Date of Decision: September 25, 2025

RISK ASSESSMENT SUMMARY

Supplemental Assessment VMF 006-378

SLICK alteration disrupting *Bos taurus* g.(NC_037347.1) fs(39099129-39099368) in exon 9 of the *PRLR* gene in *Bos taurus*

The intentional genomic alteration in the prolactin receptor gene is intended to cause prolactin receptor protein truncation at or between amino acids 433 to 497 in *Bos taurus* cattle resulting in a short, slick haircoat. A short, slick haircoat is reported to be linked to increased thermotolerance.

Developed by:

Recombinetics, Inc.

Executive Summary

Recombinetics, Inc. (via its subsidiary, Acceligen, Inc.) developed an intentional genomic alteration (IGA) in cattle using genome editing. The IGA is a targeted genomic alteration in the prolactin receptor (*PRLR*) gene that truncates the protein, resulting in a short, "slick" haircoat that has been linked to increased heat tolerance. The IGA is equivalent to genomic sequences that exist in conventional cattle with a history of safe use in animal agriculture food production. Cattle with the IGA are known as PRLR-SLICK cattle. Because the IGA conferring the slick haircoat trait is heritable, it can be passed on to offspring, allowing the trait to be propagated through breeding into a line of PRLR-SLICK cattle.

Molecular Characterization

FDA reviewed sequencing data and screening methods to confirm the IGA and screen for potential off-target genomic alterations in founder animals with the IGA. This IGA is created using CRISPR-Cas9 genome editing and is equivalent to naturally occurring variants found in certain cattle breeds adapted to tropical climates. The developer selected two genomic sites for ongoing screening for unintended alterations, and FDA concluded that the screening processes are adequate to ensure that the IGA is equivalent to natural variants found in conventional cattle.

Phenotypic Characterization / Animal Health

FDA reviewed data and information and determined PRLR-SLICK cattle demonstrate growth, health, and development comparable to conventional cattle and have the intended slick haircoat phenotype (a short, smooth coat) seen in naturally occurring slick-haired cattle breeds. FDA determined that the animal safety risks of the IGA beyond those typically seen in conventional cattle are low when introduced in additional breeds of cattle.

Human Food Safety

FDA did not identify any human food safety concerns associated with the IGA in PRLR-SLICK cattle. Furthermore, there is a history of safe human consumption of food products derived from conventionally raised cattle with slick phenotypes. FDA determined that food products (meat and milk) derived from PRLR-SLICK cattle are as safe as those derived from conventionally raised cattle with the slick haircoat that are commonly consumed by the public.

Environmental Risk

FDA determined that PRLR-SLICK cattle pose low environmental risk. Because the slick trait exists naturally in some cattle breeds, it does not introduce new characteristics that could harm the environment. Cattle are considered low risk for escaping and establishing wild populations, as they are typically recovered quickly if they escape and there are few feral bovine populations in the United States. A slick haircoat is easily identified visually, and standard cattle management practices including ear tagging provide adequate identification. No special containment, disposal, or handling procedures beyond normal farm practices are required.

Conclusions

Based on the data and information reviewed, FDA concluded that PRLR-SLICK cattle are equivalent to conventional cattle with naturally occurring slick variants and risks from generating future founder animals are appropriately mitigated. The agency does not object to the marketing of these cattle or their products (e.g., offspring, semen, embryos, meat, milk).

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A. File Number

VMF 006-378

B. Developer

Recombinetics, Inc.

C. Established Name

SLICK alteration disrupting *Bos taurus* g.(NC_037347.1) fs(39099129-39099368) in exon 9 of the *PRLR* gene in *Bos taurus*

D. Product Category

IGA in animals

E. Species

Bos taurus cattle

F. Claim

The intentional genomic alteration in the prolactin receptor gene is intended to cause prolactin receptor protein truncation at or between amino acids 433 to 497 in *Bos taurus* cattle resulting in a short, slick haircoat. A short, slick haircoat is reported to be linked to increased thermotolerance.

G. Effect of Supplementary Assessment

To expand the scope of FDA's March 7, 2022, determination (described in the original Risk Assessment Summary for VMF 006-378) such that the developer may introduce the IGA into *Bos taurus* cattle of any breed or genetic background.

II. INTRODUCTION

The intentional genomic alteration (IGA) in the prolactin receptor gene (called the *PRLR* gene) truncates (or shortens) the prolactin receptor protein (called the PRLR protein) in *Bos taurus* cattle and results in a short, slick haircoat. The cattle with the IGA are referred to as PRLR-SLICK cattle. Because the IGA conferring the slick haircoat trait is heritable, it can be passed on to offspring, allowing the trait to be propagated through breeding into a line of PRLR-SLICK cattle.

FDA previously conducted a risk review for two PRLR-SLICK cattle and their progeny. On March 7, 2022, FDA determined that the IGA in the two PRLR-SLICK cattle posed a low risk to humans, animals and the environment and FDA did not object to the developer marketing products derived from these two cattle and their progeny (e.g., live animals, semen, embryos, meat). For more information, see the March 7, 2022 Risk Assessment Summary.

In this supplementary assessment, FDA assessed the potential hazards and likelihood of harm associated with the IGA in PRLR-SLICK cattle when expanded to use in other breeds of cattle. The effect of this supplement is to allow for marketing of PRLR-SLICK *Bos taurus* cattle and products containing the IGA derived from these cattle, including meat and milk. This supplement expands upon the March 7, 2022 decision to include generation of additional founder animals and their progeny. FDA evaluated information regarding the methodology used to generate the IGA, characterization of the genomic sequence, and information addressing animal safety, food safety, and risk of impacts on the environment. FDA evaluated data including molecular characterization and animal safety information for animals generated by either introducing genome editing reagents into *in vitro*-fertilized embryos (embryo injection) or introducing genome editing reagents into cells in culture to be used for subsequent nuclear transfer (cloning). FDA evaluated data and information to determine if the developer has adequate procedures in place to characterize and screen animals with the IGA to ensure that they are equivalent to animals that contain naturally occurring slick mutations that have a history of safe use in animal agriculture food production, including the production of both milk and meat products.

III. MOLECULAR CHARACTERIZATION

The IGA is a targeted disruption of the prolactin receptor gene (*PRLR*) that truncates the expressed prolactin receptor protein (PRLR). The developer introduces the IGA in animals by CRISPR-Cas9 genome editing in either *in vitro*-fertilized embryos (embryo injection) or in cultured cells to be used for subsequent nuclear transfer (cloning). Briefly, for embryo-injected animals, zygotes are microinjected with CRISPR-Cas9 reagents to generate edited embryos. For cloned animals, bovine embryonic stem cell (bESC) lines are electroporated with CRISPR-Cas9 reagents. The subsequent edited cells are expanded and pooled for somatic cell nuclear transfer (SCNT) to generate edited embryos. For both methods, the edited embryos are then transferred to recipient cows. CRISPR-Cas9 reagents consist of Cas9 protein complexed with a single guide RNA (gRNA) to create a ribonucleoprotein (RNP) complex that is delivered to the cell either with a homology-directed repair (HDR) template (cell lines for cloning) or without an HDR template (embryo injection).

FDA evaluated genomic data from representative PRLR-SLICK cattle containing the IGA to determine if (1) the proposed product specifications for the IGA contained in PRLR-SLICK cattle ensure that it is equivalent to naturally occurring mutations that exist in conventional cattle and (2) the screening methods used to characterize the animals are sufficient to identify and mitigate risks associated with introduction of the IGA. FDA considered multiple types of sequencing data used to characterize the IGA (target site) and to screen for potential unintended alterations at off-target sites in founder animals that were generated by both editing methods. FDA also considered the information previously provided to support the March 7, 2022 regulatory determination for PRLR-SLICK cattle.

FDA reviewed the screening methods and acceptance criteria to determine if they are adequate to evaluate the IGA target site. PRLR-SLICK cattle generated from cloning will be homozygous for the IGA, which will be assessed by Sanger sequencing. PRLR-SLICK cattle generated from embryo injection may be mosaic for the IGA at the target site (more than two alleles that meet the specification for the IGA), which will be assessed by targeted amplicon next generation sequencing (Amp-seq). FDA evaluated representative raw Sanger and Amp-seq sequencing data. FDA determined that the methods and acceptance criteria to identify alterations at the target site are adequate.

FDA reviewed the methods used to identify potential off-target sites where unintended alterations may arise as a result of genome editing. FDA determined that potential off-target sites were sufficiently identified by the combination of orthogonal methods of (1) screening *in silico* predicted sites (Cas-OFFinder) with whole genome sequencing (WGS) data and (2) a saturation cell-based GUIDE-seq assay. While both methods had limitations (low coverage of control sample data in WGS; lack of replicates in GUIDE-seq), FDA determined that potential off-target sites were sufficiently identified based on the totality of the data, rather than either assay alone, given that all sites identified by the WGS method were also identified in potential off-target sites from GUIDE-seq. FDA evaluated the potential off-target sites to determine which sites occur within or near coding sequences, which could potentially alter expressed protein sequences. FDA determined that only two of the potential off-target sites are in or near known or predicted coding sequences and these two sites were therefore selected for screening in founder animals.

FDA reviewed the screening methods and acceptance criteria to determine if they are adequate to identify unintended alterations at the two potential off-target sites in or near coding sequences. As with target site characterization, founder animals generated by cloning will be assessed by Sanger sequencing and founder animals generated by embryo injection will be assessed by Amp-seq. FDA evaluated representative raw Sanger and Amp-seq sequencing data. FDA determined that the methods and acceptance criteria are adequate to identify and mitigate risks resulting from unintended alterations at the two off-target sites.

Conclusions: FDA determined that the totality of evidence demonstrates that the sequencing-based screening methods are sufficient to ensure that founder animals containing the IGA will be equivalent to those containing naturally occurring slick mutations. FDA concluded that the proposed product specifications are adequate to identify and mitigate risks, and there are no additional safety concerns related to molecular characterization.

IV. PHENOTYPIC CHARACTERIZATION / ANIMAL HEALTH

A. Animal Health

In this supplemental assessment, FDA evaluated information on the health and growth of four Holstein founder animals with the IGA produced via cloning and five Jersey and six Angus founder animals with the IGA produced via embryo injection. See the March 7, 2022 Risk Assessment Summary for information on phenotypic characterization and animal health of additional Angus cattle. The health and growth of animals with the IGA was consistent with that of conventional animals of the same breed.

Veterinary and herdsman health information was evaluated for the four Holstein founder animals. One Holstein bull calf with the IGA was observed until two years and 10 months of age and another Holstein bull calf with the IGA was observed until one year and five months of age. The growth and health parameters for these Holstein founder animals were commensurate with non-IGA comparators.

A third Holstein bull calf with the IGA died at birth following induced parturition due to hydrops complications. Hydrops is a condition that occurs at an increased rate in pregnancies with fetuses derived from nuclear transfer ("cloning'). Therefore, this is more likely a result of the cloning utilized in the production of this animal with the IGA than the IGA itself, but this cannot be definitively determined based on the available information. The fourth Holstein bull calf was observed until 10.5 months of age when it was euthanized following health complications due to athymia. The athymia was attributable to epigenetic errors from the cloning process rather than the IGA itself.

Veterinary health information was evaluated for the five Jersey founder animals. Two Jersey bull calves were evaluated to three months of age, and two Jersey heifers to 3.5 years of age. The fifth Jersey heifer calf was evaluated until 47 days of age during which time no significant health or growth abnormalities were observed. At 47 days of age this calf died during disbudding. The growth and health parameters for the Jersey founder animals were commensurate with non-IGA comparators.

Three of the five Jersey calves with the IGA underwent a disbudding procedure at the same site on the same day. During disbudding one calf died, and two others experienced nonterminal complications. The calf that died appeared restless and stressed while restrained for disbudding and collapsed and died upon release from the chute. The two other calves appeared stressed and exhibited tremors while restrained but recovered. A necropsy of the calf that died identified no significant abnormalities except those most likely associated with attempted resuscitation efforts and death. The cause of these adverse events could not be identified. No reports of similar events were identified in cattle with the naturally occurring slick phenotype, and two Holstein calves with the IGA were disbudded with a similar method at a different facility without complication.

Veterinary health information was evaluated to 3.5 years of age for 6 Angus cattle, including 4 cows and 2 bulls. One Angus heifer calf with the IGA experienced an umbilical remnant infection that responded to surgical management. The growth and health parameters for these Angus founder animals were commensurate with non-IGA comparators.

Animal health is evaluated on an on-going basis through veterinary review of health records, production history, and visual inspection. Animals are also monitored for adverse events. Both adverse events and animal health data are reported to FDA.

Conclusions: Based on the phenotypic data, animal health information, history of no known safety risks to conventionally raised cattle with the slick phenotype, and the assessments in the previous review (as further discussed in the Risk Assessment Summary for the March 7, 2022 PRLR-SLICK decision), FDA concluded that the IGA and introduction of the IGA into additional breeds of cattle for animal agriculture and food production present a low risk to animal safety. Any potential risks to animal health are further mitigated through on-going safety reporting and FDA evaluation of adverse events in order to confirm that the IGA continues to present a low risk to animal safety.

B. Intended Phenotype

The IGA is intended to produce a slick hair phenotype which is reported to be associated with improved thermotolerance in *Bos taurus* cattle (as further discussed in the Risk Assessment Summary for the March 7, 2022 PRLR-SLICK decision). The slick hair phenotype can be confirmed visually, and animals with the IGA had the expected phenotype marked by a short, slick coat, as is seen in conventionally bred animals with naturally occurring slick mutations. Because this trait is autosomal dominant, heterozygous animals will exhibit the intended phenotype but, when bred to each other, may produce offspring that do not carry the trait.

FDA assessed phenotypic characterization data for two or more founder animals from each of three different breeds, including Angus and Jersey founder animals produced via embryo injection and Holstein founder animals produced via cloning (Holstein). The developer screens animal phenotype by visual observation to confirm that animals with the IGA exhibit the slick phenotype.

Conclusions: FDA determined that the developer has adequately characterized the phenotype of PRLR-SLICK cattle produced by the described embryo injection and cloning-based methods. Based on the phenotypic data and the literature previously reviewed in the 2022 Risk Assessment (described in the Risk Assessment Summary for the March 7, 2022 PRLR-SLICK decision), FDA concluded that the founder animals produced by the developer in additional breeds exhibit the expected phenotype and that it is reasonable to expect that the cattle of any *Bos taurus* breed with the IGA will also display the same intended slick phenotype and improvement in thermotolerance as seen in conventionally bred animals with naturally occurring slick mutations.

V. HUMAN FOOD SAFETY

The slick trait in PRLR-SLICK cattle is equivalent to naturally occurring mutations found in several breeds of conventionally raised cattle, particularly those adapted to tropical or subtropical environments. FDA evaluated the human food safety aspects of PRLR-SLICK cattle and concluded that there is a reasonable certainty of no harm for human consumers of food products derived from these animals.

FDA's assessment focused on identifying any potential food safety hazards associated with human consumption of edible tissues from PRLR-SLICK cattle. The evaluation

considered the molecular characterization of the IGA, phenotypic characterization of the animals and their health status, and potential impacts on the edible tissues.

Molecular characterization of the IGA confirmed presence of the IGA at the targeted location in the *PRLR* gene. Importantly, the analysis did not reveal any potential human food safety concerns. The truncated PRLR protein encoded by the altered gene is unlikely to pose a food safety concern, as similar mutations occur naturally in conventionally raised cattle that are safely consumed by humans. DNA sequencing analysis did not identify any changes to the genome expected to alter protein expression or function beyond the intended PRLR truncation.

The phenotypic characterization and animal health records of PRLR-SLICK cattle showed no indication of effects that might raise food safety concerns. The animals' overall health and growth were comparable to conventionally raised cattle without the IGA. This similarity in health status further supports the safety of food products derived from PRLR-SLICK cattle.

FDA's assessment also concluded that the edible tissues of PRLR-SLICK cattle are comparable to conventionally raised cattle with naturally occurring slick mutations. The naturally occurring slick-coated cattle have a history of safe use in animal agriculture and food production, particularly in tropical regions. The genetic modification in PRLR-SLICK cattle mimics these natural mutations, resulting in the same phenotype.

FDA did not identify any effects resulting from the IGA that may change the compositional or nutritional content of meat or milk derived from the PRLR-SLICK cattle. Comparison of milk composition between crossbred Holstein cows with the slick phenotype to wild-type cattle showed no significant differences in the percentage of fat, protein, or lactose.[1] Crossbreeding to introduce the slick trait into dairy cattle has been practiced in various countries, including the United States. The resulting animals have been used in commercial dairy production, providing a history of safe use for milk from cattle with the slick phenotype.

Conclusions: FDA found no identifiable hazards from the *PRLR* gene truncation or the IGA on the safety of food derived from PRLR-SLICK cattle. The agency concluded that the safety of food products made from these cattle is no different than that of food products made from commercial cattle without the IGA, including those with naturally occurring slick phenotypes. This assessment supports the use of PRLR-SLICK cattle as a safe source of both meat and milk for human consumption.

VI. ENVIRONMENTAL RISK

FDA evaluated the potential risk to the environment from the marketing of the IGA in PRLR-SLICK cattle and associated products derived from them (e.g., meat, milk, semen, or embryos). The information provided by the developer is adequate to demonstrate that the IGA and alteration process do not pose a hazard to the environment and produce a phenotype that exists in other domesticated cattle present in the US. The developer did not propose specific animal containment conditions, manure/carcass disposal procedures or animal identification beyond standard farm practices. Therefore, FDA

[1] Dikmen S., et al. (2014) The SLICK hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows. J. Dairy Sci. 97:5508.

assumed that the animals with the IGA will not be held under strict containment and may be housed on any farm in the US. Cattle are considered low risk for escape and establishment in the natural environment, as they are generally expected to be recovered quickly if escape occurs and there are very few feral bovine populations in the US with which PRLR-SLICK cattle could breed. In addition, the slick phenotype can be visually confirmed, and typical cattle management practices in the US include use of ear tags for identification. Finally, no environmental hazards were identified with the IGA that would require that manure or carcasses be handled any differently from typical farm practices.

Conclusions: FDA concluded based on the available information, the development and marketing of the IGA contained in PRLR-SLICK cattle, their derivatives, and their progeny pose a low risk to the environment.

VII. AGENCY CONCLUSIONS

FDA concluded that the developer of the PRLR-SLICK IGA properly identified and appropriately mitigated the potential risks associated with the product when generated and screened according to the methods described in this supplementary risk assessment. Therefore, FDA has no additional safety concerns related to expanding the scope of the March 7, 2022 decision related to PRLR-SLICK cattle to allow the developer to introduce the IGA into *Bos taurus* cattle of any breed or genetic background. The IGA in PRLR-SLICK cattle is equivalent to naturally occurring mutations (with normal biological variability) and results in the same short, slick hair trait as in conventionally raised cattle that have a history of safe use in animal agriculture food production. Although the IGA in PRLR-SLICK cattle is not approved, conditionally approved, or index listed,[2] because FDA has determined the risks associated with the IGA are appropriately mitigated, at this time the agency does not intend to object to marketing the IGA in PRLR-SLICK cattle or associated products derived from them (i.e., offspring, semen, or embryos) or introducing milk, meat, or other food derived from the cattle containing this IGA into the food supply.

[2] See sections 512, 571, and 572 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. §§ 360b, 360ccc, and 360ccc-1].