Perspective

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Developability assessment as an early de-risking tool for biopharmaceutical development

Jesús Zurdo
Lonza Biologics plc, The Portway, Granta Park, Cambridge, CB21 6GS, UK
Tel.: +44 122 389 5105
Fax: +44 122 389 5191
E-mail: jesus.zurdo@lonza.com

Increasing attrition of therapeutic candidates during preclinical and clinical development affects productivity and causes spiraling costs, negatively impacting the development of new treatments. For biopharmaceuticals, product design, lead selection and manufacturing process development constitute significant areas of risk because of their decisive influence on product quality, biological activity and safety, as well as cost of goods. Risk-management developability assessments, introduced early on in development, can help identify and address potential causes of attrition in preclinical and clinical stages related to product manufacturing, safety, delivery and efficacy issues. This article discusses the utilization of *in silico* and *in vitro* surrogate assays early on in development as part of a comprehensive developability assessment for novel biotherapeutics, incorporating a closer interaction between discovery and development functions. It further suggests how such approaches can have a significant impact in streamlining drug development, delivering better and safer therapeutic candidates, while reducing risk and development costs.

Surviving the 'valley of death': why early de-risking is a desirable activity

R&D expenditure for new therapeutic development has seen a substantial increase that has not been accompanied by an equivalent growth in the number of new medicines. Quite the opposite has occured; the number of newly registered therapeutics per R&D billion US dollars has declined dramatically [1]. This is one of the reasons why the average cost of developing a new therapy is reaching highs not seen before. Some estimates place this cost at around US$1.8 billion [2], whereas more recent calculations suggest the real cost is more in the region of $4 billion, in some cases going as high as $11 billion [201]. Only a small fraction of the drug candidates that enter development end up becoming a commercial product. Values for clinical attrition vary among different sources, but overall seem to approach or even exceed 90% of programs entering the clinic [2–4]. The main reasons for such high drug attrition primarily include efficacy, safety and toxicology, pharmacology, commercial, and cost of goods (COGs) [5]. As we will see later on, many of these issues are related to the design and molecular characteristics of drug candidates, in addition to manufacturing and delivery strategies utilized.

Most biotherapeutic candidates will fail during what has been termed the 'valley of death' or translation gap of pharmaceutical development **(Figure 1)** [6–9]. The problem with this valley of death is double: on one hand the enormous timespan involved and, on the other, that failure tends to accumulate in later development phases, where the costs incurred are substantially higher [2]. Furthermore, in recent years attrition rates in all stages of clinical development seem to show a gradual increase [10].

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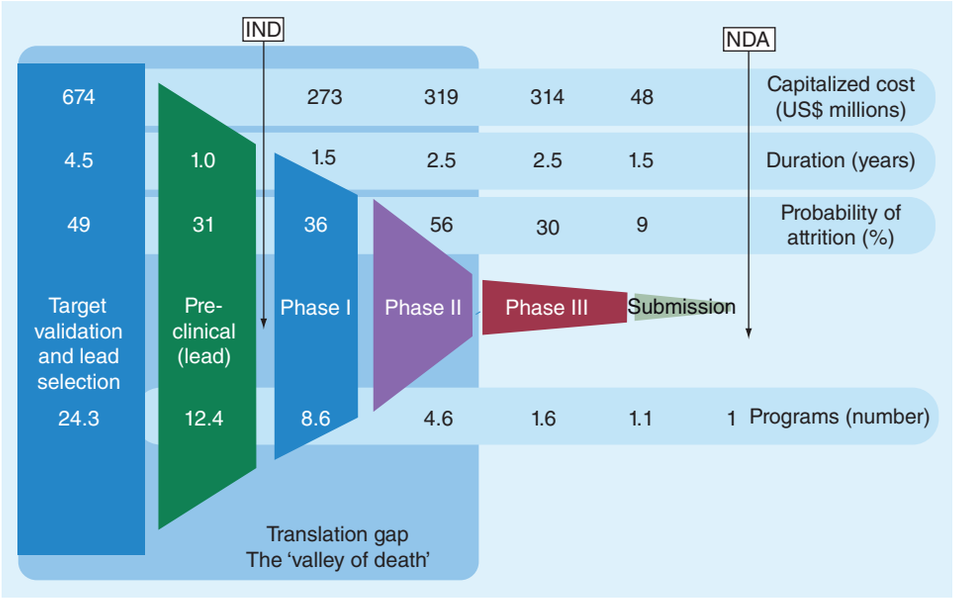

Figure 1. Drug-development cycle and the 'valley of death'. The drug-development life cycle, duration of each one of the stages, capitalized costs, and the probability of failure, which is highlighted as an 'attrition funnel' diagram in the figure. Pharmaceutical Research and Manufacturers of America's estimates indicate a significantly larger attrition during preclinical development, with only one of every 250 compounds entering preclinical development ever receiving regulatory approval [212]. Preclinical development of biopharmaceuticals (including process development and animal toxicology prior to Phase I) is typically longer, approaching 2 years.
Adapted from [1] and data taken from the Michael J Fox Foundation [211].

To further complicate the situation, the success of a drug during clinical trials and its registration does not guarantee that it will be able to recoup the investment made during development. This is mostly due to the increasingly stringent requirements from payers for new drugs to be cost effective. One example of this trend is the reduction in the number of registered anticancer therapeutics approved by the UK NICE. The proportion of approvals has decreased from 65% on average for the period 2000–2012, to just 43% during the year 2012 (reviewed on 10 January 2013) [202]. Even after approval, cost–effectiveness can be decisive in the success or not of any new therapy. Two good examples of cost pressure are the controversy surrounding the use of Avastin® as a cheaper alternative to Lucentis® for the treatment of macular degeneration [11–13], or the announcement of a substantial price reduction for Zaltrap® shortly after launch [203].

In this context, the priority has shifted towards a more pre-emptive or front-loading strategy following the 'fail early, fail cheap' motto, facilitating learning and knowledge transfer and allowing changing course, if so required, as early as possible in the development life cycle [14–16]. Some strategies to achieve this include the routine incorporation of translational medicine in drug development, facilitating the rapid transfer of information from the laboratory to patient and back, or the introduction of new clinical approaches, such as micro-dosing or Phase 0 trials [17,18]. It is expected that these approaches will facilitate the early validation of targets or identification of potential safety issues. This article will focus on strategies that, if introduced even earlier on, could provide directional information to start addressing, at least preliminary key questions for every new therapeutic candidate such as: can it be made, is it safe, will it work, and how much will it cost? This approach is called developability assessment and, if fully implemented, could substantially reduce attrition risks currently experienced by biotherapeutic candidates as they progress across the dreaded valley of death. Some of these strategies have already been applied for quite some time in the small-molecule world [14,16] but, for a number of reasons that we will address below, they have yet to be fully embraced in biopharmaceutical development.

» A very complex problem: a very compartmentalized process

Drug development in general is an expensive, highly risky and complex process requiring a large number of different types of expertise that need to be synthesized into a single final product. Its complexity is exacerbated by the fact that:

- In many cases, the biology behind the targeted disease is largely unknown (particularly in the complex context of a human being);
- The drug development process is highly regulated;
- To these two we should add the financial element that drives and regulates every single industry: the profitability requirements and the financial capacity of customers (or payers, as we will see) to reimburse both the development costs and profit levels expected by drug developers.

In this context, an important challenge in the development of new therapies is the elevated fragmentation

of expertise and, as a result, the fundamentally siloed approach to drug development that operates traditionally in the pharmaceutical and biotechnology industries [19]. It is common that research efforts during the discovery phase are primarily directed to identifying a suitable ligand molecule against a given target. This premise, therefore, often implies finding the best possible binder to the desired target (with the highest-affinity). Such a goal, however, can be complicated by various factors. Some of these include the biology of the target, including its expression in different patient backgrounds or tissue subtypes; the existence of different target subpopulations or complexes in a biological context; their interactions with other biological components or signaling molecules; their homeostasis, and so forth. Another important hurdle in the development of new biologics is defined by the inherent difficulties of 'making' and testing different therapeutic candidates or 'prototypes' and the availability of adequate and relevant assays to evaluate their biological activity and side effects.

» The urgent need for innovation

In recent years there has been substantial progress in understanding the nuances of biological activity linked to the use of biotherapeutics. However, in many cases (particularly with new targets) there are still large knowledge gaps that need to be addressed. In the case of monoclonal antibodies and their derivatives, there is an extensive body of knowledge linking molecular characteristics with biological activity and mechanism of action [20]. This makes it possible to design much better molecules with a final biotherapeutic endpoint in mind. Examples of such understanding and the design options made possible include the use of different antibody formats and scaffolds; the utilization of glycoengineering to regulate biological activity and pharmacology; the development of multivalent antibodies; or the combination of specific protein ligands with small-molecule drugs to deliver targeted payloads, as in the case of antibody–drug conjugates [21–24]. However, there are still considerable gaps in the understanding of the biology of many targets, particularly new non-validated ones. Also, the onset of new scaffolds and over-engineering of new biopharmaceuticals has created, on occasions, significant downstream issues in bioprocessing and safety for a lack of proper developability assessment.

One could argue that the dramatic evolution experienced by the biopharmaceutical industry over the last 30 years relies heavily on the innovations introduced in the way bioprocesses are developed and conducted. Productivities have increased by over 100-fold [25], from tens of milligrams to several grams per liter, and the control over product specifications has experienced a substantial progression. Among such advances one could mention:

- More robust fermentation processes;
- The use of synthetic biology applied to the development of novel cell hosts thanks to the incorporation of more advanced genome sequencing and editing technologies;
- The introduction of automation in process development, particularly in strain/cell line development and downstream process development;
- The use of miniaturized bioreactors, allowing increased understanding of process key parameters and process optimization in a much faster and more cost-effective way;
- The introduction of disposables [26–30].

This rapid evolution in bioprocessing has had a favorable impact in the reduction in COGs, currently typically below the $100/gr barrier for a typical monoclonal antibody developed in a commercial platform. This means that drug substance COGs could account for as little as 1–5% (even less in some cases) of final sales price of biopharmaceuticals [31,32]. Of course, there are numerous exceptions to this, but the improvement in efficiency is shining a light on other areas of biopharmaceutical development that are now proving to be real bottlenecks. As we will see, some of these bottlenecks are related to the design and selection of lead candidates and their impact on process output, as well as in final product quality and the resulting safety profile.

Key Terms

Valley of death: The 'valley of death' concept is frequently used in business and economics (including health economics) literature to illustrate the high-risk barriers new products and business concepts need to overcome during their development before reaching successful commercialization. In the case of pharmaceutical development the valley of death encompasses stages from discovery to translation into effective proof-of-concept, including Phase II clinical development, which accumulate the highest attrition risk.

Developability: Suitability of a drug candidate to be successfully developed attending to its ability to meet adequate quality, manufacturability, effectiveness and safety requirements.

Product Quality: According to ICH Q6A, quality can be defined as *"the suitability of either a drug substance or drug product for its intended use"* and should focus on characteristics affecting safety and efficacy of a given product, including attributes such as identity, strength and purity of the drug product.

What is wrong with our processes?

All the advances and progress described above cannot negate substantial shortcomings in the way biotherapeutics are currently developed. As it turns out, there are quite a few areas that still require attention.

» Processes are largely unpredictable

The dramatic progression in titers and quality cannot mask the fact that the way bioprocessing is currently approached is essentially obsolete, particularly

when compared with manufacturing practices used in other industries. An intrinsic difficulty in bioprocessing is the utilization of biological agents, which never evolved to function as biofactories in completely artificial environments. Their inherent complexity, and yet insufficient understanding of their biology (particularly under 'artificial' conditions subjected to multiple sources of stress), is what makes bioprocesses cumbersome and largely unpredictable. As a result, once the bioprocess has been defined, even if there is promise of better output, it is very difficult to change it without introducing complex, expensive and time-consuming robustness assessment, transferability and validation exercises.

» Resistance to adopt innovation

Another twist to this puzzle is provided, as mentioned earlier, by the logical but considerable restrictions imposed by regulators to ensure that the output of bioprocesses adheres to strict safety and quality criteria. Perversely, and despite numerous attempts from agencies to foster innovation in biopharmaceutical manufacturing, this discourages drug developers from introducing innovation and makes them reluctant to substantially alter biomanufacturing. The time required for building, validating and licensing bioproduction facilities (up to 4–5 years), and the uncertainty of the drug-development process and market uptake for a new product, also make drug developers cautious about incorporating new technologies, particularly if they have not been tested commercially [33].

» Processes take too long & are not fit for purpose

It has been proposed that the pharmaceutical industry is perhaps the only one that develops fully commercial manufacturing processes to make prototypes [34]. This practice substantially increases the investment required early on in development, and considerably extends timelines. Current bioprocesses lack agility and make it very difficult to 'rectify' or redesign molecules once the developer has committed to a given candidate and process. This 'point of no return' so early in development can have catastrophic consequences as it dramatically reduces the options available to avert failure. We should ask ourselves, how realistic is it to expect drug developers to go quickly from 'bench to bed' and then back to bench to redesign and improve, when a single iteration could take over 3 or 4 years to be completed from the designation of a lead candidate?

» Discovery & development are disconnected

Perhaps one of the most critical flaws in how drug development has traditionally been conducted is the high degree of disconnect between the discovery/design phases and the development activities that follow. Discovery scientists are often disengaged (by choice or necessity) from having to deal with quality and safety constraints that appear during biomanufacturing development, and these are frequently found to derail the progression of a given candidate to the clinic. Despite all claims of process platforms for specific product classes, the fact is that two different molecules never behave in the same way. Some products simply cannot be made or are so unstable they will never be able to become a drug.

» How can these issues be addressed?

A number of solutions could help approaching these challenges:

- Implementation of early de-risking approaches that are at the core of this article will be addressed in subsequent sections;
- Designing drugs with delivery in mind. As we will see later, this is an important and often disregarded aspect of biopharmaceutical development that can have a substantial impact on the success and cost–effectiveness of drugs;
- Integrating discovery and development activities to be more effective in developing drugs that are fit for purpose [19,34]. This is an enabling approach, as it underpins the development of the previous two solutions and fundaments the application of developability assessment strategies in biopharmaceutical development;
- Develop prototypes rather than final products for early clinical assessment [34,35] to facilitate a faster transition of drug candidates from the research laboratory to the clinic. This idea could potentially have extraordinary consequences for the development of new cures and it would need to be accompanied by new approaches to process development. Developability methods could help introduce quality and safety selection requirements, while reducing development timelines.

All research activities involved in drug development need to be better integrated if the industry is going to be able to develop better candidates in a more effective and leaner fashion [19]. As we will see later, developability technologies can help bridge previously disperse functions and provide an operational framework to integrate key aspects of quality, manufacturing and safety in the design and selection of drug candidates.

Developability: a multidisciplinary approach to understanding & de-risking drug development

» Druggability versus developability

A key consideration to define druggability is to assess whether a given target is accessible to conventional therapeutic molecules, or whether it is specifically involved in the biology of the disease, or rather whether it forms part of multiple pathways relevant for many biological functions in the organism [36–38]. If druggability looks primarily to the biological target, developability assesses the suitability of a given product to become a drug in its broadest sense. Developability looks at aspects of manufacturing, formulation, delivery/bioavailability/pharmacology, metabolism, safety/toxicology and efficacy [39–44].

In recent years, a plethora of new terminology has been created in an attempt to define the suitability of molecules for development from manufacturability or processability, to specifics such as formulability. Developability itself is a term that can induce confusion; some authors tend to restrict its application in biopharmaceuticals to aspects of product development related to the manufacturing process. Although its application to biopharmaceuticals is relatively recent, developability assessment for small molecules is a well-established practice that looks at the many aspects of drug development that could impact the future success of a given drug, including formulation, delivery, pharmacology, toxicology and so forth [39]. As suggested by such early work and for the purpose of this article I will, therefore, be using a more holistic definition of the term. The reason for doing this is, as I will argue later, that all these aspects are intricately interconnected and, for example, the way a drug product is designed can have an important influence in its pharmacology and mechanism of action in patients. Furthermore, the product quality profile, as defined during biomanufacturing, can be a determining factor in its clinical safety.

To summarize, we will consider developability as the suitability of a given molecule to satisfy a number of requirements in terms of manufacturability and processability (including productivity, stability and impurity profile), pharmacology (including bioavailability, suitability to being formulated for a specific route of administration or half-life), safety (from immunogenicity to immunotoxicology), and biological activity and mechanism of action (Table 1). Developability assessment, therefore, is the early evaluation, primarily by means of *in silico* or *in vitro* tests, of potential risks that could affect a given biopharmaceutical product during its pre-market development, from bioprocess development to clinical testing (Figure 2).

The adoption of a developability assessment element in early stages of biotherapeutic development could be considered as an extension to the implementation of quality-by-design (QbD) strategies, since it tries to understand the link between the molecular structure of the product and the quality and safety outputs

Key Terms

Druggability: Term created to define potential targets that could be approached through a pharmacological intervention. The druggability of a biological target is defined, therefore, as the feasibility to develop molecules (drugs) that would interact specifically and modulate the biology and function of such a target in order to achieve a therapeutic effect for the patient.

Formulability: Defines the suitability of a given drug candidate to be formulated for a desired route of administration or delivery method. Formulability attributes include, among others, solubility, aggregation, viscosity or product stability.

Table 1. Criteria for a developability assessment platform.

Criteria for developability assessment			
Area of development	*Scope of the assessment*	*Format of assessment*	*Questions addressed*
Manufacturability/ processability	Yield/productivity Stability (chemical/physical) Formulability	*In silico, in vitro*	Can it be made? How much will it cost?
Pharmacology Route of administration	Delivery/bioavailability Formulability PK/PD Half-life	*In vitro, in vivo*	How much will it cost?
Safety and toxicity	Immunogenicity Immunotoxicology/CRS Specificity	*In silico, in vitro, in vivo*	Will it be safe?
Mode of action	Immunomodulation Dosing and patient segmentation Efficacy	*In vitro, in vivo*	Will it work?

The assessment has been divided into four different areas: manufacturability/processability, pharmacology, safety/toxicology and mode of action, to match existing approaches to drug development and early de-risking. The format of assessment refers to the type of tools required for a developability assessment to address each specific area of development. These include *in silico* or computational (predictive) approaches; *in vitro* surrogate analytics mimicking relevant clinical or biological parameters; and *in vivo* assays making use of animal models (particularly for pharmacology, toxicity and mode-of-action studies).
CRS: Cytokine-release syndrome; PK/PD: Pharmacokinetic/pharmacodynamic.

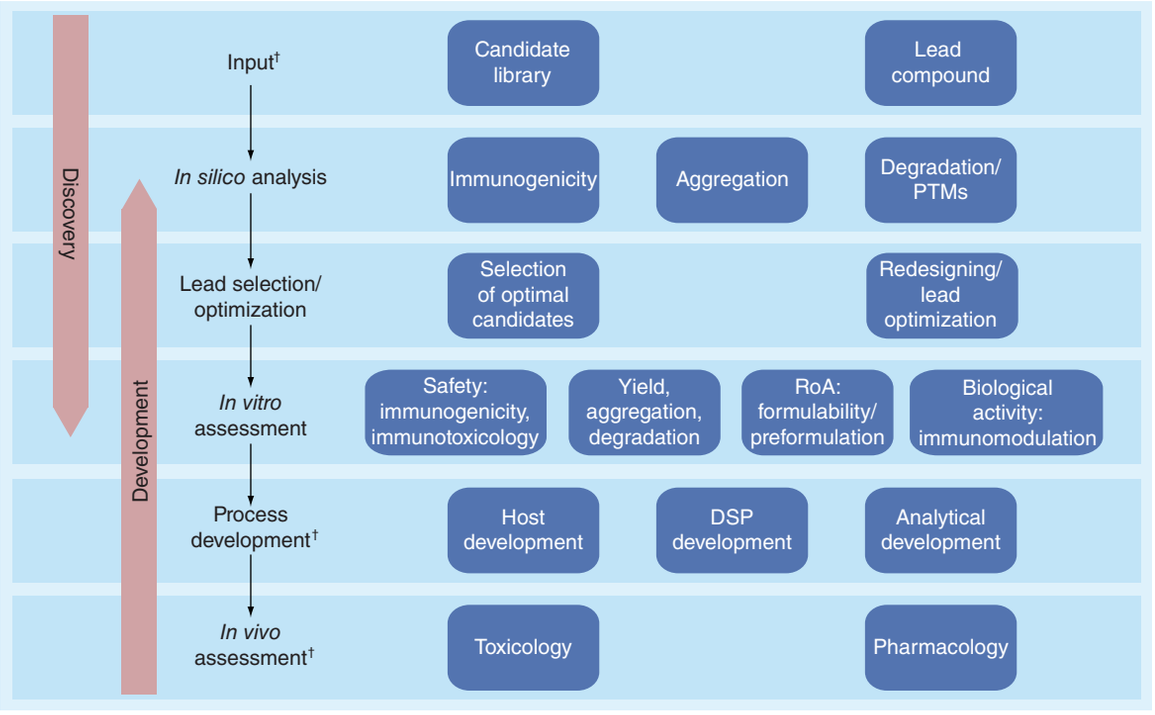

Figure 2. Suggested developability assessment workflow. How a developability assessment could be integrated within the normal drug-development cycle. This workflow requires closer overlap between discovery and development activities in order to maximize its successful application and reduce impact development timelines. The new 'intermediate' layers of assessment have several benefits: they facilitate the interaction across functions and the transition of candidates between discovery and development stages; they facilitate early and inexpensive elimination of problematic candidates; and help design required characteristics that would reduce failure later on in development, whether manufacturing or clinical.
†: The 'standard' stages in traditional biopharmaceutical development, with lead selection driven primarily by binding affinity (see text).
DSP: Downstream processing; PTMs: Post-translational modifications; RoA: Route of administration.

described above. This opens the door to selecting or engineering an optimal lead candidate before embarking on developing a given manufacturing process or designing a specific route of administration, only to name a few. So rather than engineering the manufacturing process to control the quality of the product, this approach would aim to define a better product to maximize manufacturing and clinical success [44,45]. Furthermore, developability approaches could also be utilized to facilitate the generation of biosimilar or biobetter products [46], helping to rationalize which elements are truly responsible for defining a desired target product profile or can really add value to the molecule in terms of safety, mechanism of action, patient compliance and so on.

In silico & *in vitro* methodologies to assess developability

Every method aiming to assess risk of any sort must fulfil three main requirements:

- It has to reproduce, at least to some degree, the context in which a particular problem occurs;
- It needs to provide a quick and clear answer;
- It needs to be cost effective.

These requirements can be fulfilled by implementing relevant computational models (*in silico* tools) or using surrogate assays or analytics that reproduce, at least in part, the behavior of the product in a given environment.

In silico methodologies have been hailed as an important tool to simplify and help reduce the uncertainty associated with the development of new pharmaceutical products. The Innovation Medicines Initiative, for example, has launched a number of programs aimed to develop novel approaches to drug toxicology and safety by validating computational methodologies together with *in vitro* and *in vivo* tests [47]. The National Institute of Standards and Technology, in its planning

report "Economic Analysis of the Technology Infrastructure Needs of the US Biopharmaceutical Industry" [204], restates that increasing success rates during drug development should be the main center of attention for the pharmaceutical industry, and emphasizes the utilization of computational tools for this purpose.

In silico methodologies present a number of advantages. One of them is that they allow a potentially endless throughput. Many product candidates can be tested in parallel to select those that meet a number of predefined criteria, expanding the range of variables that could be potentially explored. Another main advantage derived from the use of computational tools is the relatively low cost of implementation and the high speed of analysis they offer, which simplifies and facilitates decision making. On the negative side, computational methodologies are as good as the data used to build them and predictive accuracy can be low, particularly in cases when limited information or data are available. Bearing all this in mind, the use of computational tools early on in development offers a major advantage in facilitating the elimination of those compounds, designs or variants exhibiting a higher risk of failing later on in development [48]. From a cost-effective perspective, a small investment in computational infrastructure and/or assessment can help avert significant and expensive losses downstream in manufacturing and clinical development.

The use of computational tools alone, however, is insufficient to completely define developability risks. Therefore, it is important that predictive tools are used in conjunction with other experimental approaches. Because of the constraints imposed by availability of material or the parameter that needs to be assessed, such analytical and testing approaches will often be surrogate assessments offering a sufficient proximity to the real environment to be considered. This means, for example, that product stability characterization will need to be conducted with small amounts of material that can be obtained rapidly, and that testing allows adequate throughput for the parameter assessed. Specific requirements for testing and limitations will be addressed in subsequent sections.

Protein quality & integrity

Proteins are wonderful molecules: potent, versatile and relatively safe compared with small-molecule APIs. Proteins, however, have some drawbacks linked mostly to their complexity and inherent instability. Polypeptides have generally been evolved to perform their function in a given biological context and timeframe to be then rapidly eliminated. In their biological environment, proteins need to be stable enough to fold/assemble and perform their function when required and in a short time frame but, at the same time, they need to be unstable enough to allow their elimination and, hence, facilitate effective control of their biological activity. If one now compares the biological environment of proteins with current manufacturing infrastructure involving stainless steel, plastics and so forth, very high concentrations, extreme pH and temperature changes, long-term storage requirements (up to several years) and the lack of any repair or assistance machinery to control their stability, then we are clearly looking for trouble. To make matters worse, the biotherapeutics designed today often incorporate functionalities not directly implemented by nature before. They run the risk of not being 'compatible' with current manufacturing infrastructure, both from a host perspective as well as general process stability and degradation mechanisms.

Kozlowski and Swann have suggested that a single monoclonal antibody preparation could contain up to 10^8 isoforms, including all possible post-translational modifications (e.g., different glycosylation patterns), as well as all degradation modifications such as glycation, deamidation or oxidation [49]. Of course, it is impossible to control every single one of such forms, but it is important to understand the impact different degradation mechanisms could have in the safety and biological properties of a given biopharmaceutical. If one accepts such limitations, then the role of the process scientist is not to avoid degradation, as this would be an impossible task, but to manage risks by keeping check on the multiple degradation pathways, so they are kept within an 'acceptable' range over and over again.

So, what are all these isoforms and what is their relative risk? This is a very difficult question to answer, but all these protein modifications can be grouped into two separate categories: those defined by the host system and those defined by the manufacturing process. The host-related modifications include largely post-translational modifications such as different glycosylation patterns, phosphorylation, introduction of undesired glycosylation sites and acylation, among others [50,51]. Process-related modifications can be grouped into two major categories: those related with the chemical stability of the molecule (deamidation, oxidation, fragmentation and beta elimination) [52,53] and those related to the physical stability of the molecule, which intrinsically means aggregation potential [54–56].

Regardless of their origin, such modifications can potentially impact both biological activity of the molecule (target recognition, binding capacity and effector function) or safety (immunogenicity and anaphylactic reactions). It is, therefore, highly relevant to assess their relative importance to then either identify alter-

native products or process conditions that would minimize their impact. This is where a good risk assessment is required. Undesired glycosylation, for example, has been shown to be a significant safety issue potentially linked to the host system utilized during manufacturing [57,58]. Moveover, identification of potential degradation sites (such as deamidation or oxidation) that are more likely to impact activity, perhaps because of its proximity to binding regions, would take priority over other potential degradation sites somewhere else in the molecule. Equally, degradation pathways that could potentially impact process yield, clinical utilization of the product or even safety, such as aggregation or immunogenicity, should take priority over other risks. One of the major complications with such modifications and degradation pathways is that they are intimately linked to specific environmental conditions in the manufacturing process as well as formulation and storage. This makes testing and control of degradation pathways quite a titanic challenge, and suggests that the use of computational tools early in the process, as we have seen above, might be well placed to establish a basic developability profile for products that are to enter development. It could also help guide process scientists towards specific concerns that might appear during process development and manufacturing.

Addressing aggregation in biopharmaceuticals as a key parameter for manufacturability & safety

Protein aggregation is one of the main problems plaguing biopharmaceutical development because of its impact in the manufacturing process, target product profile, delivery and patient safety [59]. From a regulatory perspective, the presence of aggregates is a source of concern as a suspected cause of immunogenicity and side effects linked to the use of biopharmaceuticals [60–62]. One particular example of immunogenicity potentially linked to aggregates is recombinant human erythropoietin. During 2002–2003 an abnormally high number of cases of pure red cell aplasia (PRCA) were reported, eventually linked to the introduction of a new erythropoietin formulation. A number of potential causes for this increase in reported PRCA were put forward. However, there is growing support linking an increase in protein aggregates with the observed cases of PRCA. More recent data from erythropoietin biosimilars have suggested that increased aggregation, in this case due to tungsten impurities, can be linked to immunogenicity reactions [63–65].

Protein aggregation is a multifaceted problem that can manifest in multiple ways, from reduced cell viability, to low production titer, poor primary recovery, low yields in chromatographic and filtration/concentration steps or precipitation, among others. Aggregates and misfolded precursors tend to be toxic to cells to the point that they can cause cell viability problems in culture. Cells have evolved a number of strategies to cope with misfolding, misassembly and aggregation. Secretory eukaryotic cells, for example, have developed a sophisticated folding and quality control machinery to address these issues. In the case of secreted polypeptides, a complex signaling system in the endoplasmic reticulum labels those molecules that have completed proper folding and assembly to facilitate their subsequent secretion [66,67]. In practical terms this means, for example, that during eukaryotic bioprocessing (particularly mammalian secretory systems), those molecules that fail to adopt a properly folded conformation are likely to be retained within the endoplasmic reticulum and degraded, rather than being secreted [68,69]. In another extreme case, when the cellular folding machinery is unable to cope with the demands introduced by the high expression of a foreign product, the cells tend to accumulate it in the form of intracellular inclusions. This is fairly typical in prokaryotic systems, particularly when expressing heterologous complex proteins that require specific folding and secretory machinery not present in such organisms. This phenomenon can also be observed in eukaryotic systems in pathological conditions or in cases where cytoplasmic expression of a non-cytoplasmic or artificial protein is attempted [54].

Parallel to the mechanistic heterogeneity of aggregation is the broad diversity of structural species and size-distributions that can occur in different stages of development, but also in a single biopharmaceutical preparation. It is important to notice that aggregation testing is usually defined by the need to control process yield and target product profile. Full characterization of protein aggregates, particularly in reference standards or the final drug product, require the utilization of various orthogonal methodologies to map adequately the type and amount of aggregates present. Furthermore, aggregate sizes that are usually not well covered by standard analytical methods (i.e., subvisible particles in the micron/submicron range) are receiving mounting interest from regulators because of their potential safety impact [70].

» Prediction of aggregation

The structural complexity of aggregates and the multitude of mechanisms that could potentially drive their formation make the application of purely mechanistic tools to predicting aggregate formation unrealistic. This is why most predictive platforms developed to date utilize primarily phenomenological approaches combining basic physicochemical, structural and thermodynamic descriptors with experimental observations

developed in the laboratory. In this way, the degree of predictability of the tool is linked to the experimental systems utilized for its development and validation. Folding and aggregation are ultimately determined by the same physicochemical principles, and it is only the competition between different forces (whether intrinsic or extrinsic) that drive the molecule in one direction or another. Most aggregation predictive tools developed to date rely on either semi-empirical methodologies linking aggregation to physicochemical and structural parameters or use first principles based on assumptions of parameters that seem to define aggregation in proteins. Efforts on the development of aggregation predictive tools have been reviewed elsewhere [71–74]. Some of these platforms have been exemplified or validated with biopharmaceuticals, and, as we will see later, used to engineer improved versions of biopharmaceuticals with increased stability, while maintaining their biological activity intact [44,75–79]. It has also been suggested that such computational methodologies could enable the ranking and selection of biotherapeutics based on their relative predicted stability [80,81].

» Analytical tools for aggregation assessment

The previously mentioned structural heterogeneity of aggregates brings an associated substantial complexity to their analysis. This is further exacerbated by the diversity of manifestations of misfolding and aggregation observed in bioprocessing, as mentioned earlier. In this context it is extremely difficult to accurately 'measure' or 'quantify' aggregation in a meaningful way. Regulators encourage drug manufacturers to apply state-of-the-art analytical technologies to define the impurity profile of biopharmaceuticals, and aggregates in particular. The onset of new technologies for the analysis of subvisible particles has recently become the focus of attention of regulatory agencies and it is likely their use will become routine in the near future. Size-exclusion HPLC/UPLC remains the workhorse for the routine quantification of aggregates. It is a robust method, but its main drawback is that it only allows direct detection of small oligomers and, therefore, misses a large part of the puzzle. Other technologies, such as light scattering or analytical ultracentrifugation add a high degree of precision to the analysis, but their throughput is limited. In this context, surrogate analytics that can provide a basis for the assessment of the aggregation potential of a molecule in a defined context (e.g., process-wise or formulation) and at the same time afford simplicity of use, rapid data turnout, high throughput (and automation) and low sample consumption, can indeed be very valuable tools to define the developability potential of biopharmaceuticals. Some technologies based on capillary electrophoresis or immunoassays, for example, can offer greater flexibility in terms of throughput or breadth of detectable species, which could be a useful tool to define aggregation risks early on in process development [70,82,83]. One of the current challenges for developability assessment, however, remains the ability of assessing aggregation as a whole in a rapid and high-throughput manner with very little sample consumption. Several alternatives are currently in the market based on light-scattering, UPLC or capillary electrophoresis amongst others. We have recently reported the use of an immunoassay to qualitatively test aggregation. This oligomer detection assay uses standard immunoassay technologies and can be utilized to establish comparisons between different samples of a given protein (i.e., different formulations) or different biotherapeutic candidates [44,84].

Formulation & delivery

One of the most important aspects of biopharmaceutical development, and perhaps also the most widely disregarded during the early stages of development, is the delivery of the drug to the patient. At present, virtually all main biopharmaceuticals require parenteral administration, which overcomes one of the major issues in drug delivery, albeit at a cost, as we will see later. Still, the delivery of biopharmaceuticals to patients faces two main problems: half-life and dosage, both necessary to achieve a pharmacologically relevant concentration. In fact, one of the reasons why very potent bioactive compounds, such as peptide-derived drugs, have yet to live to their full potential is their poor pharmacokinetic profile.

» The importance of formulation & delivery in cost of treatment

It is interesting to see how, when addressing the question "how much will it cost?" applied to new therapeutics in development, the emphasis is more often than not in the COGs to produce the API form. Indeed, in the past COGs of biopharmaceuticals were an important element to consider when assessing their potential, as it is now with other advanced medicinal therapeutic products, and cell therapy in particular. However, the increase in efficiency experienced in bioproduction has reduced COGs to a very small fraction of the final drug price, which has to absorb the significant costs related to clinical development and high attrition rate in the clinic, which has been addressed above.

COGs are still, and will be, an important element to consider for the development of any biopharmaceutical. However, there are other aspects of drug delivery that are of equal, if not greater, importance in the developability and reimbursement-ability of any new drug candidate. One of these aspects is administration to

patients and its intrinsic connection to the formulation of the drug product. There is no systematic analysis of the impact of the medical care component in the overall cost of treatment with biopharmaceuticals, however, some specific studies suggest that this impact could be substantial, particularly in cases where specialist care is involved [205].

It would be inconceivable for any health system, for example, to organize the administration of insulin through hospitals. That would make the cost of treatment utterly unsustainable. Some recent studies have shown how bringing biological administration to patient's homes can dramatically reduce the cost of the treatment, in some cases by as much as half [85]. Clearly not all biopharmaceuticals are the same, but from a purely cost perspective, strategies aimed to simplify drug administration, reduce the time of administration and the number of doses, as well as minimizing in-hospital treatment and specialist healthcare requirements, will always be welcomed by payers and patients [86].

» Subcutaneous administration of biopharmaceuticals

Home administration programmes are one way of tackling this problem, as well as the development of sustained-release formulations of half-life extension technologies, which will not be reviewed here because of their shear volume. In any case, route of administration does play an important role in defining the pharmacological profile of biopharmaceuticals. Subcutaneous delivery, for example, has a number of advantages when compared with traditional infusion approaches. It is simpler, potentially less prone to errors or complications, and facilitates sustained release of the active form into the bloodstream. It is also potentially compatible with auto-injectors that could facilitate patient self-administration and, therefore, dramatically reduce costs associated with drug administration [87,206]. Indeed, since the onset of therapeutic antibodies there has been a gradual increase in the number of molecules that are developed for subcutaneous and intramuscular administration, currently accounting for about half of all registered products **(Figure 3)**. A similar proportion of subcutaneous products is found among all biopharmaceuticals currently in development (data not shown). Furthermore, several existing intravenous commercial antibodies are being developed for subcutaneous delivery [87]. Subcutaneous administration implies a reduced volume of injection and, as a result, requires a much higher concentration of bioactive agent than an infusion/intravenous route would need [88]. **Figure 3** shows graphically how registered antibody-related drugs formulated for subcutaneous administration are injected in much higher concentrations, typically ranging from 100 to 200 mg/ml. Such high concentrations, however, present a number of issues primarily linked to the stability and viscosity of the drug product [89]. High concentrations tend to favor the formation of protein aggregates, which can affect both the activity and safety profiles of the biopharmaceutical product (see above). At the same time, high viscosity is a significant problem for injection [90], requiring wider bore needles that cause more pain and make self-administration more difficult. Even in circumstances where formulation additives can solve such problems, once administered, small-molecule additives can diffuse much more rapidly than the biopharmaceutical molecule, therefore increasing the risk of local aggregation and precipitation, and the onset of safety and loss of efficacy issues that could derive from it. Despite the robustness and 'friendliness' of antibodies regarding their stability, solubility or half-life, not every monoclonal antibody can be formulated at concentrations of up to 200 mg/ml. This problem is perhaps becoming more acute with the growing importance of alternative scaffolds, fusion proteins and new biopharmaceuticals that are not as benign as monoclonal antibodies in terms of their developability. Another issue associated with subcutaneous administration is an increased immunogenicity risk, which can potentially be compounded with the presence of aggregates in highly concentrated formulations [91].

» Formulability & early formulation development

It is common to see biopharmaceuticals developed without giving proper consideration to the route of administration or type of formulation desired. It is true that early clinical phases allow higher flexibility in terms of dosage and route of administration, and it is common to see final formulations developed closer to late stages of clinical development. However, the main flaw in this approach is that, as we have seen, the chosen route of administration can have a significant impact on the cost of the treatment, as well as the pharmacology and potential efficacy of the product. In the current environment, where clinical trial success does not necessarily equate to market uptake, and particularly in light of mounting pressure from payers and the need to ensure healthcare affordability (see above), every single aspect of development affecting the efficacy, cost and simplicity of a given treatment can become the difference between success and failure.

In this context, early formulability assessment becomes a critical aspect in the development of any new biopharmaceutical. Either comparing the relative sta-

bility and solubility of multiple candidates or engineering biopharmaceuticals with delivery in mind, aiming to increase their stability profile can have a highly beneficial impact on the developability of new biopharmaceuticals [92]. Another complementary approach to this strategy is to start exploring suitable formulations much earlier in the process. Recent methods exploring the use of high-throughput to preformulation screening [93,94] could help to select candidates better suited for specific formulation and delivery requirements in the future, but also provid very valuable information to help designing processes better suited to them, with a clear impact on process yield and product quality. Still, further efforts are needed in order to develop reliable and sufficiently informative analytical tools for such a high-throughput approach.

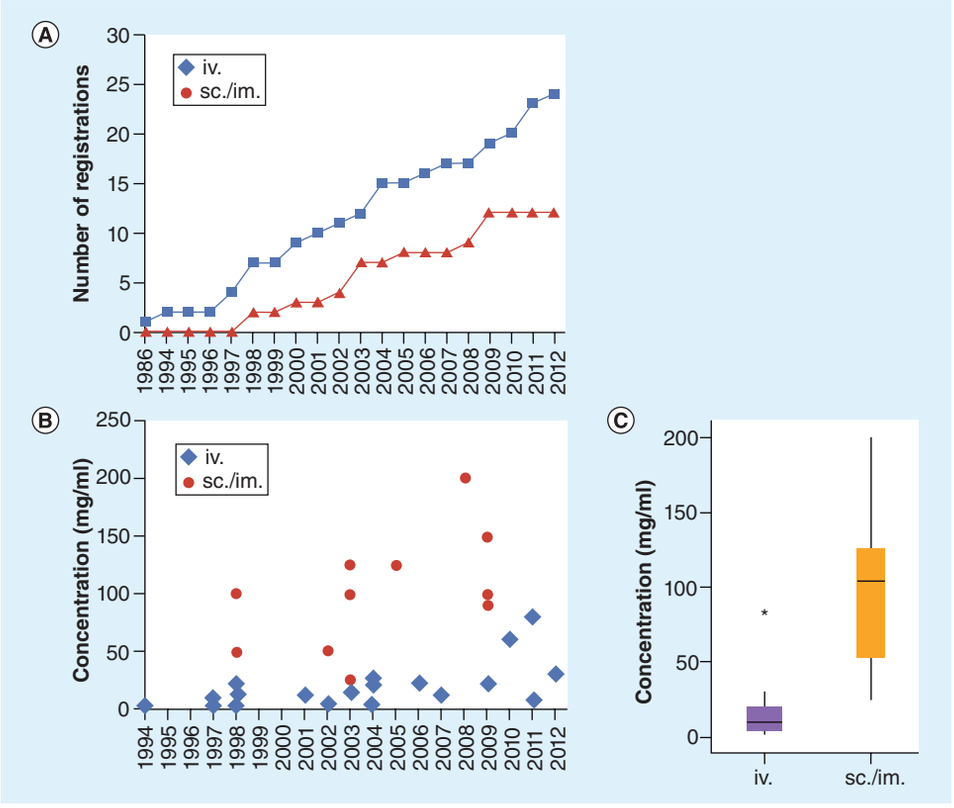

Figure 3. Route of administration for registered therapeutic antibodies and derivatives. The evolution of route of administration utilized for registered therapeutic monoclonal antibodies, antibody fragments, conjugates and fusions. **(A)** Cumulative number of registrations for both i.v. and s.c./i.m. administration. Other routes of administration, such as intra-vitreal or intra-peritoneal are not reflected here. **(B)** Concentration in mg/ml at which registered monoclonal antibodies and derivatives are administered or resuspended before infusion/administration. **(C)** Distribution of concentrations used for registered monoclonal antibodies and derivatives. *: Data point; i.v.: Intravenous; s.c./i.m.: Subcutaneous plus intra-muscular. Data taken from [132,133,213,214].

Immunogenicity assessment

Unlike small molecules, often toxic in nature, side effects due to the administration of biotherapeutics tend to be associated with their pharmacology, immunomodulatory imbalances associated to their mechanism of action, and immunogenicity reactions [95,96]. Immunogenicity is, therefore, one of the main causes of safety concern for biologics, given that most of them have the potential to cause immunogenic reactions. Still, immunogenic reactions depend on a large number of contributing factors, including disease condition, administration regime and duration of treatment, genetic background of patients, manufacturing process (including hosts) utilized in the production of the drug, additives and vialing used in the final drug product, or age of the product, among others [91]. Several examples have shown how immunogenic reactions can constitute a severe safety risk for patients. One of them, mentioned above, is the occurrence of PRCA in patients treated with erythropoietin, potentially associated with the presence of aggregates [63–65]. Another example is the incidence of IgE-mediated anaphylaxis in patients treated with Erbitux® (cetuximab) linked to the presence of the galactose-α-1,3-galactose (α-Gal) antigen in the drug, a known cause of hypersensitivity [57].

» Immunogenicity of biopharmaceuticals

Immunogenic reactions can manifest as allergic reactions or, more frequently, as antidrug antibodies. Antidrug antibodies can, on occasion, reduce the efficacy of biotherapeutics or mediate other immunogenic reactions, and are one of the principal elements in the safety assessment of any new biopharmaceutical in development. However, it is often desirable to have some degree of information about the immunogenicity risk of any given biotherapeutic product before it reaches the clinic. The main problem behind immunogenicity assessment is the sheer complexity of the human immune system and vast differences with that of other animals, even primates.

Key Term

3Rs (Refinement, reduction and replacement): Initiative aimed to improve the utilization of animal models in pharmaceutical development. Addresses refinement and development of better and more relevant animal models for disease modeling or toxicology studies; reduction in animal experimentation; and replacement of animal testing with *in vitro* or *in silico* assays that can produce an equivalent output.

To make things even more complicated, humans have a very diverse genetic repertoire encoding for key components of the immune system, that are likely to influence the specific responses observed in the clinic. The major histocompatibility complex (MHC), also known in humans as the human leucocyte antigen, is one such key component that helps determine what is self and what is foreign, among other roles. There are also many factors that can influence a potential immunogenic reaction against a given biopharmaceutical besides the genetic makeup of the patient. These include, among many other factors, the ability of antigen-presenting cells (APCs) to capture, process (proteolyze) and present antigens, and the recognition of MHC–antigen complexes by T-helper cells and their activation, which is related to the presence of T-cell epitopes in the presented sequence.

To describe the immune response in a nutshell, antigens are processed and presented by APCs as MHC–antigen complexes. APCs degrade pathogens or antigens and cleave them into small peptides that interact with the MHC in the endoplasmic reticulum (class I) or endosomes (class II). MHC–antigen complexes are then exported to the cellular membrane where they are recognized by T cells that mediate the immune response against the antigen or pathogen. In the case of intra-cellular antigens, virtually every cell in the body can process and present epitopes via the MHC Class I complex, whereas extracellular antigens are predominantly presented by dendritic cells via the MHC Class II complex. In essence, the relative prominence of some epitopes is linked to their relative binding affinity to the MHC. Predictive models, hence, either rely on statistical analysis derived from MHC binding assays, *in vitro* T-cell proliferation or cytokine secretion assays, or in cases where mechanistic approaches are used, structural determinants defining the binding of different peptide sequences to MHC complexes.

Immunogenicity is one of the key risk factors for biopharmaceuticals. Most therapeutic proteins are, to a variable extent, immunogenic. Clinical immunogenicity of biotherapeutic drugs can compromise drug safety, alter its phamacokinetics and reduce efficacy. It is, therefore, highly desirable to assess and manage potential immunogenicity issues during the early development stages. An immune response usually occurs via two different mechanisms: breaking tolerance and reaction against non-self. In the case of biopharmaceuticals there is a large collection of factors that are known to contribute to biotherapeutic drug-induced immunogenicity, including intrinsic factors, such as T-cell epitope content and protein structure or glycosylation patterns, as well as extrinsic factors such as degradation products, production contaminants from hosts or otherwise, protein aggregates and formulation. As a result, stability issues that might be present in a given biopharmaceutical can be prominent in exacerbating immunogenicity, primarily via breaking tolerance. For example, protein aggregation is believed to increase the immunogenicity of biotherapeutics, and one of the mechanisms proposed suggests increased APC uptake [60]. Together with aggregation, degradation of the sequence via deamidation, oxidation or abnormal post-translational modifications such as hydroxylation or glycosylation can increase immunogenicity risks [57,97–99].

» Preclinical immunogenicity risk assessment

Immunogenicity assessments performed in early stages of preclinical development have been recognized in recent years as a very relevant aspect of the developability and risk-evaluation of biopharmaceuticals. Such assessments can usually employ one, or a combination of, *in silico*, *in vitro* (*ex vivo*) and *in vivo* methodologies. Indeed, integrated preclinical immunogenicity assessment through prediction, detection and characterization of product-induced immune responses can help to address immunogenicity risks [100–104]. Because of the difficulties in reproducing a human immune response in animal models [105], two different complementary strategies have been developed. One of the approaches involves the use of computational platforms relying on statistical analysis and/or structural parameters to identify potential T-cell epitopes in the context of MHC Class II binding specificity. As we have seen above, the use of computational or *in silico* platforms allows extremely large throughputs; it is also cost effective and can be a very useful tool to facilitate lead selection with low immunogenicity risks.

The second approach relies on *in vitro* (*ex vivo*) assays using blood from human donors to evaluate the immunogenic potential of biotherapeutics. This analysis directly measures T- and B-cell responses using high-quality PBMCs from specific human target cohorts that are representative of world, disease or ethnic populations. Such responses can be evaluated by studying, among others, T- and B-cell activation and proliferation, cytokine secretion, specific antidrug antibody secretion assays [103,104]. Latest-generation cellular assays are considered to offer one of the closest surrogate approaches to assessing immunogenicity in humans prior to the first clinical administration. Such

assays also allow evaluation of the potential impact of specific formulations, impurities or modifications present in the drug product, helping to avert problems that could be extremely serious and costly if they happened to occur in the clinic.

Finally, it is also important to realize the potential of immunogenicity assessment tools as a key comparability parameter that could be utilized in batch-to-batch consistency assessment, or even in areas such as the development of biosimilars and biobetters [46,106].

Immunomodulation: when biological activity is more than ligand binding

Many of the biopharmaceuticals currently licensed and most therapeutic antibodies and fragments approved for human use have an immunomodulatory activity, particularly in cancer and inflammatory or autoimmune disease [107]. One of the main challenges affecting these products is related to the difficulty of validating their mechanism of action during preclinical development. This is usually problematic in many disease conditions, given the lack of relevant animal models that reproduce with sufficient fidelity a given disease phenotype. However, modeling the human immune system in an animal is substantially more challenging. Even primates, despite being very close genetically to humans, exhibit substantial differences in their immune components compared with humans. Considerable advances have been made in the development of animal models that reproduce at least some of the key components of the human immune system [108]. However, it is still virtually impossible to reproduce all the nuances and specific pathways that are present in a human subject. Furthermore, even if that hurdle was ever overcome, it would require dealing with the vast genetic and phenotypic diversity that exists among human beings from different ethnic, geographical and pathophysiological backgrounds. As a result, most biopharmaceuticals carry over a considerable level of uncertainty well into their clinical development, particularly around essential aspects of their biological activity. In turn, such uncertainty complicates the selection of suitable values for key pharmacology parameters and increases potential safety risks.

» Alternatives to the use of animal models

One alternative to the use of animal models is the development of relevant '*ex vivo*' assays using tissue samples, usually blood, from human donors. The main benefits of utilizing this approach are the broad coverage of genetic and disease backgrounds allowed by sampling tissue from multiple donors and the possibility of assessing the impact of such different backgrounds in specific biological parameters or cellular responses. Furthermore, it fits with '3Rs' initiatives aiming to refine, reduce and replace animal experimentation [109,110].

» Benefits & limitations of '*ex vivo*' methods

From a purely mechanistic perspective, these assays can help assess multiple interactions and complex pathways that more basic binding or activation assays would miss. This type of approach can help in validating the biopharmaceutical mechanism of action in a human-relevant system. Because of the genetic and phenotypic diversity allowed, it is also possible to identify discriminators of effectiveness in different populations, opening the door to patient segmentation, which could be extremely valuable to define target patient populations for clinical trials. Furthermore, they can assist in the identification or validation of potential molecular and cellular biomarkers for both efficacy and safety that could be utilized during clinical trials. Finally, this type of assays could also potentially be used to define a suitable dosage to be used in clinical development, based on more robust parameters of observed efficacy and safety in relevant tissues [107].

It is true, however, that such an approach cannot reproduce in its entirety the complexity of a human subject and the pharmacology of a therapeutic intervention; for example, the impact of the route of administration, presentation, pharmacodynamics and distribution, bioavailability or half-life. In such cases, the use of animal models would still be required. However, '*ex vivo*' assays could indeed introduce very valuable information about dosage, responsiveness, patient segmentation or potential biomarkers.

Immunotoxicology & CRS

It is infrequent for biopharmaceuticals to present safety issues derived from metabolism and excretion, given that they can be broken down into single amino acids. Usually, only biopharmaceutical drugs consisting of some kind of conjugate or adduct involving a chemical moiety require a more thorough assessment, given their potential for accumulation in specific organs or generation of toxic species through their catabolism. Therefore, the most frequent causes of toxicity observed in biopharmaceuticals, besides immunogenicity, are linked to the risk of biopharmaceuticals causing immunotoxicology. This includes autoimmune, allergic, inflammatory or even immunosuppressive reactions that can be caused by exaggerated pharmacology and overstimulation of specific immune components, off-target recognition or secondary issues associated with the biological activity of the biotherapeutic molecule (i.e., immunosuppression) [111]. These events are rare, but when occurring as a result of a clinical intervention they can be life-threatening. A notorious example of such a type of uncontrolled reac-

Key Term

Cytokine storm: Also known as cytokine-release syndrome. Severe immunogenic reaction observed occasionally in patients in response to the administration of therapeutic candidates, particularly those with an immunomodulatory mode of action.

tion was observed during the Phase I trial of the superagonistic anti-CD28 monoclonal antibody TGN1412. During this trial, six healthy volunteers experienced a cytokine-release syndrome (cytokine storm) shortly after being administered the drug. All six volunteers required immediate admission into intensive care after suffering multiple organ failure [112]. As a result of this tragic event, the way in which first-in-human trials are conducted has been thoroughly revised and now require, particularly in cases of potential high risk, specific protocols for number of subjects dosed and acceptable dosing intervals [113,207]. Irrespective of the organization of the trial itself and the dosage regime, at the time following the trial, the absence of any negative observations in any of the animal studies undertaken seemed surprizing [114,115]. This reinforces the fact that differences in the immune system between humans and animal models are essential in defining not only the biological validity of a given biotherapeutic, but also the risks associated with it.

» Making a (cytokine) storm in a teacup: surrogate tests to assess immunotoxicology

In subsequent studies, it became apparent that the 'presentation' of the TGN1412 molecule also had a major impact on the ability of triggering a CRS. For some time researchers were only able to reproduce this response when the TGN1412 antibody had been immobilized by drying [116]. More recently, alternative approaches including pre-incubation of PBMCs [117], whole blood assays [118], or the incorporation of endothelial cells [119], have been reported to successfully reproduce the effects of a CRS in a test tube. These studies once more show how subtle variations in experimental models can have a significant impact on the responses observed. In this context, '*ex vivo*' tissue-based systems offer a suitable alternative to assess immunotoxicity risks at a very early stage and offer a number of advantages:

- They allow the segregation of patients based on their genetic, ethnic and geographical background;
- They allow testing biopharmaceuticals in individuals with specific disease backgrounds or differing responsiveness (e.g., immunosuppressed vs immunoreactive patients);
- They make it possible to evaluate interaction of various treatments and identify potential pathophysiological risks;
- They reduce the utilization of animals following 3Rs initiatives [109,110].

Engineering developability in biopharmaceuticals

We have seen that it is possible to assess a variety of risks that could compromise the success of a given biopharmaceutical during development. The key question then is: "what to do with that information?" The first area where developability assessment has a very important role to play is in the selection of optimal biotherapeutic candidates. On many occasions, biopharmaceuticals are developed using large libraries of compounds obtained from display technologies. These large collections of candidates are usually screened through binding assays; however, such assessment does not necessarily incorporate other key parameters that can make the difference between success and failure. For example, the incorporation of *in silico* developability methodologies can help identify not only good binders, but also sequences with a lower risk of immunogenicity, aggregation, degradation, and so forth. A much smaller group of sequences can then be further assessed using *in vitro* tests to validate the initial screening and home in on an ideal lead candidate, or group of them, to take forward into development.

Unfortunately, there are occasions where it is not possible to choose from a collection of different candidates. This could be due to the nature of the biotherapeutic agent under development, for example, a variant of an existing human protein or peptide. Alternatively, the programme might have already progressed into early development stages where a number of problems could have been identified. For example, aggregation might have been detected during process or formulation development, or perhaps even immunogenicity issues could have been observed in early clinical assessment. In such circumstances, protein re-engineering can help bring a problematic biotherapeutic candidate back into development. Different computational approaches can be utilized to identify the root of the problem in either the protein sequence or structure and propose suitable modifications that could solve the problem or problems. Candidates can then be assessed using a variety of tests to guarantee that the redesigned variants have improved their behavior **(Figure 4)**. Ideally, both stability and safety (immunogenicity) should be tested alongside biological activity. As we have seen before, stability and immunogenicity can be linked and sequence modifications can inadvertently introduce T-cell epitopes or modify the biological activity of re-engineered molecules. These developability re-engineering approaches can

also be used alongside other protein engineering; for example, during antibody humanization, affinity maturation or reformatting of antibodies into bispecifics or fusions. A number of examples have been reported describing molecules that have been successfully designed to reduce stability and immunogenicity concerns [44,75,76,78,120–125].

Conclusion & future perspective

Decreasing productivity and growing development costs are putting pressure on the pharmaceutical industry to rethink the way in which new therapeutic treatments are developed. The introduction of a developability assessment during lead selection and optimization has the potential to reduce drug attrition in later development stages by addressing important quality aspects relevant to the manufacturability, safety and pharmacology of new drug candidates. The use of developability assessment tools has the potential to save time, money and effort required to develop new therapies.

» The cost of failure

The extremely low probability of success of drug candidates in development make them akin to 'walking dead', however, the 'cost of risk' has been largely neglected, particularly among early drug developers. In fact, the cost–effectiveness of introducing additional early risk-assessment stages in the development of new drugs has often been questioned. This leaves the management of safety and quality to later stages of the development process, when the costs and risks associated with failure are much higher. This perception is changing rapidly, and the industry is becoming more sensitive to quality risks and their potentially severe financial implications on both internal and partnered programmes. The increasing number of observed recalls, products with substantial manufacturability and process robustness issues, in addition to quality flaws, seems to be chang-

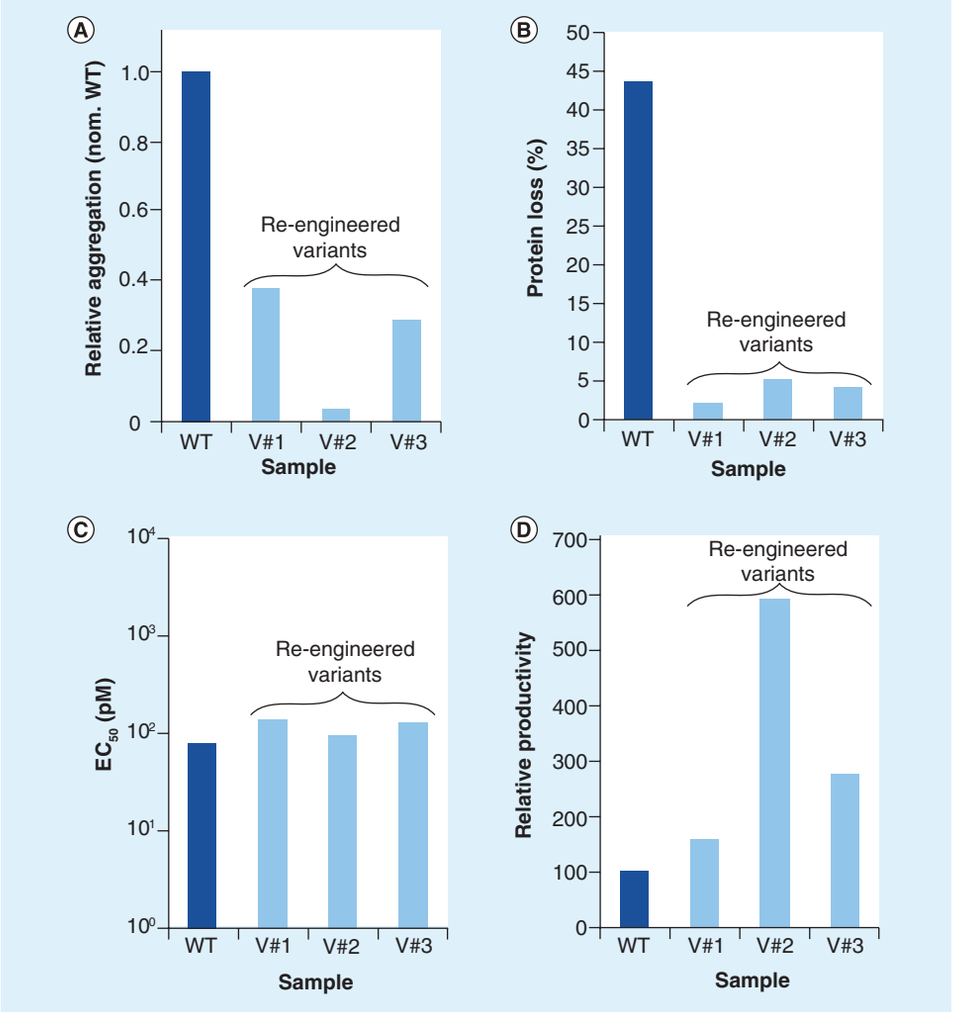

Figure 4. Example of lead optimization in a therapeutic monoclonal antibody by means of molecular re-engineering. Comparison of a 'parental' (WT) monoclonal antibody exhibiting aggregation problems with three re-engineered variants. Sequence of the control (parental) antibody was analyzed using *in silico* computational tools to identify regions of the molecule posing a stability risk. Specific residues in those regions were modified for amino acids that would increase stability of the molecule. A number of variants were produced by transfecting genes encoding for the control (parental) sequence and re-engineered sequences into mammalian cells. Proteins were tested for a number of developability parameters including productivity, aggregation, stability and biological activity. **(A)** Relative aggregation as determined by gel-permeation HPLC. The three re-engineered variants show reduced aggregation compared with WT sequence. **(B)** Assessment of percentage total monomer loss (primarily due to aggregation) during accelerated stability studies by incubating the different variants for 2 h at 60°C. The three re-engineered variants show a substantial reduction in protein loss compared with WT. **(C)** The re-engineered variants show comparable biological activity to that of the WT, assessed as equilibrium binding constant (EC_{50}). **(D)** Re-engineered variants also show an improvement in productivity, assessed as antibody titer in culture supernatants, when compared with WT. WT: Wild type.

ing the tide. It is far too common to find drug candidates unable to transition from late discovery stages to the clinic because of manufacturability concerns. It is not infrequent either to see programs discontinued or stuck, sometimes for several years, in early clinical or preclinical development because of safety or quality issues [126,127]. Indeed, the real cost of such late 'surprises' could potentially reach the multibillion dollar range if one takes into consideration the additional investment and time needed to correct these issues, lost opportunity to develop another candidate and lost sales due to the erosion of the market exclusivity period. In some cases this could make the development of a new drug economically unfeasible. In this scenario, the cost of failure clearly outweighs the relatively modest investment required to introduce early predictive and de-risking measures.

» Developability as an extension of QbD guidance

There is a growing realization that critical quality attributes can be more effectively managed by engineering them into the product in the first place. Indeed, it has been recognized that the obvious way of 'designing quality' into a drug is by designing an optimal molecular candidate with a defined target product profile [128]. Developability approaches can in fact expand the design space of a given candidate and, therefore, can be considered fully in symphony with the ultimate motivations behind QbD strategies (ICH Q8 and subsequent guidance – these are to facilitate the development of new drugs and improve their quality, safety and efficacy) [208]. Unlike current QbD exemplification, developability places the focus on the product-design stages of development and on the nature of the product itself, rather than on the control of the manufacturing process.

The routine implementation of early de-risking approaches such as those presented here lacks a fully structured framework of reference. New regulatory guidance extending the scope of ICH Q8 to include in depth pre-manufacturing risk management could be very valuable. This is particularly important for strategies requiring the utilization of *in silico* and surrogate *in vitro* analytics as predictors of quality and safety risks. Some steps in this direction have recently been taken by regulators, for example, by encouraging the use of new preclinical immunogenicity assessment options (utilizing *in silico* and *in vitro* platforms) as predictors of immunogenicity risks [104,209,210]. It is expected this trend will be further strengthened in the near future as part of the effort by regulatory bodies to fully develop QbD and translational medicine initiatives.

» Uptake & impact of developability approaches

Despite the clear benefits of early developability assessment, uptake by industry as a whole is still in its early days. There are several reasons for this. One of them is that the science is still emergent (particularly around predictive computational tools), for example, predictive immunogenicity approaches have so far only achieved circumstantial clinical validation. In addition, there is insufficient alignment around the different early risk-assessment methodologies currently utilized across the industry, and their application is restricted mostly to a few disconnected tests. Furthermore, there is a lack of guidance to articulate and structure such methodologies into an integrated workflow. Finally, the implementation of these new technologies is a key element, but culture and workflow evolution within organizations will also be important. Among others, segregation between discovery and development will have to be addressed. There are signs this change is already happening in the industry. For example, new hybrid departments or multidisciplinary teams are being created to address a variety of quality and efficacy issues affecting therapeutic development. One could envision some of the current discovery and development functions morphing into 'drug design' workforces with competencies to incorporate a wide scope of desired quality attributes into drug candidates. This could include safety, mode of action, delivery, or even elements of product life cycle management, such as patient- or disease-management approaches.

Predictive tools are becoming more sophisticated in the description of processes and biology. Their cost–effectiveness is likely to become a powerful argument to favor their routine application in the selection of suitable development candidates, perhaps also providing a 'development footprint' or signature for each compound that would facilitate the design of a specifically optimized process. It is also likely that the combination of *in silico* and *in vitro* early risk-mitigation will become a value-enhancing activity for programmes that are to be invested in, co-developed or partnered, essentially as a sort of 'insurance policy'. Interestingly, developability strategies have been postulated as a key approach in the development of biosimilar and biobetter products [106].

» Two-tier development processes

The need for condensing development timelines is fundamental in the development of translational medicine in a meaningful way [129]. As we have seen already, current development processes are far too rigid, complex and slow to make this possible. As a response to this, the concept of developing drug candidates as prototypes rather than final (commercial) products is likely to gain ground

in coming years [34]. In fact, some authors suggest prototypes should follow an entirely different, more adaptive, development protocol using iterative cycles to introduce changes or improvements instead of the currently standard linear development process [35]. This scenario has important consequences. An obvious one would be the consolidation of an alternative two-tier process development approach separating prototypes and commercial products. Recent reports on new host development initiatives seem to approach this philosophy [130]. If this strategy were to be developed further, such 'prototypes' would use a simpler, more agile and cost-effective manufacturing route that would facilitate a very fast transition of candidates to the clinic [131]. Perhaps several different candidate variants could be developed in parallel. Early developability screening could help select candidates with the desired quality attributes and higher probabilities of success without costly and time-consuming process-development qualification. This would simplify the validation of new targets and mechanisms of action, and

Executive summary

Surviving the 'valley of death': why early de-risking is a desirable activity

» Costs for developing new drugs and low R&D productivity require new approaches to reduce current attrition rates and produce cost-effective medicines.

What is wrong with our processes?

» The current development process is complex and extremely compartmentalized across a multitude of poorly connected silos.

» Quality and safety, rather cost of goods, are becoming central areas to be addressed in biopharmaceutical development.

» Implementation of early de-risking strategies as well as incorporating delivery into the drug-design process, could potentially have a significant impact on biopharmaceutical development.

Developability: a multidisciplinary approach to understanding & de-risking drug development

» Developability assesses the suitability of a given molecule to be developed based on parameters of quality, manufacturability, pharmacology, safety and efficacy. It can be used to select or engineer better lead candidates before developing a given manufacturing process.

» Developability tools combine *in silico* predictive methodologies, offering high-throughput and cost–effectiveness, and *in vitro* surrogate assays that are used as validation tools.

Protein quality & integrity. Aggregation as a key parameter for manufacturability & safety

» Protein degradation can affect process yield, biological activity and safety.

» Aggregation is a major quality concern and it is suspected to cause immunogenicity and safety issues.

» Several computational methods have been developed to predict aggregation in proteins and some of them have been exemplified with biopharmaceutical products. New analytical tools need to be developed to assess the aggregation potential of biopharmaceuticals in a simpler and high-throughput manner.

Formulation & delivery

» Formulation and drug-delivery strategies can have a significant impact on cost of treatment.

» Subcutaneous formulations can facilitate patient self-administration, but place considerable constraints in terms of stability, aggregation and viscosity.

» Early formulability assessment and x could reduce risks emerging later on in manufacturing and clinical development.

Immunogenicity assessment

» Immunogenicity reactions can reduce the efficacy of the drug (neutralizing response), but can manifest as allergic or immunotoxic reactions, which can potentially be life threatening.

» Preclinical immunogenicity tools predict patient responses to a given biopharmaceutical by using computational methodologies and cell-based assays utilizing blood samples from human donors.

Immunomodulation & immunotoxicology

» Many of the biopharmaceuticals currently licensed have an immunomodulatory activity.

» In extreme cases, biopharmaceuticals can trigger severe immunotoxic reactions, such as cytokine-release syndrome, which can be potentially fatal.

» Animal models are poor descriptors of the human immune system. Cell-based assays are being developed as alternatives to model biological activity and as surrogate immunotoxicology markers.

Engineering developability in biopharmaceuticals

» The use of *in silico* developability methodologies can help identifying candidates with a lower risk of immunogenicity, aggregation, degradation and so forth.

» Developability platforms to engineer important quality attributes in a given candidate, producing an improved version with reduced development risks.

could accommodate re-engineering treatments if so required. Furthermore, this scenario achieves compliance with true translational medicine endeavors. Following first-in-human or proof-of-concept results, a fully defined manufacturing process could then be developed that is based on earlier findings. At this stage, the risk of failure is lower and process robustness and quality requirements are more stringent. Here, the application of developability and QbD approaches would reduce the demand for expensive testing to qualify process scalability, robustness and quality assurance. The progressive nature of this approach, facilitated by its speed and flexibility, would significantly reduce the commitment of high-risk, heavy upfront investment for the development of new candidates and could be instrumental in reducing the development costs of new drugs.

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J Zurdo is an employee of Lonza Biologics plc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

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