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The art of antibody process development

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Biopharmaceutical drug development is an intricate path that spans a dozen years from discovery through registration. The development of a therapeutic antibody presents substantial challenges, particularly with respect to the creation and implementation of manufacturing process technologies. Process development and large scale biotherapeutic manufacturing is an art generally only practiced within industry. As a consequence, these technologies may be seen as something of a 'black box' by many in the medical community. This article provides insight into the current art of antibody process development leading to market entry of novel, life-saving medicines.

Monoclonal antibodies can have exquisite and diverse activities, such as interfering with protein–protein interactions, selectively delivering a payload, or modulating the interplay between specific cells [1–3]. Since they can be readily used to both identify and drug a target, antibodies offer the potential to simplify the transition between discovery and development and are, therefore, especially attractive as first-in-class compounds. Because roughly 3200 of the 5200 pharmacologic targets in the human genome are amenable to protein therapeutic interventions (1700 exclusively so), antibodies have broad utility [4]. Their high specificity translates into a lower likelihood of non-mechanism-based toxicity, which is one reason that they are approximately twice as likely to progress through development (18–29% historical approval success rate) than small molecules and that the cost to bring an antibody to market is manageable [5–7]. All of these factors underwrite the extraordinary current interest in antibodies as medicines.

Still, the development of a therapeutic antibody presents substantial challenges, particularly with respect to the creation and implementation of manufacturing process technologies. While significant effort is required to identify an antibody with the appropriate antigen specificity and create supplies of sufficient quality and quantity to initiate clinical testing, it is a much greater challenge to develop and license a commercial process technology that results in the potentially favorable outcomes that an antibody offers. Process development and large scale biotherapeutic manufacturing is an art generally only practiced within industry. As a consequence, these technologies may be seen as something of a 'black box' by many in the medical community. We provide herein a look at some of the issues relating to the current art of antibody process development and discuss its application to the development of a novel medicine, the immunomodulatory antibody tremelimumab (CP-6 75 206; formerly ticilimumab), which is in clinical development for use in melanoma and a variety of oncology indications.

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The antibody development paradigm

Biopharmaceutical drug development is an intricate process that typically spans about a dozen years from discovery through registration and involves the closely coordinated activities of a large number of individuals in disciplines as diverse as clinical, regulatory, toxicology, manufacturing, and the process development sciences [8,9]. These individuals can all exist within a single company and organization but more often reside in different organizations and in multiple firms. A development program is structured around a series of evaluations of a potential medicine that determine whether it is safe, at which dose it should be used and whether it is sufficiently effective in the therapeutic setting. The overall cost to develop and register a new biotechnology product is currently estimated to exceed $1.2 billion, partly because the majority of compounds that begin clinical development do not progress through to registration [6,8].

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Efficiency in development

The development of a manufacturing process is, of course, a key activity in the creation of a new medicine. In addition to creating the process technology, the technology development team must do so in synchrony with the overall development effort in support of the goal of allowing the overall project timeline to be governed by the clinical development timeline, to the degree possible. Clearly, however, the number of new medicines that can be brought to patients will be a function of how well development resources are utilized in any given project and how they are distributed across a portfolio of programs. An efficient process development organization must optimize the activities of individual project teams through such measures as ensuring that the teams are doing the right activities at the right time, minimizing iterative work and seeking to avoid unnecessary costs and likewise applying capabilities and systems across projects [10,11].

An antibody medicine

Human monoclonal antibodies consist of a complex of four covalently linked amino acid chains, at least two of which usually bear some degree of glycosylation (Figure 1). Subtle differences in structure, as minor as the presence or absence of a single sugar residue on one sequence, can have important effects on antibody function [1]. Antibody manufacturing processes, as with any bioprocess technology that utilizes living cells as the biosynthetic machine, produce not a single fully definable molecular species, but rather a sizable family of closely related structures [5,12]. It is the sum of the activities of this large number of related species that collectively provides the pharmacologic effect. This is a key difference between small molecules and biologics. Because manufacturing process variables can substantially influence the types and numbers of the different molecular species that are created, the central goal of biologics process development is to establish manufacturing technologies that will generate product that has the same composition from one batch to the next and that reflects the materials used in clinical safety and efficacy trials [12].

Robust production technologies

Antibody process development begins after the discovery and engineering of a gene sequence that will provide an antibody with the desired attributes. It encompasses the creation, robustness testing, and transfer of process technologies into manufacturing facilities of various scales, as well as the creation of limited quantities of clinical trial material (Figure 2). In brief, antibody process technologies include (1) mammalian cells that have been genetically modified so that they are able to produce the desired antibody; (2) cell culture procedures that provide an environment in which these cells propagate and produce the correct forms of the antibody; (3) chromatography and (4) formulation steps that purify the antibody and then put it into a dosage form that maintains quality and meets patient needs and (5) analytical testing, which is used to monitor the processes and evaluate the intermediates and final product. A detailed review of the antibody process technologies is outside of the

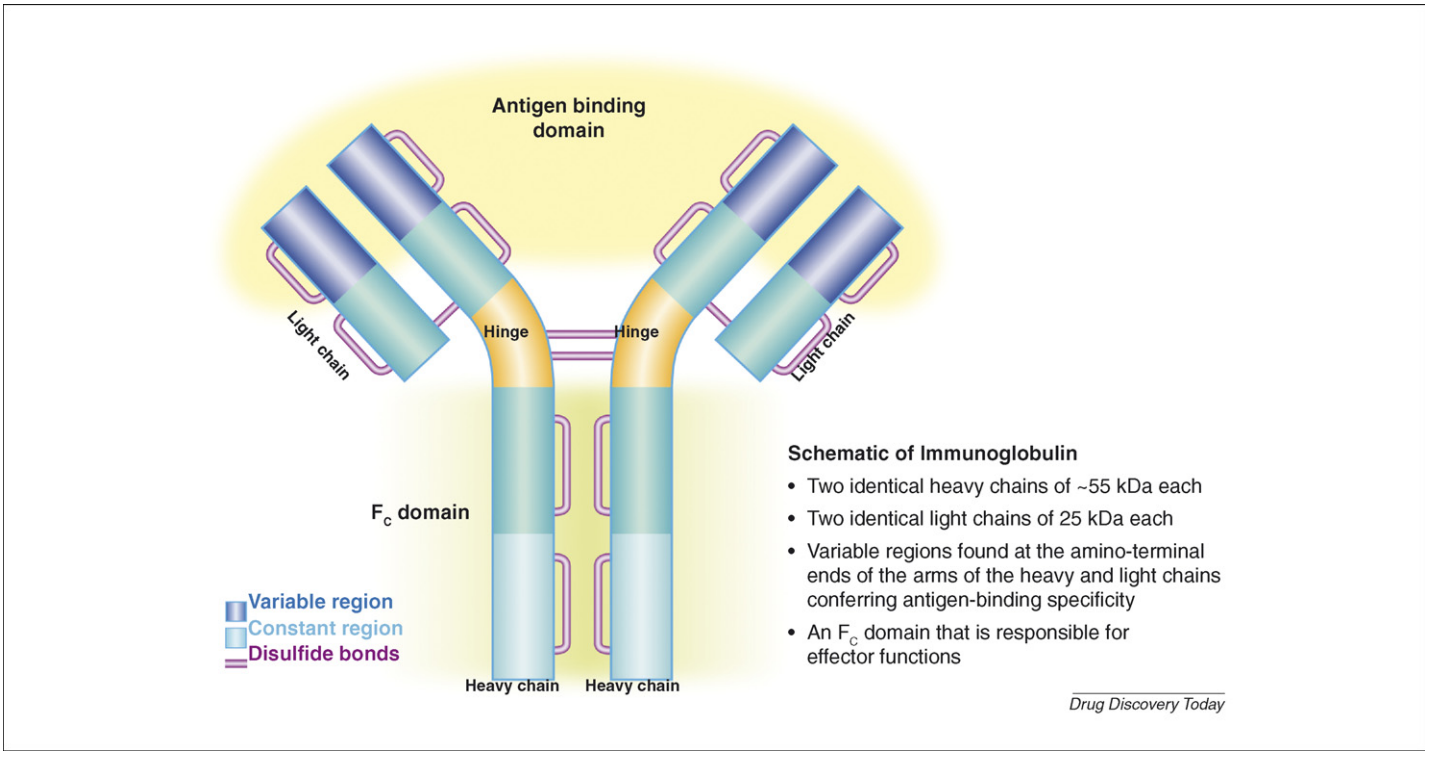

FIGURE 1

Schematic of an antibody. Pfizer's developmental compound tremelimumab is an antibody of subclass IgG2 and is composed of four proteins linked by disulfide bonds. Many antibodies are glycosylated, meaning that polysaccharide structures are covalently attached to the proteins at distinct sites. An antibody can have several domains that have activity, including the variable regions on the arms of the heavy and light chains, which bind to antigens, and the Fc domains, which can mediate effector functions. Adapted with permission from Pfizer, Inc.

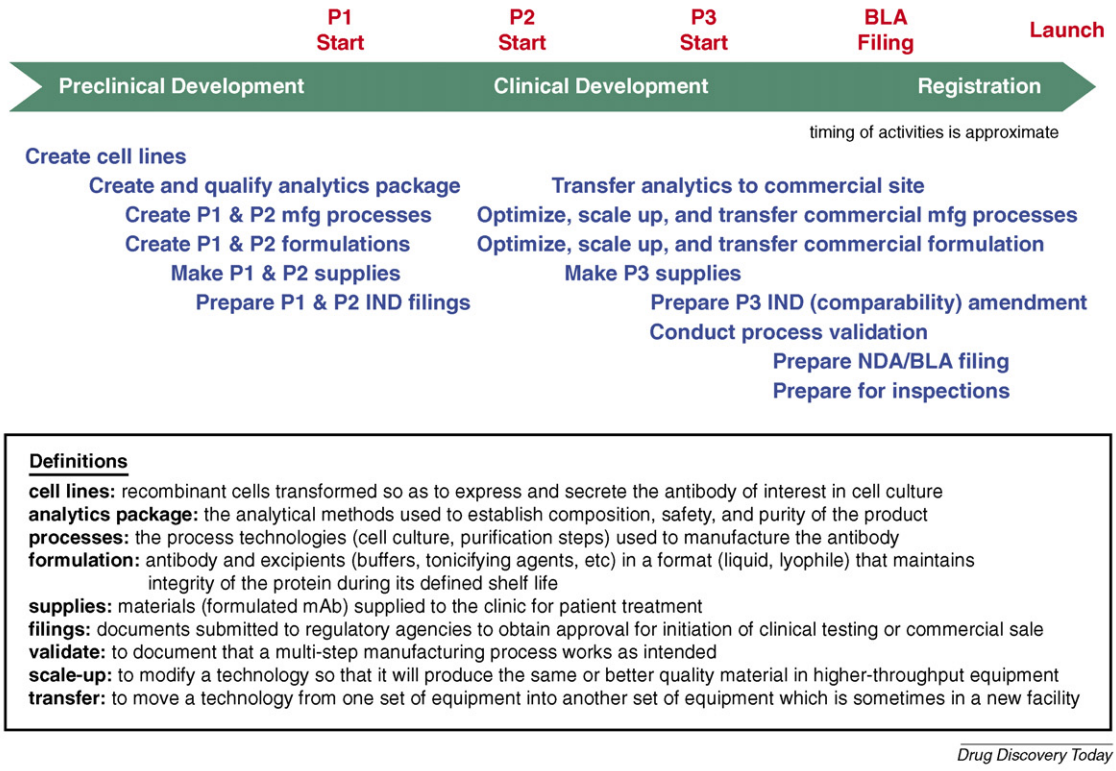

FIGURE 2

The activities of process development and their approximate alignment with the clinical program. Process development activities are very interdependent. For example, each cell clone will exhibit unique behavior during cell culture, and purification processes need to be tailored to both the chemistry of the antibody but also to the milieu of impurities created by the clone growing in a certain set of conditions. As part of development, technologies are transferred into facilities for use in the production of clinical supplies. Significant changes to technologies late in development can trigger a cascade of additional work, costs, and timeline impacts on other parts of the development program. Planning and good communication are central to efficient process development. NDA, New Drug Application; IND, Investigational New Drug; BLA, Biologics License Application.

scope of this commentary, but many excellent recent reviews are available [1,13–19].

While small molecules and non-antibody protein therapeutics typically are unique and require highly individualized process technologies, any given antibody concept will have varying degrees of relatedness to other antibodies, and often a process technology that is appropriate for use with one antibody can be successfully utilized with a closely related antibody. The so-called 'platform' process technologies utilized to produce antibodies include host systems (e.g. Chinese hamster ovary or mouse myeloma-derived cell lines) capable of suspension cell culture, pre-engineered transformation/selection vectors (e.g. glutamine synthetase and dihydrofolate reductase selection systems), standardized high performance purification systems (e.g. protein A affinity chromatography), and certain analytical methods [14,15,20]. In addition, combinations of these technologies have emerged as platform processes [16,21–23] (Figure 3). A key historical challenge in antibody process development has been to relieve manufacturing supply constraints and lower manufacturing costs by endeavoring to rapidly create, productive, stable host–vector systems, an activity that is now aided through the use of highly automated, robotic cell culture and evaluation machinery analogous to the high throughput screening tools used to discover small molecules.

Managing intellectual property issues

The management of intellectual property (IP) is also important in the development of protein therapeutics, particularly antibodies. An innovative drug developer will need access to IP on the drug, the target and on the drug mechanism of action. In the antibody field, however, much of the crucial process technology, including some platform technologies, is new and still covered by third-party IP. If a developer needs to purchase access to antibody discovery tools, as well as process technologies, this accumulation of royalties paid to third parties (the so called 'royalty stack') can be substantial and has the potential to render a drug concept non-viable [24,25]. With an understanding of the costs and benefits of the IP in the antibody process technology space, the process developer can thoughtfully choose which costs to incur and avoid certain costs altogether.

The right facilities

Development of an antibody for clinical applications requires the use and availability of specialized facilities, including laboratories to conduct process development and scale-up studies, pilot facilities to produce gram-scale quantities of preclinical and early clinical supplies, and ultimately commercial manufacturing facilities to produce potentially hundreds of kilograms of product on an annual basis [22] (Figure 4). Laboratory and pilot facilities are,

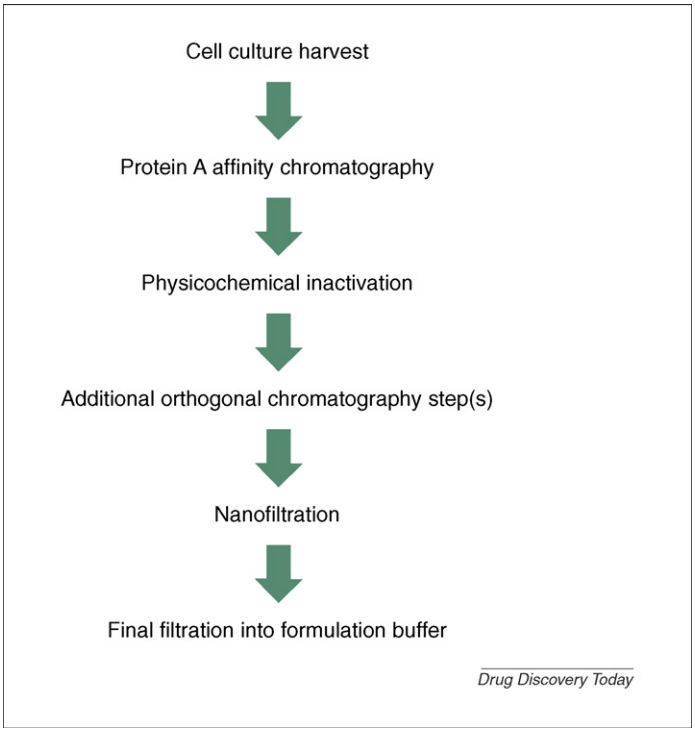

FIGURE 3

A typical commercial manufacturing purification process for a monoclonal antibody (mAb) product. Chromatography is the key technology in mAb purification processes. In terms of scale, a 13 000 L cell culture batch might yield 10–25 kg of mAb in a typical process, which could then be split into several aliquots for further processing in chromatography columns containing a few hundred liters of chromatography resin. The noted 'physico-chemical inactivation' is often a low pH hold step intended to facilitate removal and destruction of impurities. The use of similar or 'platform' technologies across mAb programs simplifies development and manufacturing facility decisions.

by their nature, flexible and able to support a variety of programs. Commercial manufacturing facilities are typically more tailored toward specific processes so as to provide maximum operational efficiency and precision. Since commercial manufacturing facilities require roughly five years to construct and validate and must be operational preferably at the time of the manufacture of BLA/NDA registration materials, commercial facility decisions must be made in the midst of development, well before clinical outcome is known [26]. In addition, new biologics manufacturing facilities can cost hundreds of millions of dollars. Facility investment risks can be mitigated by launching a product from the pre-existing facilities of a contract manufacturer, rather than in-house facilities, but doing so creates additional program complexities [26,27]. The use of platform technologies also simplifies the manufacturing equation by creating the opportunity to more easily run the corresponding processes of different projects in the same facilities [22,27].

Teamwork

A process development team for an antibody project typically includes a project manager, geneticists and cell biologists, biochemists and biochemical engineers, analytical chemists, formulation scientists, regulatory and quality specialists, and manufacturing and supply chain specialists. The composition, structure and size of the team will change as a project moves through the development program, but typically hundreds of individuals, again often within different organizations and firms, will contribute in some way to the process development effort. As with the overall development program, thoughtful planning, abundant communication, the right tools, and stamina are all crucial to the process development effort, the material product of which is to provide

FIGURE 4

Going from one scale to another while maintaining quality. The process development challenge can be appreciated by considering what would be required to produce antibody of the same or better quality as the scale of operation is increased. The 13 000 L bioreactors (commercial manufacturing scale) shown in the picture represent a scale-up factor of 10^5 from the 150 mL flasks used to screen clones. Photo is courtesy of Boehringer Ingelheim Pharma GmbH.

doctors with new, potentially life-changing options to offer their patients.

Case study: development of tremelimumab

Tremelimumab is a fully human monoclonal antibody currently in development for use in melanoma and a variety of oncology indications [28]. Administration of the antibody enhances the anti-tumor immune response [29]. This antibody acts as an immunostimulant by binding to the CTLA4 (CD152) receptor on T cells and antagonizing interaction between CTLA4 and its natural ligand B7, thereby preventing a negative signal that downregulates T cell activation. Tremelimumab was created as an IgG2 to allow it to bind to T lymphocytes with a lower likelihood of effector function-mediated T cell depletion than might be expected with other IgG subtypes, such as IgG1. Likewise, a fully human sequence was chosen to minimize the potential for anti-antibody responses [1,2,13].

Clinical testing of tremelimumab began in 2002. Results of early clinical trials (Figure 5) were encouraging and compelled Pfizer to accelerate the overall program, with the goal of starting pivotal trials within one year of obtaining the proof-of-concept data that would justify these expanded trials. This accelerated timeline created significant challenges for the process development team, including the need to introduce process changes in a conservative, stepwise fashion and produce clinical testing supplies in a series of facilities of increasing capacity.

A central issue during scale-up was, therefore, to manage modifications to the process technology, including changes associated with scale-up and process fitting, such that they could be implemented in a way that provided the necessary comparability of materials produced at all scales and across succeeding versions of the process. Additionally, a key timeline constraint resulted from the need to produce pivotal trial materials at the commercial manufacturing site, again with a process that would be as close as possible to the process planned to be used for commercial supply.

The process technologies that have been used with the tremelimumab program are based on platform-type technologies, such as a robust, standard cell line, conventional antibody purification procedures and orthogonal, template analytical characterization and testing procedures. Key process-specific changes, implemented during the development effort, included the creation of clonal cell banks, the introduction of more practical powdered cell growth media and the optimization and alignment of the analytical testing package to the characteristics and mechanism of the molecule. Additionally, several cell harvest mechanisms were developed and applied as needed during the program to support operations in different facilities. Crucial path activities associated with producing materials for the pivotal trials included transfer of the process and facility fit to the commercial manufacturing site, finalization of the commercial formulation, and qualification or validation of the analytical methods package.

Scales of operation during the development program for tremelimumab increased from the few hundred microliters of the first multi-well cloning plate to the 13 000 L bioreactor scale that will ultimately be used for commercial supply. Clinical supplies production facilities included internal pilot plants and the intermediate and commercial scale facilities of two contract manufacturing organizations (CMOs). The use of the CMO facilities was facilitated by the creation of strong working relationships between organizations, including the sharing of regulatory and process development knowledge and experiences. Partnership with the

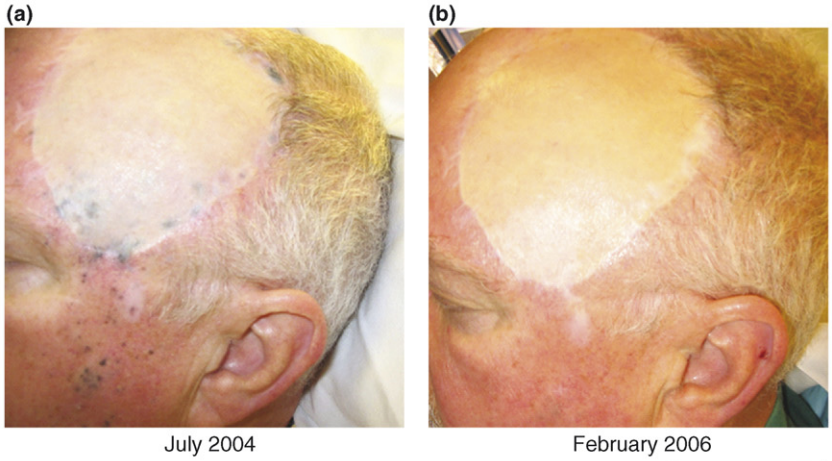

FIGURE 5

The product of innovation: induction of cellular immune response against melanoma by anti-CTLA4 monoclonal antibody. (a) This patient is a 67-year-old male, diagnosed in July of 2003 with melanoma in the forehead. Shortly after a wide excision, satellite lesions appeared on the scalp. In December of 2003, a larger excision was done and a skin graft was implanted. The following month, melanoma lesions again appeared on the scalp. The patient was then treated with chemotherapy (temozolomide), local immunotherapy (imiquimod), and radiation to the scalp, all of which was followed by the appearance of new lesions. (b) In July of 2004, the patient began treatment with tremelimumab, 15 mg/kg intravenous q 3 months; and by October, a brisk $CD8^+$ T cell infiltrate was demonstrated in the sites of scalp metastases, and he achieved a pathological complete response. The patient currently remains without evidence of disease. Photos are courtesy of A. Ribas, University of California, Los Angeles.

commercial scale CMO, Boehringer Ingelheim, was a key factor in enabling the final commercial process to be fully validated at the commercial scale within one year from its initial pilot scale demonstration.

Despite the acceleration of timelines and the need to adapt to multiple production facilities, the manufacturing process technology development effort for tremelimumab has been kept off the crucial path of the overall development program through the use of platform technologies, an intense development effort and strong collaboration with contract manufacturers. The compound is currently in clinical development for use in melanoma and a variety of oncology indications.

Conclusion

Antibodies represent an exciting and attractive means of treating many ailments, and pharmaceutical research and development organizations worldwide have greatly expanded their efforts to bring these and other new types of biotherapeutic molecules to market. The manner in which the manufacturing processes for these molecules are created and implemented will continue to be central to the delivery of the greatest number of effective new medicines to patients as expeditiously and economically as possible.

Competing interest statement

The authors declare competing financial interest. The authors are employed by Pfizer Inc., BBI.

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