repurposed FDA-approved drugs for COVID-19: speed is important, not haste,» Agents Chemother., vol. 64, p. e000857, 2020.

[2] F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart and et al., «Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine,» N. Engl. J. Med., vol. 383, pp. 2603-2615, 2020.

[3] L. R. Baden, M. El Sahly H, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert and et al., «Efficacy and safety of the mRNA-1273 SARS-COV-2 vaccine,» N. Engl. J. Med., vol. 384, pp. 403-416, 2021.

[4] M. Voysey, S. A. Costa Clemens, S. A. Mahdi, L. Y. Weckx, P. M. Folegatti, P. K. Aley and et al., «Safety and efficacy of the ChAdOx1 nCOV-19 vaccine (AZD1222) against SARS-COV-2: an interim analysis of four randomised conrolled trials in Brazil, South Africa, and the UK,» The Lancet, vol. 397, no. 10269, pp. 99-111, 2021.

[5] R. L. Gottlieb, A. Nirula, P. Chen, J. Boscia, B. Heller, J. Morris and et al., «Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19,» JAMA, vol. 325, no. 7, pp. 632-644, 2021.

[6] D. M. Weinrich, S. Sivapalasingam, T. Norton, S. Ali, H. Gao, R. Bhore and et al., «REGN-COV2, a neutralizing antibody cocktail, in outpatients with Covid-19,» N. Engl. J. Med., vol. 384, pp. 238-251, 2021.

[7] TICO Study Group, «Efficacy and safety of two neutralising monoclonal antibody therapies, sotrovimab and BRII-196 plus BRII-198, for adults hospitalised with COVID-19 (TICO): a randomized clinical trial,» The Lancet, vol. 22, pp. 622-635, 2022.

[8] H. Montgomery, R. Hobbs, F. Padilla, D. Arbetter, A. Templeton, S. Seegobin and et al., «Efficacy and safety of intramuscular administration of tixagevimab-cilgavimab for early outpatient treatment of COVID-19 (TACKLE): a phase 3, randomized, double blind, placebo-controlled trial,» The Lancet, vol. 10, no. 10, pp. 985-996, 2022.

[9] J. A. Grobler, A. S. Anderson, P. Fernandes, M. S. Diamond, C. M. Colvis, J. P. Menetski, R. M. Alavarez, J. A. Young and K. L. Carter, «Accelerated preclinical paths to support rapid development of COVID-19 therapeutics,» Cell Host Microbe, vol. 28, no. 5, pp. 638-645, 2020.

[10] Food and Drug Administration, «Coronavirus treatment acceleration program,» 2022. [Online]. Available: https://www.fda.gov/drugs/coronavirus-covid-19-drugs/coronavirus-treatment-acceleration-program-ctap.

[11] R. Agostinetto, M. Rossi, J. Dawson, A. Lim, M. H. Simoneau, C. Boucher, B. Valldorf, A. Ross-Gillespie, J. G. Jardine, D. Sok, D. R. Burton, T. Hassell, H. Broly, W. Palinsky, P. Dupraz, M. Feinberg and A. K. Dey, «Rapid cGMP manufacturing of COVID-19 monoclonal antibody using stable CHO cell pools,» Biotechnol. Bioeng., vol. 119, pp. 663-666, 2022.

[12] B. Kelley, «Developing therapeutic monoclonal antibodies at pandemic pace,» Nat. Biotechnol., vol. 38, no. 5, pp. 540-545, 2020.

[13] A. Lehman, V. A. Muniz, R. Chaney, J. Pimentel, J. Mattila and S. Lawence, «Speed and need: twin development challenges in rapid response for a SARS-COV-2 antibody cocktail,» Curr. Op. Biotechnol., vol. 76, p. e102715, 2022.

[14] Z. Zhang, J. Chen, J. Wang, Q. Gao, Z. Ma, S. Xu, L. Zhang, J. Cai and W. Zhou, «Reshaping cell line development and CMC strategy for fast responses to pandemic outbreak,» Biotechnol. Prog., vol. 35, no. 5, p. e3186, 2021.

[15] B. Kelley, P. De Moor, K. Douiglas, T. Renshaw and S. Traviglia, «Monoclonal antibody therapies for COVID-19: lessons learned and implications for the development of future products,» Curr. Op. Biotechnol., vol. 2022, p. e102798, 2022.

[16] D. M. Morens and A. S. Fauci, «Emerging pandemic diseases: howwe got to COVID-19,» Cell, vol. 182, no. 5, pp. 1077-1092, 2020.

[17] J. Fieder, P. Schulz, I. Gorr, H. Bardl and T. Wenger, «A single-step FACS sorting strategy in conjunction with fluorescent vital dye imaging efficiently assures clonality of biopharmaceutical production cell lines,» Biotechnol. J., vol. 12, p. e1700002, 2017.

[18] K. Zingaro, D. Shaw, J. Carson, A. Mayer-Bartschmid, C. Bender, C. Alves, D. McVey, N.-X. Qian, Q. Wei, M. W. Laird, Y. Zhu, R. Emmins, J. A. Follit, A. Porter, A. J. Racher, S. Milne, I. Carubelli, Z. Du, A. Khatri, M. Failly, H. Broly, F. Lee, M. Reeser, J. Spidel, K. Anderson, C. Demaria, J. Di-Carlo, J. Gill, A. Lundquist, S. R. Kumar and T. Gill, «Implementation of plate imaging for demonstration of monoclonality in biologics manufacturing development,» PDA J. Pharm. Sci. Tech., vol. 72, pp. 438-450, 2018.

[19] J. T. Welch and N. S. Arden, «Considering «clonality»: A regulatory pespective on the importance of the clonal derivation of mammalian cell banks in biopharmaceutical development,» Biologicals, vol. 62, pp. 16-21, 2019.

[20] M. Stettler and M. Minakowski, «Going from DNA to IND in 11 months,» 2022. [Online]. Available: https://dam.lonza.com/dmm3bwsv3/assetstream.aspx?assetid=15476&mediaformatid=10061&destinationid=10016.

[21] C. Wright, C. Alves, R. Kshirsagar, J. Pieracci and S. Estes, «Leveraging a CHO cell line toolkit to accelerate biotherapeutics into the clinic,» Biotechnol. Prog., vol. 33, pp. 1468-1475, 2017.

[22] M. Stuible, F. van Lier, M. S. Croughan and Y. Durocher, «Beyond preclinical research: production of CHO-derived biotherapeutics for toxicology and early-phase trials by transient expression or stable pools,» Curr. Op. Chem. Eng., vol. 22, pp. 145-151, 2018.

[23] S. M. Noh, S. Shin and G. M. Lee, «Comprehensive characterization of glutamine synthetase-mediated selection for the establishment of recombinant CHO cells producing monoclonal antibodies,» Sci. Rep., vol. 8, no. 1, p. e5361, 2018.

[24] International Council on Harmonisation, «ICH Q5D Derivation and characterization of ell substrates used for production of biotechnological/biological products,» 1997. [Online]. Available: https://database.ich.org/sites/default/files/Q5D%20Guideline.pdf.

[25] Food and Drug Administration, «Points to consider in the manufacture and testing of monoclonal antibody products for human use,» 1997. [Online]. Available: https://www.fda.gov/media/76798/download.

[26] European Medicines Agency, «Guideline on development, production, characterisation and specification for monoclonal antibodies and related products,» 2016. [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-production-characterisation-specification-monoclonal-antibodies-related_en.pdf.

[27] International Council on Harmonisation, «ICH Q11 Development and manufacture of drug substances (chemical entities and biotechnological/biological entities),» 2012. [Online]. Available: https://database.ich.org/sites/default/files/Q11%20Guideline.pdf.

[28] International Council on Harmonisation, «ICH S6(R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals,» 2011. [Online]. Available: https://database.ich.org/sites/default/files/S6_R1_Guideline_0.pdf.

[29] Y. Rajendra, S. Balasubramanian, N. A. McCracken, D. L. Norris, Z. Lian, M. G. Schmitt, C. C. Frye and G. C. Barnard, «Evaluation of piggyBAC-mediated CHO pools to enable material generation to support GLP toxicological studies,» Biotechnol. Prog., vol. 33, pp. 1436-1448, 2017.

[30] Z. Hu, W. Hsu, A. Pynn, D. Ng, D. Quicho, Y. Adem, Z. Kwong, B. Mauger, J. Joly, B. Snedecor, M. W. Laird, D. C. Andersen and A. Shen, «A strategy to accelerate protein production from a pool of clones in Chine hamster ovary cells for toxicological studies,» Biotechnol. Prog., vol. 33, pp. 1449-1455, 2017.

[31] L. Fan, G. Rizzi, K. Bierilo, J. Tian, J. C. Yee, R. Russell and T. K. Das, «Comparative study of therapeutic antibody candidates derived from mini-pool and cloncal cell lines,» Biotechnol. Prog., vol. 33, pp. 1456-1462, 2017.

[32] J. J. Scarcelli, T. Q. Shang, T. Iskra, M. J. Allen and L. Zhang, «Strategic deployment of CHO expression platforms to deliver Pfizer's monoclonal antibody portfolio,» Biotechnol. Prog., vol. 33, pp. 1436-1467, 2017.

[33] L. Zhang, M. C. Inniss, S. Han, M. Moffatt, H. Jones, B. Zhang, W. L. Cox, J. R. Rance and R. J. Young, «Recombinase-mediated cassette exchange (RMCE) for monoclonal antibody expression in the commercially relevant CHOK1SV cell line,» Biotechnol. Prog., vol. 31, pp. 1645-1656, 2015.

[34] P. Bolisetty, G. Tremmi, S. Xu and A. Khetan, «Enabling speed to clinic for monoclonal antibody programs using a pool of clones for IND-enabling toxicity studies,» mAbs, vol. 12, no. 1, p. e1763727, 2020.

[35] R. Vazquez-Lombardi, D. Nevoltris, A. Luthra, P. Schofiled, C. Zimmermann and D. Christ, «Transient expression of human antibodies in mammalian cells,» Nature Protocols, vol. 13, pp. 99-117, 2018.

[36] J. D. Budge, R. J. Young and C. M. Smales, «Engineering of Chinese hamster ovary cells with NDPK-A to enhance DNA nuclear delivery combined with EBNA-1 plasmid maintenance gives improved exogenous transient reporter, mAb and SARS-COV-2 spike protein expression,» Front. Bioeng. Biotechnol., vol. 9, p. e679448, 2021.

[37] G. Xu, C. Yu, W. Wang, C. Fu, H. Liu, Y. Zhu, Y. Li, C. Liu, Z. Fu, G. Wu, M. Li, S. Guo, X. Yu, J. Du, Y. Yang, M. Duan, Y. Cui, H. Feng and L. Wang, «Quality comparability assessment of a SARS-COV-2 neutralizing antibody across transient, mini-pool-derived and single-clone CHO cells,» mAbs, vol. 14, no. 1, p. e2005507, 2022.

[38] S. Rodriguez-Conde, S. Inman, V. Lindo, L. Amery, A. Tang, U. Okorji-Obike, W. Du, B.-J. Bosh, P. J. Wichgers Schreur, J. Kortekaas, I. Sola, L. Enjuanes, L. Kerry, K. Mahal, M. Hulley and O. Daramola, «Suitability of transiently expressed antibodies for clinical studies: product quality consistency at different production scales,» mAbs, vol. 14, no. 1, p. e2052228, 2022.

[39] I. C. o. Harmonisation, «ICH Q5A(R1) Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin,» 1999. [Online]. Available: https://database.ich.org/sites/default/files/Q5A%28R1%29%20Guideline_0.pdf.

[40] G. Xu, C. Yu, W. Wang, C. Fu, H. Liu, Y. Zhu, Y. Li, C. Liu, Z. Fu, G. Wu, M. Li, S. Guo, X. Yu, J. Du, Y. Yang, M. Duan, Y. Cui, H. Feng and L. Wang, «Quality comparability assessment of a SARS-COV-2 neutralizing antibody across transient, mini-pool-derived and single-clone CHO cells,» mAbs, vol. 14, no. 1, p. e2005507, 2022.

[41] R. Novak, «Regulatory perspective on the evaluation of clonality of mammalian cell banks,» in CASSS Forum 2017, Washington DC, 2017.

[42] F. M. Wurm and M. J. Wurm, «Cloning of CHO cells, productivity and genetic stability - A discussion,» Processes, vol. 5, no. 2, p. e20, 2017.

[43] F. M. Wurm, «CHO Quasispecies - Implications for manufacturing processes,» Processes, vol. 1, no. 3, pp. 296-311, 2013.

[44] C. Frye, R. Deshpande, S. Estes, K. Francissen, J. Joly, A. Lubiniecki, T. Munro, R. Russell, T. Wang and K. Anderson, «Industry view on the relative importance of «clonality» of biopharmaceutical-producing cell lines,» Biologicals, vol. 44, pp. 117-122, 2016.

[45] F.-T. Kao and T. T. Puck, «Genetics of somatic mammalian cells, VII. Induction and isolation of nutritional mutants in Chinese hamster cells,» Natl. Acad. Sci. USA, vol. 60, pp. 1275-1281, 1968.

[46] G. M. Adair, R. L. Stalling and M. J. Siciliano, «Chromosomal rearramgements and gene expression in CHO cells: Mapping of alleles for eight enzyme loci on CHO chromosomes Z3, Z4, Z5 and Z7,» Somat. Cell Mol. Genet., vol. 10, no. 3, pp. 283-295, 1984.

[47] S. Vcelar, V. Jadhav, M. Melcher, N. Auer, A. Hrdina, R. Sagmeister, K. Heffner, A. Puklowski, M. Betenbaugh, T. Wenger, F. Leisch, M. Baumann and N. Borth, «Karyotype variation of CHO host cell lines over time in culture characterized by chromosome counting and chromosome painting,» Bioecthnol. Bioeng., vol. 115, pp. 165-173, 2018.

[48] Y. Rouiller, B. Kleuser, E. Toso, W. Palinsky, M. Rossi, P. Rossatto, D. Barberio and H. Broly, «Reciprocal translocation observed in end-of-production cells of a commercial CHO-based process,» PDA J. Pharm. Sci. Technol., vol. 69, no. 4, pp. 540-552, 2015.

[49] P. Ko, S. Misaghi, Z. Hu, D. Zhan, J. Tsukuda, M. Yim, M. Sanford, D. Shaw, M. Shiratori, B. Snedecor, M. Laird and A. Shen, «Probing the importance of clonality: Single cell subcloning of clonally derived CHO cell lines yields widely diverse clones differing in growth, productivity and product quality,» Biotechnol. Prog., vol. 34, pp. 624-634, 2018.

[50] J. Woodcock, «The concept of pharmaceutical quality,» Amer. Pharm. Rev., vol. November 2004.

[51] Food and Drug Administration, «Guidance for industry - Process validation: General principles and practices,» 2011. [Online]. Available: https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf.

[52] J. C. Burns, T. Friedmann, W. Driever, M. Burrascano and J.-K. Yee, «Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: Concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells,» Proc. Natl. Acda. Sci., vol. 90, pp. 8033-8037, 1993.

[53] G. T. Bleck, «An alternative method for the rapid generation of stable, high-expressing mammalian cell lines,» Bioproc. J., vol. 5, no. 4, pp. 36-42, 2006.

[54] G. T. Bleck, «Flexible methodology for developing mammalian cell lines,» BioPharm Int., vol. 2006 Supplement, no. 1, 2006.

[55] Catalent, «Catalent Biuologics launches new GPEx lightning cell line expression technology to shorten drug substance development timeleines by up to three months,» 2021. [Online]. Available: https://www.catalent.com/catalent-news/catalent-biologics-launches-new-gpex-lightning-cell-line-expression-technology-to-shorten-drug-substance-development-timelines-by-up-to-three-months/.

[56] J. G. Mikkelsen, S. R. Yant, L. Meuse, Z. Huang, H. Xu and M. A. Kay, «Helper-independent Sleeping Beauty transposon-transposase vectors for efficient nonviral gene delivery and persistent gene expression in vivo,» Mol. Ther., vol. 8, no. 4, pp. 654-665, 2003.

[57] C. S. Miskey, Z. S. Izsvak, R. H. Plasterk and Z. Ivics, «The Frog Prince: A reconstructed transposon from Rana pipiens with high transpositional activity in vertebrate cells,» Nucleic. Acids Res., vol. 31, no. 23, pp. 6873-6881, 2003.

[58] S. Rajendran, S. Balasubramanian, L. Webster, M. Lee, D. Vavilala, N. Kulikov, J. Choi, C. Tang, M. Hunter, R. Wang, H. Kaur, S. Karunakaran, V. Sitaraman, J. Minshull and F. Boldog, «Accelerating and de-risking CMC development with transposon-derived manufacturing cell lines,» Biotechnol. Bioeng., vol. 118, pp. 2301-2311, 2021.

[59] J. Carver, J. Ng, M. Zhou, P. Ko, D. Zhan, M. Yim, D. Shaw, B. Snedecor, M. W. Laird, S. Lang, A. Shen and Z. Hu, «Maximizing antibody production in targeted integration host by optimization of subunit gene dosage and position,» Biotechnol. Prog., vol. 36, no. 4, p. e2967, 2020.

[60] X. Chi, Q. Zheng, R. Jiang, R. Y. Chen-Tasi and L.-J. Kong, «A system for site-specific integration of transgenes in mammalian cells,» PLoS ONE, vol. 14, no. 7, p. e0219842, 2019.

[61] K. Nehlsen, R. Schucht, L. da Gama-Norton, W. Krömer, A. Baer, A. Cayli, H. Hauser and D. Wirth, «Recombinant protein expression by targeting pre-selected chromosomal loci,» BMC Biotechnol., vol. 9, p. e100, 2009.

DRUG PRODUCT PROCESS DEVELOPMENT: ENSURING A CONSISTENT, HIGH-QUALITY BIOLOGIC

by Catalent

The development process for a biologic is generally divided into two major parts: drug substance development and drug product development. Within those is cell line development, upstream and downstream development, process development, and formulation development. Each development process holds its own purpose along a biologic's journey to commercialization. For drug product process development, it generates the required knowledge to ensure manufacturability of a consistent, high-quality drug product. When biopharma companies choose a partner to develop and manufacture their biologic, they sometimes run into challenges associated with drug product process development. It's critical that biopharma companies work with a partner that has the proven expertise to conduct drug product process development on their innovator or biosimilar drugs.

One Catalent Biologics expert shares his expertise with drug product process development challenges, risks and how to mitigate them, the difference between innovator and biosimilar drug product process development, and what to expect from the development and manufacturing partner during the drug product process development phase.

What are the biggest challenges when conducting drug product process development for a biologic?

Drug product process development can be a very costly initiative, however if sufficient planning is put in place, then the benefit outweighs the cost. Investing adequate resources in the development stage, especially in the advanced analytics of product characterization, gives a drug higher probability of success. If sponsor companies and their partners don't do enough development research before the drug product process development phase, then challenges may arise, prolonging development timelines. However, it's unrealistic for every company to be able to put unlimited money and other resources towards such research. For example, a small biotech company may not have the same resources available compared to a large pharma company for extensive development work. Many small/virtual biotechs even up to mid- to large pharma, opt to partner with an experienced contract development and manufacturing organization who can build and execute necessary protocols. Clearly, an efficient, customized, thoughtful drug product process development program must be built and followed to help take the biologic molecule to the next level.

Is there a difference in process development challenges for innovator biologics versus biosimilars?

They each have their own set of challenges. For innovators, the challenge is timeline and the costs associated with the basic research. A novel biologic requires extensive research to understand the therapeutic effect, side effects, duration of action, elimination, and many more properties. The innovative biologic must demonstrate proof of efficacy for an intended indication and safety in randomized, controlled, clinical trials. Biosimilars on the other hand are less challenging than biologics purely because the competitor of said biosimilar drug is already out in the market. The research has already been done and is available for that biosimilar, either through the company marketing it or through various market and scientific channels. The main challenge for biosimilars is that they must be carefully engineered to match the reference products. However, a biosimilar does have the opportunity to differentiate itself against an established competitor by leveraging new or different delivery methods. From a process development perspective, the challenge resides within physical and chemical characteristics of a product.

The understanding of these characteristics is very important as that will help determine which filling mechanism and filtration process will be more efficient during drug product manufacturing.

What should sponsor companies expect from their development and manufacturing partner when they're ready for drug product process development?

We find the chances of success of a project increase when the sponsor company secures knowledge of the physical properties of the molecule, route of administration and targeted patient population, which are covered during the technology transfer phase. When drug scale-up is initiated for clinical studies, your development and manufacturing partner should look to understand the formulation, the excipients, and stability at the current scale of your biologic drug. This information should then be used to formulate, fill, inspect, package and ship product at commercial scale. Most importantly, the sponsor company should look to partners who have the proven expertise with industry rules and regulations to help move drugs through the "Stage Gate" process. The earlier the sponsor company engages with its partners, the more information that is known about the drug substance, and the better chance the drug will reach patients.

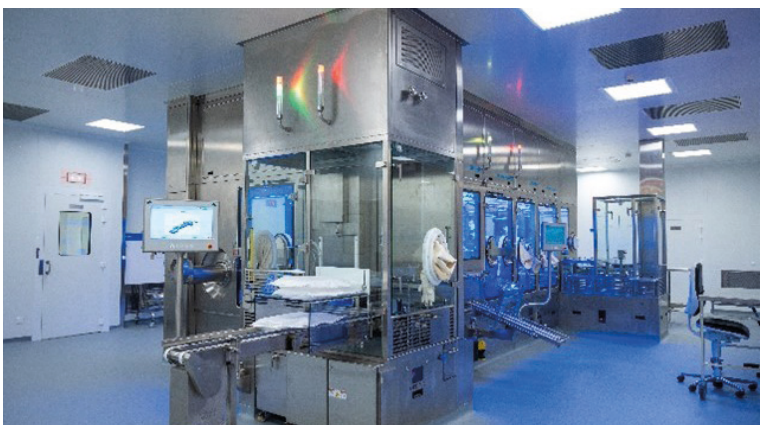
What mistakes do sponsor companies tend to make during drug product process development? How do we mitigate those?

I can't stress enough that there is no such thing as too much data. Not having enough data or not performing sufficient stability studies can really hurt a company's product going into the next phase. To mitigate this, it's important to understand the formulation and how the product reacts to different conditions or different environments, which is accomplished through a robust study design.

As soon as a company brings us their product, that's when we explore those details with them—because getting this information sooner can alleviate headaches for the sponsor company down the road by making good decisions for manufacturing early in the process. As a development and manufacturing partner, Catalent has technical transfer templates that we customize with the sponsor company to capture the type and extent of data collected to date by the sponsor. This will ensure that we minimize challenges during our formulation, filling, or inspection processes, and it helps us to conduct a smooth and successful tech transfer or scale-up.

About the Limoges site

Catalent recent completion of a $30 million (€27 million) project at its facility in Limoges, France, transformed the site into a European center of excellence for biopharmaceutical development, drug product fill/finish services, and packaging. The site further expand Catalent Biologics' global network, with early phase integrated clinical development through to clinical supply services and small-scale commercial manufacturing, allowing seamless tech transfer of projects as they progress to late-stage and larger-scale commercial supply from other Catalent manufacturing facilities in Europe and North America.The project has seen a complete modernization of the Limoges site, to handle large molecule programs, with additional capacity for small molecule injectable dosage form development. A new small-to-mid-scale flexible filling line has been installed, capable of handling vials, syringes or cartridges under barrier isolator technology, and enhancements have been made to analytical and quality control laboratories, supporting clinical packaging, cold storage, and regulatory capabilities.



"This investment has transformed the Limoges site into a world-class facility to support the development of early phase and small-scale commercial biologic drugs, and offers customers integrated services to accelerate programs towards and through the clinic, and ultimately to market. Even prior to completion, multiple clinical and commercial customers have already signed contracts for programs to be undertaken at the site," commented Mike Riley, Catalent's President, Biotherapeutics. "Limoges will now work closely with other Catalent facilities in Europe and the U.S. to provide globally integrated solutions for a range of therapies."

With over 40 years of experience and expertise in supplying life-saving injectable medicines, Catalent Biologics' approximately 56,000 square-foot (5,200 square-meter) Limoges site currently employs over 170 staff.

About Catalent Biologics

For 30+ years, Catalent Biologics has built comprehensive capabilities and proven expertise in development, manufacturing, and analytical services, spanning new biological entities, biosimilars, plasmid DNA, cell and gene therapies, vaccines, sterile injectables, mRNA, and antibody-drug conjugates. It has developed 600+ antibodies and 80+ recombinant proteins, with 120+ active clinical trials and 16 biotherapeutics products using GPEx® cell line engineering technology. An additional 45+ commercially-approved products have employed Catalent Biologics' manufacturing and packaging capabilities. Catalent Cell & Gene Therapy is an industry-leading technology, development, and manufacturing partner for advanced therapeutics. With a comprehensive cell therapy portfolio and deep expertise in viral vector development, scale-up and manufacturing, Catalent is a full-service partner for plasmid DNA, adeno-associated viral (AAV) and other viral vectors, viral vaccines, iPSCs and autologous and allogeneic cell therapies. Using advanced technologies and tailored solutions from clinical to commercial supply, Catalent brings better biologic and advanced treatments to patients, faster.

For more information, please contact solutions@catalent.com

KEY CONSIDERATIONS TO ENSURE A SUCCESSFUL TECH TRANSFER FOR INNOVATIVE BIOPHARMACEUTICAL

by Hervé Ginisty[1]

[1]Chief Scientific Officer, GTP Bioways, herve.ginisty@gtp-bioways.com

A technology transfer consists in transferring process knowledge from development to manufacturing or between different manufacturing sites, with the transfer happening within the same company or between different companies.

A tech transfer involves multiple partners including the sending unit (defined as the organisation a designated process or method is expected to be transferred from) and the receiving unit (defined as the organisation a designated process or method is expected to be transferred to and where it will be executed).

The importance of a dedicated team

As the tech transfer stakes are high, and unforeseen challenges may arise, the creation of a dedicated team able to address multiple areas of the process to be transferred is a good starting point. The tech transfer team may include experts from development, production, quality assurance, regulatory affairs, quality control, and qualification/validation from the sending and receiving units, as well as from the **biopharmaceutical company**.

To operate a smooth tech transfer, the tech transfer team will have to overcome two major challenges:

- Replicate operating procedures with receiving unit equipment
- Complete a full knowledge transfer

Why are tech transfers so challenging?

Even if two sites use the same equipment and operate the same procedures, it cannot be guaranteed that the processes will behave identically. Even if we can assume that the more similar the equipment train between the two sites, the closer the drug substances & the drug products across sites will be, potentially reducing the regulatory burden of filing a site change, this has to be validated through analytical methods. Those analytical methods also have to be transferred from the sending unit towards the receiving unit.

The importance of ensuring knowledge transfer between staff can also not be over-emphasized. It's frequent for key details of a process to be unclear, or difficult to find when the receiving unit gets the complete standard operating procedure (SOP).

Key parameters to consider for a tech transfer between different CDMOs

Over the years, we have managed multiple tech transfers, either as a sending or receiving unit. This experience has taught us that expertise, equipment, and process should be considered equally before entering a tech transfer process.

• **Equipment.** One of the first parameters to examine is the capacities of the receiving site being considered. This is not as simple as seeking the latest technologies - it is important to check equipment compatibility between the sending and receiving facilities.

• **Process.** The process may have been developed by a partner without a clear understanding of GMP requirements. It is important that the receiving unit determines whether the process can be transferred to its facilities as is, and if it meets GMP requirements. A gap analysis is usually helpful to assess potential process parameter changes during the transfer and to evaluate the impact and risks of such changes.

• **Expertise.** Transferring to a team with a strong expertise in process development is an asset for a smooth process transfer as the combination of huge scientific expertise and operational skills will allow to identify potential tech transfer risks and/or redesign a process based on minimal information.

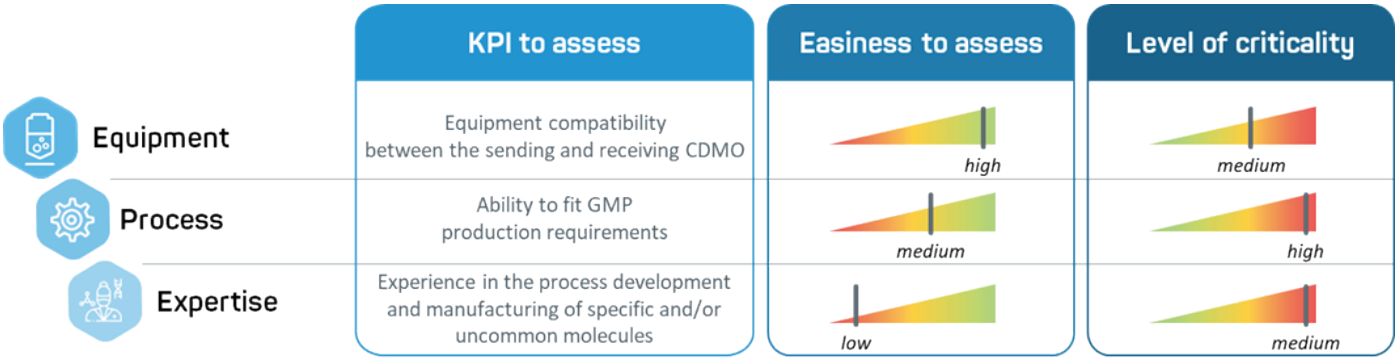


FIGURE 1 - COMPARATIVE TABLE OF THE KEY PARAMETERS TO ASSESS WHILE TRANSFERRING BETWEEN CDMOS

Process cross-analysis should not be overlooked

Once the tech transfer team is established, the next key step is to proceed to a complete cross-analysis of the processes. A detailed and relevant documentation of the processes should be carried out and provided by the transferring site. It will be critical to perform the risk evaluation and gap analysis according to the International Council for Harmonisation (ICH) Q8 guideline. To ensure the tech transfer leads to a robust and reproducible manufacturing process at the receiving facility, critical quality attributes (CQAs) should be identified and then controlled and assessed to achieve the target product profile.

Parameter	Facility A	Facility B	Change Y/N	Impact on CQAs	Potential risk
Centrifugation clarification	Centrifuge	No centrifuge	Y	High	No go for project
SDS page	Coloration duration: x minutes	Coloration duration: y minutes	Y		High if there is a specification for lot release
Nitrogen flush	Nitrogen flush installed	Nitrogen flush installed	N	CQA (oxygen level in the syringe head space)	High, stability is affected, efficacy of the nitrogen flush is unknown, additional studies required

FIGURE 1 - EXAMPLES OF DIFFERENCES BETWEEN THE SENDING AND RECEIVING UNITS ENCOUNTERED OVER THE YEARS BY GTP BIOWAYS DURING TECH TRANSFERS

Although transfer runs have the disadvantage of additional cost and time, their benefits are many including better process understanding and performance, demonstrating product comparability, and verifying CQAs, among others. Transfer runs are also a chance to mitigate risks, solve issues and improve manufacturing instructions.

A few years ago, GTP Bioways was involved in a tech transfer but only as 'facilitator'. A biopharmaceutical company had promising results during preclinical phase with a biotherapeutic protein, produced using CHO cells as an expression system. Their CRO partner had recognised expertise in process development, however they did not anticipate all the GMP constraints while developing an efficient process.

During the early process development phase, a GMP manufacturing partner had not been identified. Once the clinical trial partner was identified, a high-yield process was developed. All protocols and required equipment were exchanged. However, the downstream process required high volumes of buffer solution, which meant the receiving CDMO was not able to handle the required quantity of bioprocessing containers in its DSP room. They needed the biopharmaceutical company to downscale the purification process in order for them to be able to handle it in their GMP facility.

GTP Bioways collaborated with the sending and receiving units to adjust the bioprocess to GMP requirements. We had to downscale the bioprocess twice to manage an acceptable quantity of bioprocessing containers in the DSP area. Additionally, we had to implement two chromatography cycles instead of one (in the original bioprocess designed by the CRO). Therefore, we had to investigate the stability of purification intermediaries, develop and validate methods compatible with GMP constraints. Despite a few changes in the process, the project was successfully transferred to GMP manufacturing and passed phase I/II.

Do not underestimate human expertise

A tech transfer implies having a certain expertise on both sides – the sending unit and the receiving unit. One of the key success factors is the expertise of the tech transfer teams. Managing successful tech transfers between organisations requires talented people with proficient project management and operational skills combined with scientific expertise.

Communication is the key for knowledge transfer

An additional challenge, often neglected, is clear and effective communication. From the earliest stages of the project until its completion, project management and communication among the project team play a key role. Lack of early and effective coordination between the receiving and sending sites is further complicated by lack of clearly defined roles and responsibilities, lack of communication and poor visibility on timelines, progress, and results. People should be introduced right away, to help build as cohesive a team as possible, so everyone is familiar with who their technical and nontechnical counterpart is and can build bridges among those teams (i.e., upstream, downstream, quality, analytical, etc.).

Alongside bringing together the technical teams of the sending and the receiving units, the project team of the sponsor should dedicate time to ensuring a smooth tech transfer. It can indeed provide guided readings of the important documents to make sure that valuable information is not disclosed too late in the transfer process, avoiding situations where technical solutions need to be found in a short time period, and making it possible to pre-empt possible issues. A strong involvement of the project managers, with dedicated time to prepare meetings, prepare guided batch record readings and provide alerts at early stages is key for a seamless tech transfer. For this, a transfer plan must be set up including a steering committee once a month and working sessions once a week with a defined agenda and appropriate experts invited to move the tech transfer forward.

At GTP Bioways, when we operate a tech transfer, we always allow a “person in plant” during the optimisation of the development process as well as during manufacturing, to observe and guide in-plant activities. By asking questions and hearing the logic behind all the decisions being made, the receiving team can gain a deeper understanding of the process in real time. Additionally, it gives the transferring facility a chance to jump in when critical or even non-critical decisions need to be made, as well as to help troubleshoot.

Conclusion

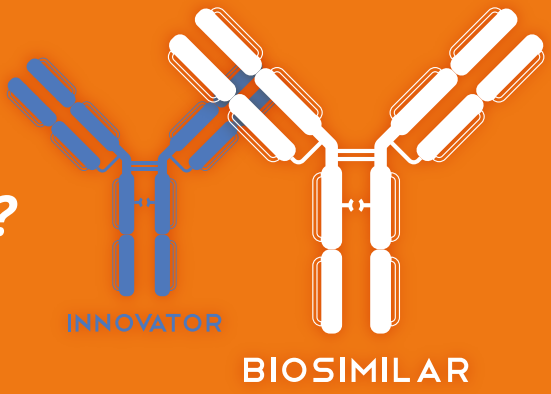
Drawing on our experience gained through multiple processes transferred, we have built a checklist of the key questions to be answered in light of your reasons for transferring your project and of your project development stage.

- Has the receiving unit got a defined, proven technical transfer plan?
- Are the teams responsive, expert and engaged?
- How easily can open discussions be organised between all involved parties?
- Does the receiving unit demonstrate cost-effective planning and project handling experience?
- Does the receiving partner demonstrate excellence evaluating, assessing, managing, and mitigating risk?
- Does the receiving unit demonstrate stewardship of time, expenses, and schedules?

When you find these attributes and the technical fit to meet your drug's particular needs, your programme's success is more likely to follow. Moreover, partners with project managers that demonstrate proficiency in tech transfer and scale up are likely to provide a more solid fit over the long run.

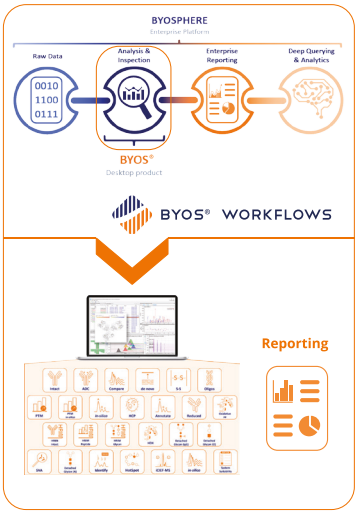
For more information, please contact Séverine Hountondji, Director of Business Development & Marketing, severine.hountondji@gtp-bioways.com

Are you comparing Apples to Apples?



Biosimilar Comparability made easy

To analyse biosimilars you will have data from many workflows and many different vendor instruments, Byosphere can help you with how to get the most out of your data with the least challenges.



For ID (MW) and primary structure

Glycosylation profile
Intact, Peptide, Released Glycan

PTMs
deamidation, oxidation, glycation, PTM mapping

Disulfide bonds mapping

Charge variant analysis

Higher order structure

Vendor neutral

Compliance ready

Cloud or on-premise

Complete suite of analytical workflows

Architected for the Enterprise, it helps break down silos across your organization.

With innovative advanced tools like Dashboards powered by Deep Query you can get insights rapidly on across studies and projects.

PROTEIN METRICS by Dotmatics

Biosimilar resource page

Upcoming Events

8TH BIOPRODUCTION CONGRESS

5-6 October 2023 - Lyon - France

http://www.biopcongress.com

mabdesign

PARTNERING EVENT

MAY 2023 - TOURS

https://mabdesign-partnering-day.mabdesign.b2match.io/

https://aiscongress.com/

https://www.i4id.org/

Next on BioprocessWatch

- **BioprocessWatch - Special edition on the BIOPC2022**
- **Analytical tools in bioprocessing**

mabdesign
THE BIOTHERAPY EXPERT

Follow us on
@Mabdesign_fr
MabDesign

Bât. L'Initial
17 , rue Crépet
69007 Lyon
Tél. 04 78 02 39 88
contact@mabdesign.fr
www.mabdesign.fr