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Current and future issues in the manufacturing and development of monoclonal antibodies☆

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Abstract

Despite a slow beginning, monoclonal antibodies have had many successes over the past decade. It is important that these successes continue, bringing more products for more indications to market. Although manufacturing is not the most common cause of product failure, product quality issues can delay antibody development. Manufacturing has depended on the triad of process validation, process control and product testing. Applying product knowledge proactively to manufacturing (quality by design) may allow greater flexibility and maintain or improve product quality. An integrated approach to biological characterization is an important aspect of product knowledge. Greater product knowledge also facilitates development in other disciplines. Independent of manufacturing strategy, there are a number of regulatory hurdles in initial and ongoing antibody development. These are described to help prevent unnecessary delays.
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Keywords: Monoclonal antibodies; Manufacturing; Product testing; Biological characterization; Quality by design

Contents

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1. Introduction

The generation of monoclonal antibodies through somatic cell hybrids was published in 1975 [1]. The development of this technology allowed for generation of proteins that could specifically bind almost any target. Although the clinical possibilities were immediately obvious, development of monoclonal antibodies into marketed products took some time. The first therapeutic monoclonal antibody, Muromonab-CD3, targeted a T-cell receptor complex protein and was approved in 1986. In 1992, a monoclonal imaging agent, In-111 Satumomab, was approved by the FDA and the second therapeutic antibody, Abciximab, was approved in 1994. Abciximab, an antibody fragment, was chimeric with the constant regions having human sequences. In 1997, the first whole antibody that was chimeric, Rituximab, and the first "humanized" antibody, Daclizumab, were approved. Humanization further reduced the murine sequences in monoclonal antibodies by engineering them into the complementarity determining sequences of human variable region genes. This often led to appropriate antibody binding with very limited murine sequences in the framework of the human genes. Decreasing the level of murine sequences was important in reducing immune responses [2] to antibody products and thus facilitating chronic use. A variety of strategies were used in humanization [3] and many humanized antibodies have been approved. In addition antibody conjugates, fragments, fusion proteins and a fully human monoclonal antibody have been approved and marketed.

The points to consider for monoclonal antibodies was published in 1994 and revised in 1997 [4]. Under the leadership of Dr. Kathryn Stein, this guidance was written to provide an overview of the major issues in developing monoclonal antibody products. This document along with other FDA and ICH guidance facilitated product submissions to the agency. Until October 1st, 2003, regulatory review activities for antibody products occurred in the Division of Monoclonal Antibodies within the Center for Biologics Evaluation and Research (CBER) Office of Therapeutics Research (OTRR). At that time, OTRR officially transitioned to the Center for Drug Evaluation and Research (CDER) and was split into the Office of Drug Evaluation 6 (ODE6) and the Office of Biotechnology Products (OBP). ODE6 was made responsible for clinical and pre-clinical issues and ODE6 personnel and products were distributed to other ODEs throughout the Office of New Drugs at CDER as of October 2005. Although many monoclonal antibodies have indications for oncologic and inflammatory diseases, antibodies are used for a variety of indications and were distributed throughout the Office of New Drugs. OBP was made responsible for chemistry, manufacturing, control (CMC) and related issues and OBP will continue to review these issues for the ODE6 products as they are distributed throughout CDER.

Throughout all the organizational changes, the agency remained focused on ensuring antibody quality and facilitating product development. Twenty-six monoclonal antibody or related products have been approved to date. The time from submission of an investigational new drug (IND) to licensed product has ranged from more than 13 to less than 5 years. The majority of licensed antibodies have had single cycle reviews. Since 1994, an average of greater than

two monoclonal antibodies has been approved per year. Although this is a great advance on an 8-year lag between the first two therapeutic antibody products, there is always room for improvement. We discuss here a variety of issues that have led to product delay or failure and strategies to facilitate the development of therapeutic monoclonal antibodies.

2. Manufacturing issues in product development

2.1. Overview of product failure

Areas of product failure align with the three dimensions of the critical path [5]: medical utility (efficacy), safety and industrialization (manufacturing and quality). One frequent reason for product failure is that the product does not show efficacy in clinical trials. Although ideally this failure would be picked up early in development, in many cases, it has taken a pivotal study to reveal lack of efficacy. Choosing the right indication is critical and successful products have failed in their initial indication [6]. Even if the product works in the chosen indication, a poor study design may fail to reveal or support true benefits. Safety concerns from clinical trials may stop or delay product approval. Although manufacturing issues are usually not the cause of product failure [7], manufacturing problems can significantly delay product approval for complex biotechnology drugs. The lack of manufacturing consistency or the initiation of manufacturing changes late in product development [8] has delayed approval of monoclonal antibody products.

2.2. Manufacturing process overview for monoclonal antibodies

Antibody drug substance is a critical source of product variability. For current monoclonal antibody products, the source material is usually generated in mammalian cell culture. The type of bioreactor, media composition, culture duration and parameters can all impact on the nature of the desired product and product-related impurities. Other source materials, such as cultures of yeast or bacteria, transgenic plants or animals, also impact on the characteristics of the product. The source material for drug substance and its initial processing (harvesting for cell culture) can significantly impact the level and type of impurities that challenge downstream purification steps.

For monoclonal antibodies, purification often consists of multiple chromatography columns. Each of these columns performs multiple functions. They may select for a desired product with a particular charge range, remove product-related impurities such as aggregates, process-related impurities such as host cell proteins and model endogenous retrovirus. Column type, such as affinity or ion-exchange, column parameters, product and impurity loads and buffer compositions can all impact the purification. The purification will also have other steps such as a low pH incubation or nano-filtration primarily designed to remove or inactivate endogenous retrovirus. Parameters for these steps are critical for successful viral clearance but also may secondarily impact the product. Purification processes may have multiple filtrations, including ultra- and dia-filtration steps. Each of these purifications steps may introduce new process-related impurities such as filter and column leachates, or buffer components. Thus, downstream processing must be designed as a sequence of steps that ensures removal of process-related impurities to safe levels in the final drug product. A number of monoclonal antibodies are conjugated to radionuclides or other small molecules. The steps used in generating these antibody conjugates are sensitive to a variety of factors and often lead to introduction of new impurities such as linkers, chelators, inactivating chemicals and other reagents. These are often introduced late in the process and appropriate removal of these impurities needs careful planning.

2.3. Manufacturing control

A complex multistep process with so many variables that can impact the product and/or impurities needs to be carefully controlled. Process controls include raw material acceptance criteria, in-process testing, defined setpoints and operating ranges for process parameters and defined process and hold times. The conditions for reprocessing or reworking intermediates need to be evaluated and defined. The assays used for testing need to be sensitive and reliable.

However, there is no way to control every parameter in such a complex process. Critical parameters may be far fewer but identifying these parameters that

affect product quality is difficult. Thus, an important strategy used in controlling manufacturing is validation [9–11]. Validation assesses the ability of a unit operation to do what it is supposed to do. Generally, validation involves repetitions of a step or assay under a variety of defined conditions. The success in meeting specific criteria provides evidence that the unit operation or method will perform similarly if done the same way. Process validation has been successful in demonstrating the removal of impurities and thus removing routine end product testing for that impurity. Robustness in a validation evaluates the sensitivity of the step or method to changes. Development studies done prior to formal validation may provide important information on a process step or assay. This information may suggest parameters that are important to carefully control during a manufacturing or testing method. Often the parameters evaluated are limited and combinations of parameters that could impact product quality are not considered in a comprehensive manner. The range of output parameters from a prior step should also be considered in evaluation of a unit operation.

The triad of process control (e.g. raw material acceptance criteria, in-process testing, defined setpoints and operating ranges for process parameters and defined process and hold times), process validation and product testing are the basis for current manufacturing of monoclonal antibodies and the majority of pharmaceuticals. This combination, along with product knowledge, is also critical for establishing biochemical comparability between products after a manufacturing change. Despite the success of this manufacturing strategy in bringing quality monoclonal antibodies to market, there have been problems. There have been cases where manufacturing has been inconsistent at scale even though no new processes were introduced. More commonly, problems have been seen with changes in the manufacturing process. Scale ups in manufacturing where careful process validation has been performed and the process is carefully conserved are often successful. Similarly, establishing new manufacturing facilities for a product can be successful when the process undergoes as little change as possible. However, changes that perturb the process have had unforeseen consequences on the product. Thus, conservatism in manufacturing changes is warranted. On the other hand, efficient development and meeting market demand requires flexibility in scale up and manufacturing capacity [12]. Furthermore, flexibility allows for changes to improve product quality. This creates a tension regarding industry decisions.

2.4. Manufacturing principles

The real goal of manufacturing is to consistently provide product that reflects the material used in clinical trials regarding safety and efficacy. Ideally, any manufacturing process that consistently produced such product and controlled impurities would be appropriate. If one could test every dose of product for every clinically relevant attribute, impurity and contaminant, the manufacturing process could have very wide boundaries as long as these attributes are controlled. However, since this is currently not possible, the only approach felt to achieve these goals is, as described above, is a combination of process control, process validation and product testing. In Fig. 1, the desired product is displayed as an iceberg. Routine product testing, or lot release, reveals the tip of the iceberg. Process control and validation control deal with the base of the iceberg, product attributes that cannot currently be evaluated. The middle space reflects product attributes that can be revealed by extensive characterization but are not evaluated on a lot-to-lot basis. This middle space is shared with

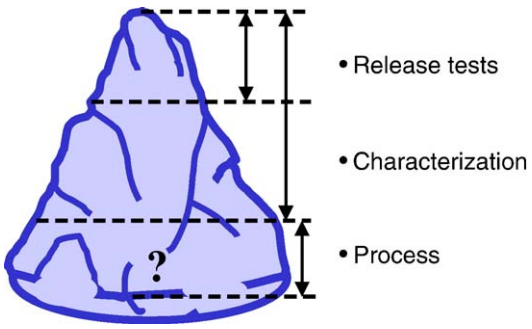

Fig. 1. How much of the desired product can we evaluate? A schematic iceberg is used to represent the desired product, and the role of release testing, characterization and process consistency in evaluating product is illustrated by arrows between dotted lines. The question mark is used for the area covered by process since, without a thorough understanding of the manufacturing process, there is uncertainty in the level of product consistency insured by the process.

process validation and control. Since these attributes are not evaluated on a regular basis, we depend on process consistency to ensure they are unchanged.

Although we are currently locked into the triad of process validation, process control and product testing, the role each plays in revealing the iceberg or desired product can change significantly. Greater process control may diminish the role of historical validation. Improved product characterization may shrink the base of the iceberg and minimize unknown attributes. Advances in structure function relationships and product biology may restrict the attributes important for the desired product. All of these changes would lead to greater flexibility in manufacturing without an increased risk to product quality. Even with these changes, product quality will depend on some combination of product testing and process control in the short and intermediate time frame.

3. Product testing

3.1. Specifications

The tip of the iceberg or specifications are the most visible aspect of quality control. The certificate of analysis, listing the specifications and results, is the first place usually examined to assess the quality of a lot. A specification is defined as a list of tests, references to analytical procedures and appropriate acceptance criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use [13]. The difficulty is in knowing what tests, what acceptance criteria and what sampling to use. Specifications are generally a subset of the tests used for characterization. Specifications should assess relevant attributes. The acceptance criteria should reflect values that maintain the safety and efficacy of the product. The sampling should be appropriate for the variability of the attribute. In the absence of information on clinical relevance and process control, many specifications are instituted with narrower than necessary acceptance ranges based on manufacturing experience. Specifications lead to a trade off between the likelihood a consumer will acquire a suboptimal dose and the likelihood a lot will fail release. Increasing the number of tests increases the likelihood of failing good lots of product. In theory, performing 30 truly independent tests with acceptance criteria of plus or minus three S.D. would fail more that 25% of acceptable lots ($(0.99)^{30}=0.74$). The lots would fail based on the expected test variability of acceptable material. Narrowing the acceptance criteria also increases the number of acceptable lots that fail. Ideally, by focusing on the attributes that are relevant and setting acceptance criteria that are relevant to risk, consumers can receive high quality product without manufacturers incurring unnecessary failed lots.

Phil Krause [14] has divided specifications into three classes: those that confirm safe levels of critical impurities/contaminants; those that confirm manufactured lots are similar to clinical trial lots; those that confirm the manufacturing process is under control. The third category is one being currently being reconsidered. In process tests and controls may replace these specifications. Acceptance criteria that have been narrowed to reflect process capability may be widened if the process can be shown to be controlled by other means. The first type of specification for safe levels of impurities/contaminants is generally necessary. However, in some cases, such as removal of model endogenous retrovirus [4,15] or evaluating sterility through parametric release, process validation may replace a specification for contaminants. The second category, confirming that manufactured lots reflect clinical lots, is the most complex and highlights the dilemma of selecting specifications. For biotechnology products there may be an extremely large number of attributes to consider. How many of these attributes need to be evaluated in evaluating similarity of a lot to clinical trial material? How close do they need to be to clinical trial material? As suggested above, these attributes are a subset of a detailed product characterization. Physiochemical characterization may provide a large set of attributes to choose from but does not necessarily establish those attributes with clinical relevance.

In addition, a relevant subset of product attributes needs to be selected for evaluation of product stability. Certain studies for stability are broadly applicable and/or based on route of administration and dosage form (e.g. container closure integrity, excipients in some cases, moisture for lyophilized products). For evaluation of the active pharmaceutical ingredient, accelerated stability and stressed samples can provide

information on the degradation of product and help select stability indicating assays. However, stability-indicating attributes do not necessarily equate with clinical relevance. In general, stability protocols for monoclonal antibodies include assessments of a variety of product attributes such as potency, denatured and native size and charge distribution. Since it is generally not clear exactly which product attributes are critical for clinical performance, the panel of assays chosen for stability may not always be ideal.

Although many monoclonal antibodies share large stretches of sequence, especially antibodies of the same class, differences have been noted between antibodies with very similar frameworks [16]. If an antibody mechanism of action depends on effector functions, certain post-translational modifications may have greater impact [17–20]. Thus, an appropriate understanding of the structure and function of each antibody product is crucial. Although general lists of tests appropriate for antibody characterization and testing are of value [21], the most effective quality control strategy will derive from a through understanding of the specific product under development.

3.2. Characterization

3.2.1. Biochemical characterization

As described above, a thorough understanding of product attributes will facilitate product quality throughout development. The more complete the characterization of the physio-chemical attributes of the product, the more solid the basis for product development. Early understanding of the range of product attributes facilitates evaluation of the importance of specific attributes. For example, defining a methionine oxidation that may impact antibody binding would allow for evaluation of formulation buffers and/or storage conditions to avoid later stability issues. Although insufficient to replace immunogenicity testing, in silico approaches to assess the impact of deamidation on a product peptide-major-histocompatibility-complex-molecule interaction may suggest changes that would be important to control to avoid alterations in immunogenicity. Characterization of product variants with glycoform changes that may impact pharmacokinetics (PK) could facilitate early development of a manufacturing process that avoids variability in the relevant glycoforms.

There are certainly economic forces that appear to work against early characterization. The majority of products do not make it to market so anything that front loads costs is undesirable. There is also a view that larger companies are driving the regulatory expectations for characterization and this will set a bar unachievable by other companies [12]. This is not the case. For entry into the clinic, a basic level of product characterization is needed (see later section on clinical holds for monoclonal antibodies). The regulatory agency needs to know the sponsor has made the product they have described and that the product used in toxicology studies is related to that intended for use in the clinic [22]. The ability to further characterize the product is an advantage that may smooth development and help avoid pitfalls, but not a requirement. Although avoiding upfront costs is important since many products fail to make it to market, monoclonal antibody products have achieved impressive success rates compared to other products. Chimeric and humanized monoclonals have had a success rate of approximately 20%, while the overall success rate for new chemical entities has been 11% [23]. The success rate for monoclonal antibodies is expected to improve. Since manufacturing and quality issues can hold up biotechnology products late in development and at great cost, investment in a thorough early characterization may be of greater value than industry currently perceives. There is also the impression that more information shared with regulatory agencies will lead to more questions and potential barriers. However, new agency initiatives, such as current good manufacturing practices (cGMPs) for the 21st century [24], process analytical technology [25], risk-based and quality by design approaches [26], intend to utilize greater product and process information to support regulatory flexibility.

Although protein characterization [27] has been a mainstay of product development, many new approaches to characterization are available. Peptide mapping [28] with mass spectrometry [29] and glycoform analysis [30–32] have significantly improved resolution and identification of product isoforms. Evaluation of higher order structure, with tools such as multi-dimensional nuclear magnetic resonance (NMR) spectrometry is improving and may be

currently utilized for antibody fragments. Advances in protein characterization using mass spectrometry and NMR merited the Nobel prize in chemistry [33]. Structure can also be assessed by electron microscopic tomography of molecules [34,35] and other novel techniques. Signature assays using a variety of technologies from circular dichroism and ultrasound [36] to partition coefficients are being developed. Aggregation and size can be evaluated using analytical ultracentrifugation [37], field flow fractionation [38] and other approaches. A full accounting of characterization technologies would be too large to describe here and lists of antibody characterization tools are available [21] although no list is complete and alternative strategies are always being developed. The tools described above are mentioned as examples of technologies, however the best methodologies for characterization of a particular monoclonal antibody will depend on a variety of considerations and should be discussed with the appropriate regulatory agency.

The use of orthogonal methods can lead to resolution of many variants. A method such as a charge separation followed by mass spectrometry may reveal combinations of product variations. Theoretical numbers of potential monoclonal antibody variants are suggested in Fig. 2. The 10^8 possibilities described are only a subset of theoretical antibody combinatoric variants. Only a limited number of glycoform variants are included in this calculation. Although the changes described are probably not all independent and the true number of variants is probably far smaller, there are still a very large number of potential structural variants. Currently, characterization does not assess all these combinatoric variants and instead assesses the levels of individual changes. However, if for particular attributes, it becomes clear that combinations of attributes have a functional impact, characterization may need to resolve these combinations. For example, consider a theoretical glycoform variant that is known to have an effect only when both heavy chains of the same antibody molecule have this variant. If 50% of heavy chains have the variant, the % of product showing this effect could vary from 0% to 50% depending on the distribution. It would be important to assess this and determine the variability of the distribution of these isoforms.

3.2.2. Biological characterization

The limits of biochemical characterization are advancing rapidly. However, the results of biochemical characterization for complex proteins such as monoclonal antibodies reveal heterogeneous mixtures. As suggested by the above discussion on combinatorics, the greater the resolution of characterization methods, the more heterogeneity will be apparent. The critical issue is which variants matter and at what levels do they matter. We are concerned with the relevant attributes and these are defined in ICH Q6B

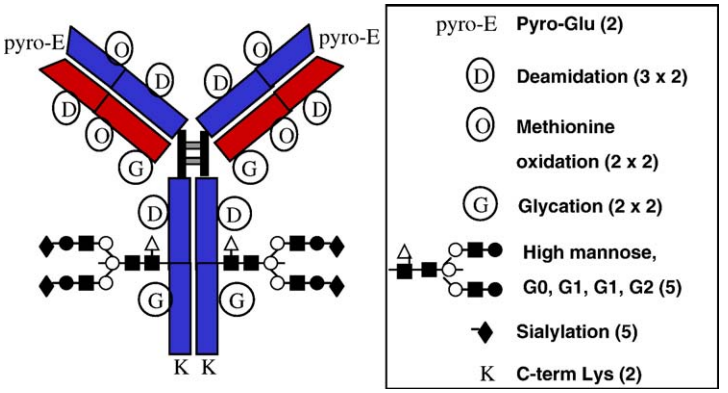

Fig. 2. Potential monoclonal antibody variants. An IgG antibody schematic is shown with some potential structural variations indicated by symbols. Each symbol is noted in the legend with a list of variations. The number of variation sites in each half-antibody × the number of possible variations at each site is in parenthesis. Not all possible variants are described. For example, there are fucosylation variants in glycosylation that were not counted. If one assumes these variants are independent and considers combinations, each half-antibody has $2\times6\times4\times4\times5\times5\times2=9600$ possible states. If one assumes both halves of the antibody are independent, there are $(9600)^2 \approx 10^8$ possible states.

[13] as those molecular and biological characteristics found to be useful in ensuring the safety and efficacy of the product.

As the biology of antibody function is better understood and clinical experience with antibodies grows, knowledge regarding variants and their effects will accumulate. Although extrapolation is difficult, prior knowledge is an important component in considering potential structure/function relationships. The biological and functional characterization of selected biochemical variants in combination with prior knowledge may allow a risk-based approach to changes in product attributes. No one assay or approach may be convincing but a panel of biological studies may be of great value both in assessing the importance of an attribute and evaluating appropriate ranges for an attribute. Guidance on such "biological characterization" is limited. The guidance for IND meetings [39] suggests that by the end of phase 2 meeting there should be "adequacy of physicochemical and biological characterization (e.g., peptide map, amino acid sequence, disulfide linkages, higher order structure, glycosylation sites and structures, other post-translational modifications and plans for completion, if still incomplete)". It is clear that the examples given are for physiochemical characterization. However, the guidance does suggest a bioassay and evaluation of bioactivity of product-related substances and product-related impurities relative to desired product. If one considers bioactivity to be a set of biological activities rather than a single bioassay, this can be a very useful approach. Although any *in vitro* or *in vivo* bioassay will have limitations, a set of assays can be complementary. Models of biological activity can occur at many levels (Table 1) and similar patterns at different levels can be very convincing.

Table 1
Levels of biological characterization

Characterization level	Relevant models[a]
Binding; conformational changes	Receptors
Signal transduction	Pathways
Cell culture effects	Cellular targets
Tissue studies	Tissues
In vivo studies	Animal model
Clinical studies	Biomarkers pharmacodynamics

[a] The aspects of the model system that must relevant to the product and/or disease being characterized.

Although the levels of biological characterization can seem overwhelming, many of these models may also be used in drug discovery and pharm-tox evaluations. Prior knowledge may be of value in assessing what levels may be the most revealing for any given product. To be useful in defining risk, the assays should be relevant to the product mechanism of action. This may not always be easy since products may have multiple mechanisms that impact efficacy, safety and PK. Relevant models are critical and depending on the level of the characterization, the correct receptor, pathway, cells, tissues or species must be evaluated (Table 1).

Biological characterization is further complicated by the product-specific nature of most bioassays. This differs from the majority of physiochemical assays, which can be applied to many products with minor changes in methodology. However, some bioassays, such as those for antibody effector function, may share common approaches and reagents. Binding assays for different products may also share approaches. Binding studies using technologies such as surface plasmon resonance [40] or calorimetry [41] may provide important functional information in the form of binding kinetics and/or thermodynamics.

Biological characterization is non-linear since choosing the correct models is based on knowing the relevant mechanisms and knowing the relevant mechanisms is based on data from models. Thus, a systems approach is needed with a variety of evaluations. Data on product attributes from use of product lots in clinical and pre-clinical studies should be combined with data generated in simpler models where purified variants and material that has been degraded can be specifically evaluated. The use of all this information in a matrix (Fig. 3) combined with prior knowledge may facilitate broader specifications and greater flexibility. In Fig. 3, a variety of studies relevant to protein biology from *in vitro* assays to clinical data is integrated with product lots and their attributes over development. A hypothetical example of how such a matrix could be used follows. A variant has appeared to increase during stability for an antibody product. Therefore, the biological activity of clinical lot extremes containing this variant would be assessed as described in the second column in Fig. 3. Specifically, product containing a high level of this variant was administered to patients with an early lot

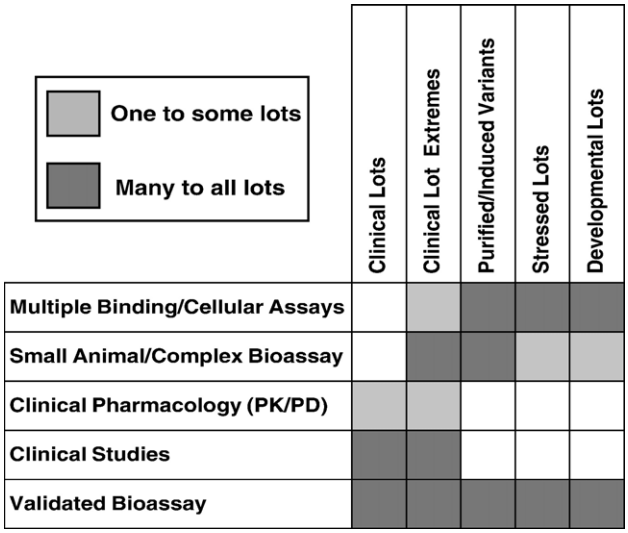

Fig. 3. Biological activity matrix. Since no one bioassay, animal model or pharmacodynamic marker is likely to define all the relevant activities of an antibody, a combination of studies is likely to provide the most useful information. This matrix lists a variety of studies that are complementary. If such studies are performed with product variants during product development, the integration of study results may provide important information on product structure function relationships.

in a dose escalation study so there was limited exposure to high levels in a clinical study. In addition, product containing very high levels of the variant (purified variants as in the third column or stressed lots as in the fourth column of Fig. 3) exhibited acceptable activity in binding/bioassays. The product mechanism of action is fairly well understood; the change is far away from known active sites and a similar change in another product in the literature had little impact on activity or pharmacokinetics. Further assessment regarding the likelihood of immunogenicity suggests a low risk since this variant occurs in native circulating immunoglobulin. Putting all this information together would support a broader specification for this attribute in evaluating product stability without additional studies, however, should there be a reason for additional concern, appropriate clinical studies can assess this risk.

Although immunogenicity is beyond the scope of this paper, it warrants some comment here. Immunogenicity cannot be currently predicted based on structure although new strategies are being applied to this problem that may be of value. Thus, as described above, the impact of structural attributes on immunogenicity always needs to be a consideration in setting ranges for them. Mostly, human or human antibodies have unique advantages in this respect. They are primarily human proteins that exist at high levels in circulation with a wide variety of structural variants. This favors tolerance to the antibody shared structure and its natural variants. Antibodies to these products are thus primarily directed against unique regions such as the complementarity determining regions (CDRs). This facilitates an assessment of the immunologic impact of specific changes and their locations. Furthermore, antibodies are generally not structural mimics of non-redundant self-proteins, a high-risk category for immunogenicity adverse events. However, structural mimics of non-redundant self-proteins linked to Fc as an Fc fusion protein would be in such a high-risk category. Also an antibody targeting a non-redundant protein may lead to immunization against that protein through an anti-idiotype network internal image [42]. As this is a low probability event, it would only be a concern for a protein target that poses a very high risk. Despite the above advantages, immune responses to antibody CDRs are often neutralizing and immune response to antibodies have been associated with adverse events [43]. Thus, depending on the nature of an attribute change and the history of the antibody product, changes in antibody attributes (or their

acceptable ranges) may need an evaluation of immunogenicity with appropriate studies and assays [44].

Clearly, this type of biological matrix analysis cannot be done for all products and all attributes. As discussed above, immunogenicity concerns may be limiting. Assessing potential PK effects may be difficult even if there is comfort over attribute effects on mechanism and vice versa. Bioassays may have high variability and are expensive. However, an effective drug development program utilizes a variety of assays in target selection. Choosing appropriate assays can avoid development of product that will fail or is being developed for an inappropriate indication. If the expertise in development assays can be strategically applied to assessment of product attribute effects, biological characterization may be a valuable strategy. Certainly, biological knowledge is growing along with physiochemical technologies and further development in systems biology may facilitate biological characterization.

A biological characterization is only as good as the experimental data supporting it. If regulatory decisions are impacted by biological characterization, a framework for the appropriate interpretation of cell and molecular biology information is needed. If sponsors begin to take advantage of biological characterization in requesting regulatory flexibility, regulatory agencies will need expertise in cutting edge cell and molecular biology and the ability to communicate relevant knowledge throughout the review teams. A systems review approach is needed to interpret biological characterization since it involves data in all the review disciplines.

Biological characterization is an important component of any risk-based approach to regulation of antibodies or drugs in general. Since FDA initiatives such as CGMPs for the 21st century [24], process analytical technology (PAT) [25] and quality by design [26] depend on assessing characteristics relevant to safety and efficacy and defining the process parameters that control these attributes, biological characterization will become more important over time. Understanding the relevant product attributes and how important attributes may interact with each other in specific cases can lead to a design space for product attributes (Fig. 4A). The default to biological characterization is looking at a very large number of attributes and controlling them to levels based on manufacturing capability.

4. Quality by design

Ideally, once product attribute targets and ranges have been defined, a manufacturing process can be

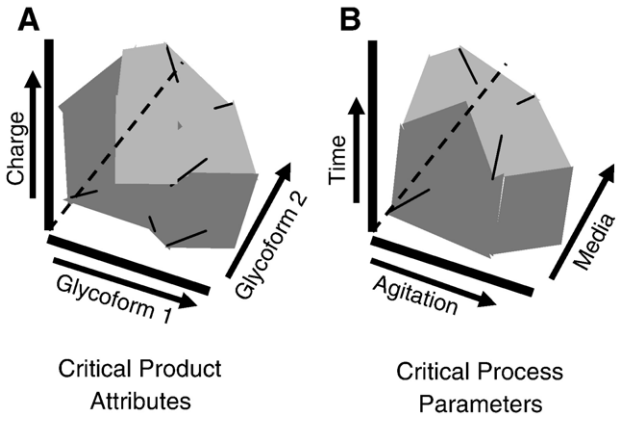

Fig. 4. Use of design space in product manufacture. Attributes or parameters that may interact should be controlled as a multi-dimensional space rather than as independent ranges. Panel (A) describes a three dimensional space for acceptable combinations of antibody charge and two defined glycoforms. Such a space would be defined by a matrix of data from in vitro assays to clinical studies. An ideal process control system would directly measure these attributes in real time. Panel (B) describes a three dimensional space for acceptable combinations of time to harvest, agitation and media composition in a bioreactor. Such a space would be defined by studies linking these parameters to acceptable product attributes such as defined in panel A.

implemented or modified to control for these attributes. Quality by design [26] strategies would encourage this. Quality by design is an approach in which product and process performance characteristics are scientifically designed to meet specific objectives, not merely empirically derived from performance of test batches [45]. Since characterization and stability studies are not complete when early manufacturing schemes are implemented, this is an iterative process. However, the aggressive gathering and communication of this information may be of value in process design.

New selective technologies in antibody development such as phage, ribosome and yeast display [46] allow for functionality by design. Adaptive evolution and selection can maximize critical product functional attributes such as binding constants, cross-linking, internalization and effector functions. Additional criteria may reduce the potential for adverse events through selection against immunogenic epitopes or cross-reactivity. Thus, such technologies bring about the potential for better "efficacy and/or safety by design". However, these technologies can also be used to select against heat-sensitivity, aggregation or other undesirable characteristics. Selection can also avoid amino acids prone to modifications in important binding sites such as antibody CDRs. This suggests a role for quality by design in very early product discovery that may avoid costly stability and formulation issues. This type of strategy combined with further developmental data may facilitate confidence in manufacturing and regulatory flexibility.

5. Enhanced role of manufacturing process control

In order to consistently manufacture a product that meets appropriate targets for attributes, a robust process with appropriate controls should be developed. Examples of unit operations in current manufacturing processes for monoclonal antibodies are shown in Fig. 5. Just as there can be a design space for critical product attributes, a design space can be developed for the process parameters needed to achieve the critical quality attributes (Fig. 4B). Although many biotechnology manufacturing steps are controlled by volume or time, some steps are currently controlled by process parameters (Fig. 5) such as dissolved oxygen and pH in bioreactor media. Harvest turbidity and buffer conductivity are also used in controlling process steps. Chromatography elution may be controlled by protein concentrations using 280 nm absorbance.

Ideally, process steps are controlled by measurements of relevant material attributes. Assessing relevant material attributes can facilitate use of appropriate raw materials or intermediates. Measurements of material attributes can also feedback on a process to control duration of a step or other process parameters. If this is done at near real time and the correct material attributes are assessed for each operation, product quality can be controlled and monitored during the manufacturing process. Endpoints can be based on desired material attributes rather than fixed parameters. At or on-line measurements allow more complete evaluation of a batch. These represent key parts of PAT [25].

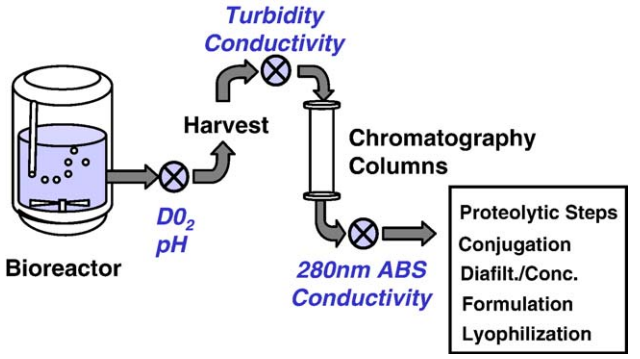

Fig. 5. Current biotechnology process control. Schematics of a bioreactor and chromatography column are annotated at a number of process control points (indicated by X in circles). Process decisions are modified by process parameters or product concentration (in blue italics) at these steps. A variety of other downstream steps are indicated in the box. Most of these steps are controlled by pre-defined times, volumes or temperatures.

If one is willing to call 280 nm ABS a relevant product attribute, the control of fraction collection through 280 nm ABS is a very basic PAT-like operation. Bioreactor feedback often is based on measures of process parameters rather than material attributes but advances in biosensors and/or spectral analysis may incorporate product attributes into bioreactor control. Biotechnology products, such as monoclonal antibodies, are amenable to feedback control of steps such as lyophilization and container closure interactions. One issue regarding PAT of biotechnology products is the multiple roles associated with unit operations. In addition to concentrating the desired product and removing product-related impurities, a chromatography column may remove specific impurities (e.g. DNA, endotoxin, host cell proteins) and clear virus particles. Each of these roles may be impacted differently by changing conditions (e.g. buffers, product load, etc.). Although the literature suggests column failure for some of these roles can be modeled by other parameters [47,48], any range of column process parameters used during PAT would need to be shown not to impact any important column functions. A design of experiment (DOE) approach [49] might be needed to establish the parameter ranges used to control column performance. However, within those ranges, material attributes may be used to control column performance.

Although instituting quality by design and PAT approaches to biotechnology is complex, it affords some real opportunities for industry. PAT case studies for biotechnology unit operations (UF/DF, chromatography, protein refolding) have been presented. Even if limited initially to defined unit operations, the agency can provide regulatory relief for changes in appropriately controlled and understood operations. A well justified design space, for process parameters or product attributes, may eliminate the need for some supplements. For this approach, defining critical product attributes is important and a systems approach to biological characterization as discussed above may be needed. This approach may be more amenable to less complex proteins with a well-defined mechanism of action. Any history of prior changes and/or experience with lots that have variable attributes would contribute. The more *in vitro*, pre-clinical, clinical, PK and pharmacodynamic data linked to these lots, the more knowledge available to provide flexibility.

In addition to reducing regulatory burdens, these approaches may allow manufacturing strategies that have not been previously possible. For combinations of antibodies, combined process streams either at the cell-culture side or purification side have not been encouraged. Each component has needed to be separately manufactured. The inability to control the manufacturing of each component has been an important factor in concerns for such one-pot approaches. One could imagine a biosensor-based monitoring of multiple products and their variants in the process alleviating much of this concern. One pot manufacturing strategies could lower the cost and increase flexibility in the manufacture of combination therapies.

Not all products or manufacturers may wish to take advantage of these strategies and there is a history of quality products using more classical approaches. The disadvantage of classical approaches is limited flexibility. Independent of the broad approach to manufacturing of antibodies, a company must pass through a number of regulatory hurdles to successfully develop their product.

6. Avoiding CMC-related clinical holds

In pharmaceutical development, failing early is better than failing late. However, no one wants products to be held up for avoidable reasons. We have put together experience at the division of monoclonal antibodies to highlight pitfalls in product development. We have performed a retrospective analysis of clinical holds based on CMC issues for monoclonal antibodies and related products (fragments, Fc fusion proteins, etc.). These were based on evaluation of approximately fifty hold letters dating from 1997 to 2005. These may not include every antibody CMC hold issue but are a reasonable sample. Common issues were combined into general categories that could apply to many antibody products and do not identify specific products or sponsors.

In Table 2, original submission hold issues are listed in order of frequency. Lack of adequate data on viral clearance of model endogenous retrovirus leads the list. There is clear guidance on these issues [4,50] and these holds should be avoidable. Lack of appropriate specifications is another common issue.

There is resistance by industry to locking themselves in to meaningless narrow specifications and that is appropriate. However, for clear safety issues, such as endotoxin, a safe limit should be set. Attributes that may impact immunogenicity, such as aggregates, should have reasonable limits. Attributes that do not have a clear link to safety may have broad limits and ideally, regulatory agencies should allow some changes in these limits as development progresses. Potency is a critical component of antibody assessment and for any dose-related information on a product to be meaningful (e.g. dose escalation studies), a potency assay should have reasonable limits. Although detailed characterization is not necessary for an IND, some basic characterization of product size (native and denatured), charge, purity and functionality is needed. Again, although a detailed description of the manufacturing process is premature, the manufacturing steps and reagents should be described. For biotechnology products, many potential safety issues are related to the process. A regulatory issue over the lack of any information on product, product components or high-risk raw material safety data is without need of explanation. Stability is generally not an early issue, however the inability to convince a regulatory agency that the product will be stable throughout the planned studies is a problem. In addition, other hold items have been specific to products with a high risk for antibody mediated adverse events or patient-specific products. There have been holds for lack of data on materials other that product used in a clinical trial (e.g. viral challenge material), lack of appropriate cross-reference letters or lack of other necessary documentation.

Table 2
Hold categories for IND original submissions

Hold category	Frequency[a]
Lack of data on clearance of endogenous retrovirus	7
Lack of appropriate specifications for critical tests (e.g. sterility, endotoxin, free isotope)	5
Lack of basic product characterization	4
Lack of potency assay or appropriate potency assay limits (e.g. safety, futility)	4
Lack of information on product manufacture	3
Lack of information on product or critical drug product components	3
Lack of safety data on human or animal derived material (e.g. source, viral testing)	3
Lack of appropriate cell line testing for viruses or adventitious agents	2
Lack of stability data regarding appropriateness of product use	2

[a] The frequency of hold categories for original IND submissions from approximately 50 letters with CMC hold issues for monoclonal antibody-related products. Each letter may have had more than one CMC hold issue.

Table 3
Hold categories for in-effect INDs

Hold category	Frequency[a]
Stability issues regarding appropriateness of product use	3
Unexplained increase in adverse events (possible CMC or immunogenicity cause)	3
Damaged container closure	1
Lack of appropriate potency assay for phase 3 trial	1
Lack of evidence for consistently manufactured lots	1

[a] The frequency of hold categories for in-effect INDs submissions from approximately 50 letters with CMC hold issues for monoclonal antibody-related products. Each letter may have had more than one CMC hold issue.

For INDs that are already in effect, product contamination and expired material are common reasons for CMC-based clinical holds. Table 3 describes other categories of CMC issues, in order of frequency, that have led to clinical hold of an antibody product under IND. Of note stability issues are more common in later development. Adverse events that may have a product quality relationship can lead to holds until that relationship is defined or ruled out. Lack of container closure is clearly a safety issue. It is of note that the last two categories in Table 3 relate to the inability to evaluate and demonstrate lot consistency as development progresses. Clearly, process advances as described above would avoid these issues.

7. Conclusions

Many monoclonal antibody products have been successfully manufactured and marketed using the combination of process validation, process control and product testing. There are a number of common pitfalls in early antibody development that can be avoided by referring to guidance and appropriate planning. Advances in approaches to specifications and manufacturing may speed up industrialization of

monoclonal antibodies and other biotechnology products. The key to the use of these approaches is defining critical product attributes and linking them to critical process parameters. Product complexity and heterogeneity suggest critical product attributes can only be defined with a better knowledge of product biology and structure function relationships.

Although biological characterization is challenging, the benefits are not restricted to manufacturing. In addition to defining physiochemical attributes relevant for FDA initiatives such as cGMP for the 21st century [24] and PAT [25], careful biological characterization facilitates product development in other disciplines. An understanding of molecular mechanisms using a variety of models can inform decisions on toxicity and efficacy. In the past, cellular and molecular biology have primarily played a prominent role in drug discovery and early development. However, there is now a growing role for cellular and molecular biology in choosing relevant toxicology models, designing the best clinical studies and assessing the potential impact of safety concerns across related products. Cellular and molecular biology are important in selecting appropriate treatment regimens for defined populations. For antibodies in particular, the role of Fc receptor polymorphism [51,52] on clinical outcomes is an important example as well as the presence or absence of the antibody target antigen [53]. As drug development embraces a systems approach to biology utilizing pharmacogenomics, proteomics and metabolomics, industry and regulatory agencies will be faced with patterns of markers and limited statistical data. One factor in weighing these patterns or other biomarkers is biological plausibility. Knowledge of cellular and molecular biology facilitates both design and assessment of experiments to support biological plausibility. Cellular and molecular biology have moved into the critical path [5] and biological characterization leads to a multiplicity of benefits across all disciplines (Fig. 6).

There has been great success in development of monoclonal antibodies as pharmaceuticals. A proactive science-based approach can facilitate greater manufacturing flexibility and yield benefits in other

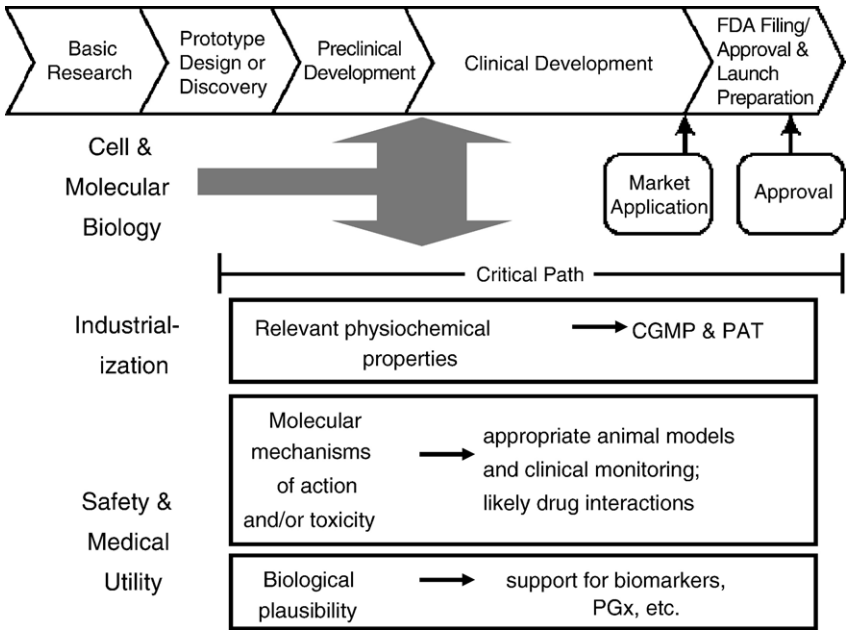

Fig. 6. The role of cellular and molecular biology in the critical path. Cell and molecular biology will move from early to later stages in drug development as indicated by the grey double arrow. The linkage of biological functions to measurable physiochemical properties is critical for new initiatives in product manufacture, such as PAT and risk-based CGMP. The same biological characterization that facilitates industrialization through defining relevant quality attributes can also play a role in the safety and medical utility dimensions of the critical path. Better understanding of product mechanisms leads to better choices of animal models and clinical study design. Better understanding of product biology can be used in support of individual biomarkers and systems biology approaches such as pharmacogenomics (PGx).

disciplines. This can facilitate antibody development and increase the availability of these important therapeutic products. Although this discussion has focused on monoclonal antibody products in particular, many of these principles will also apply to other therapeutic proteins.

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