Review

Advancing pharmaceutical quality: An overview of science and research in the U.S. FDA's Office of Pharmaceutical Quality

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A R T I C L E   I N F O

Article history:
Received 7 September 2016
Received in revised form 17 October 2016
Accepted 18 October 2016
Available online 20 October 2016

Keywords:
Pharmaceutical quality
Regulatory science
Emerging technology
Quality standards
Policy

A B S T R A C T

Failures surrounding pharmaceutical quality, particularly with respect to product manufacturing issues and facility remediation, account for the majority of drug shortages and product recalls in the United States. Major scientific advancements pressure established regulatory paradigms, especially in the areas of biosimilars, precision medicine, combination products, emerging manufacturing technologies, and the use of real-world data. Pharmaceutical manufacturing is increasingly globalized, prompting the need for more efficient surveillance systems for monitoring product quality. Furthermore, increasing scrutiny and accelerated approval pathways provide a driving force to be even more efficient with limited regulatory resources. To address these regulatory challenges, the Office of Pharmaceutical Quality (OPQ) in the Center for Drug Evaluation and Research (CDER) at the U.S. Food and Drug Administration (FDA) harbors a rigorous science and research program in core areas that support drug quality review, inspection, surveillance, standards, and policy development. Science and research is the foundation of risk-based quality assessment of new drugs, generic drugs, over-the-counter drugs, and biotechnology products including biosimilars. This is an overview of the science and research activities in OPQ that support the mission of ensuring that safe, effective, and high-quality drugs are available to the American public.

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Abbreviations: ADF, abuse deterrent formulation; API, active pharmaceutical ingredient; T-DM1, Ado-Trastuzumab Emtansine; ADC, antibody-drug conjugate; CDER, center for drug evaluation and research; CBRN/EID, chemical, biological, radiological/nuclear and emerging infectious disease; CMC, chemistry, manufacturing, and controls; DMD, Duchenne muscular dystrophy; DLS, dynamic light scattering; FDA, food and drug administration; FTIR, Fourier transform infrared spectroscopy; GA, glatiramer acetate; IVIVC, in vivo/in vitro correlation; MHC, major histocompatibility complex; MVDA, multivariate data analysis; NMR, nuclear magnetic resonance; OBP, office of biotechnology products; OPQ, office of pharmaceutical quality; OTR, office of testing and research; PAT, process analytical technology; QbD, quality by design; SLEP, shelf-life extension program; TNF, tumor necrosis factor; VLP, virus-like particle.

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http://dx.doi.org/10.1016/j.ijpharm.2016.10.038
0378-5173/Published by Elsevier B.V.
1. Introduction

For over a decade, the Center for Drug Evaluation and Research (CDER) in the U.S. Food and Drug Administration (FDA) has followed a vision to modernize pharmaceutical development and manufacturing in order to enhance product quality (FDA, 2004). Over the same time span, there have been increasing challenges with regard to drug shortages and recalls that reflect failures in pharmaceutical quality. Nearly two-thirds of all drug shortages can be attributed to quality failures, particularly due to issues in facility remediation and product manufacturing (FDA, 2013b). In addition, there is increasing scrutiny from the industry and lawmakers, coupled with a greater desire for engagement and dialogue among the regulatory agencies, industry, and patients. At the same time, major technical and scientific advancements have challenged existing regulatory paradigms. Such advancements have impacted biosimilars, precision medicine, combination products, emerging manufacturing technologies, and the use of real-world data.

Pharmaceutical manufacturing is globalizing at an unprecedented pace, making product quality surveillance even more challenging. Furthermore, there is pressure to implement new regulatory mandates (e.g., accelerated approval pathways) with limited resources. To strengthen pharmaceutical quality in the face of present and future challenges, the FDA established the Office of Pharmaceutical Quality (OPQ) within CDER on January 11, 2015 (Yu and Woodcock, 2015). The science and research program within OPQ is built to support the mission and priorities of the FDA related to pharmaceutical quality (FDA, 2013a). Science and research in OPQ forms the foundation for risk-based quality evaluation including review, inspection and surveillance, as well as for quality-related standard and policy development for all drug product areas — new drugs, generic drugs, over-the-counter drugs, and biotechnology products. In this review, OPQ science and research includes: (i) testing and scientific investigation of methods and data that aid drug quality evaluation and (ii) proactive research for development of scientific tools and approaches for evaluating the safety, performance and quality of products.

There is a mandate to better utilize science and research to advance public health by improving access to safe, effective, and high-quality drugs. From the product quality perspective, the FDA needs science and research to keep pace with rapid advances in technology and increasing complexity of FDA-regulated products. For example, since the dawn of OPQ, approvals have been seen for products of new manufacturing paradigms including the first 3D printed product (Norman et al., 2016), the first three biosimilars (FDA, 2016b; Holzmann et al., 2016; von Schaper, 2016), the first new drug product made using continuous manufacturing (Vertex, 2015), and the first switch from a batch process to a continuous process for a previously FDA-approved product (PharmaTech, 2016). OPQ science and research helps the FDA, once perceived as a potential obstacle to innovation, actively promote and support new technology paradigms with the potential to improve overall drug manufacturing quality and reliability (Yu et al., 2016). The information gained supports review and inspection, as well as accelerated development timelines for new drugs and biotechnology products, including biosimilars.

OPQ’s designation as a super-office indicates that multiple offices operate within its purview (Fig. 1). Under this organizational structure, OPQ can integrate the science and research findings into review, inspection, and surveillance across the product lifecycle. The laboratories of OPQ — established in the Office of Biotechnology Products (OBP) and the Office of Testing and Research (OTR) — conduct mission-directed, collaborative laboratory-based science and research activities to support the development of scientific standards and policies for safe and effective quality drug products. OPQ also collaborates with other Centers and stakeholders (e.g., academia) to conduct research to advance pharmaceutical quality as appropriate. These efforts collectively build the capacity for evaluation and monitoring, address mission-critical science matters, and maintain a state of research readiness that anticipates FDA needs while allowing for rapid response to emergent regulatory issues. There are seven key areas in OPQ’s science and research portfolio:

- Manufacturing Science and Innovation.
- Drug Quality Standards.
- Advanced Characterization of Complex Mixtures and Biologics.
- Physicochemical Characterization of Complex Formulations and Dosage Forms.
- Post-Market Product Quality and Public Health Issues.
- Immunogenicity and Immunology.

Fig. 1. Organization of CDER’s Office of Pharmaceutical Quality (OPQ). There is an immediate office and eight sub-offices within the purview of OPQ. The immediate office consists of Program Management Analysis Staff (PMAS), providing administrative services, and Science and Research Staff (SRS), who help coordinate scientific activities within OPQ. Governance of scientific and research work in OPQ is the mission of OPQ’s Research Review Coordinating Committee which supports, promotes, priorities and funds OPQ laboratory research that will have an impact on review and policy decisions. Committee membership is drawn from OPQ’s immediate office and 8 sub-offices.
2.1. Manufacturing and controls for small molecule drugs

Batch manufacturing is the predominant method for the production of small-molecule drugs (i.e., drug substances and finished dosage forms). However, there is often insufficient systematic understanding of batch processes, particularly with respect to manufacturing scale up and especially for more complicated unit operations. To address these problems, the FDA’s research effort in manufacturing science is focused on improving the understanding of how process parameters affect drug product critical quality attributes across scales for various batch processes.

Recognizing the limitations of current batch manufacturing processes (e.g., difficult scale up, long total processing times due to storage and shipping of intermediates, and large manufacturing facilities needed for large-volume product production), the FDA places an emphasis on advancing new pharmaceutical manufacturing technologies. Advances in manufacturing science have the potential to reinvigorate the pharmaceutical manufacturing sector in the U.S. by stimulating adoption of emerging technologies that are innovative in the pharmaceutical domain and expected to improve product quality (NSTC, 2016). For example, 3D printing technology enables previously inaccessible dosage forms (e.g., rapidly dissolving oral dosage forms) and has the potential to advance precision medicine (e.g., by enabling patient-specific drug manufacturing). Continuous manufacturing can result in better efficiency and improved product quality, benefiting both the industry and patients (O’Connor et al., 2016).

The FDA has projects — several in partnership with external collaborators – that focus on emerging technologies including process analytical technology (PAT), advanced process controls, real-time release testing, and process simulation and modeling for continuous manufacturing (FDA, 2016a).

Integrated process models for continuous manufacturing can support a quantitative initial risk assessment through sensitivity analysis by examining the relative magnitude of the impact of variations in process parameters and/or material attributes on quality attributes. Process models can also be tools to assess the

![Graph](image-url)

**Fig. 2.** Modeling robustness of continuous direct compression with respect to the disturbance in the feeding of active pharmaceutical ingredient (API) for two different control strategy approaches. The amount of time for which the blender/tablet API concentration exceeded limits is plotted in relation to the magnitude and the duration of pulse disturbance. The incorporation of ratio feed-rate controls is able to increase the robustness of the system.
effectiveness of a proposed control strategy through the use of case studies, such as examining the ability to mitigate the impact of disturbances (e.g., feeder refills) (Escotet-Espinosa et al., 2015). Therefore, these modeling approaches can aid and streamline regulatory review and inspection activities. For example, the process modeling of continuous direct tablet compression was used to identify: (i) types of disturbances that can have a significant impact on product quality and (ii) the effectiveness of an active control system to minimize these disturbances for a continuous process (Fig. 2). Furthermore, process models may be directly incorporated into the control strategy for a continuous manufacturing process (e.g., models that track non-conforming material to downstream diversion points). Systematic risk assessments and failure mode analysis from process modeling simulations can inform feedback and recommendations to manufacturers during the development of continuous manufacturing processes. This research ensures that advances in small-molecule drug manufacturing will be understood by regulators and can be used to support advanced technology in the industry.

2.2. Manufacturing and controls for biotechnology products

Biotechnology products are large, complex molecules typically produced in living cells. For these products there is a general desire to advance manufacturing, both upstream and downstream, to reduce shortages and variability, allow for cost savings and production flexibility, and simplify scale up procedures (Konstantinov and Cooney, 2015). To this end, the pharmaceutical industry is transitioning toward continuous processes in biomanufacturing to improve product quality, reduce facility footprints, increase productivity, and reduce production costs (Walther et al., 2015). Continuous processes decrease bioreactor residence time and eliminate intermediate hold steps while minimizing manual operations and human decision making. These advancements may reduce the risk of modification or degradation of product, and potentially decrease impurity levels by allowing drug production at high cell densities.

FDA research in biomanufacturing includes work to improve process analytical technology (PAT) for the production of biotechnology products. Research to develop PAT for bioreactor optimization evaluated a Fourier transform infrared spectroscopy (FTIR) method for measuring key metabolites (Wu et al., 2015) and a dielectric spectroscopy-based method for measuring viable cell density (Lee et al., 2015a). These methods were determined to be suitable for monitoring cell culture process dynamics in real time. Another recent project used a quality by design (Qbd) approach to assess specific parameters (e.g., temperature and nonessential amino acid supplementation) that significantly influence glycoform profiles of a monoclonal antibody (Agarabi et al., 2015). Multivariate data analysis (MVDA) of this process was used to correlate product quality attributes (e.g., specific glycan abundance) with process variables, particularly levels of amino acid supplements in the cell culture media (Rathore et al., 2015) (Fig. 3).

In addition to work on upstream processes, downstream processes are also a focus of FDA research, especially with respect to the critical area of viral safety. FDA efforts in the area of viral safety include understanding failure modes for viral filters (LaCasse et al., 2016), evaluating new chromatographic methods for viral clearance (Iskra et al., 2015), evaluating viral clearance as a function of resin age, and introducing concepts to assure viral safety for continuous processes (Johnson et al., 2016). FDA efforts on downstream processes also include work on downstream processes such as lyophilization (Awotwe-Otoo et al., 2015) and optimizing formulation buffers for stability (Chavez et al., 2016). Biomanufacturing research in the FDA supports chemistry, manufacturing, and controls (CMC) decisions on biotechnology products, and aids in the formulation of guidance and policy development.

FDA research includes investigations into types of biomolecules other than recombinant proteins. For example, FDA scientists develop chemical methods for the synthesis, purification, and delivery of synthetic nucleic acids. Amphiphatic phosphorothioate DNA elements were recently demonstrated by FDA researchers to transport uncharged nucleic acid sequences into mammalian cells (Jain et al., 2015). This work may enable the development and manufacture of therapeutic uncharged and negatively charged nucleic acid-based drugs and streamline the FDA review process for these products.

![Fig. 3. Quality by design (Qbd) optimization of bioreactor manufacturing for monoclonal antibodies. A Plackett-Burman screening design was applied to laboratory-scale parallel cultures to study the effects of 11 process variables on critical quality attributes of a monoclonal antibody. Engineering changes related to culture temperature and nonessential amino acid supplementation significantly impacted glycan profiles associated with fucosylation, galactosylation, and sialylation. Figure adapted from Agarabi et al., 2015.](image-url)
3. Drug quality standards

Efficient drug evaluation and lifecycle management require clear standards (i.e., tests or other measures) to ensure product identity, strength, purity, quality, and potency. As such, the development and evaluation of science-based, clinically-relevant standards is a focal point of the FDA product quality assessment. FDA science and research on drug quality standards aims to inform industry, standard setting organizations including compendia, and generates recommendations that facilitate the regulatory quality assessment and product approval.

With respect to drug quality standards, of particular importance is work focuses on providing standards for abuse deterrent formulations (ADF) of opioid products. Such products are important for improving the quality of life for patients who need them, but they can be misused and abused, potentially leading to addiction (Meyer et al., 2014). The issue of drug abuse becomes more prominent when the dosage forms can be easily manipulated to release or extract a high amount of opioid. One approach to deter opioid abuse is by providing scientific standards for ADF properties that make abuse of the product more difficult. For example, as the highest mortality rates for opioid abuse are associated with parenteral and nasal routes, this may include increasing the difficulty of crushing a tablet in order to snort the contents or dissolving a capsule in order to inject the contents (Katz et al., 2011). With limited history and a lack of compendial standards, evaluation of ADF opioid products can be challenging. An FDA laboratory recently developed a risk-based, standardized in vitro approach for testing abuse deterrent features for all ADF products, including the solubility in common solvents and assessment of particle size after manipulation which may influence the rate of opioid extraction from a product or the potential for abuse by insufflation (Xu et al., 2016b) (Fig. 4). This and other work on the impact of formulation and process variables on ADF (Rahman et al., 2016) enhances the understanding of ADF and supports guidance development and the quality review of ADF products (FDA, 2016c).

Knowledge surrounding the quality of well-established drugs across the lifecycle is evolving. For example, a new product failure mode was identified for warfarin sodium. A significant FDA research effort has focused on understanding and detecting this undesirable phase transformation of the crystalline solid to a glue-like substance at high humidity (Korang-Yeboah et al., 2015; Nguyenpho et al., 2015; Rahman et al., 2015b; Siddiqui et al., 2015). It is important to understand this phase transformation to properly evaluate the stability of warfarin sodium drug products during use and handling, especially to ensure the consistent therapeutic efficacy a drug with such a narrow therapeutic index. Research into this failure mode resulted in post-marketing corrections of the approved products, impacted regulatory decisions for pending warfarin sodium applications, and led to a better quality control of this drug product (Rahman et al., 2015a).

FDAs researchers are working to provide clinically-relevant specifications for complex dosage forms and formulations, including products containing ophthalmic ointments and transdermal drug delivery systems (i.e., patches). An important scientific challenge for complex products is correlating in vitro drug release of a formulation to the in vivo drug profile. Such an in vivo/in vitro correlation (IVIVC) can be used to ensure product quality and performance during product development and when facing scale-up and post-approval changes. An FDA laboratory recently developed an IVIVC for estradiol transdermal drug delivery systems using in vitro skin permeation studies for marketed products (Yang et al., 2015) (Fig. 5). This is the first IVIVC model for transdermal systems using marketed estradiol as a model drug product. The IVIVC may prove useful as a performance predictor for ensuring product quality, as it passed both internal and external validations. An FDA laboratory also developed an in vitro release testing method suitable for ointment formulations using acyclovir as a model drug (Xu et al., 2015). This model included a novel transient-boundary layer which challenges the well-established Higuchi model for kinetic release from oily ointment bases. This work provides a needed and improved understanding of product quality and performance for locally acting complex topical drug products, including ophthalmic ointments.

4. Advanced characterization of complex mixtures and biologics

The regulation of drug substances consisting of complex heterogeneous mixtures (Lee et al., 2015b) or biomolecules (Ghasrani et al., 2016) poses a pressing regulatory challenge. In addition, there is a need to keep pace with advancements in analytical technology in the face of accelerated clinical development and approval timelines, such as the breakthrough therapy designation (Sherman et al., 2013). Drug substance characterization is required for all product areas, including new drugs, generic drugs, new biotechnology products, and biosimilars. Equivalence and similarity comparisons by analytical means constitute a significant basis of the evaluation of manufacturing and formulation changes, generic complex drug substances, and biosimilars. In addition, analytics can identify chemical markers that are sensitive to changes in raw materials and manufacturing process.

![Fig. 4. A risk-based in vitro performance assessment of extended release abuse deterrent formulations. A model compound, sotalol, that resembles the physicochemical properties of certain opioids, was manipulated (e.g., splitting, crushing, grinding, etc.) to determine particle size and size distribution. For example, (A) one sotalol test formulation could easily be manipulated into smaller particles; over 20% were below 500 μm. (B) Another sotalol test formulation showed relatively high resistance to physical tampering and was very difficult to break into sizes smaller than 3 mm. Figure adapted from (Xu et al., 2016b).](image-url)
parameters, which allow for improved development of quality controls. Impurities are a significant concern for all product areas, but particularly for generic complex mixture drug substances. Sensitive characterization can provide information on the identity and quantity of individual impurities. Therefore, it is necessary to develop and use an orthogonal set of advanced analytical methods for characterization of complex drug substances and biomolecules, and for linking these attributes to safety, quality, and clinical performance.

In this context, FDA science and research supports equivalence or similarity evaluation by identifying highly specific product attributes and aiming to achieve “fingerprint-like” characterizations of heterogeneous mixtures. Recent FDA research has shown how so-called “modern or advanced analytics” can be used to address challenges in characterizing:

- **Biosimilars** (e.g., filgrastim (Ghasrani et al., 2016) or monoclonal antibodies (Ferguson and Gucinski-Ruth, 2016; Schiel et al., 2015)).
- **Complex mixtures**, either naturally derived, (e.g., protamine sulfate (Gucinski et al., 2015)) or synthetic (e.g., glatiramer acetate (Rogstad et al., 2015)), and

- **Modifications to therapeutic proteins that are post-translational** (e.g., glycosylation (Zhang et al., 2016)) or occur during manufacturing/storage (e.g., oxidation (Kryndushkin and Rao, 2016; Uehara and Rao, 2015)).

For example, the drug substance glatiramer acetate is a mixture of synthetic copolymers consisting of four amino acids marketed for the treatment of multiple sclerosis that is highly heterogeneous (Jalllian et al., 2012). The FDA developed an independent, sensitive analytical method (i.e., LC–MS with digestion) coupled with principal component analysis to characterize the sameness of glatiramer acetate products (Rogstad et al., 2015). The result of this analysis, coupled with orthogonal techniques such as nuclear magnetic resonance (NMR) or multivariate light scattering, together with information from the application helped inform the regulatory decision to approve the first generic glatiramer acetate product in 2015 (Anderson et al., 2015) (Fig. 6).

The FDA was also part of an international collaborative effort to determine the precision and robustness of 2D-NMR for the structural assessment of filgrastim biosimilars (Ghasrani et al., 2016). This was the first reported laboratory study of a high-resolution 2D-NMR method to assess higher-order structure of a biotherapeutic. In this study, the U.S. FDA-approved originator product and three international filgrastim products were compared using six spectrometers in four different laboratories. Results showed that minimal measurement drift was observed over the nine-month period of the study. The resolution of the 2D-NMR method was shown to allow visualization of the degree of similarity between filgrastim products (Fig. 7). In addition, traceable reference values were established which could allow for comparisons to the NMR signature of a comparator product from a validated database. Recently, a filgrastim product became the first U.S. biosimilar approval, followed by an infliximab and an etanercept product in 2016 (FDA, 2016b; Holzmann et al., 2016; von Schaper, 2016). Further, the presence of trace amounts of copper and the presence of ascorbate in protein formulations was found to increase levels of carbonylation, a type of oxidative modification, in therapeutic proteins and, in some instances, lead to aggregation and loss of function (Kryndushkin and Rao, 2016). This research has informed risk assessment strategies on the impact of trace metals, certain excipients, and storage conditions on the stability, purity, and potency of protein therapeutics.

5. Physicochemical characterization of complex formulations and dosage forms

Complex dosage forms and formulations, such as transdermals, ointments, emulsions, and products containing nanomaterials, are developed to achieve specific functionalities including enhanced bioavailability, reduced side effects, and/or improved drug targeting and drug concentration at the site of action. Physicochemical characterization of these products is particularly useful to support the evaluation of equivalence of generic products to their innovator counterparts. For example, for generic drug products with a complex formulation, the goal is to use physicochemical characterization to capture key molecular features that result from unique formulation and process designs to ensure delivery of the same clinical performance as the innovator product (Chang et al., 2013). Such sameness determinations can be particularly important because they may eliminate the need for a
6. Post-market product quality and public health issues

As the FDA is committed to assuring quality throughout the drug product lifecycle, assessing or monitoring post-market product quality is a key aspect of oversight. FDA laboratory science supports the surveillance of product quality over product lifecycle, especially when potential safety signals (e.g., patient complaints or abnormal occurrence of adverse events) arise for specific products. FDA laboratories play an important role in investigating the root cause of such signals and determining whether they are related to product quality issues. The result of such investigations helps inform appropriate regulatory actions. In addition to post-market product quality which impacts public health, the FDA’s diverse knowledge and expertise are positioned to help address broad public health issues when needed, including providing assistance in the development and evaluation of medical countermeasures. This includes work to minimize domestic vulnerability to chemical, biological, radiological/nuclear and emerging infectious disease (CBRN/EID) threats.

An example of FDA laboratory contributions to the above areas is the maintenance of a national stockpile of oseltamivir phosphate, a critical medical countermeasure for a national influenza pandemic (Patel and Gorman, 2009). The Shelf-Life Extension Program (SLEP), which extends the useful shelf life of federal stockpiles of critical drugs through periodic testing (Khan et al., 2016), saves the federal government large sums of money by reducing stockpile replacement costs (Courtney et al., 2009). In addition to maintaining the stockpile, it is important to eliminate counterfeit products that may contain unapproved antibiotics and analgesics rather than the FDA-approved drug substance (FDA, 2010). The FDA recently developed means to predict qualitative and quantitative critical quality attributes of oseltamivir phosphate using principal component analysis and a Raman spectroscopy-based method that could be deployed in the field (Lothen and Rodriguez, 2015) (Fig. 9). This field-deployable method enables enhanced capability to monitor and assess product quality more rapidly than laboratory-based tests, which require days to transport samples and many hours to perform the analyses (e.g., assay, dissolution, and impurities).

When needed, there is capacity in the FDA to help protect the public by minimizing domestic vulnerability to emerging
infectious disease (EID) threats. Emerging public health threats of infectious disease include Ebola virus and Zika virus (Russek-Cohen et al., 2016). The recent Ebola virus outbreak has emphasized the need to better understand filovirus pathogenesis and the necessity for rapid development and evaluation of preventive and therapeutic agents. The limited number of certified biosafety level 4 (BSL-4) facilities to conduct experiments with filoviruses prompted the FDA to develop replication incompetent virus-like particles (VLPs) with filovirus surface glycoproteins (unpublished data). Since VLPs cannot replicate, they allow scientists to study filovirus entry into cells without the risk of productive infection. VLP studies will be helpful in supporting regulatory submissions for therapeutic proteins against filoviruses, including monoclonal antibodies. In anticipation of the need for assessing therapeutics for Zika virus infections, the FDA has developed a novel mouse model that allows for testing of potential drug candidates (unpublished data). Unlike existing Zika infection models, the new mouse system allows for exploration of long-term effects of the virus on sites where the virus is known to persist in infected patients, such as the central nervous system, the eye, and the reproductive system. 

Protecting the public from infectious disease and bioterrorism threats is accomplished in part by developing medical countermeasures to these threats. In the case of anthrax, monoclonal antibodies have been shown to increase survival in animal models and have been approved for use in patients with inhalational anthrax (Fox, 2013). Nevertheless, questions surround the pathogenic effects of anthrax toxin, meaningful biomarkers are needed to assess potential therapeutics, and gastrointestinal anthrax remains unaddressed by therapeutics. Moreover, the FDA recently demonstrated that passive immunotherapy with an anthrax toxin neutralizing antibody also protects against enteric invasion in a mouse model of gastrointestinal anthrax (Huang et al., 2015) (Fig. 10). This suggests a new medical countermeasure and a potential new role for existing products licensed for treatment of inhalation anthrax in the treatment of gastrointestinal anthrax.

7. Immunogenicity and immunology

A pressing issue with regard to safety and efficacy in relation to pharmaceutical quality is characterizing and predicting potential interactions between components or attributes of drug products and the human immune system (Catalfamo et al., 2015). Proper targeting to and/or avoidance of certain elements of the innate and adaptive immune system is often key to meeting clinical expectations. Within the FDA, immunology research is carried out to better understand the relationship between pharmaceuticals and specific components of the immune system in order to aid the FDA’s immunogenicity risk assessment of products. This can be achieved by, for example, deciphering the mechanisms of adverse immune events (Rosenberg et al., 2012). Beyond general mechanisms, human immune responses are a complicated field of study as they are a highly personalized result of an individual’s genetic polymorphism and previous exposure to antigens, including both vacaval and pathogens. It is important to understand product attributes that positively or negatively influence immune response by both the innate and adaptive immune systems. Such information is critical for evaluating product quality attributes, particularly for protein therapeutics (e.g., structure, aggregation, posttranslational modifications such as glycosylation, and impurities such as host cell proteins).

Mechanisms that underlie immune responses are an important focus of immunology research in the FDA, including receptors and regulators such as FcγRI (Swisher and Feldman, 2015), the SRC kinase family (Boekhoudt et al., 2015), receptor chain IL-13Rα1 (Sheikh et al., 2015), and Fc receptor-like 5 (Damdinsuren et al., 2016). To this end, an FDA laboratory recently showed the first elucidation of the expression and function of the receptor CD300c on natural killer cells (Dimitrova et al., 2016; Li et al., 2016). Furthermore, peptide loading of major histocompatibility complex class I (MHC-I) molecules is a key to antigen presentation in the immune system and to immune-mediated adverse drug reactions. FDA research recently showed the molecular workings of peptide loading of MHC-I by the TAP binding protein (Morozov et al., 2016). The knowledge gained from this work could have implications for understanding the molecular basis of MHC-linked drug hypersensitivity.

FDA immunology research also aims to develop techniques to predict immunogenicity and modulate immune response. For example, an FDA laboratory developed a cell-based in vitro approach, using immune cell lines (human and mouse) to detect receptor-specific agonists, to evaluate impurities that impact immunogenicity risk (Haile et al., 2015) (Fig. 11). Such an approach may allow for an immunogenicity comparison between products by, for example, comparing cellular gene expression fingerprints. This may help in the evaluation of trace impurities with immunogenicity risks, an issue particularly important for generic peptides, naturally-derived products, and biosimilars. FDA research in immunology has highlighted immunological safety issues that need to be addressed during product development and informed the risk assessment for certain products, such as low molecular weight heparin (FDA, 2016d).

Research in immunology also allows the FDA to address potential medical interventions and serious public health issues related to the immune system. With respect to medical interventions, there is a desire to modulate pathological antibody response, for example in patients with adverse responses to
therapeutic proteins or autoimmune diseases. FDA scientists hypothesized that targeting long-lived plasma cells may result in immune tolerization in several clinical scenarios (Rosenberg et al., 2016). This concept was supported by recent studies of immune tolerance to enzyme replacement therapies (Kazi et al., 2016). With respect to human disease, Duchenne muscular dystrophy (DMD) is a degenerative muscle disorder in boys that eventually leads to paralysis and death. Advancing treatments for patients with DMD is a public health priority because there are currently no treatment options (FDA, 2015b). Research on mechanisms of inflammation have aided in the understanding of the immune-mediated pathology of DMD and clarified opportunities for clinical intervention (Rosenberg et al., 2015). In this context, FDA laboratories contributed by collaborating with internal and external stakeholders to facilitate the development and validation of robust methodologies and assessment criteria for reviewing biomarker data obtained from these methods. Method validation expertise developed through FDA laboratory and regulatory experiences have been applied to facilitate biomarker and drug development in DMD and other clinical conditions.

8. Linking biomarkers and drug attributes to safety and efficacy

The FDA emphasizes the importance of linking both biomarkers and drug quality attributes to the clinical performance, safety, and efficacy of a drug product. Biomarkers are characteristics measured as an indicator of normal biological processes, pathogenic processes, or responses to exposures or interventions (Robb et al., 2016). Biomarkers can provide insights into, among other things, whether a particular drug will be safe or effective for a particular patient. Precision medicine is an approach to disease prevention and treatment that takes into account differences in people’s genes, environments, and lifestyles. As precision medicine continues to develop, it will become critical to better understand biomarkers, therapeutic targets, and pathology at the individual patient level.
A large and rapidly growing number of FDA-regulated products have an oncological indication (Beaver et al., 2015; Casak et al., 2015; de Claro et al., 2015; Khizin et al., 2015; Kim et al., 2015; Miller et al., 2015; Nair et al., 2015; Przepiorka et al., 2015). The use of monoclonal antibodies has proven to be particularly useful in the treatment of cancers, as these drugs can provide a mechanism for targeting treatment on cancerous lesions while sparing normal cells. The approval of trastuzumab, a monoclonal antibody directed against the biomarker HER2, along with a diagnostic kit to detect breast cancers positive for HER2, advanced a new era of precision medicine (Collins and Varmus, 2015). It is anticipated that incorporation of biomarkers into clinical care will facilitate smarter, personalized treatment of individual cancer patients (Blumenthal et al., 2016). With the establishment of the National Cancer Moonshot Initiative, there is an opportunity to change the paradigms of preventing, diagnosing, and treating cancer (Lowy and Collins, 2016). With the dawn of this era come new opportunities to identify cancer biomarkers to ensure that drug products are deployed appropriately. Recent research in the FDA used RNA sequencing to identify a distinct RNA profile of metastatic colorectal carcinoma by comparing colorectal tumor tissue with adjacent healthy tissue from the same patient (Xu et al., 2016a). This biomarker could potentially assist in determining the risk of colorectal carcinoma metastasis. Another FDA study found that a protein expressed in primary breast tumors, RhoGDI, was significantly decreased during tumor progression from benign to malignant and metastatic lesions (Bozza et al., 2015). In addition to identifying a potential cancer biomarker, this study may also suggest that RhoGTPase inhibition could be explored as a therapy for advanced breast cancer. This type of research into cancer biomarkers supports the evaluation and approval of oncological drug products with cancer indications.

FDA researchers also investigate drug quality attributes and their effect on safety and efficacy. For example, the clinical administration of monoclonal antibodies often involves dilution in a 5% dextrose-saline solution for intravenous administration. FDA research recently identified the cause of monoclonal antibody aggregation when antibody-dextrose solution mixtures with human plasma. In this case, pH-dependent precipitation of plasma proteins, including complement proteins, resulted in insoluble aggregates containing antibody (Luo and Zhang, 2015) (Fig. 12). As the aggregation of monoclonal antibodies in human plasma may adversely impact product safety and efficacy (Rosenberg, 2006), this finding improves the ability to assess monoclonal antibody product development regarding compatibility with diluent and human plasma. FDA laboratories also seek to better understand quality attributes relevant to clinical performance, and mechanism of action, to improve the FDA’s risk-based approach for evaluating biotechnology products. For example, trastuzumab is a monoclonal antibody indicated for HER2-positive breast and metastatic gastric cancer (Mohan and Wu, 2015). However, trastuzumab has also shown unintended impact to the heart (cardiotoxicity). The FDA investigated the mechanisms underlying trastuzumab-induced cardiotoxicity and revealed that it is due to HER2 expressed by cardiac muscle cells (cardiomycocytes) (Seidman et al., 2002). Binding of the antibody to HER2 expressed on the cell surface dysregulates autophagy in these cells (Mohan et al., 2016). Similarly, FDA scientists have studied unintended liver injury (hepatotoxicity) induced by ado-trastuzumab emtansine (T-DM1), a trastuzumab antibody-drug conjugate (ADC). Hepatotoxicity is a serious adverse event associated with T-DM1 therapy (Dieras et al., 2014). Recent FDA research demonstrates that T-DM1 is internalized in hepatocytes following binding of HER2, leading to damage to the microtubule network, nuclear fragmentation, and

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**Fig. 11.** Detecting complex impurities in therapeutic protein products using human monocytic cell line Macrophage-like-MonoMac6 (MM6) cells stimulated with the indicated concentration of endotoxin, a synthetic triacylated lipopeptide (Pam3CSK4), and a synthetic lipoprotein (FSL-1). Levels of IL-8 mRNA (relative expression vs media) was measured in triplicate. Data points represent mean ± SD. (*p < 0.05 vs media control). Red points show the limit of detection for each individual impurity. Figure adapted from (Haile et al., 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 12.** Micro-flow imaging analysis to examine insoluble aggregates in the mixture of bevacizumab, 5% dextrose, and human plasma. (A) Representative images of particles (>5 μm) formed in the mixture. (B) Particle counts in the sizes of 1–70 μm were determined for the resulting mixtures before and after centrifugation at 21,000 g for 3 min. Shown are representatives of 3 independent experiments. Figure adapted from (Luo and Zhang, 2015).
production of tumor necrosis factor (TNF-α) [Yan et al., 2016]. Both of these studies unveil mechanisms that explain significant risks associated with the use of trastuzumab therapies and inform the regulatory review of these types of products. These findings may also present insight useful for the development of monoclonal antibody products with fewer associated side effects.

9. Summary

Industry trends are expanding the regulatory challenges pertaining to quality, manufacturing, and drug product development. Manufacturing technologies are becoming more complex (e.g., 3D printing, continuous manufacturing). Clinically-meaningful specifications are increasingly needed to support diverse dosage forms, including abuse deterrent formulations of opioid products, ointments, and emulsions. Analytical methods are advancing and being used for the characterization of complex mixtures, biologics, and complex formulations. The immunogenicity risk for naturally-derived products, protein therapeutics, and biosimilars needs to be better understood so both the regulatory agency and industry can utilize the newly generated knowledge to better manage or mitigate potential safety risks. Science-based informatics systems are needed to analyze “big” datasets to predict manufacturing outcomes, assess equivalence/sameness, and identify sources of risk. Bioanalysts need to be identified and qualified to advance the era of precision medicine. Meanwhile, clinical designs are evolving and development timelines are accelerating.

The pharmaceutical industry is globalizing at an unprecedented pace. The promising era of biosimilars, precision medicine (National Precision Medicine Initiative), the microbiome (National Microbiome Initiative), and the eradication of cancer (National Cancer Moonshot Initiative) has just begun. The resulting scientific and regulatory challenges must be met with science- and risk-based decisions. In addressing these challenges, OPQ has built its science and research program to: (i) maintain and establish collaborations with external stakeholders, (ii) prepare to understand and evaluate new technologies, (iii) respond quickly to drug-related emergencies or public health issues (e.g., Ebola and Zika viruses), and (iv) provide drug product quality surveillance testing and science-based investigational activities. It is also critical to train and expose the next generation of regulatory scientists to pharmaceutical quality through external collaborations. OPQ science and research is the foundation that underlies the FDA’s ability to meet mounting regulatory challenges and supports the mission of ensuring that safe, effective, high-quality drugs are available to the American public.

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