Developability Assessment During the Selection of Novel Therapeutic Antibodies

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ABSTRACT: Therapeutic antibodies and antibody derivatives comprise the majority of today's biotherapeutics. Routine methods to generate novel antibodies, such as immunization and phage-display, often give rise to several candidates with desired functional properties. On the contrary, resource-intense steps such as the development of a cell line, a manufacturing process, or a formulation, are typically carried out for only one candidate. Therefore, "developability," that is, the likelihood for the successful development of a lead candidate into a stable, manufacturable, safe, and efficacious drug, may be used as an additional selection criterion. Employing a set of small-scale, fast, and predictive tests addressing biochemical and biophysical features, as well as *in vivo* fate can help to identify a clinical candidate molecule with promising properties at an early stage of drug development. This article gives an overview of existing methods for developability testing and shows how these assays can be interlaced in the lead selection process. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Therapeutic proteins are an important class of newly approved drugs in indications as diverse as oncology, inflammation, virology, ophthalmology, and certain rare diseases. Currently, more than 75 therapeutic proteins are approved worldwide, and more than 500 biopharmaceuticals are currently in development.[1,2] Most of these therapeutic proteins are monoclonal antibodies or antibody fragments.[1,2] Nowadays, protein engineering allows the design of biopharmaceuticals such as bispecific antibodies or fusion proteins with activity profiles not accessible with traditional antibodies. In contrast to small molecule drugs, biopharmaceuticals often provide superior target specificity, low toxicity, and long-acting pharmacokinetics.[3,4]

Novel therapeutic antibodies are initially raised by immunization of animals with the respective target molecule or by *in vitro* display approaches. Antibodies raised in animals are often humanized, that is, the nonhuman constant parts and the framework regions in the antibody variable (Fv) region are exchanged for their human counterparts in order to reduce the risk of immunogenicity in humans.[5] Antibody discovery is accompanied by extensive functional profiling to ensure that the selected candidate fulfills all desired functional properties dictated by the respective disease biology. This functional testing is a multistaged approach and may include target-binding assays, cell-based assays, and *in vivo* models.[6–9]

Traditionally, antibodies were chosen predominantly based on their functional characteristics. This led to some therapeutic proteins on the market that need to be supplied as lyophilized products because of stability issues.[10] Lyophilization entails extra effort and extra costs during drug product manufacturing and is not readily compatible with certain dosage forms, such as prefilled syringes.

Modern techniques for antibody generation can create an immense number of different antibodies so that often more than one candidate can be identified, which fulfills the target candidate profile. In such cases, additional selection criteria pointing to aspects of technical development, long-term stability, and DMPK properties may be considered. By addressing such developability aspects early, the success rate and the speed of preclinical and clinical development can be enhanced, because liabilities such as product heterogeneity, stability, and unfavorable *in vivo* behavior are avoided. In cases where potential liabilities cannot be avoided, for example, because of the limited number of available candidates, their identification offers the chance to re-engineer such candidates or to adapt bioprocessing and formulation development accordingly.

Next-generation biotherapeutics include bispecific or multispecific antibodies, antibody-drug conjugates, glycoengineered antibodies, antibodies or antibody domains fused to cytokines, and nonantibody scaffolds.[11–13] These formats tend to have an architecture that is more complex than traditional monoclonal antibodies (e.g., two different antigen-binding domains, several different polypeptide chains to be assembled in the correct stoichiometry, etc.). This more complex architecture might require an even more careful selection of lead candidates regarding their biochemical, biophysical, and *in vivo* properties. Another aspect in drug development posing particularly strong demands on the biophysical properties is high-concentration applications, for example, for subcutaneous delivery or in long-acting devices. Only a subset of antibodies is sufficiently soluble and has sufficiently low aggregation tendency under these conditions.[14–16]

In this commentary, we describe factors considered during developability assessment to facilitate a smooth technical development and ensure favorable *in vivo* properties of

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therapeutic antibodies and next-generation biotherapeutics, and we discuss approaches to include this assessment in the lead generation process. The developability assessment workflow and the tests performed presented here may need to be modified to suit different approaches of drug development taken by other organizations.

DESIRED DEVELOPABILITY FEATURES

From a development standpoint, an "ideal" protein drug candidate can be produced with high yields and high quality using a standard bioprocessing platform. An ideal candidate shows low product heterogeneity, consistent manufacturability, and is stable over a long time (ideally several years) in a liquid formulation. It does not show inacceptable signs of potency loss, chemical degradation, fragmentation, and aggregation. *In vivo*, it does not have atypical pharmacokinetics, for example, because of off-target binding or impaired recycling mediated by the neonatal Fc receptor (FcRn). Moreover, an ideal candidate is chemically stable *in vivo* and exhibits low immunogenicity. In practice, we find that many therapeutic protein lead candidates are not optimal in each of the aspects mentioned above. Therefore, potential weaknesses should be determined early in the lead generation phase so that either an alternative candidate can be chosen or a re-engineering and redesigning step can be included before further activities such as process and formulation development are initiated. The desired molecular features depend to a certain extent on project-specific demands, for example, if high-concentration formulations or long-acting dosage forms are envisioned.

INTEGRATION OF THE DEVELOPABILITY ASSESSMENT IN THE LEAD GENERATION PROCESS

At early stages of technical development, the aim is not for a thorough characterization of biophysical properties and degradation pathways in order to achieve sufficiently fast project timelines. Instead, based on our experience with antibody drug development, we focus on prevalent or severe liabilities with a designated set of *in silico* and *in vitro* methods.

Our process to generate novel therapeutic antibodies typically starts with immunization and/or an *in vitro* display approach, followed by screening of a large number (often 10^3 to 10^4) of candidates for functional properties in high-throughput assays (Fig. 1). In a screening cascade, which typically comprises different kinds of functional assays, antibodies are successively deselected to identify a small number of promising candidates. If these candidates are derived from nonhuman-transgenic animals, the nonhuman sequence parts are replaced by their human counterparts in a process termed humanization. Humanization gives rise to a set of protein variants (typically in the order of 10^2) by a systematic combination of several heavy-chain and light-chain alternatives. In a second round of candidate screening, we search for a single candidate that eventually enters the next steps of development. It is important to note that the development steps after this candidate has been selected are very specific, which means that any change of the protein sequence would necessitate a complete restart of these activities.

According to our experience, not all of the most potent candidates are equally well suited for further development. Therefore, developability assessment is a further dimension of profiling of functionally suitable candidates, which aims at the identification of potential liabilities. During "developability assessment 1," we assess predominantly the CDR regions (Fig. 1; Table 1). As soon as the sequences of the variable domains are available, *in silico* methods are used to assess, for example, potential degradation sites in the CDRs. If no candidate is available that is completely free of predicted hotspots (which is, in our experience, rather the rule than the exception), stressed samples are generated and analyzed (Table 1). This workflow is streamlined to require approximately 2 mg of sample and is aimed to be completed within 3 weeks including 2 weeks of incubation at elevated temperatures. It is crucial to limit the time and material amounts used for this purpose because larger-scale production at this point requires significant efforts and lead selection tends to be on the critical path in many projects. If available, a stable candidate is selected for humanization/engineering; otherwise, humanization/engineering offers a chance to remove potential liabilities from CDRs.

"Developability assessment 2" examines the whole molecule (Fig. 1; Table 1). At this stage, potential PTM or degradation sites and the charge distribution are identified *in silico* in the context of the final (i.e., humanized or re-engineered) molecule. In addition to a stress test analogous to the stress test mentioned above, additional tests are employed that are intended to address what we deem the most common or severe liabilities. Focusing on the most common liabilities and learning from previous projects is believed to enhance—but not to guarantee—the likelihood for future success. In our approach, timelines and sample demands are similar to "developability assessment 1."

DESIRED MOLECULAR FEATURES AND TEST METHODS USED DURING DEVELOPABILITY ASSESSMENT

A multitude of *in silico* and *in vitro* methods have been published to describe and categorize the biochemical and biophysical features of proteins in general and therapeutic antibodies in particular.[18] In this section, we share our perspective of the set of methods that can be used for antibody lead candidate

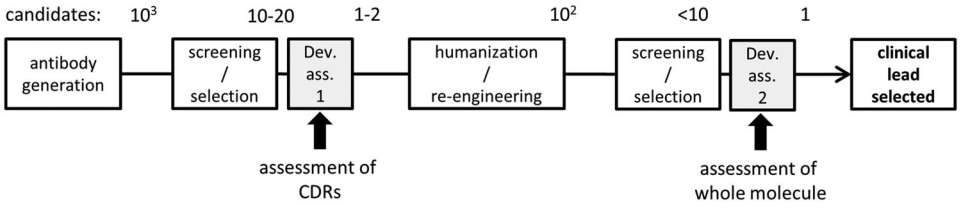

Figure 1. Developability workflow. The various stages of protein drug discovery are shown as boxes with the number of candidates typically tested indicated above. Arrows mark the time points at which developability assessment is performed.

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