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Special Topic Commentary

# Shifting Paradigms Revisited: Biotechnology and the Pharmaceutical Sciences

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ABSTRACT

In 2003, Crommelin et al. published an article titled: "Shifting paradigms: biopharmaceuticals versus low molecular weight drugs" (https://doi.org/10.1016/S0378-5173(03)00376-4). In the present commentary, 16 years later, we discuss pharmaceutically relevant aspects of the evolution of biologics since then. First, we discuss the increasing repertoire of biologics, in particular, the rapidly growing monoclonal antibody family and the advent of advanced therapy medicinal products. Next, we discuss trends in formulation and characterization as well as summarize our current insights into immunogenicity of biologics. We spend a separate section on new product(ion) paradigms for biologics, such as cell-free production systems, production of advanced therapy medicinal products, and downscaled production approaches. Furthermore, we share our views on issues related to reaching the patient, including routes and techniques of administration, alternative development models for affordable biologics, biosimilars, and handling of biologics. In the concluding section, we outline outstanding issues and make some suggestions for resolving those.



## Introduction

In 2003, a number of us published an article titled: "Shifting paradigms: biopharmaceuticals versus low molecular weight drugs."[1] We outlined paradigm shifts in the pharmaceutical world as a result of the introduction of biological products (from hereon referred to as biologics). Those paradigm shifts would impact both the pharmaceutical sciences and pharmacy practice. Now, 16 years later, we discuss various pharmaceutical(ly relevant) aspects of the evolution of biologics since then: The fast growing repertoire of biologics, the increasing understanding of the potential and limitations of these biologics, and the change in views over time regarding, for example, the emergence of biosimilars. Obviously, a commentary does not permit a comprehensive and complete description of all relevant developments of this fast-growing field. Thus, we had to be selective and were somewhat subjective in picking the topics.

This commentary is divided into 4 main sections (cf. Fig. 1): (1) new biologics; (2) designing a biological drug product: formulation and immunogenicity aspects; (3) new product(ion) paradigms; (4) reaching the patient. The concluding section outlines outstanding issues and possible marching routes for solutions.

## New Biologics

The FDA web site defines biological products-biologics as follows: "Biological products include a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins. Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances or may be living entities such as cells and tissues. Biologics are isolated from a variety of natural sources—human, animal, or microorganism—and may be produced by biotechnology methods and other cutting-edge technologies."[2] We limit the discussion to a subset of innovative biologics: (1) mAb-based biologics and (2) advanced therapy medicinal products (ATMPs). mAb medicines were already available in 2003. But, could we foresee that this group of biologics

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would outgrow all other medicinal product groups over the next 16 years? And what about further modifying their structures to generate various types of mAb-derived molecules and conjugates? ATMPs are a "mixed bag" of innovative biologics, different from the classical pharmaceutical proteins. Whereas in 2003 they were promising new features at the horizon, nowadays the ATMP field is booming. Thus, it is time for a status update.

## *mAb-Based Biologics*

### *Monoclonal Antibodies*

Beyond doubt, mAbs are the most successful family of biologics that evolved in the period 2003-2019. They offered new and successful therapies in cancer, infectious diseases, autoimmune diseases, osteoporosis, macular degeneration, and migraine.[3] In 2002, 10 mAbs were marketed.[4] That number grew to 75 in 2018. In that year, 6 out of the 10 highest selling medicines were mAbs.[5] Over time, the subcutaneous route of administration became more prominent. This led to extensive studies on highly concentrated mAb products because some mAbs are dosed in the >100 mg range and the maximum injection volume for subcutaneous injection is about 1.5 mL; stable and injectable (acceptable viscosity) formulations with mAb concentrations up to 200 mg/mL had to be designed.[6] Alternatively, subcutaneous administration of high doses of mAbs in larger volumes has been made possible by including recombinant human hyaluronidase in the formulation. This enzyme degrades hyaluronan—a major building block of the extracellular matrix, allowing subcutaneous delivery of injection volumes far beyond 1.5 mL (cf. Rituxan Hycela® and Herceptin Hylecta®).[7]

The mAb family provides an excellent example of the required shift in thinking about quality aspects of biologics compared to classical low-molecular-weight medicines. For the latter, we expect purity levels close to 100% with high batch-to-batch consistency. For mAbs, a different situation exists. In one mAb batch, the protein molecules may contain several different post-translational modifications, such as glycosylation and deamidation variants. An illustrative example is trastuzumab where over 25% of the drug substance consists of acidic variants of the main component.[8] Moreover, physicochemical characteristics may vary considerably from batch to batch, in particular if changes in the production process occur.[9] This new quality paradigm sparked discussions on what degrees of variation of which characteristics are acceptable. What are the critical quality attributes (CQAs) and what is the related, acceptable design space? An important step in answering these questions was made with the publication of the A-mAb case

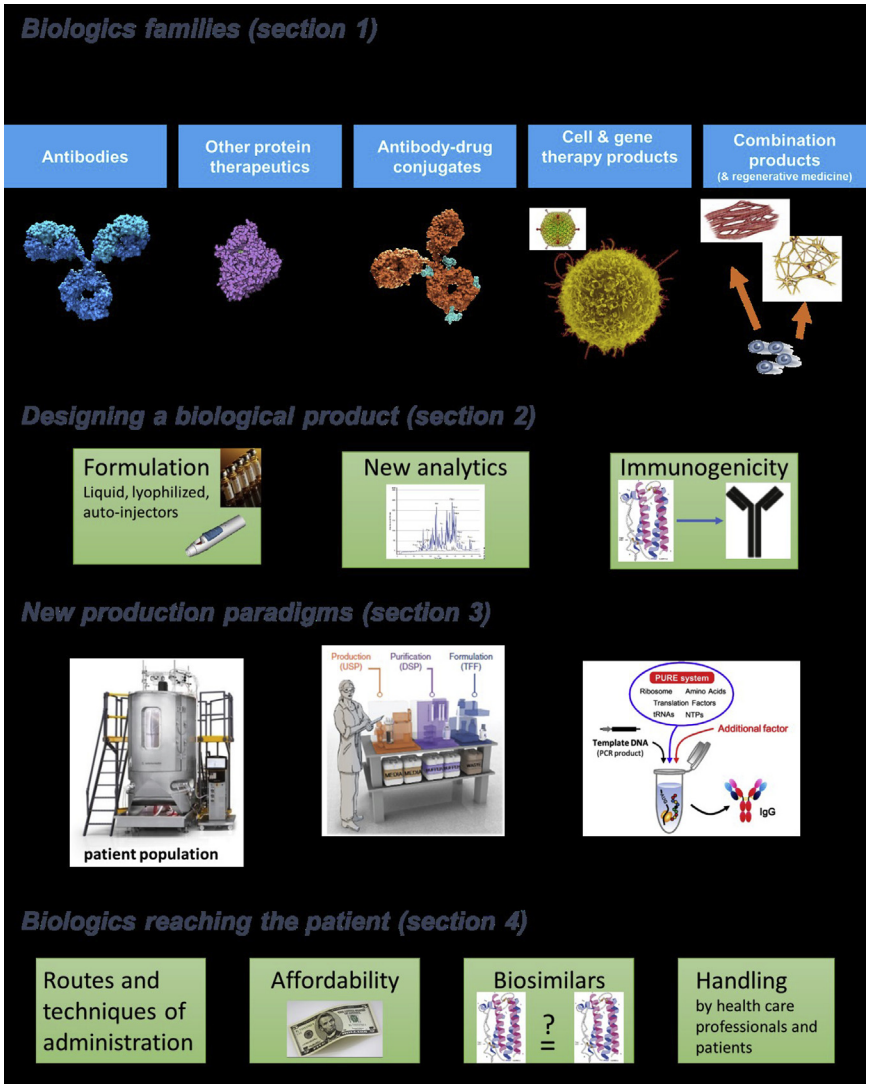


**Figure 1.** Topics dealt with in this commentary.

report by an industrial consortium in 2009.[10] Herein, acceptable ranges for CQAs, such as the percentage of aggregates, galactose, and sialic acid content, based on quality attribute risk assessment are listed. But the discussion continues.[11,12]

In spite of their therapeutic success, there is still a long existing desire to improve the clinical performance of mAbs. Presently, mAbs exert their pharmacological action by neutralizing ligands, blocking or downregulating receptors, or via the antibody-dependent cell-mediated cytotoxicity, and complement-dependent cytotoxicity pathways. Whereas for antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity the Fc part of the molecule is essential, it is not needed (or may lead to unwanted effects) for mAbs that owe their effect to ligand neutralization or receptor downregulation. This has led to the development of mAb-based products without Fc portion (e.g., certolizumab pegol, ranibizumab, abciximab). Below, we discuss 2 other successful approaches: antibody-drug conjugates (ADCs) and bispecific antibodies.

*Antibody-Drug Conjugates*

Already in the 1980s, a great interest existed in coupling toxins to mAbs, but no ADC was approved until 2000. In that year, FDA approved Mylotarg® (gemtuzumab ozogamicin). The difficulties around Mylotarg may be illustrative for the challenges ADCs face. In 2010, Mylotarg was taken off the market by the supplier because of lack of efficacy and it was reintroduced in 2017 only after extensive clinical testing and changes in dosing strategies.

All currently approved ADCs are designed for anticancer therapy. They consist of 3 components: the mAb which improves delivery at the site of action, the bioactive, and the linker. A mAb typically binds to its ligand that is either circulating or exposed on the outer membrane of the target cells. For an ADC, the conjugate has to be internalized, for example, by endocytosis. The choice of the bioactive is crucial as well. In the past, existing cytostatics such as doxorubicin were attached to mAbs. These ADC turned out to lack efficacy. The bioactives of choice are those that are too toxic in clinical practice because of off-target toxicity. Three highly toxic families are used in the presently approved ADCs: maytansines, auristatins, and calicheamicins. Regarding the linker, one can choose a noncleavable one—the bioactive is then released in the lysosomes where the mAb is degraded—or a cleavable one, where the bioactive splits off in the endosomes or lysosomes, for example, an acid-labile, peptide-based, or reducible linker.[13] The drug-to-antibody ratio typically is 3-4. Higher loading rates will compromise the long circulation time and receptor binding.[14] The 4 ADCs approved at the moment are as follows: anti-CD22: inotuzumab ozogamicin, anti-CD30: brentuximab vedotin, anti-CD33: gemtuzumab ozogamicin and anti-HER2: ado-trastuzumab emtansine.

*Bispecific Antibody–Based Products*

The idea to bring 2 ligands together by engineering bispecific antibodies with 2 antigen-binding sites with different binding specificity was first launched in the 1980s as well. T-cell recruitment to tumor cells by a T-cell binding site and a tumor cell–specific binding site in one mAb received early attention. Yang et al.[15] and Carter and Lazar[3] described the myriad of options and challenges bispecific antibody technology offers to combat cancer and other diseases.

When considering the extensive efforts made in the past to develop therapeutic bispecific antibodies, their success in the clinic is still modest. In 2019, only 2 bispecific antibody-based products have been approved by the FDA. One of those is blinatumomab, a bispecific T-cell engager (BiTE), linking T-cell receptor CD3 and B lymphocyte antigen CD19. In 2014, it was approved for certain forms of leukemia. This BiTE molecule (55 kDa) consists of 2 single-chain variable fragments (scFvs), one for the CD3 and one for the CD19 receptor, held together by a short peptide chain that leaves enough freedom for the scFVs to orient themselves freely in space. The small size and lack of the Fc part leads to a short half-life. Consequently, blinatumomab is administered by continuous intravenous infusion.

An example of an application outside the cancer field is emicizumab, a bispecific antibody for the treatment of hemophilia, approved in 2017. It connects activated factor IX (factor IXa) and factor X and thereby induces the cascade of the coagulation reaction.

*Advanced Therapy Medicinal Products*

Gene therapy products, cell-based products, and tissue-engineered products, together called ATMPs or cell and gene therapy products, represent a heterogeneous group of innovative biologics, which can be classified in many different ways.[16] ATMPs are based on viable cells, tissue, or genetic material. The cells and tissues can be derived from a patient (autologous), from a healthy donor (allogeneic) or (less common) from an animal (xenogeneic). Genetic materials are typically RNA or single- or double-strand DNA and delivered to the patient via plasmids, a nanoparticulate delivery system, a viral or bacterial vector system, or cells (i.e., *ex vivo* gene therapy) (see Table 1). ATMPs are administered via parenteral routes, usually by intravenous infusion or local (e.g., intratumoral, intraocular) injection.[16]

In our article from 2003, there is no section on ATMPs simply because the field was still in its very early days. The approval of the first gene therapy product (Gendicine) in China in 2003 represents an important turning point in the area of these innovative biologics. Currently, there are more than 15 ATMPs approved in the USA and Europe and the list is growing (Table 2 and Cuende et al.[24]).

ATMPs, in particular cell- and tissue-based products, differ in several aspects from classical biologics. For instance, animal models for preclinical safety and efficacy testing are lacking, traditional pharmacokinetic-pharmacodynamic (PK/PD) studies are often not possible, dose definition is very challenging, structure-function relationships and immunogenicity risks are largely unknown, orthogonal and stability-indicating product characterization techniques have hardly been established, (large-scale) production platform technologies are not available, and sterile filtration of cells and tissues is not possible. Moreover, formulation development of ATMPs is still in its infancy, as will be discussed in detail elsewhere (Hoogendoorn et al., manuscript in preparation). Briefly, several marketed cell-based products are formulated in cell culture medium, that is, a complex mixture of multiple components, and are stored for a limited period (hours to days) in the liquid state; some other products that are stored frozen contain a cryoprotectant (typically dimethyl sulfoxide) and have a longer shelf-life.

Since the first gene therapy clinical trial in 1990, the field has encountered various setbacks.[25] Problems with vector safety and immunogenicity has led to 2 casualties in clinical trials but also resulted in an intensified effort to improve the safety of viral vectors for gene delivery. The first gene therapy product that obtained marketing approval was gendicine in 2003 by the Chinese FDA. Gendicine is a recombinant adenoviral vector that expresses recombinant p53 for the treatment of head and neck squamous cell carcinoma.[22] Not until 2012, the first gene therapy product was approved in Europe: Glybera® for the treatment of lipoprotein lipase deficiency. Despite this hallmark, the high price (1.4 MEuros per treatment) and lack of demand resulted in market withdrawal of this product in 2016. Other currently more successful gene therapy products are Strimvelis® (for treatment of ADA-SCID), Imlygic® (oncolytic virus for treatment of melanoma),

**Table 1**
DNA/RNA: Delivery Systems, Characteristics, and Examples

| Delivery Technology | Characteristics | Stage of Development | Example(s) of Products/Indications |
|---|---|---|---|
| Naked DNA | Chemically modified ssON to increase stability and cellular uptake; no carrier required although cellular uptake is rather inefficient | Approved | Spinraza, Eteplirsen |
| Nanoparticulate delivery systems | | | |
| Liposomes | Mostly cationic, ionizable lipids that form complexes with negatively charged nucleic acids; particle size range 70-200 nm | Approved | Onpattro |
| Micelles | Polymeric micelles that can entrap ssON as well as siRNA | Preclinical | Cristal Therapeutics Cripec CPC879 |
| Cationic polymers | Cationic, biodegradable, and often pegylated polymers that can condense nucleic acids | Phase II | Inodiftagene vixteplasmid (Anchiano Therapeutics) NCT00595088, NCT03719300<br>Poly(ethylene imine)-based delivery of pDNA encoding diphtheria toxin alpha chain to treat superficial bladder cancer |
| Solid lipid nanoparticles | | Preclinical | |
| Dendrimers | Highly branched polymers with modifiable surfaces | Preclinical | PAMAM dendrimers for delivery of siRNA. Palmerston Mendes et al.[17] |
| Physical methods | | | |
| Microinjection | Direct injection of nucleic acids in cytosol or nucleus: 100% delivery | *In vitro* fertilization, approved application | Not a medicinal product, but *in vitro* fertilization practice |
| Gene gun | Biolistic delivery of nucleic acids coated on gold nanoparticles | Preclinical, mostly used to transfect plant cells and for genetic vaccination | Jinturkar et al.[18] |
| Electroporation | High-voltage electric pulses enable DNA cell entry by transiently breaching the cell membrane | Early clinical trials | Intramuscular pDNA delivery and *in vivo* electroporation to treat patients with cervical intraepithelial neoplasia grade 2/3.[19] |
| Bacterial vector system | Modified *Lactococcus* sp, *Listeria* sp, *Streptococcus* sp. | Preclinical and early clinical | Cancer treatments[20] |
| Viral vector systems | | | |
| Retroviral vector system | ssRNA (+); enveloped virus; particle size range 80-130 nm; infects dividing cells; integration in host genome; long-lasting effects; packaging capacity of 8 kb | Preclinical and early clinical trials | *In vivo* gene therapy: local administration in tumor to treat advanced melanoma; local administration |
| Lentiviral vector system | See retrovirus system | Preclinical and early clinical trials | *In vivo* gene therapy: local administration in brain to treat Parkinson's disease.[21] |
| Adenoviral vector system | dsDNA; naked virus; 70-90 nm; infects dividing and nondividing cells; no integration in host genome; transient effect; packaging capacity of 7.5 kb | Approved<br>Late clinical trials | *In vivo* gene therapy: Gendicine (China)[22]<br>Vaccine approach: systemic administration of viral vector–based product to treat infectious diseases, such as Zika, RSV, Ebola, HIV |
| Herpes simplex vector system | dsDNA; enveloped virus; 150-200 nm; infects dividing and nondividing cells; no integration in host genome; potential long-lasting effects; packaging capacity of >30 kb | Approved | *In vivo* gene therapy: Imlygic (see Table 2) |
| Adeno-associated viral system | ssDNA; naked virus; 18-26 nm; infects dividing and nondividing cells; no integration in host genome; potential long-lasting effects; packaging capacity of >4.5 kb | Approved | *In vivo* gene therapy Glybera, Luxturna (see Table 2) |
| *Ex vivo* genetically modified cells | | | |
| CAR-T | Autologous T cells, genetically modified *ex vivo* with a lentiviral vector system | Approved | Kymriah, Yescarta (see Table 2) |
| TCR | Autologous T cells, genetically modified *ex vivo* with a lentiviral vector system | Preclinical and early clinical trials | Melanoma treatment |
| Dendritic cells | Autologous DCs, genetically modified *ex vivo* with various methods (viral vector, plasmid + liposome) | Preclinical and early clinical trials | Cancer and other indications[23] |
| Natural killer cells | Autologous and allogeneic DCs and DC cell line genetically modified with lentiviral and α-retroviral vector systems | Preclinical and early clinical trials | Cancer treatment (systemic and solid tumors) |

ssON, single-stranded oligonucleotides; ssRNA, single-stranded RNA; RSV, respiratory syncytial virus; dsDNA, double-stranded DNA; CAR-T, chimeric antigen receptor T-cell therapy; DC, dendritic cell.

and Luxturna™ (Leber's congenital amaurosis). Kymriah™ and Yescarta™ consist of genetically modified autologous T cells expressing a chimeric antigen receptor (CAR) on their cell surface that recognizes the cell surface marker CD19 on malignant B cells. Despite their success, the high costs of these products remain a serious issue and limit global access. It is therefore important to explore novel regulatory frameworks that would enable alternative development pathways and innovative reimbursement strategies to ensure a sustainable health care system (see section Alternative Development Models for Affordable Biologics).

*mRNA-Based Medicines*

In recent years, mRNA-based medicines have gained significant momentum.[26,27] Although the first proof-of-concept publication of exogenous mRNA delivery dates back to 1978, these initial studies suffered from instability and immunogenicity of the mRNA molecules, as well as lack of availability of efficient delivery systems. Clinical translation of mRNA has been made possible through chemically modifying mRNA to make it more resistant to nucleases and at the same time less immunogenic. Moreover, improved delivery systems for mRNA, such as lipid nanoparticles, have now

**Table 2**
ATMPs Approved in the EU and the USA (2008 to May 2019)

| Product and Classification | INN/Description | Therapeutic Indication | Company |
|---|---|---|---|
| ChondroCelect[a] (TEP) EU | Characterized viable autologous cartilage cells expanded *ex vivo* expressing specific marker proteins | Cartilage defects of the femoral condyle of the knee | TiGenix NV |
| Glybera[2] (*in vivo* GTMP) EU | Alipogene tiparvovec (AAV1 vector) | Hyperlipoproteinemia Type I | uniQure Biopharma BV |
| MACI[2] (TEP) EU & USA | Autologous cultured chondrocytes | Fractures, cartilage | Genzyme Europe BV |
| Provenge[1] (SCTMP) EU & USA | Sipuleucel-T; autologous peripheral blood mononuclear cells activated with PAP-GM-CSF | Prostatic neoplasms | Dendreon UK Ltd. |
| LaViv (SCTMP) USA | Azficel-T, autologous cellular product | Improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults | Fibrocell Technologies, Inc. |
| Gintuit (SCTMP) USA | Allogeneic cultured keratinocytes and fibroblasts in bovine collagen scaffold | Topical (nonsubmerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults | Organogenesis Inc. |
| Imlygic (*in vivo* GTMP) EU & USA | Talimogene laherparepvec | Regionally or distantly metastatic melanoma in adults | Amgen Europe BV |
| Holoclar (TEP) EU | *Ex vivo* autologous corneal epithelial cells including stem cells | Corneal diseases stem cell transplantation | Chiesi Farmaceutici SpA. |
| Strimvelis (*ex vivo* GTMP) EU & USA | Autologous $CD34^+$ cells transduced with retroviral vector containing the adenosine deaminase gene | Severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID) | GlaxoSmithKline Trading Services Ltd. |
| Zalmoxis (*ex vivo* GTMP) EU | Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2) | Haploidentical hematopoeitic stem cell transplantation | MolMed SpA |
| Spherox (TEP) EU | Spheroids of human autologous matrix—associated chondrocytes | Cartilage defects of the femoral condyle of the knee | Co.don AG. |
| Alofisel (SCTMP) EU | Darvadstrocel; allogeneic expanded adipose stem cells | Complex perianal fistulas in adult patients with nonactive/mildly active luminal Crohn's disease | TiGenix NV |
| Kymriah (*ex vivo* GTMP) EU & USA | Tisagenlecleucel | B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse in patients up to 25 y | Novartis Pharmaceuticals Corporation |
| Yescarta (*ex vivo* GTMP) EU & USA | Axicabtagene ciloleucel; CD19-directed genetically modified autologous T cells | Relapsed or refractory large B-cell lymphoma in adult patients | Kite Pharma Inc. |
| Luxturna (*in vivo* GTMP) EU & USA | Voretigene neparvovec-rzyl (AAV2 vector) | Inherited retinal disease in patients who have a biallelic mutation of the RPE65 gene | Spark Therapeutics |
| Zynteglo (*ex vivo* GTMP) EU | Autologous CD34 + cells encoding $\beta^{A\text{-}T87Q}$-globin gene | Beta-thalassemia | Bluebird bio BV |
| Zolgensma | Onasemnogene abeparvovec | AAV9 gene therapy to treat spinal muscular atrophy (SMA) | AveXis/Novartis Pharmaceuticals Corporation |

AAV1, adeno-associated virus serotype 1; TEP, tissue-engineered product; GTMP, gene therapy medicinal product; SCTMP, somatic cell therapy medicinal product; PAP-GM-CSF, pulmonary alveolar proteinosis—granulocyte macrophage—colony-stimulating factor.
[a] Withdrawn from the EU market.

become available.[28,29] To date, several clinical trials with mRNA are ongoing of which most applications are on tumor immunotherapy, protein replacement, vaccination, and gene editing (mRNA encoding Cas9 nuclease). The future will tell whether these trials will lead to a first-in-class medicine.

*Oligonucleotide-Based Medicines*

Oligonucleotide (ON)-based medicines, including antisense ONs, differ from gene therapy products in that they are chemically synthesized. Hence—in a strict sense—they are not biologics. However, they share a number of characteristics of ATMPs and are therefore discussed here. Whereas small-molecule medicines mostly act at the protein level, ONs act at the DNA or RNA level, enabling the modification of cellular pathways that cannot be easily modulated by small-molecule medicines. Furthermore, ON medicines are generally more specific for their target compared to more broadly acting small-molecule medicines.

ON-based medicines have been in development for several decades with the first experiments starting back in 1978 and the first human trial in 1993.[30] Despite some successes (e.g., approval of fomivirsen in 1998 to treat cytomegalovirus infections of the eye), their development has been slow and troublesome, mainly due to poor stability of the drug substance, rapid clearance, toxicity issues, difficulties in delivering ONs to the right tissue and into cells, and high production costs. The development of better synthetic pathways and discovery of the RNA interference strategy as well as the parallel development of delivery systems to target ONs to specific tissues or organs has led to a revival of ON therapeutics.[31] As of April 2019, 9 ON products have gained marketing authorization—only one with a drug delivery system—with >20 in late-stage clinical development.

Two interesting ON medicines are inotersen (Tegsedi®, Akcea Therapeutics, Boston) and patisiran (Onpattro®, Alnylam, Boston), both for the treatment of hereditary transthyretin-mediated amyloidosis, but with different modes of action. Inotersen is an aqueous solution of antisense ON administered subcutaneously to target the degradation of mRNA encoding both the mutant and wild-type transthyretin.[32] Patisiran is an small interfering RNA (siRNA) targeting the degradation of the same mRNA through RNA interference and is formulated in lipid nanoparticles for targeting it to the liver.[33] Both medicines reduce disability and increase quality of life; however, there remain questions about their cost-effectiveness.[34]

*CRISPR/Cas Genome Editing Technologies*

Besides gene therapy through gene addition, the precise editing of the genomes has gained increased popularity as this would in principle enable correction of monogenetic diseases

with only minor changes to the genome. Engineered nucleases have been developed for this purpose, including zinc finger nucleases, meganucleases, TALENs, and the CRISPR/Cas system. The latter has as main advantage that the nuclease is directed toward a specific genome sequence by virtue of a guide RNA that can be easily synthesized, in contrast to the other nucleases that require engineered protein domains for sequence-specific interaction. For this reason, the CRISPR/Cas system has become the most popular tool for genome editing. The first application of CRISPR/Cas in humans was performed in 2016 in China in which a team of scientists led by the oncologist Lu You administered genome edited immune cells of a cancer patient in which the programmed cell death protein 1 (PD-1) had been knocked out to prevent tumor-instructed silencing of these immune cells.[35] In 2017, CRISPR Therapeutics and Vertex Pharma were the first to start clinical testing of CRISPR/Cas in Europe with the aim to edit blood stem cells from patients with beta thalassemia. The first direct in-body application of CRISPR/Cas is expected to start Q2/3 2019 after Editas received green light from the FDA for clinical testing of their drug candidate EDIT-101 in patients with Leber's congenital amaurosis type 10, the most common form of inherited childhood blindness.[36] These pioneering clinical trials will also be important to establish safety of CRISPR-mediated gene editing as it is at present unknown how the preclinical observations of immunogenicity and off-target genome edits translate to clinical settings.

## Designing a Biological Drug Product: Formulation and Immunogenicity Aspects

Formulation strategies keep pace with the aforementioned widening of the arsenal of biologics and with the increasing quality requirements for the drug substance and excipients. Formulation of ATMPs will be covered elsewhere (Hoogendoorn et al., manuscript in preparation).

Below, we discuss some of the trends in protein formulation development and new analytical approaches for assessing protein structure and stability. Moreover, we provide an update about the critical importance of formulation strategies in minimizing immunogenicity risks of biologics.

### *Trends in Protein Formulation Development*

Key to transforming a novel molecule into a stable and safe drug product is the development of a suitable formulation. Similar to 2003, most formulations of “classical” biologics are composed of pharmacopeial-grade excipients traditionally used in small molecule drug products; novel excipients are hardly used. An exception is the introduction of recombinant human serum albumin (Albumedix®), as a better defined and potentially safer excipient than albumin derived from human donors. It is included as a stabilizer in a few marketed vaccines and in several other biologics under development. Furthermore, the Chinese Pharmacopeia has introduced stricter requirements for polysorbate (PS) 80 quality, that is, $\geq$98% of the fatty acid content should be oleic acid (all-oleic acid PS), compared to $\geq$58% according to USP/Ph. Eur. The implications of this higher purity for drug product stability are currently unclear and under investigation.

Since 2003, there is a tendency toward more aggressive development timelines, in particular with respect to the time to enter clinical phase I. This can be achieved for well-characterized molecule classes, such as mAbs, by testing only a few candidate formulations or standard formulation screening approaches, and only performing additional studies if this approach is unsuccessful. In this context, lyophilization to reduce the risk of failure (because of instability) is often performed. Furthermore, protein formulation development based on prior knowledge is an approach becoming more feasible with the advancement of data science and artificial intelligence. For novel molecule families, however, the risk of failure when relying on platform approaches is still high. Another drawback of a fast formulation development approach is that a formulation for clinical phase I studies may be unsuitable for later clinical stages and commercialization.

The regulatory requirements for product characterization have become stricter since 2003: a larger analytical portfolio is expected to demonstrate drug product stability (see New Analytical Approaches for Monitoring Protein Structure and Stability Section). Furthermore, not only the stability of the drug substance itself, but also that of the excipients—and its impact on protein product stability—needs to be addressed. A prominent example is PS and its instability (see New Analytical Approaches for Monitoring Protein Structure and Stability Section).

In addition, the current arsenal of biologics covers a wide protein concentration range (ca. 4 orders of magnitude). For subcutaneous application of mAbs and other molecules, concentrations up to >200 mg/mL are targeted (see above), whereas highly active molecules (such as BiTEs, see above) require very low concentrations (μg/mL). Both bring challenges with respect to manufacturing and product characterization, including long-term and in-use stability testing. For instance, highly concentrated protein solutions may become too viscous for accurate fill and finish as well as administration. For low concentration products, loss due to adsorption and quantification assay sensitivity (in particular for very low concentrations during in-use stability testing upon dilution into a carrier solution) may become problematic.

A larger assortment of primary packaging materials has become available since 2003. For instance, an increasing number of products are administered subcutaneously via prefilled syringes, dual-chamber cartridges, or autoinjectors. If the primary packaging material is changed, for example, from a vial to prefilled syringe, additional studies are required to demonstrate that this change does not compromise product quality, safety, and efficacy.

### *New Analytical Approaches for Monitoring Protein Structure and Stability*

The analytical portfolio required for the assessment of (critical) quality attributes of biologics has substantially increased since 2003. Higher order structure determination of secondary, tertiary, and quaternary structure is getting more sophisticated, owing to fast-evolving innovations in nuclear magnetic resonance, hydrogen deuterium exchange mass spectroscopy, and other mass spectroscopy-based higher order structure analysis of proteins besides the continued use of established methods, such as circular dichroism, infrared and fluorescence spectroscopy.

In 2003, light obscuration (Ph. Eur.2.9.19/USP<788>) for subvisible particles >10 μm and >25 μm, and visual inspection (Ph. Eur. 2.9.20/USP<1>) were applied as quality control (QC) methods to monitor particles in protein drug products, whereas size exclusion chromatography was used to analyze relatively small aggregates (dimers, oligomers). While this is still the case, these classical QC methods may miss important categories of submicron- and micron-size aggregates, as pointed out in critical commentaries by John Carpenter, Ted Randolph and colleagues.[37,38] Therefore, efforts to reduce and control aggregate and particle contents in biologics should be guided by complementary and orthogonal analytical methods, that is, beyond the routine QC methods. During the past 15 years, several analytical tools for aggregate/particle characterization in the micrometer-size range (in particular flow imaging microscopy) and the submicron-size

range (e.g., resonant mass measurement, nanoparticle tracking analysis, microfluidic resistive pulse sensing) have been introduced for the orthogonal analysis and quantification of particles. However, until today, the robust quantification of submicron particles remains challenging.[39]

Another trend is the application of mass spectrometry–based multiattribute methods for the simultaneous monitoring of multiple relevant product attributes, instead of applying a number of separate methods for the different attributes (e.g., capillary electrophoresis–sodium dodecyl sulfate, ion-exchange high-performance liquid chromatography, peptide mapping with UV, glycan analysis, immunoassays). Through this approach, one can monitor several quality attributes by only one method, thereby reducing the number of assays required for QC release testing.[40]

Besides the stability of the drug substance, it has been clear that excipients can have their own quality and stability issues, potentially compromising product quality; see Table 3 for examples. This implies that also the excipients should be characterized during product development. For instance, qualitative and quantitative analysis of PS 20 and 80 and their degradation products by methods such as LC-CAD, LC-ELSD, and LC-MS has received major attention, because of the incidence of fatty-acid related particles (subvisible and visible particles) being formed in biological drug products due to enzymatic hydrolysis and oxidation.[44,49]

In all fields of analysis, there is a shift toward miniaturization, reduction of analysis time per sample, and automation of sample preparation and analysis, by using autosamplers, plate reader–based systems, use of ultra-performance liquid chromatography instead of HPLC in the reversed-phase or size-exclusion mode, or capillary-based systems, such as capillary gel electrophoresis–sodium dodecyl sulfate (CG-SDS), or capillary isoelectric focusing instead of classical gel electrophoresis. In 2016, a new protein-specific USP<787> chapter was introduced to allow light obscuration measurements using lower test volumes (1-5 mL in USP<787> instead of 25 mL as in USP<788>).

Enhanced computing power enables the application of more sophisticated statistical models (e.g., ultrascan analysis for analytical ultracentrifugation, multivariate statistical analysis, design of experiment tools). Moreover, artificial intelligence is emerging to address analytical questions, for example, machine learning tools for image-based particle analysis.[50]

## *Immunogenicity*

### *Immunogenicity of Classical (Protein-Based) Biologics*

Unwanted immunogenicity of therapeutic proteins, factors that modulate it, and clinical consequences were already pointed out extensively in the 2003 commentary. Since then, the problem of protein immunogenicity has not disappeared. Immunogenicity can lead to several adverse effects, such as the formation of neutralizing antibodies, hypersensitivity reactions, antibody-dependent cellular cytotoxicity, and complement-dependent cytotoxicity. Unfortunately, although *in silico*, *in vitro*, and *in vivo* models to assess the relative immunogenicity of protein drug substances and drug products have become available,[51] we still do not have reliable tools that accurately predict the incidence and—more importantly—clinical relevance of the immunogenicity of a certain protein product in a certain patient population, let alone in a single patient. As a result, despite increased awareness and monitoring of immunogenicity during clinical development and postmarketing, we are still confronted with unexpected events. One example is the withdrawal of a PEGylated peptide product, peginesatide (Omontys), from the market after postmarketing reports about hypersensitivity reactions in 2013.[52] Another one is the discontinuation of the late-stage clinical development program for bococizumab in 2016 because of the formation of anti-drug antibodies in a large proportion of patients, associated with a significant attenuation of its therapeutic effect as well as a higher rate of injection-site reactions.[53]

Major efforts have been made to improve the assessment and reporting of protein immunogenicity.[54,55] For instance, the development of more sensitive anti-drug antibody assays has taught us that products such as Humira (adalimumab) and other TNF-blocking biologics that were initially considered poorly immunogenic are in fact highly immunogenic (resulting in blockage of the therapeutic effect in a substantial number of patients).[56,57] This illustrates that one has to be cautious when interpreting reported immunogenicity levels, such as those mentioned in the literature or package inserts. Moreover, protein immunogenicity does not only depend on the product but also on clinical factors.[58]

Since 2003, a lot of preclinical research has been devoted to better understand the potential role of product-related factors, such as protein aggregates and particles, protein structure, and host cell proteins, in protein immunogenicity. From an immunological perspective, it is well known that protein aggregates present in a drug product may increase the risk of protein immunogenicity.[38,59] Furthermore, results from preclinical studies suggest that aggregates in the size range between ca. One hundred nanometer and ten micrometer, the so-called gap range, are potentially more immunogenic than smaller ones.[60-62] Moreover, nonproteinaceous particles (e.g., metal, glass) and silicone oil droplets in the same size range have been shown to potentially increase the immunogenicity of proteins, especially if the protein adsorbs to those.[63-66] Therefore, from a formulation perspective, it is sensible to avoid as much as possible the introduction or formation of such particulate impurities.

**Table 3**
Examples of Instability Issues Encountered With Common Excipients Used in Formulations of Biologics and Potential Consequences

| Excipient | Instability Issue | Potential Consequences | Reference |
|---|---|---|---|
| Citric acid/citrate | Formation of covalent bonds with peptide | Chemical modification of peptide/protein | 41 |
| Histidine | Oxidation | Protein oxidation | 42 |
| Sodium phosphate | Acidification during freezing | Protein unfolding<br>Protein aggregation | 43 |
| Polysorbate 20 & 80 | (Enzymatic) hydrolysis | Formation of fatty acid–related particles | 44 |
| | Oxidation | Loss of functional polysorbate; consequently protein instability<br>Formation of fatty acid–related particles<br>Protein oxidation | |
| Sucrose | Inversion to glucose and fructose under acidic conditions, followed by Maillard reaction with primary amines<br>Nanoparticulate impurities | Chemical modifications of protein<br>Protein aggregation and fragmentation | 45<br>46,47 |
| Tris | Formation of formaldehyde, reacting with amino acid residues | Chemical modifications of peptide/protein | 48 |

*Immunogenicity of New-Generation Biologics*

To date, relatively few patients have been treated with ATMPs, and hence immunogenicity risks are relatively unexplored. Moreover, appropriate animal models are lacking and immunogenicity is not routinely assessed as part of clinical programs. Nevertheless, addressing these issues is critical for developing safe and effective advanced therapies.

The particulate nature of viruses and cells may be a risk factor by itself, as antigen-presenting immune cells have evolved to readily recognize and take up nanoparticles and microparticles.[67] Other examples of product-related factors probably affecting immunogenicity are the nature of the viral vector, cell source (e.g., autologous vs. allogeneic), and cell maturation state (e.g., induced pluripotent stem cells or fully differentiated cells). However, like for therapeutic proteins, the immunogenicity risk of these products is multifactorial, that is, it depends not only on the product but also on the patient population, disease state, comedication, route of administration, dose and dosing regimen, and so on.

The immunogenicity of viral vectors and oncolytic viruses is well recognized and can be a major obstacle to successful gene transfer in humans.[68-70] One problem is that many patients may already have preexisting antibodies against viral vectors such as AAV. Moreover, immunogenicity against viral vectors has multiple levels: it can be directed against the viral capsid, against the genetic material (DNA or RNA), and against the protein encoded by the transgene. In addition, not only antibody responses but also cytotoxic T cell responses should be considered and monitored. Practically, all patients develop neutralizing antibodies against viral vectors, even against those that are considered to be poorly immunogenic such as AAV, which likely precludes systemic readministration, as these antibodies will neutralize the vector before gene expression can occur. Another potential risk is immunotoxicity caused by cytotoxic T cells directed against *in vivo* transduced cells, such as hepatocytes. Immune responses against the transgene products can also pose serious problems, especially in patients with a mutational genotype that results in complete loss of expression of a particular protein and thus loss of immunological tolerance against that protein.[71,72] Solutions to overcome AAV immunogenicity, such as immunosuppressive comedication, local administration, and genome editing technologies, are being explored and hopefully will result in safer and more efficacious therapies in the near future.

Cells are much larger and even more complex than viral vectors. Upon administration, cells interact with their environment, including the host immune system. This can be intended (e.g., immune-modulatory effects of tolerogenic DCs and CAR-T cells) or unintended.[73,74] For example, patients have shown immune reactions against nonhuman structures, such as the murine scFv region of the anti-CD19 CAR, expressed on the surface of *ex vivo* transduced T cells. However, long-term safety and efficacy implications are largely unknown. Furthermore, we cannot assume that autologous cells or human leukocyte antigen−matched allogeneic (stem) cells are not immunogenic, as even much more simple (recombinant) human protein products may be immunogenic.

## New Product(Ion) Paradigms

The increasing demand for manufacturing of biologics and the broadening of the spectrum of diseases, often with an orphan disease status, drives innovations in manufacturing processes with the intention to simplify these, as discussed in the section "Cell-free production." For ATMPs, questions related to the small-scale, often for the individual patient, production of these complex medicines have to be answered (Section "Production of Cell- and Tissue-Based Products"). The interest in individualizing the production of biologics is further worked out in the Section "Biologics and Precision Medicine: Scaling Down Production Batch Size."

*Cell-Free Production*

Biosynthesis of biologics is a complex process, requiring living cells, expensive and time-consuming production processes, and purification techniques. Cell viability is often the limiting factor in reaching high production yields. Furthermore, it is challenging to control all production parameters necessary to obtain batch-to-batch consistency. The ideal situation would be the possibility to produce complex proteins by chemical synthesis with full control over structure and post-translational modifications. Despite some successes with native chemical ligation to assemble small proteins from peptide fragments, this technology is still in its infancy and yields are generally poor.[75,76] However, alternative production processes without the need to use living cells for on-demand, small-scale production of biologics are under development. An example is the use of cell-free production systems (CFPSs). These involve extracts derived from living cells, containing all the necessary components for transcription, translation, and energy regeneration in a single vial. Protein production is initiated simply by adding a gene construct encoding the desired protein. Although CFPSs have been around for decades, they were primarily used for research purposes. The gradual improvement in production yields, which at present reach the g/L scale, make them a serious alternative for production in living cells, especially for small-scale on-demand production settings (see below). Advantages of a CFPS over production in cells include speed of production (1 day compared to several weeks), the possibility to produce cytotoxic proteins or proteins containing nonnatural amino acids, and the integration of production and purification in a single device.[77,78] Several companies have started to offer cell-free production platforms for the generation of biologics, albeit still at a relatively small scale.[79]

*Production of Cell- and Tissue-Based Products*

For the production of cell- and tissue-based products, there are basically 2 approaches: off-the-shelf (always allogeneic source of cells/tissue) and patient-specific products. Off-the-shelf production processes are similar to those for protein production where one production batch can treat multiple patients. Hence, there is a wealth of engineering and process knowledge as well as technologies that can be leveraged to support the manufacture of these products at increasing scale. However, because the cell/tissue culture is the product of interest, retention of cell viability, phenotype, and function is of primary importance for product safety and efficacy. This means that the desired quality of the cells/tissue must be maintained through the entire manufacturing process, including fill & finish, storage, shipment, and delivery to the patient. This will require the development of scalable harvesting, purification, and formulation technologies to cope with the large batch size produced.

Patient-specific advanced therapies offer a new challenge for process scalability, that is, for each patient, a single-product batch will be manufactured. Here, the cost of production per batch cannot be reduced by exploiting an increasing economy of production scale. Reducing the cost of these patient-specific ATMPs must therefore be achieved by advances in engineering and manufacturing technology, reducing the number of complex, labor-intensive, and open-process steps. The development of closed and automated processes using innovative systems, such as the Octane Technology and CliniMACs Prodigy, as well as process simplification and new and rapid process analytical tools, are key factors for

commercial success, as this will allow multiple batches to be produced in parallel.

*Biologics and Precision Medicine: Scaling Down Production Batch Size*

Since 2003, the emergence of validated biomarkers has given a strong impetus to further stratify patient populations. The introduction of precision therapy for patients implies a reduction of the patient population size if a target specific medicine is available. One of the consequences is that smaller amounts of a biological are required to provide the selected patient population with the medicine. This approach also applies to the production of biologics for orphan diseases. Smaller needs will affect economy of scale for manufacturing. However, this argument of cost price increase may be offset by the development of new, more efficient manufacturing processes that already led to very high gross margins between cost of goods of biologics and actual selling prices (between 1% and 4%).[80] Schellekens et al.[81] pointed out that innovations in manufacturing technology open up the possibility of bedside or magistral biologic preparation by pharmacists for the individual patient. Crowell et al.[82] described protocols to actually build a small-scale production unit for on-demand manufacturing of biologics, thereby making the first steps to realize bedside-patient-specific treatment with biologics.

## Reaching the Patient

This section introduces the patient as a recipient of a biologic. The relationship between a biologic and the patient can take different forms and shapes. The following topics are briefly discussed in the sections below: (1) the routes and techniques of administration; are there alternatives to "the needle"?, (2) the high costs of biologics and consequently the question of patient access to these medicines, (3) the advent of biosimilars, and (4) the way the patient and the health care professional handle these rather unstable products in real-life situations.

*Routes and Techniques of Administration*

*The Oral Route*

Oral administration of peptides and proteins leads to extremely low and variable bioavailability. The gastrointestinal environment is hostile to these compounds. The whole physiological machinery is geared to cut peptides into their amino acids building blocks and absorb those by an active transport mechanism. Passive transport of peptides, proteins, and even amino acids through the intact gut wall is minimal. The only biological drug products that are orally administered are a number of live attenuated vaccines (e.g., oral polio and Salmonella vaccines) for which the oral route is the natural route of infection.

In our 2003 review, we wrote "In spite of tireless efforts of a number of groups, … oral delivery of proteins and peptides never became a success." Since then, research in this field flourished. For instance, when searching the Scopus database for "oral AND absorption AND insulin," the number of publications grew from 45 in 1990 to 109 in 2005 and 125 in 2018. However, in 2016, Aguirre et al.[83] came to the conclusion that even the most advanced clinical studies with peptides led to bioavailabilities of only about 1%. Nevertheless, there is one product in a late stage of clinical trials: the antidiabetic semaglutide. An absorption enhancer is included in the oral formulation. In a phase 3a clinical trial, the chosen daily dose of semaglutide was 14 mg. As the dosing scheme for the subcutaneous formulation varies between 0.5 and 1 mg per week, this 100-fold difference in dose is an indicator for the low bioavailability of this glucagon-like peptide-1 (GLP-1). The company expects to launch this oral formulation before mid 2019.[84,85]

*The Dermal Route*

Does the dermal route offer opportunities to deliver biologics for systemic use? Up until now, all attempts of transporting therapeutically relevant doses of proteins through the intact skin by using patch-type devices have failed. The intact skin turned out to be a formidable barrier. Two other approaches for macromolecule delivery have been demonstrated to offer potential, at least for vaccines: needle-free injection techniques and microneedle technologies.

Needle-free injection techniques have been around for many years. Multiuse nozzle jet injection systems have been used for mass vaccination but were discarded because of the risk of cross-infection with hepatitis B virus. Nowadays, high-speed disposable cartridge devices are being used to exclude the possibility of cross-infection. Depending on fluid velocity and nozzle design, the vaccine is deposited intradermally or dispersed deeper, that is, subcutaneously or intramuscularly. New insights and technologies, such as flow speed modulation, may help optimizing the dermal delivery of vaccines.[86,87]

Then, there are the microneedle technologies for dermal delivery of biologics. Microneedles are about 150-1000 μm in length and are typically placed in arrays on a solid patch surface. Three types of microneedles are being studied: (1) solid microneedles on which the protein of interest is coated, (2) hollow microneedles where a liquid formulation is administered via a syringe of micropump, and (3) dissolvable polymer- or sugar-based microneedles containing the drug substance. The microneedles dissolve and release the bioactive *in situ*. The site in the skin where microneedles deliver their payload (epidermis/dermis) and the limitations in size of the dose make them a logical choice for modulation of the immune system for vaccination or tolerization.[88] No commercial microneedle system made it to the market yet, but especially for vaccines the concept holds promise.

*The Pulmonary Route*

The nasal, buccal, rectal, and pulmonary route have been studied for systemic protein delivery as alternative to the parenteral and oral routes. So far, only for the pulmonary route, 2 products were approved: Exubera® and Afrezza®. Both products contained insulin for inhalation. Uptake of insulin via the lung was fast. Exubera bioavailability was about 10% with a reproducibility similar to that of subcutaneously administered insulin. It received EMA and FDA approval in 2006 but was taken off the market in 2008, probably mainly because of poor market penetration. The reasons were as follows: concerns about lung function, costs, and the bulky device for administration. In 2014, MannKind received approval from the FDA to market Afrezza, a powder-based insulin formulation for pulmonary delivery. Market penetration is low up until now.[89] To date, one biologic is taken via inhalation for local delivery in the lungs: dornase alfa (Pulmozyme®) breaks down DNA in sputum of cystic fibrosis patients.[90]

*Intraocular Administration*

Intraocular administration of biologics has evolved at a rapid pace over the last 20 years. The predominant therapeutic target is age-related macular degeneration. In the eye, vascular endothelial growth factor (VEGF)-mediated angiogenesis is an important driver in the pathogenesis of posterior segment intraocular diseases such as age-related macular degeneration. Intraocular administration of VEGF-binding mAbs or mAb fragments slows down progression of this disease. A Fab' fragment (ranibizumab, Lucentis®) was approved by regulatory bodies throughout the world for treating

macular degeneration. Bevacizumab is a VEGF-binding mAb originally developed to be used in oncology. It is administered (off-label use) into the eye as a much less expensive alternative to ranibizumab. Head-to-head comparison in a clinical trial did not demonstrate significant differences in efficacy or safety between ranibizumab and bevacizumab.[91] Later, aflibercept (Eylea®) was approved. This is a recombinant fusion protein consisting of VEGF-binding sections fused to the Fc portion of the human IgG1 immunoglobulin. The dosing interval is typically 1 month and controlled release systems or alternative routes (transscleral or uveal) to achieve longer dosing intervals and "avoid the needle" would be welcomed.[92]

Interestingly, the first EMA-approved stem cell product (Holoclar®) is administered into the eye to replace damaged corneal tissue. And finally, voretigene neparvovec (Luxturna®) is a gene therapy product approved for treatment of a rare disease, inherited retinal dystrophy. This product has to be injected subretinally.

### *The Parenteral Route: Half-Life Extension and Rate-Controlled Delivery*

Despite all efforts to develop noninvasive administration technologies, still the vast majority of biologics is administered via the needle. The pharmacokinetic profiles of therapeutic proteins vary widely. Many protein medicines have a short half-life and are administered via frequent subcutaneous injections or intravenous infusion(s), associated with patient discomfort. Three options are available to optimize their delivery regimen: (1) modification of the protein structure resulting in a longer half-life; (2) the use of pumps with biofeedback loops, (3) controlled release formulation design. In 2003, these 3 strategies were already known, but since then, new clinical experience has been gained, the original technologies have matured, and new concepts have been introduced.

*Modification of the Protein Structure.* Successful examples of various approaches to prolong the action of proteins can be found in protein-based products originally used to treat diabetes: insulin and GLP-1; both proteins have a short half-life. Traditionally, onset and duration of insulin action was controlled by forming amorphous or crystalline complexes with zinc, phenols, or protamine. Later, exchange of amino acids in the insulin molecule led to either rapid onset, short duration, or slow onset, long-acting analogs. A successful example of creating protracted action is insulin glargine (Lantus®). Adding 2 arginine units to the chain increased the isoelectric point from 5.4 to pH 6.7, causing the modified protein, in solution formulated at pH 4, to precipitate at the injection site, resulting in a once-a-day dosing interval. Another more recent development to prolong insulin action is the use of human serum albumin (HSA) as an endogenous carrier system. HSA has a long half-life and a high binding affinity for fatty acids such as myristic acid. In insulin detemir (Levemir®), lysine replaces threonine at the C-terminus of the insulin molecule and myristic acid is then attached via this lysine. After subcutaneous injection, the myristic acid—insulin combination reaches the blood circulation, binds to HSA, and is subsequently slowly released from this carrier protein, prolonging the half-life from a few minutes for insulin to over 5 h for the detemir variant. A similar approach is used with GLP-1 (7-37). Myristic acid is covalently coupled to GLP-1 (7-37) (liraglutide marketed as Victoza®). This modification prolonged the half-life from 2 min to over 10 h. Later, semaglutide (Ozempic®) was introduced with both a stearic acid and aminobutyric acid attached to the amino acid chain. This aminobutyric acid protects against dipeptidyl peptidase-4 attacks. Semaglutide has a half-life of 1 week.

Just like HSA, antibodies are physiological molecules with a long plasma half-life. This feature was used to genetically modify proteins with a short half-life by integrating them with parts of mAbs. Early examples of fusion proteins with Fc-parts are etanercept with the TNF-alpha receptor, FDA approved in 1998. A later example is aflibercept, a fusion protein comprising vascular endothelial growth factor receptor (VEGFR1) domains and the Fc region of a human IgG1.

A "traditional" chemical modification approach to extend dosing intervals and plasma half-lives is the covalent attachment of polyoxyethylene glycol (PEG) to proteins. Examples are PEGylated interferon alfa-2a and -2b and PEGylated human granulocyte colony stimulating factor, later followed by a PEGylated mAb Fab fragment (certolizumab pegol) specific for tumor necrosis factor alfa (TNF-α) and PEGylated epoetin analogs. Recently, several other conjugation technologies using unstructured peptides have emerged as promising alternatives to PEGylation, such as XTENylation and PASylation.[93]

*Pumps With Biofeedback Loops.* Biofeedback-loop technology has been mainly developed for the controlled delivery of insulin in diabetic patients. Blood-glucose level control requires a flexible input rate. Basically, a biofeedback system has 3 active units with different functions: (1) a biosensor, measuring the (plasma) concentration of the biomarker (glucose); (2) an algorithm, to calculate the required input rate for the delivery system; and (3) a pump system, to administer the protein (insulin) formulation at the required rate.

The realization of a fully integrated closed-loop delivery of insulin comes closer and closer. In 2016, FDA approved a hybrid diabetes management system (MiniMed 670G) consisting of an insulin pump, a continuous glucose-monitoring biosensor, and diabetes therapy management software.[94] Every 5 min, the biosensor measures interstitial fluid glucose levels. The outcome is sent via a wireless connection to a therapy management algorithm. This adjusts the insulin pump settings to an appropriate input rate for insulin to sustain basal glucose levels. However, the patient still has to inject a bolus before meals. That is why it is called a "hybrid" closed loop. Trevitt et al.[95] described the ongoing activities in this fast-moving field. Biosensor stability, robustness, absence of histological reactions by the sensor, and handling postprandial highs are outstanding challenges in the design of fully integrated closed-loop systems for chronic use.

*Controlled Release Formulation Design.* In spite of major efforts to design a controlled release system for proteins, the clinical success of sustained release technologies has been rather disappointing.[1] Expectations were high as therapeutic peptides such as leuprolide, a luteinizing hormone-releasing hormone agonist, formulated as implant, microspheres, or gel, have proven to be highly successful in the therapy of prostate cancer with dosing intervals up to 6 months.[96,97] More recently, one microsphere-based system for a synthetic version of a natural protein made it to the market: a GLP-1 agonist (exenatide, 39 amino acid residues) slow release formulation (Bydureon™) based on PLGA microspheres for once-a-week administration to type II diabetics was approved by the FDA in 2012.

### *Alternative Development Models for Affordable Biologics*

In our 2003 article, we did not mention pharmacoeconomic aspects of biologics. In 2019, the increasing number of highly priced biologics puts health care systems in the western world under pressure. Apart from quality, safety, and efficacy considerations, health economic outcomes including cost-utility analyses decide whether a patient will have access to a therapeutic intervention. For example, the recent addition to the list of marketed ON

medicines, Spinraza®, for the treatment of children and adults with spinal muscular atrophy will cost €640,000 per patient per year. Similar price ranges apply to ATMPs, such as Kymriah. Keeping health care affordable should be a priority of all stakeholders, including payers, pharmaceutical companies, and policymakers. Some scenarios are as follows:

(a) For biologics, the advent of biosimilars definitely led to competition and prices dropped considerably with published reductions (in the Netherlands) of 85% of the original price for adalimumab. That trend will continue. The argument of high manufacturing costs for biologics is contradicted by Kelley et al.[98] and Gal et al.[80] Their analysis shows that manufacturing costs make up, on the average, 3% of the price of a mAb. There is definitely a chance for high-price erosion when multiple competitors enter the market. For ON, manufacturing costs are not expected to make up a substantial part of the product price either, even in case of small batch sizes for orphan indications.
(b) A WHO-related organization is developing an alternative model to market affordable, life-saving biologics for use in less affluent countries. Here the clinical costs for the development of a biosimilar are shared by a number of companies working for local or regional markets, leading to a significant reduction of the costs for clinical trials.[99]
(c) For patient-specific ATMPs, the production costs are generally high.[100] As mentioned previously, a conversion of these products to off-the-shelf products may lead to upscaling of manufacturing processes and lowering of the costs.
(d) Expedited regulatory pathways, including fast-track designation, priority review, accelerated approval, and breakthrough designation, also foster earlier patient access, which potentially leads to lower drug prices. In addition, adapted legislative frameworks for cell- and tissue-based products may decrease development costs.[101]
(e) Stakeholders are experimenting with alternative reimbursement strategies. Among those: "pay for performance" reimbursement, that is, on a "no cure no pay" basis, or "results-driven installment" payment plans.

### *Biosimilars*

In the 2003 article, it was stated that "it is very unlikely that generic versions of biologics will enter the market along the same regulatory pathways as low molecular weight generic products do."[1] This statement was based on discussions in the scientific community and among regulators about the possibility to introduce generic versions of large proteins such as mAbs.

Interestingly, already in 2005, the EMA issued the first overarching guideline on biosimilar drug products: CHMP/437/04.[102] It was revised in 2014. In 2006, the EMA started issuing general and protein-specific guidance documents on the regulatory pathway for biosimilars. Guidance documents followed for specific protein families. Up until August 2018, EMA had approved 49 biosimilars (based on proprietary names) for the EU market.[103]

Biosimilar uptake and resulting price "erosion" vary per product and country. Lately, up to 85% price reduction was reported for Humira®, the innovator version of adalimumab.

Some regulatory bodies in countries outside the EU followed suit and are using the principles of the EMA legal framework. Others, such as the US FDA, introduced a different system. At present, 17 biosimilars have been approved in the USA, but their actual market launch is delayed because of intellectual property issues.

Over the years, publications appeared where efficacy, safety, and immunogenicity of biosimilar and innovator products were compared and no major red flags were raised.[104-107] Kurki et al.[106] conclude their article with the following phrase: "In the authors' opinion, biosimilars licensed in the EU are interchangeable if the patient is clinically monitored, will receive the necessary information, and if necessary, training on the administration of the new product."

An issue yet to be resolved is the "drift, evolution, and divergence" of biologics over time. Changes in production processes for biologics—both for the innovator and biosimilar product—occur on a regular basis, potentially leading to small but detectable changes in the performance of the biologic.[9,108] This may lead to non-similarity of innovator and biosimilar product over time.[109]

One can conclude that since 2003, the biosimilar concept is developing at a rapid pace. The EMA led the way by developing (science-based) regulatory policies and its cautious approach resulted in a set of efficacious and safe alternatives to the innovator products in the EU. However, a number of outstanding issues still have to be resolved before the same level of maturity is reached as we have with the evaluation of small-molecule generics.

### *Handling of Biologics in Real-Life Situations*

In a special issue of the European Journal of Hospital Pharmacists in 2003, the critical importance of proper handling of biologics in a hospital setting was outlined. The hospital pharmacist is charged with the task to ensure integrity of the product and educate those who are involved in the logistics and administration of biologics to the patient. Essential elements are maintaining the storage conditions as indicated by the manufacturer and avoiding heat shocks and shaking when preparing the product for injection.[110] Over time, subcutaneous injections and patient self-injection schemes won in popularity. Here, again, the pharmacist or another health care professional has to instruct the patient how to store and administer the biologic. In particular, the chance of increasing immunogenicity through the forming of protein aggregates by inappropriate storage and administration is a concern. In addition, mishandling may compromise container closure integrity, and thereby product quality, in particular sterility.[111]

Up until a few years ago, hardly any real-world data were available on handling conditions in hospital or patient settings. Paul et al. published on the stability of a diluted mAb in their hospital pharmacy and the same group studied the effect pneumatic tube transport on antibody stability.[112,113] Jiskoot et al.[114] published observations on handling biologics in hospitals and listed a number of irregularities that could jeopardize the quality of the drug product. Nejadnik et al[115] then wrote a commentary summarizing the "state of the art" and actions to be taken. Vlieland et al.[116] monitored storage temperatures for biological drug products at patient's homes and reported major deviations from the prescribed temperature range. Both freezing-thawing excursions and excursions to ambient temperature for prolonged periods were observed. Subsequently, simulation studies were carried out in the laboratory and the possible impact on the physicochemical properties of the proteins under these conditions was established.[117] Clearly, this issue of proper storage and handling of biologics in real-life situations needs more attention in educational programs for health care professionals and more hard data are urgently needed to assess the real risk patients run.

## Concluding Remarks

In this commentary, we have drawn an impressionist picture of the progress made in the field of biologics over the last 16 years.

Here, we point out some of the outstanding issues that still have to be resolved.

It has been the era of the breakthrough of ATMPs as a therapeutic approach. They are often targeted for use in an orphan or individualized drug setting. The introduction of these products asks for new, adapted drug development approaches, including good clinical and good manufacturing practices. The number of patients in clinical trials for a particular ATMP program may be very small. What statistics should one use in such situations? And what QC program may be realistic considering the lack of test material and the short shelf-life (hours to days) of many ATMPs? One of the consequences of custom-made treatments is that access of patients to these novel therapeutic approaches may be jeopardized by the extra financial burden that these novel biologics bring to the already overstretched health care budgets. The introduction of biosimilars, advanced production platforms, expedited regulatory pathways, and alternative reimbursement strategies may offer some relief in the long run, but currently, the rapid pace of the introduction of new biological medicines and the accompanying price tags fuel concerns about patient access in the years to come.

An ongoing concern with the use of biologics is that they tend to be immunogenic. We need predictive tools for development of unwanted immune responses and robust monitoring programs for assessment of the associated clinical risks, both at a patient population and an individual patient level. Moreover, mitigating immunogenicity of biologics is another requirement for a successful further expansion of the field, for example, by taking out murine sequences in CAR-T cells, optimizing mRNA sequences, and minimizing aggregate formation.

For gene therapy, viral vectors turned out to be superior to synthetic nonviral vectors, which is reflected by the fact that all gene therapy products currently on the market are based on viral vectors. Synthetic, nonvectors still suffer from the transient nature of gene expression as well as the poor transfection efficiency for genetic material that needs to be delivered into the nucleus. Whereas viral vectors have built-in mechanisms to enable active transport of the genetic material into the nucleus, all attempts to copy these into synthetic vectors have been largely unsuccessful. Nevertheless, for applications where cytosolic delivery of genetic material is required (e.g., siRNA, mRNA) and where repeated administrations are needed for prolonged therapeutic effects, nonviral vectors have a major advantage over viral vectors whose immunogenicity often prevents repeated administrations.

Biologics vary in complexity. Small proteins are relatively easy to characterize and formulate. On the other end of the scale, one finds highly complex ATMPs where there is a clear need for the development of novel affordable, heat-stable formulations. Liquid formulations are preferred for several reasons such as cost, no reconstitution/less handling issues. However, in many cases, lyophilization (for protein formulations) or cryopreservation (for ATMPs) is still needed to reach the required level of stability. Formulation development remains a challenging field, as high concentration protein solutions, without aggregates and with a low viscosity, are needed and new biological entities that do not fit in existing formulation platforms are introduced.

Progress made in analytical techniques for characterizing complex medicines such as biologics in great detail is impressive. New techniques continue to be developed and are important in assessing the level of heterogeneity, demonstrating batch-to-batch consistency as well as identifying the CQAs of biologics and assessing the related design space through risk assessment analyses.

Interestingly, there is a blurring borderline between biologics and nonbiological products. For example, siRNA products are considered biologics, while the siRNA is not produced by “natural sources” but synthetically. The same is true for mRNA products. An interesting regulatory case came up when a dossier for a marketing authorization of a synthetic generic version of a peptide (i.e., teriparatide) was submitted. The originator product is produced by recombinant technology; the generic product was accepted via the abbreviated new drug application route and not as a biosimilar.[118] In the future, we can expect more of these “border-crossing” examples to occur.

When considering the extensive quality assurance and QC measures taken during the manufacturing process of biologics and the strict adherence to storage conditions by the manufacturer, the lack of information on the fate of biologics after leaving the manufacture's premises is remarkable. Only recently, findings were published on the handing of biologics in real-life situations in hospitals and in the hands of patients. It is clear that storage and handling of biologics in the investigated situations were far from optimal and more attention should be paid to ensure the preservation of the efficacy and safety of these expensive medicines in those situations.

Finally, we can only speculate about what the coming 16 years will bring to the field of biologics. Progress definitely has not slowed down since 2003, the pipeline is well filled, and novel technologies are being developed. Therefore, we foresee a bright future for biologics, expect major new therapies to come and anticipate that paradigms will continue to shift.

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