Date of Approval: April 29, 2025

FREEDOM OF INFORMATION SUMMARY

ORIGINAL APPLICATION

NADA 141-609

DELETION OF EXON 7 OF *CD163* GENE IN DOMESTIC PIGS

Deletion of exon 7 of the *CD163* gene (abbreviated CD163$^{\Delta E7}$) in domestic pigs (*Sus scrofa domesticus*) is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in homozygous pigs. Pigs carrying one or two copies of CD163$^{\Delta E7}$, and their offspring, are intended for breeding or to be used as sources of food.

Sponsored by:

Genus plc

Executive Summary

The deletion of exon 7 of the *CD163* gene in domestic pigs (*Sus scrofa domesticus*) is approved to confer resistance to the porcine reproductive and respiratory syndrome virus (PRRSV) in homozygous pigs. The altered gene is abbreviated CD163$^{\Delta E7}$, and pigs carrying one or two copies of CD163$^{\Delta E7}$, and their offspring, are intended for breeding or to be used as sources of food. The pigs contain an intentional genomic alteration (IGA) that was accomplished using a genome editing process that deleted a specific DNA sequence (exon 7) from the *CD163* gene. (An exon is a DNA sequence within a gene that contributes to the expression of a protein, often coding for amino acids that form part of the final protein.) Pigs carrying two copies of CD163$^{\Delta E7}$ are homozygous for the IGA, and pigs carrying one copy of CD163$^{\Delta E7}$ and one normal copy of *CD163* without the genetic alteration are heterozygous for the IGA.

The *CD163* gene codes for the CD163 protein, and the portion of the protein encoded by exon 7 plays a crucial role in PRRSV infections by allowing the virus to bind to it. In homozygous pigs, this portion of the CD163 protein is not expressed because exon 7 is deleted, making the pigs resistant to PRRSV. The remainder of the CD163 protein is still expressed normally.

PRRSV causes reproductive failure in sows and respiratory disease in young pigs. The virus is found throughout the United States (US) and causes significant economic losses to the swine industry.

Safety and Effectiveness

The pigs themselves are not a drug and are not subject to FDA approval; rather, the IGA (the deletion of exon 7 of the swine *CD163* gene) which is contained within the pigs is the regulated product subject to FDA approval. For approval, the sponsor must show that the IGA is safe and effective for its intended use.

The sponsor demonstrated that this IGA is safe and effective by confirming that (1) the intended alteration to the pigs' genome was achieved; (2) any unintended alterations resulting from the genome editing process were identified and removed via selective breeding; (3) pigs containing the IGA are as healthy as pigs without the IGA; (4) pigs containing the IGA are resistant to PRRSV; and (5) there are no food safety concerns to people or animals who consume food products derived from pigs containing the IGA.

The sponsor introduced the IGA into breeding lines of pigs by conducting genome editing in fertilized eggs (also known as zygotes) and then implanting the resulting embryos into surrogate sows. The resulting offspring were the first generation to contain the IGA (called founder pigs), and they were adequately characterized using multiple techniques to show that the IGA was present in their genome as intended. The sponsor confirmed that the target alteration (the deletion of exon 7) was achieved and there were no unintended off-target alterations to the *CD163* gene. DNA sequencing data from the founder pigs and two subsequent generations of offspring demonstrated that the sponsor's characterization methodology is sufficient to identify unintended genomic alterations. The sponsor used selective breeding to eliminate any unintended alterations from the population of pigs containing the IGA. The sponsor also confirmed that when the *CD163* gene is expressed, the portion encoded by exon 7 is absent. Therefore, the amino acid sequence of the expressed CD163 protein would join exon 6 directly to exon 8, skipping the sequence normally encoded by exon 7. In addition, the molecular weight of the expressed CD163 protein (i.e., with exon 7 deleted) is decreased as expected.

To evaluate the safety of the IGA to the pigs containing it, the sponsor compared pigs that were homozygous for the IGA to pigs that were heterozygous for the IGA and to pigs without the IGA. There were no differences in morbidity or mortality at numerous life stages or in post-mortem assessments between the three groups of pigs. No animal safety concerns were noted for pigs containing the IGA beyond those that would be expected in well-managed, commercial swine operations.

The sponsor conducted a food safety assessment to determine if the IGA impacts the safety of food derived from pigs containing the IGA. This evaluation included a compositional analysis study that looked at the composition and nutritional content of muscle and the carcass and meat quality from pigs that were homozygous for the IGA, pigs that were heterozygous for the IGA, and pigs without the IGA. There were no significant differences in any of these variables between the three groups of pigs. No potential food safety concerns were identified for people or animals who consume food products derived from pigs containing the IGA. The US Department of Agriculture (USDA) has regulatory oversight over the labeling and processing of most food products made from pigs containing the IGA.

To confirm that the IGA is effective and confers resistance to PRRSV, the sponsor conducted multiple viral challenge studies where pigs that were homozygous for the IGA, pigs that were heterozygous for the IGA, and pigs without the IGA were exposed to PRRSV. In the studies, pigs were inoculated with PRRSV and resistance was evaluated 21 days later by detecting viral RNA and by quantifying the presence of antibodies that bind to PRRSV. Pigs were considered resistant if they had undetectable viral RNA and undetectable PRRSV-binding antibodies at 21 days post-inoculation. The sponsor used multiple strains of PRRSV in the studies, and the results showed that pigs that were homozygous for the IGA were resistant to all but one strain of the virus, referred to as KS06. FDA determined that the risk of a lack of effectiveness associated with the KS06 strain is low, as it is not a strain that is currently circulating at an appreciable level in the US. Pigs that were heterozygous for the IGA and pigs without the IGA were not resistant to any of the strains of PRRSV in the studies. Overall, based on the data and information provided by the sponsor, FDA concluded that the IGA is effective at conferring resistance to PRRSV in pigs that are homozygous for the IGA.

FDA determined that the sponsor has validated methods and procedures in place to ensure that the IGA remains safe and effective. In addition, the sponsor is required to promptly report to FDA any information regarding safety concerns or a lack of effectiveness after approval (for example, if there is a breakthrough PRRSV infection in pigs that are homozygous for the IGA, and therefore, expected to be resistant).

Conclusions

Based on the data submitted by the sponsor for the approval of the deletion of exon 7 of the *CD163* gene in domestic pigs, FDA determined that the IGA is safe and effective.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-609

B. Sponsor

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Drug Labeler Code: 086205

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C. Proprietary Name

DELETION OF EXON 7 OF *CD163* GENE IN DOMESTIC PIGS

D. Species

Domestic Pigs (*Sus scrofa domesticus*)

E. Indication

Deletion of exon 7 of the *CD163* gene in domestic pigs (*Sus scrofa domesticus*) is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in homozygous pigs. Pigs carrying one or two copies of CD163$^{\Delta E7}$, and their offspring, are intended for breeding or to be used as sources of food.

II. PRODUCT DEFINITION

Deletion of exon 7 of the *CD163* gene (abbreviated CD163$^{\Delta E7}$) in domestic pigs (*Sus scrofa domesticus*) is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in homozygous pigs. Pigs carrying one or two copies of CD163$^{\Delta E7}$, and their offspring, are intended for breeding or to be used as sources of food.

III. MOLECULAR CHARACTERIZATION OF THE ALTERED GENOMIC DNA

The intentional genomic alteration (IGA) contains a deletion of a 414 base pair (bp) sequence encompassing exon 7 of the swine *CD163* gene, which is located on chromosome 5. The CD163 protein is a macrophage differentiation antigen belonging to the scavenger receptor cysteine-risk domain (SRCR) family of proteins and is considered the primary binding target for PRRSV, the causative agent of porcine

reproductive and respiratory syndrome.[1] Deletion of exon 7 of the *CD163* gene leads to the deletion of the scavenger receptor cysteine-rich domain 5 (SRCR5) with a single amino acid change. Previous studies indicate that CD163 SRCR5 mediates PRRSV entry into the cell.[2,3]

The sponsor introduced the IGA in animals by using a genome editing technology called CRISPR-Cas9. CRISPR-Cas9 utilizes a site-specific nuclease designed to induce a double-strand break at a target site in the genome. A pair of guide RNAs (gRNAs) were selected based on their efficiency and specificity to target the introns flanking exon 7 of *CD163*. Double-strand breaks created by CRISPR-Cas9, upstream and downstream of *CD163* exon 7, are repaired by endogenous DNA repair pathways such as non-homologous end-joining resulting in a deletion of a sequence encompassing exon 7.[4] While this repair can result in different insertions, deletions, or rearrangements at the target site(s), this IGA is defined as the specific sequence resulting from cut-site-to-cut-site repair, leading to a 414-bp deletion.

To characterize potential unintended off-target alterations, a list of possible off-target sites to screen was generated using an *in vitro* biochemical assay, SITE-Seq®.[5] The assay was performed in triplicate with four ribonucleoprotein concentrations for both gRNAs. A total of 182 off-target sites (plus the on-target sites) were identified for the pair of gRNAs at the two lowest ribonucleoprotein concentrations. The sites were annotated for overlap with NCBI[6] and Ensembl[7] genes. Most of the sites are located at intergenic regions, and eighteen sites are located at coding regions of protein coding genes. Further analysis of the 182 sites in porcine fetal fibroblasts was conducted by next generation sequencing (NGS) following the introduction of ribonucleoprotein by nucleofection. The analysis verified alterations at two off-target sites, indicating that the method can identify a true off-target.

The methods and materials used to generate the IGA and the information provided by the sponsor are appropriate to support the molecular characterization of the altered genomic DNA.

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1 Welch SK, Calvert JG. A brief review of CD163 and its role in PRRSV infection. Virus Res 2010. https://doi.org/10.1016/j.virusres.2010.07.018

2 Burkhard C et al. Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. PLoS Pathog. 2017. https://doi.org/10.1371/journal.ppat.1006206

3 Wang H et al. Deletion of CD163 exon 7 confers resistance to highly pathogenic porcine reproductive and respiratory viruses on pigs. Int J Biol Sci 2019. https://doi.org/10.7150/ijbs.34269

4 Nesbitt, et al. Pigs lacking the SRCR5 domain of CD163 protein demonstrate heritable resistance to the PRRS virus and no changes in animal performance from birth to maturity. Frontiers in Genome Editing 2024. https://doi.org/10.3389/fgeed.2024.1322012

5 Cameron, *et al*. Mapping the genomic landscape of CRISPR-Cas9 cleavage. Nature Methods 2017. https://doi.org/10.1038/nmeth.4284

6 https://www.ncbi.nlm.nih.gov/datasets/gene/GCF_000003025.6/

7 https://www.ensembl.org/Sus_scrofa/

IV. MOLECULAR CHARACTERIZATION OF THE LINEAGE OF ANIMALS WHOSE GENOMES HAVE BEEN INTENTIONALLY ALTERED

Fertilized zygotes were microinjected with CRISPR-Cas9 reagents, and the subsequent edited embryos were then transferred to recipient gilts. CRISPR-Cas9 reagents include two ribonucleoprotein complexes, each consisting of Cas9 protein and gRNA.

The sponsor provided a description of the breeding strategy to generate pigs with the IGA and methods to characterize the IGA as well as potential unintended alterations. Pigs with the IGA were generated in four genetic lines. Founder (E0) generation pigs were screened for the presence of the IGA and possible unintended alterations using three NGS methods: 1) amplicon-based short-read sequencing to query the target site (i.e., *CD163* locus), 2) amplicon-based long-read sequencing to query a larger window around the target site, and 3) hybridization-based sequence capture analysis to examine the target site as well as the 182 potential off-target sites identified by SITE-Seq®. Two of the verified potential sites are the same locations where off-target edits were observed in at least one of the E0 pigs. The identified unintended alterations are not expected to affect protein function based on locations and available gene annotation data. The first site occurs in an intronic region (non-coding) and the second site occurs in an intergenic region (not within a gene). Subsequent breeding and screening were used to eliminate transmission of these unintended alterations from the E0 pigs to the population of pigs intended for commercialization.

Because embryos were edited, E0 pigs can have mosaic alterations (where multiple populations of cells with distinct alterations exist within one founder pig), but only two alleles are present in the pig's germline and passed on to progeny (E1 pigs). NGS analysis demonstrated that one of the selected E0 pigs had an unintended inversion at the target site that was not inherited by its E1 progeny.

To generate a representative population of pigs to support molecular characterization, a total of eight E0 founder pigs containing the IGA were bred with wild-type (WT) gilts to produce the E1 generation. E0 progeny (E1 pigs) were selected for the presence of the IGA and absence of unintended off-target alterations by using 1) amplicon-based short-read NGS to query the targeted *CD163* locus and 2) hybridization-based short-read NGS to examine the target site as well as potential off-target sites identified by SITE-Seq®. NGS data from E1 pigs, including capture-based sequencing data of the target site as well as the potential off-target sites, confirmed the inheritance of the IGA and presence/absence of off-target alterations. Previously identified off-target alterations were detected at two different genomic locations in some of the E0 founder pigs. Only E1 animals free from off-target alterations were selected for further breeding.

Selected E1 pigs (heterozygous for the IGA and without off-target alterations) were intercrossed within their respective E0 founder populations to generate the E2 progeny. The final product does not have off-target alterations as they were bred out selectively.

The sponsor provided a detailed description and representative data on the following molecular analyses performed with E2 pigs: 1) TaqMan® genotyping assay to determine zygosity, 2) transcript analysis using Nanopore sequencing, and 3) western blot analysis to examine CD163 protein, respectively.

The TaqMan® genotyping assay was designed to produce amplicons from both the IGA and WT alleles. TaqMan® assay results from more than 300 E2 pigs showed a segregation pattern close to the expected Mendelian ratio 1:2:1. Note that a similar TaqMan® genotyping assay is also used to support genotypic durability (Section VII). To determine the splicing of the *CD163* transcripts in pigs with and without the IGA (e.g., splicing of exons 6 to 8 when the IGA is present), RT-PCR followed by Nanopore sequencing confirmed the lack of *CD163* exon 7 RNA expression in E2 pigs homozygous for the IGA. For WT pigs, the expression level of exon 7 was consistent with the neighboring exons; for all heterozygous pigs, the expression level of exon 7 was approximately half of the neighboring exons. Western blot analysis using antibodies against CD163 was performed to compare the sizes of the CD163 proteins produced by pigs with the IGA to pigs without the IGA. The provided data and information demonstrated that the sizes of *CD163* RNA transcripts and protein were shortened in E2 pigs containing the IGA, compared to WT, as expected.

The data and information the sponsor provided in support of the Molecular Characterization of the Altered Genomic DNA and the Molecular Characterization of the Lineage of Animals Whose Genomes Have Been Intentionally Altered adequately characterized the IGA as well as potential unintended alterations that could impact safety and effectiveness. No hazards were identified intrinsic to the deletion of exon 7 of the *CD163* gene.

V. PHENOTYPIC CHARACTERIZATION OF ANIMALS WITH THE IGA

A. Phenotypic Characterization Study

The sponsor conducted a phenotypic characterization study evaluating target animal safety at multiple life stages, including reproductive function and post-mortem assessments.

Study Animals: Homozygous IGA and heterozygous IGA pigs from the E2 generation were used for the phenotypic characterization studies. The homozygous null pigs from the E2 generation were used as study controls. WT animals of the same genetic lines that were not derived from the E0 founder pigs but obtained from the commercial breeding population were also used as study controls. Data from the study were compared to data from commercial pig populations of the same line where available.

Measurements and Observations: Animal management allowed for evaluation of pigs at each life stage with approximately equal numbers of each genotypic group, line, and sex. Differences in numbers of pigs observed between parameters was related to the number of pigs available for evaluation at each life stage and if the parameter is sex-linked (e.g., scrotal hernia). Neonatal parameters including birthweight, teat count, and congenital hernias were evaluated in 113 to 298 pigs of each genotype of each genetic line. Between 30 and 67 pigs of each genotype from each genetic line were evaluated for body weight at 140 days of age, back fat, loin depth, leg conformation, and lifetime daily gain. Six pigs of each genotype of each genetic line were evaluated for female reproduction parameters, and between 6 and 8 pigs of each genotype of each genetic line were evaluated for male reproductive parameters. Morbidity and mortality were monitored for all study pigs at all life

stages. Postmortem data was evaluated from 12 pigs of each genotype of each genetic line.

Results: The birthweight and teat number of the pigs with the IGA were comparable to their WT and commercial production pig comparators. The incidence of umbilical hernias, scrotal hernias, and cryptorchidism in the pigs with the IGA was comparable to the WT and commercial pig comparators. The 140-day weight and lifetime daily gain of the pigs with the IGA were comparable to their commercial pig comparators. Differences in 140-day weight and rate of weight gain between some homozygous IGA pigs and WT pigs were likely due to differences in selective breeding pressure between animal groups and not associated with an increased safety risk to pigs with the IGA. The backfat and loin depth in the pigs with the IGA was comparable to WT and commercial pig comparators, as was leg conformation. Both female and male reproductive performance in pigs with the IGA were comparable to their WT comparators and similar to industry standards and expected performance reported in literature.

Morbidity for pigs with the IGA in the phenotypic characterization study was typical of commercial pig production and did not indicate an increased safety risk to pigs with the IGA. Overall morbidity was based on animal health observations and recorded according to system/category such as the following: reproductive, lameness, respiratory, gastrointestinal, skin/integument, or central nervous system. Morbidity in the phenotypic characterization study occurred only during the nursery stage (Table V.1), an approximately 6-week period immediately following weaning which included randomly assigned grouping of 15 to 20 pigs by sex per pen. The predominant morbidity noted was skin/integument abnormalities attributed to an outbreak of exudative epidermitis, a common bacterial dermatitis of young pigs housed in groups characterized by high morbidity. Only two of the pigs exhibiting morbidity out of a total of 96 pigs observed with morbidity were for reasons other than exudative epidermitis and both were classified as “poor condition” (1 homozygous IGA pig and 1 WT pig). No morbidity was noted during the pre-weaning, finishing, or reproductive stages.

Table V.1. Summary of morbidity of nursery stage pigs by IGA zygosity and nursery stage WT pigs in the phenotypic characterization study.

Observation	Homozygous IGA pigs	Heterozygous IGA pigs	Homozygous null pigs	WT pigs
Total pigs observed (n)	192	453	218	133
Total pigs treated (n)	25	30	17	24
Morbidity rate (%)	13	7	8	18

The mortality reported for pigs with the IGA in the phenotypic characterization study was consistent with industry and literature reports of mortality in commercial pig production and did not indicate an increased safety risk to pigs with the IGA. Mortality, inclusive of animals found dead and humanely euthanized for health reasons, was reported for each life stage and is summarized in Tables V.2 - V.5.

Table V.2. Summary of pre-weaning mortality by IGA zygosity and WT pigs in the phenotypic characterization study.

Observation	Homozygous IGA pigs	Heterozygous IGA pigs	Homozygous null pigs	WT pigs
Total pigs observed (n)	199	496	238	154
Total pigs died (n)	5	26	11	20
Mortality rate (%)	3	5	5	13

Table V.3. Summary of nursery stage mortality by IGA zygosity and WT pigs in the phenotypic characterization study.

Observation	Homozygous IGA pigs	Heterozygous IGA pigs	Homozygous null pigs	WT pigs
Total pigs observed (n)	192	453	218	133
Total pigs died (n)	15	43	20	12
Mortality rate (%)	8	9	9	9

Table V.4. Summary of finishing stage mortality by IGA zygosity and WT pigs in the phenotypic characterization study.

Observation	Homozygous IGA pigs	Heterozygous IGA pigs	Homozygous null pigs	WT pigs
Total pigs observed (n)	110	126	121	86
Total pigs died (n)	1	1	1	0
Mortality rate (%)	1	1	1	0

Table V.5. Summary of reproductive stage mortality by IGA zygosity and WT pigs in the phenotypic characterization study.

Observation	Homozygous IGA pigs	Heterozygous IGA pigs	Homozygous null pigs	WT pigs
Total pigs observed (n)	32	17	36	22
Total pigs died (n)	1	1	4	0
Mortality rate (%)	3	6	11	0

Twelve pigs of each study group (homozygous IGA, heterozygous IGA, homozygous null, and WT) from two genetic lines were evaluated postmortem at a meat processing laboratory at approximately 180 days of age. The assessment included measures for anatomic pathology. The postmortem findings for the pigs with the IGA were consistent with industry and literature reports and did not indicate an increased safety risk to pigs with the IGA.

B. Morbidity and Mortality Rates for Pigs with the IGA

After the phenotypic characterization study, morbidity and mortality rates for homozygous IGA pigs were calculated from data recorded over several months on two farms and are summarized by life stage in Table V.6 and Table V.7 below. Mortality rate was calculated as the number of mortality events (not including voluntary culling) in the group divided by the total number of pigs in the group per week. The morbidity and mortality rates for the pigs with the IGA were consistent with industry and literature reports and did not indicate an increased safety risk to pigs with the IGA.

Table V.6 Morbidity rate (%) summary statistics for homozygous IGA pigs

Life Stage	Mean (%)	Standard deviation
Breeding	0.8	0.3
Pre-Weaning	12.5	10.8
Post-Weaning	0.1	0.1

Table V.7 Mortality rate (%) summary statistics for homozygous IGA pigs

Life Stage	Mean (%)	Standard deviation
Breeding	0.2	0.2
Pre-Weaning	5.0	1.3
Post-Weaning	0.7	0.6

C. Phenotypic Characterization Conclusion

Based on the data provided by the sponsor, no animal safety concerns were noted in pigs with the IGA beyond those that would be expected in pigs without the IGA under conventional swine management practices.

VI. FOOD SAFETY

The food safety assessment was conducted to identify potential hazards that may impact food safety for food derived from the lineage of pigs with the IGA relative to a conventional counterpart without the IGA. This assessment considered intended and unintended effects, as well as their relevance to human health, based on review of submitted information regarding the product characterization, animal health records and durability, and tissue composition analysis. FDA concluded that there are no food safety concerns to human consumers who would consume the edible tissues of food derived from lineage of pigs with the IGA. Additionally, no adverse impacts were identified for the use of the lineage of pigs with the IGA in animal food.

A. Product Characterization

Based on the conclusions in the Molecular Characterization of the Altered Genomic DNA (Section III) and the Molecular Characterization of the Lineage of Animals Whose Genomes Have Been Intentionally Altered (Section IV), FDA concluded that the sponsor has adequately characterized the IGA as well as potential unintended alterations that could impact safety. No hazards were identified intrinsic to the deletion of exon 7 of the *CD163* gene, described above in Section IV.

Expression data demonstrated that the sizes of *CD163* RNA transcripts and protein were shortened in animals containing the IGA, compared to WT, as expected. The CD163 protein lacking SRCR5 was analyzed to determine if the truncated protein and single amino acid change could cause a potential allergy concern to humans. Incubation of CD163 full-length or truncated CD163 (deletion of SRCR5 domain) from lung macrophage lysates obtained from WT and homozygous IGA animals with simulated gastric fluid and pepsin resulted in rapid enzymatic degradation. No differences in thermal stability were observed for WT CD163 protein compared to the truncated CD163 protein.

An *in silico* analysis of the sequence using predictive analysis tools and comparison to known and putative allergen databases resulted in no matches to known allergens of human consumers. The analysis was conducted using three publicly available allergen databases (AllergenOnline, Comprehensive Protein Allergen Resource, [COMPARE 2023], and the Structural Database of Allergenic Proteins). The information does not indicate that the truncated CD163 protein expressed in animals with the IGA would warrant further allergenicity assessment.

An evaluation of potential DNA sources that could result from contamination of either the DNA template used for transcription of gRNA or bacterial/plasmid DNA carryover during purification of Cas9 was conducted. PCR analysis of the founder animals from four genetic lines was also conducted to examine for potential random incorporation of gRNA by reverse transcription into the pig genome. These results together with the bioinformatic data support that no exogenous DNA is likely to be found in the pig genome that could impact food safety.

Therefore, based on the product characterization, there are no risks identified that may impact food safety.

B. Phenotypic Characterization and Health Status of the Animals with the IGA

Animals with acceptable health status are generally considered an essential factor to determine if the food derived from the animal is suitable for human consumption. Therefore, an evaluation of the health of the animals with the IGA, compared to the health status of animals without the IGA was conducted. The phenotypic characterization data related to animal health, safety, and reproduction for various life stages were evaluated in four genetic lines. As predicted based on the molecular and phenotypic characterizations, the pigs with the IGA were as healthy as pigs without the IGA; as described above in Section V.

Animal health records and durability information did not suggest that there may be a concern for unintended effects in the lineage of pigs with the IGA. Data were provided to show that the genotype and phenotype were durable over multiple generations of pigs (see Section VII). The IGA is stably inherited, and the phenotype is consistent and predictable over the product lifespan.

There were no animal safety concerns noted in pigs with the IGA beyond those that would be expected in standard pigs under conventional swine management practices. Additionally, the sponsor will continue to monitor animal health through post-approval reporting.

In addition to the postmortem animal health assessment, meat carcass and quality were assessed. The final carcass and meat quality data were collected from 40 randomly selected carcasses from E2 pigs from one genetic line at approximately 205 days of age (10 per study group; equal number of boars and gilts). The carcass and quality data values for fresh meat (e.g., muscle pH, temperature, color, marbling, hot carcass weight, muscle composition) from homozygous IGA pigs were similar to US commercial finishing pigs. All parameters measured for the homozygous IGA pigs were not different when compared with the WT except for the least squares means for muscle temperature at 35 minutes postmortem ($P<0.05$), and the last and 10^{th} rib fat thickness ($P<0.05$). Despite the statistical differences, all group means were within the standard measurements for pork quality in the US.[8] All carcass measures were indicative of normal growth, muscle, and fat partitioning, as compared to swine industry values expected for commercial finishing pigs. Meat quality traits were also analyzed for three other genetic lines and there were no biologically significant differences between the pigs with the IGA and the WT pigs.

Based on the information provided, the acceptable health status of the animals and lack of identifiable concerns over multiple generations indicate that the food derived from the pigs with the IGA is expected not to be harmful to consumers.

C. Composition of Edible Tissues

A tissue compositional analysis was conducted in order to determine if the muscle composition was affected by the IGA. The objective of the study was to support that the edible tissues of the pigs with the IGA are comparable to pork products derived from conventional pigs that are commonly consumed by the public.

Title: Compositional Analyses and Long Term Storage of Pork Tissue for Line 03. (Study No. 221009)

Report Date: January 25, 2023

Study Locations: Indiana (in-life), Wisconsin (analytical)

Study Design: Adult E2 generation pigs (205 ± 1.61 days) were slaughtered using standard industry practices from four study groups: homozygous IGA, heterozygous IGA pigs, homozygous null (control), and a separate maternal line as reference pigs without the IGA (WT). Postmortem organ inspection at slaughter was conducted and a carcass inspection by the state health inspector was performed. Carcass and meat quality parameters were also measured as described above in Section V. Loin samples (5 boars and 5 gilts) were collected to conduct a muscle compositional analysis.

Frozen tissues (~500 g) were ground and analyzed to determine the composition for 80 different analytes (proximates, minerals, amino acids, lipids, carbohydrates, and vitamins). Samples were analyzed following standard published methods according to GLP regulations (21 CFR Part 58). Statistical analyses were carried out to

[8] Huff-Lonergan E, et al. Correlations among selected pork quality traits. J Anim Sci. 2002. https://doi.org/10.2527/2002.803617x

estimate least squares means, standard errors of the mean, and tests of significance at $P<0.05$.

Results and Conclusions: Postmortem findings for the animals with the IGA were consistent with industry and literature reports and were not indicative of an animal safety risk associated with the IGA. The carcass inspection concluded that no humane handling violations were found, and no abnormalities were found. Carcass and meat quality information from pigs with the IGA were similar to standard commercial finishing pigs (see Section V). For the majority of the nutritional parameters analyzed, the values fell within the range noted in the USDA's Food Composition Databases (FoodData Central).[9] For all other parameters, the values were within 25% of the analyte range or target value reported by USDA and consistent with natural variation, therefore a food safety concern was not anticipated. There were no biologically significant differences for muscle compositional parameters between the homozygous IGA compared to other groups.

No significant changes in the composition of the muscle derived from the homozygous IGA and heterozygous IGA pigs were found in the study. The values also were within the normal range of acceptability for the analyzed pork muscle compositional parameters.[10] Composition of loin muscle for three other genetic lines (E2 generation pigs homozygous for the *CD163* gene edit and WT pigs) was also measured (5 boars and 5 gilts per genotype per genetic line). Results showed no statistically significant differences between the homozygous IGA and WT groups for protein, moisture, ash, carbohydrates, calories, and total fat content. Based on the information, no substantial differences in chemical composition for any of the genetic lines and crosses of pigs are anticipated due to the IGA. The information supports that the muscle composition shows typical natural pork variation and the pigs with the IGA are not nutritionally inferior to standard pigs.

The muscle compositional findings are consistent with the assessment conducted on the molecular and phenotypic characterization and with the information from the animal health records, that there are no increased safety risks from the IGA in the animal. Therefore, considering all the information provided regarding the pigs with the IGA, potential human food safety concerns were not identified. The information also indicates that there are no concerns when the pigs with the IGA are used in animal food.

VII. GENOTYPIC AND PHENOTYPIC DURABILITY

Data were provided to demonstrate that both genotype and phenotype are conserved over multiple generations of pigs with the IGA. The TaqMan® genotyping assay cited in Section IV above was used to evaluate genotypic durability, and PRRSV challenge studies cited in Section VIII below were used to evaluate phenotypic durability. These data adequately demonstrate that the genotype and phenotype of these pigs are durable, the deletion of exon 7 of the *CD163* gene is stably inherited, and the phenotype is consistent and predictable.

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[9] https://fdc.nal.usda.gov/

[10] Realini CE, et al. Characterization of *Longissimus thoracis*, *Semitendinosus* and *Masseter* muscles and relationships with technological quality in pigs. 2. Composition of muscles. Meat Sci. 2013, 94:417-23. https://doi.org/10.1016/j.meatsci.2013.03.007

The sponsor provided a plan to ensure that future pigs with the IGA will continue to meet the product definition. This plan included the sponsor's: (1) methods and sampling plan for monitoring genotypic and phenotypic durability after approval, (2) procedures for addressing genotypic and phenotypic durability failures, (3) recordkeeping and reporting plans as a means of documenting and communicating to FDA observations related to durability and animal health/safety, and (4) contingency/disaster preparedness and recovery procedures for maintenance and/or re-derivation of pigs with the IGA. The sponsor will monitor genotypic and phenotypic durability using a qualitative TaqMan® PCR assay and qualitative PCR assay, respectively, on an ongoing basis after approval.

Together, the data and information the sponsor provided assure that pigs with the IGA will continue to be genotypically and phenotypically equivalent to those pigs evaluated prior to approval.

VIII. EFFECTIVENESS

To validate the claim that the homozygous pigs are resistant to PRRSV, the sponsor provided data from multiple live animal viral challenge studies using several PRRSV Type I and II isolates. The PRRSV isolates selected for these studies represent the genetic diversity of the virus as well as the most common viral lineages circulating in the US commercial pig population at the time of approval. The results of these studies support the claim that homozygous IGA pigs are resistant to PRRSV infection.

A. Substantial Evidence of Effectiveness

Three studies were performed to demonstrate substantial evidence of effectiveness of the IGA to confer resistance to PRRSV infection. The studies were conducted at three different locations in the US between July 2022 and April 2023, and shared a common study design:

Objective: To evaluate the resistance of homozygous IGA animals to infection with PRRSV Type I and Type II in contrast to heterozygous IGA, homozygous null, and WT pigs.

Study Animals: Homozygous IGA and control (heterozygous IGA, homozygous null, and WT) pigs were 3 to 5 weeks of age at the start of the studies. The desired phenotype (PRRSV infection resistance) is intended to be conferred only to homozygous IGA animals. Therefore, the heterozygous IGA, homozygous null, and WT pigs served as the control group for claim validation, as they would be expected to be susceptible to infection with PRRSV.

Experimental Design: Pigs were divided into groups for placement into separate rooms. Each group included homozygous IGA and control pigs challenged with a specific PRRSV inoculant. Pigs containing the IGA were screened for presence of the IGA prior to study enrollment or were generated by breeding homozygous IGA boars with homozygous IGA sows and homozygous null boars with homozygous null sows to generate offspring of the same zygosity as the parent.

Viral Challenge Administration: All pigs in each group were challenged with 3 mL inoculum containing 10^4 – 10^5 median tissue culture infectious dose ($TCID_{50}$) of one isolate of PRRSV intranasally or both intramuscularly and intranasally.

Measurements and Observations: PRRSV infection in animals with and without the IGA was assessed by detection of PRRSV ribonucleic acid (RNA) in serum samples via PCR and by antibody seroconversion indicative of an active PRRSV infection via enzyme-linked immunosorbent assay (ELISA). The assays were conducted at an independent veterinary diagnostic laboratory.

Success Criteria: Success was defined as resistance to infection by the PRRSV challenge as indicated by undetectable PRRSV RNA in serum samples and undetectable PRRSV antibody seroconversion 21 days post-inoculation.

The studies differed by study site, study dates, and PRRSV isolates used for viral challenge.

Study 1

This study of 40 homozygous IGA and 56 control pigs was conducted in July and August 2022 in Illinois and included viral challenge with the following PRRSV inoculants: Type I – SD13-15, Type I – SD01-08, Type II – NVSL97-7895, and Type II – KS06-72109. Table VIII.1 below summarizes the number of pigs of each group challenged with each PRRSV inoculant in this study and the corresponding success rates.

Table VIII.1. Study 1 Live animal viral challenge results

PRRSV inoculant	Number of homozygous IGA pigs challenged	Success rate (%)* in homozygous IGA pigs	Number of heterozygous IGA / homozygous null pigs challenged	Success rate (%)* in heterozygous IGA / homozygous null pigs
Type I – SD13-15	10	100	14	0
Type I – SD01-08	10	100	14	0
Type II – NVSL97-7895	10	100	14	0
Type II – KS06-72109	10	0	14	0

* Success rate is the proportion of pigs with undetectable PRRSV RNA in serum samples and undetectable PRRSV antibody seroconversion on study day 21.

Study 2

This study of 24 homozygous IGA and 12 control pigs was conducted in January and February 2023, in Illinois and included viral challenge with the following PRRSV inoculants: Type II – UIL21-0712 (144-1C), and Type II – KS06-72109. Table VIII.2 below summarizes the number of pigs of each group challenged with each PRRSV inoculant in this study and the corresponding success rates.

Table VIII.2. Study 2 Live animal viral challenge results

PRRSV inoculant	Number of homozygous IGA pigs challenged	Success rate (%)* in homozygous IGA pigs	Number of heterozygous IGA / homozygous null pigs challenged	Success rate (%)* in heterozygous IGA / homozygous null pigs
Type II – UIL21-0712	8	100	4	0
Type II – KS06-72109	16	20	8	0

* Success rate is the proportion of pigs with undetectable PRRSV RNA in serum samples and undetectable PRRSV antibody seroconversion on study day 21.

Study 3

This study of 50 homozygous IGA and 50 control pigs was conducted in March and April 2023, in Nebraska and included viral challenge with the following PRRSV inoculants: Type I – SD13-15, Type I – SD01-08, Type II – NVSL97-7895, and Type II – UIL21-0712 (144-1C). Table VIII.3 below summarizes the number of pigs of each group challenged with each PRRSV inoculant in this study and the corresponding success rates.

Table VIII.3. Study 3 live animal viral challenge results

PRRSV inoculant	Number of homozygous IGA pigs challenged	Success rate (%)* in homozygous IGA pigs	Number of heterozygous IGA / homozygous null pigs challenged	Success rate (%)* in heterozygous IGA / homozygous null pigs
Type I – SD13-15	12	100	13	0
Type I – SD01-08	12	100	13	100
Type II – NVSL97-7895	13	100	12	0
Type II – UIL21-0712 (144-1C)	13	100	12	0

* Success rate is the proportion of pigs with undetectable PRRSV RNA in serum samples and undetectable PRRSV antibody seroconversion on study day 21.

Results: All homozygous IGA pigs demonstrated resistance to PRRSV infection when challenged with the Type I – SD13-15, Type I – SD01-08, Type II – NVSL97-7895, and Type II – UIL21-0712 (144-1C) PRRSV inoculants in these viral challenge studies.

Table VIII.4 summarizes the success rate observed in the groups successfully challenged (i.e., the control group pigs were successfully infected) with various

PRRSV isolates, organized by the PRRSV isolate. This table combines results across studies 1, 2, and 3 to provide a more comprehensive perspective of PRRSV resistance by viral isolate.

Table VIII.4. Live animal viral challenge results organized by PRRSV isolate (from studies 1, 2, and 3)

PRRSV inoculant	Number of homozygous IGA pigs challenged	Success rate (%)* in homozygous IGA Pigs	Number of heterozygous IGA / homozygous null pigs challenged	Success rate (%)* in heterozygous IGA / homozygous null pigs
Type I – SD13-15	22	100	27	0
Type I – SD01-08	10	100	14	0
Type II – NVSL97-7895	23	100	26	0
Type II – KS06-72109	26	8	22	0
Type II – UIL21-0712(144-1C)	21	100	16	0

* Success rate is the proportion of pigs with undetectable PRRSV RNA in serum samples and undetectable PRRSV antibody seroconversion on study day 21.

The results from the Type I-SD01-08 isolate challenge in study 3 are removed from Table VIII.4 because that challenge was determined to be unsuccessful based on the lack of infection in the control group.

Pigs challenged with the Type II – KS06-72109 isolate in studies 1 and 2 failed to demonstrate resistance to PRRSV infection regardless of genotype. In contrast to the other Type II isolates used in these viral challenge studies, the KS06-72109 strain is not currently circulating among the US commercial swine population at an appreciable level.[11,12]

Adverse Reactions: No safety risks to pigs with the IGA were identified when reviewing the animal observation data associated with these studies.

Conclusions: The results of these studies provide substantial evidence of effectiveness of the IGA in animals to confer resistance to PRRSV infection in homozygous pigs. The Type II – NVSL97-7895, and Type II – UIL21-0712 (144-1C) PRRSV inoculants used in these viral challenge studies represent two of the most prevalent lineages of PRRSV in the US commercial swine population. The risk of a

[11] Paploski IAD, et al. Phylogenetic Structure and Sequential Dominance of Sub-Lineages of PRRSV Type-2 Lineage 1 in the United States. Vaccines 2021. https://doi.org/10.3390/vaccines9060608

[12] Zeller MA, et al. ISU PRRSView (data retrieved 22 Dec, 2023) https://prrsv.vdl.iastate.edu

lack of effectiveness associated with the KS06-72109 isolate is low; it is unlikely to be encountered as a source of natural infection due to the low prevalence in the US.

B. Additional Effectiveness Evaluation

Three additional studies were conducted to support validation of the claim with regard to PRRSV resistance for additional viral isolates. These smaller live animal viral challenge studies were conducted between July 2023 and May 2024, at the same study site in Nebraska as study 3 described above. These studies used the same study design as studies 1, 2, and 3 but the pigs were challenged with different PRRSV isolates.

Study 4

This study of 15 homozygous IGA and 15 control pigs was conducted in July and August 2023 in Nebraska and included viral challenge with the following PRRSV inoculants: Type II – USA/NE/26342-1/2022 (1-8-4 L1H), Type II – USA/IN/65239-GA/2014 (1-7-4 L1A), and Type II – USA/OK/27915-12/2022 (1-4-2 L1E). All homozygous IGA pigs demonstrated resistance to PRRSV infection when challenged with all studied isolates in contrast to no control pigs demonstrating resistance.

Study 5

This study of 5 homozygous IGA and 5 control pigs was conducted in September and October 2023, in Nebraska and included viral challenge with a modified-live vaccine for PRRSV of the L1D lineage of PRRSV Type II. The viral challenge of this study involved inoculation of the pigs with the vaccine according to label directions (1 mL intramuscular injection). All homozygous IGA pigs demonstrated resistance to PRRSV infection when challenged with this inoculant in contrast to no control pigs demonstrating resistance.

Study 6

This study of 5 homozygous IGA and 5 control pigs was conducted in April and May 2023, in Nebraska and included viral challenge with PRRSV Type II – USA/IL/23295-GA/2002 (2-5-2 L5). All homozygous IGA pigs demonstrated resistance to PRRSV infection when challenged with this isolate in contrast to no control pigs demonstrating resistance.

IX. AGENCY CONCLUSIONS

The data submitted in support of this original application satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that the DELETION OF EXON 7 OF THE *CD163* GENE IN DOMESTIC PIGS is safe and effective for the conditions of use in the General Information Section above. Additionally, data demonstrate that consumption of food products derived from domestic pigs with the deletion of exon 7 of the *CD163* gene will not represent a public health concern.

A. Exclusivity

The exclusivity provisions of section 512(c)(2)(F) of the Federal Food, Drug, and Cosmetic Act do not apply to the DELETION OF EXON 7 OF THE *CD163* GENE IN DOMESTIC PIGS because under section 106 of the Generic Animal Drug and Patent Term Restoration Act (Pub.L. 100-670), FDA cannot approve an abbreviated application for a product that is primarily manufactured using recombinant DNA, recombinant RNA, hybridoma technology, or other processes involving site specific gene manipulation techniques. Therefore, a sponsor cannot submit an abbreviated application to market a generic version of the DELETION OF EXON 7 OF THE *CD163* GENE IN DOMESTIC PIGS.

B. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.