CRISPR, animals, and FDA oversight: Building a path to success

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Technological advances, such as genome editing and specifically CRISPR, offer exciting promise for the creation of products that address public health concerns, such as disease transmission and a sustainable food supply and enable production of human therapeutics, such as organs and tissues for xenotransplantation or recombinant human proteins to treat disease. The Food and Drug Administration recognizes the need for such innovative solutions and plays a key role in bringing safe and effective animal biotechnology products to the marketplace. In this article, we (the Food and Drug Administration/Center for Veterinary Medicine) describe the current state of the science, including advances in technology as well as scientific limitations and considerations for how researchers and commercial developers working to create intentional genomic alterations in animals can work within these limitations. We also describe our risk-based approach and how it strikes a balance between our regulatory responsibilities and the need to get innovative products to market efficiently. We continue to seek input from our stakeholders and hope to use this feedback to improve the transparency, predictability, and efficiency of our process. We think that working together, using appropriate science- and risk-based oversight, is the foundation to a successful path forward.

CRISPR | animals | FDA | CVM | genome editing

Last December 2019, scientists gathered at the National Academy of Sciences colloqium, "Life 2.0: The Promise and Challenge of a CRISPR Path to a Sustainable Planet," to present research and discuss breakthrough genome-editing technology with the potential to have wide-ranging societal benefits. The presentations and discussions addressed the great promise of genome editing, and CRISPR in particular, to provide solutions to myriad challenges.

The Food and Drug Administration's (FDA's) Center for Veterinary Medicine (CVM) has long recognized and supports the potential of genome editing in animals to deliver transformative solutions to challenges in both human and veterinary medicine, agriculture, and more. For example, researchers are using genome editing to create alterations that target diseases with a significant impact on agricultural production, such as African swine fever or bovine respiratory disease (1–3), that prevent serious human diseases such as dengue or malaria (4, 5), and that enable production of safer xenotransplantation products for humans with less potential for immune rejection (6–9). Genomic alterations also offer unique solutions to agricultural production challenges, such as the development of animals with increased food yield (10, 11) or animals with improved tolerance to certain environmental conditions, such as warmer weather conditions (12).

While we (the FDA/CVM) are enthusiastic about the possibilities, we also recognize that new technological advancements, like genome editing, and the products they help create need to meet the standards for safety and effectiveness. The research community and the industry that are using advanced techniques like CRISPR to develop intentional genomic alterations (IGAs) in animals share the enthusiasm for using existing and newer biotechnology techniques to produce innovative products. They are eager to move forward with the fewest hurdles possible to provide breakthrough solutions. This sense of excitement and urgency can lead to a view that regulation hampers progress and that regulators are unjustifiably cautious and risk-averse. We believe products created using new technologies should strike a careful balance between fostering innovation while ensuring the products work and are safe.

State of the Science

Among the reasons that regulation is necessary is the fast-changing state of the science. Science is rapidly evolving and technological capabilities now exist that we never imagined in the past. Genome-editing technology, such as CRISPR/Cas9, has revolutionized the ability to make targeted changes to an animal's genome. However, as with any new technology, there are limitations and, despite its use for over a decade, there are still a lot of unknowns. What is known is that error can occur, such as off-target alterations due to mistargeting by the guide RNA (gRNA) (13–15). Furthermore, genome editing at the target site is not always precise due to different modes of DNA repair machinery, including nonhomologous end-joining resulting in variable length indels. Indels can promote internal ribosomal entry, produce aberrant proteins, or induce exon skipping by disruption of the alternative splicing mechanism (16–18). Unintended on-target effects, such as large-scale deletions and complex genomic rearrangements, as well as unintended integration of donor plasmid and multiple template insertions, have also been observed in different cell types, including human embryos (19–29). These errors could result in loss of heterozygosity, genome instability, or the unintended activation/inactivation of genes. There has been tremendous work in the field to mitigate these unintended outcomes by optimizing experimental design [e.g., improved gRNAs (30–35), limiting Cas nuclease exposure (36–38)], engineering new genome-editing systems with improved nuclease fidelity, or developing alternatives to DNA cleavage-induced editing (39–41). Furthermore, researchers are

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also enabling better detection of these events through the development of novel, unbiased detection methods (42, 43).

Researchers have used several Cas9 proteins and variants with improved on-target specificity, such as SaCas9 (44, 45), CjCas9 (46), eSpCas9 (47), HFCas9 (48), HypaCas9 (49), and Sniper Cas9 (50). They have also generated a variety of CRISPR systems, such as Cas12a (Cpf1) (51), Cas13 (52), CAST (CRISPR-associated transposase) (53), or Cascade (54) to overcome the limitations of the CRISPR/Cas9 system. And they recently have used prime-editing (55) or base-editing systems (56, 57) to make single nucleotide changes without generating double-stranded DNA breaks. Researchers are also developing new variants of base editors with reduced off-target activities (58–62); recent studies suggest that some base editors result in off-target RNA, in addition to DNA, editing (63, 64), and off-target alterations in a gRNA-dependent but also in a random gRNA-independent manner (64, 65).

Various off-target detection methods are in development, and each has its own strengths and weaknesses (43). Researchers have developed several bioinformatics-based tools (66–73) to rapidly and efficiently predict potential off-target sites, which can be subsequently validated via methods such as PCR or sequencing. The major limitation associated with such targeted methods is that the criteria for selecting for off-targets can introduce bias and some errors may get overlooked. Unbiased biochemical and cell-based methods, such as GUIDE-seq (74), HTGTS (75), BLESS (76), IDLV (77), CIRCLE-seq (78), SITE-seq (79), Digenome-seq (80), DISCOVER-seq (81), and CHANGE-seq (82), have been developed to assess off-target alterations at a genome-wide level. The limitations of these methods include complex protocols, applicability across cell types, and low sensitivity, among others. Whole-genome sequencing is also an effective unbiased method to detect alterations, including structural variants; however, it requires suitable controls (e.g., a high-quality reference genome or unedited controls for comparison) and when sequencing depth is low, the detection of low frequency (in bulk cell populations) and large or complex alterations can be challenging (64, 83–85).

For the detection of unintended on-target effects, a comprehensive on-target analysis, using methods such as long-range PCR or long-read sequencing, could identify large-scale unintended on-target effects that may go undetected using conventional methods, such as short-range PCR. Additional methods are also under development, such as UDiTas (86), to measure large deletions, inversions, and translocations.

Moving Beyond the Bench to a Commercial Setting

The specificity of the CRISPR/Cas system and other genome-editing tools will continuously improve over time with the rapidly growing body of knowledge in the field. In addition to the discovery of higher-fidelity nucleases, improvements include development of measurements and standards (e.g., standardized off-target detection methods, controls, and so forth), such as the effort led by the National Institute of Standards and Technology Genome Editing Consortium*, which are important for the characterization of potential commercial IGA products developed using genome editing. As such improvements are still under development, commercial developers ("developers") can employ careful experimental design (e.g., selection of a high-fidelity nuclease, inclusion of proper controls, adequate characterization methods, and so forth) to mitigate any potential unintended effects.

In contrast to genome editing in somatic cells, IGAs made in the germline of animals will be passed on to their offspring along with any unintended on- and off-target alterations, whether beneficial or detrimental. While it is important for developers to identify unintended effects and be alert to their potential risks, the presence of unintended effects in a genome-edited animal does not mean that the IGA is necessarily unsafe or should not enter the market.

Both phenotypic and genotypic characterization of IGAs and their potential unintended effects are important because genetic changes can impact safety. Asking certain questions can help to determine whether any unintended alteration results in such an impact. For example, where is it located? Is it in a region with known biological importance or function that could have an impact on animal safety? Are there any identified biological or physiological impacts on animal health (e.g., differences in morbidity/mortality, disease prevalence/susceptibility, routine health observations, and so forth, between animals with the alteration and comparator animals)? Developers rely on phenotypic characterization not only to ensure that they have successfully produced the intended trait for commercialization, but also to evaluate any unintentional effects of the IGA and the impact of any unintended alterations. For example, some CRISPR/Cas9-mediated myostatin-deficient animals exhibit the intended double-muscling phenotype but also exhibit unintended effects, such as stillbirth, early-stage death, spinal deformity, and abnormal fat, sugar, and protein metabolism (87–89). While it might seem that detrimental changes to the genome would result in a product that is not viable for the commercial market or would obviously impact animal health and well-being, changes that impact safety are not always so evident. For example, genetic changes in animals at certain receptors may increase susceptibility to other viruses, e.g., genetic alteration in the chemokine receptor CCR5 intended to yield protection against HIV infection may increase the risk of developing West Nile virus infection (90, 91). Tumor suppressor genes may be affected by CRISPR/Cas, as shown with p53, increasing the potential risk for cancer (92, 93). There also are other factors to consider aside from animal safety concerns, such as whether the IGA or any unintended alterations impact gene/protein expression, resulting in a change in the composition or nutritional content of food from the animals (if the animals will be used as food). For example, IGAs and any unintended alterations that result in changes to the composition of milk could impact the health of humans, and in particular infants. Identification of potential hazards is facilitated by the combination of both genotypic and phenotypic analyses, offering focus for the characterization of the resulting type expressed by the genetic alteration in the animal and its potential contribution to animal or human risk.

Science-Based, Risk-Appropriate Regulation

We are committed to pursuing an approach that strikes a careful balance between sufficient oversight to ensure products are safe and achieve their intended effect and minimizing regulatory burden. The goals of this approach are to protect consumers, enable developers to bring innovative products to market, and expend the FDA's limited resources prudently. In practice, what a risk-based approach means is that where the risk posed by the potential hazard(s) resulting from an IGA is low, we do not expect developers of those products to submit and obtain FDA approval of an application in order to market their product. Our assessment of the potential risk profile of a product is not based simply on the technology used (e.g., genome editing or recombinant DNA technology) or the size or type of the intended genomic alteration (e.g., single base pair changes versus large deletions; insertions versus deletions) but rather also on factors, such as the intended use, characteristics of the IGA, preexisting knowledge regarding safe use, and animal containment level. Furthermore, the FDA does not regulate based on whether a product is a genetically modified organism (GMO).

*83 Fed. Reg. 1335, 1335-36 (January 11, 2018).

Agricultural products containing foreign DNA and called GMO are ubiquitous[†] and all of those we are aware of on the market are safe. People and animals have been consuming them for decades. Since these foods were introduced in the 1990s, research (94) has shown that they are just as safe as non-GMO foods. Moreover, altering animals with techniques that result in incorporation of "foreign" DNA in the animal's genome, while admitting some additional risk, allows for a greater range of alterations that can produce the most innovative and beneficial qualities, such as disease resistance.

Regardless of the technology used to create the IGA, we evaluate based on risk. Where the risk is lowest, we do not expect developers to come to the FDA prior to marketing either with an approval application or with any risk data or even a notification. These types of IGAs include those in highly contained laboratory animals intended for research, such as mice and rats. The FDA believes that these IGAs are either already adequately regulated or that they pose negligible risk because the animals containing them are not likely to end up in the food supply or to get out into the environment. For those products that do not fit into this lowest risk category, the FDA/CVM may not expect developers to submit an application for approval in order to market their product if we review product risk data and conclude that the product is, in fact, low risk. Such products currently include IGAs in animals of food-producing species intended for use as models of human disease and could potentially include IGAs in animals that are for use as food.

For those products that may pose greater risks and that we, therefore, expect to go through the FDA approval process, the FDA/CVM is committed to streamlining that process so that it functions efficiently and transparently, thereby enabling developers to have a roadmap of the FDA/CVM's data and information submission expectations. Clarity about the process will help IGA sponsors to make product development plans well in advance and to prepare high-quality FDA/CVM submissions that allow the approval process to operate most efficiently without the delays resulting from incomplete submissions.

Among the steps we have taken to support this goal is establishment of the Veterinary Innovation Program (VIP; https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/vip-veterinary-innovation-program). The VIP is available for developers of certain innovative products, including most IGAs in animals. It offers benefits, including a dedicated review team, the ability to stop and restart the review clock, pre- and post-review feedback, hands-on assistance, and senior management involvement. The goal of the VIP is to facilitate innovative animal product advancements by providing greater certainty in the regulatory process and supporting an efficient and predictable pathway to approval. We understand that for first time sponsors, who are often small start-up companies or academically based research initiatives, the approval process can be difficult to understand and plan for; the VIP is intended to help them through the process. As of the date of writing, the FDA/CVM has 25 products enrolled in the program and this number is continuing to grow.

[†]For example, in 2018, 94% of the soybeans and 92% of the corn planted in the United States were genetically engineered (https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx).

FDA/CVM's Steps to Building a Path to Success

We intend to publish updated guidance for industry in keeping with our goal of greater transparency. We plan to make a number of changes in response to the feedback we have already received and continue to receive from academic researchers, small biotech companies, and other stakeholders, many of whom are currently developing IGAs in animals. In the meantime, we are continuing to engage with product developers early and often about their IGA products and how they can prepare for the approval process so they can successfully achieve product approval in the least amount of time possible.

One way we are engaging with stakeholders is through a series of outreach meetings. While we had hoped to hold in-person meetings in the spring/summer of this year, due to the COVID-19 pandemic we are planning to hold meetings later, on-line. Several stakeholders suggested that they would find case-study examples helpful to understanding how the FDA's regulatory program works in practice and the data the FDA would expect for hypothetical but representative products, so we have prepared several case studies of hypothetical products that we walk through in a webinar (https://www.fda.gov/animal-veterinary/animals-intentional-genomic-alterations/fda-cvm-animal-biotechnology-webinar-developers). We plan to collect feedback from the webinar and, depending on interest, follow the webinar with a series of small meetings to address the received feedback. We will also use the feedback we receive to revise the approach described in the case studies, as well as to inform our approach for existing and future guidance documents.

While we are planning to make changes that respond to comments and make the regulatory process more efficient, significantly, the statutory standards for approval and for environmental review will remain the same. IGAs in animals must be safe for the animal, safe for consumption (where relevant), and effective (i.e., the IGA does what the developer claims it will do), and the FDA/CVM must assess whether potential environmental impacts of an approval are significant, in accordance with the National Environmental Policy Act. However, the type and amount of data required to meet these standards may vary based on a product's risks. For example, if an IGA is in an animal of a food-producing species but will not be marketed for food use, the data expectations relating to food safety will be less than for an IGA in an animal that will be consumed.

Conclusions

The FDA/CVM, those developing IGAs in animals, and consumers each may have a different perspective. Ultimately, we believe that we share the same end goal: The availability of innovative products that can improve human and animal health, animal well-being, and food production and quality. Appropriate, science-based, and risk-based regulation is key to realizing this goal and building a path to success. We are eager to hear from those developing these products about the innovative solutions they are creating to address the challenges we face.

Data Availability. There are no data underlying this work.

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