EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR POMC/PCSK1/LEPR CDx Panel DECISION SUMMARY

I Background Information:

A De Novo Number

DEN200059

B Applicant

PreventionGenetics, LLC

C Proprietary and Established Names

POMC/PCSK1/LEPR CDx Panel

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QRV	Class II	21 CFR 862.1164 - Setmelanotide eligibility gene variant detection system	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for POMC/PCSK1/LEPR CDx Panel

B Measurand:

Germline variants in genes in human genomic DNA

C Type of Test:

Next generation sequencing

III Indications for Use:

A Indication(s) for Use:

The POMC/PCSK1/LEPR CDx Panel is a next generation sequencing (NGS)-based in vitro diagnostic test that analyzes genomic DNA isolated from blood or saliva. Specimens used with the test are K_2EDTA blood collected using certain indicated K_2EDTA blood collection devices and saliva collected using ORAcollect-Dx™ OCD-100 devices. The test detects germline nucleotide substitutions, short insertions and deletions, and copy number variants (CNVs) within the following 3 genes:

- Pro-opiomelanocortin (*POMC*)
- Proprotein Convertase Subtilisin/Kexin type 1 (*PCSK1*)
- Leptin Receptor (*LEPR*)

The test is a companion diagnostic device intended to select adult and pediatric patients 6 years of age and older who have obesity and certain variants in *POMC*, *PCSK1* or *LEPR* genes for treatment with IMCIVREE® (setmelanotide) in accordance with the approved therapeutic product labeling. The POMC/PCSK1/LEPR CDx Panel is a single-site assay performed at PreventionGenetics, LLC (Marshfield, WI).

B Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only.
- For in vitro diagnostic use.
- Therapeutic decisions must be based on the independent medical judgement of the treating physician, taking into consideration the test results and all applicable information concerning the patient's condition, clinical history, and other findings.
- This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations. The classification and interpretation of all variants identified reflects the current state of scientific understanding at the time the result report is issued.
- When NGS does not reveal any difference from the reference sequence, or when a sequence variant is homozygous, we cannot be certain that we were able to detect both patient alleles. Occasionally, a patient may carry an allele which does not capture or amplify, due for example to a large deletion or insertion.
- Test reports contain no information about other portions of the gene, such as regulatory domains, deep intronic regions or any currently uncharacterized alternative exons.
- The POMC/PCSK1/LEPR CDx Panel is not intended to detect mosaic variants.
- We cannot be certain that the reference sequences are correct. Genome build hg19, GRCh37 (Feb2009) is used as reference for this assay.
- Insertions and deletions larger than 18 base pairs in the variable number tandem repeat (VNTR) region in exon 3 (NM_000939.3) of the POMC gene with coordinates chr2:25,384,457-25,384,474 (GRCh37/hg19) containing repeated sequence "AGCAGCGGC" were not validated with this device and will not be reported.
- Two runs of intronic mononucleotide repeats [e.g., (A)n or (T)n] are excluded from the analysis. The excluded regions reside in LEPR (NM_002303.5) intron 4 (chr1:66,037,998-66,038,001) and post-coding (chr1:66,096,092-66,096,098) (GRCh37/hg19).

- Balanced translocations or inversions within a targeted gene, or large unbalanced translocations or inversions that extend beyond the genomic location of a targeted gene are not detected.
- In nearly all cases, our ability to determine the exact copy number change within a targeted gene is limited. In particular, when we find copy excess within a targeted gene, we cannot be certain that the region is duplicated, triplicated, etc. In many duplication cases, we are unable to determine the genomic location or the orientation of the duplicated segment with respect to the gene. In particular, we often cannot determine if the duplicated segment is inserted in tandem within the gene or inserted elsewhere in the genome. Similarly, we may not be able to determine the orientation of the duplicated segment (direct or inverted), and whether it will disrupt the open reading frame of the given gene.
- The performance of the POMC/PCSK1/LEPR CDx Panel was assessed for single nucleotide variants (SNVs), insertions and deletions < 50 base pairs, and a single copy number variant (CNV; a homozygous deletion in exons 6, 7, and 8 in the LEPR gene). Due to limitations in the POMC/PCSK1/LEPR CDx Panel for CNV detection, any CNV duplication or deletion ≥ 50 base pairs reported by the device may not be accurate other than the homozygous deletion in exons 6, 7, and 8 in the LEPR gene.
- The accuracy of the POMC/PCSK1/LEPR CDx Panel was not assessed for PCSK1 exon 2 and LEPR exon 7.
- The POMC/PCSK1/LEPR CDx Panel is for use only with whole blood collected in K_2EDTA blood collection tubes or saliva specimens collected in DNA Genotek ORAcollect Dx™ OCD-100 saliva collection devices.
- The assay has been validated with the Illumina NovaSeq6000.
- By definition, there is not sufficient scientific information available to make a pathogenicity assignment to variants of uncertain significance (VUS/VOUS). All variants and these variants in particular could change classification as new scientific information becomes available, which may impact patient eligibility for IMCIVREE (setmelanotide) injection.
- The pathogenicity assignments determined with the POMC/PCSK1/LEPR CDx Panel are intended to predict response to therapy with setmelanotide and are not intended for diagnostic purposes.

C Special Instrument Requirements:

Illumina NovaSeq6000 Sequencer (qualified by PreventionGenetics, LLC)

IV Device/System Characteristics:

A Device Description:

The POMC/PCSK1/LEPR CDx Panel is a next generation sequencing (NGS) assay for the detection of germline variants in three genes (pro-opiomelanocortin (POMC), leptin receptor (LEPR), and convertase subtilisin/kexin type 1 (PCSK1)). The POMC/PCSK1/LEPR CDx Panel is performed in a single laboratory (PreventionGenetics, LLC in Marshfield, WI).

B Principle of Operation

Saliva samples collected into DNA Genotek ORAcollect Dx OCD-100 collection devices (K152464) and K_2EDTA whole blood samples collected into certain indicated K_2EDTA blood collection devices (as described in the labeling) are shipped to the Prevention Genetics (PG)

laboratory (Marshfield, WI). The laboratory utilizes NGS technologies to cover the full coding regions of the genes plus ~10 bases of noncoding DNA flanking each exon. DNA is captured using an optimized set of DNA hybridization capture probes and then sequenced using Illumina's Reversible Dye Terminator (RDT) platform (Illumina, San Diego, CA). Variant interpretations are based on the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines.

C Instrument Description Information

1. Instrument Name:

   Illumina NovaSeq6000 Sequencer (qualified by PreventionGenetics, LLC)

2. Specimen Identification:

   A maximum of (b)(4) POMC/PCSK1/LEPR CDx Panel samples can be prepared on (b)(4) assay at a time. A (b)(4) and at least (b)(4) positive human controls are processed along with patient samples with each library preparation of the POMC/PCSK1/LEPR CDx Panel. (b)(4)

3. Specimen Sampling and Handling:

   Specimens are collected and shipped to PreventionGenetics, LLC where they are accessioned upon receipt. Specimens may be stored according to the conditions described in Specimen stability (section F below) prior to processing.

4. Calibration:

   Calibration is performed after installation and may be performed after instrument repairs are performed. Calibration checks are performed regularly.

5. Quality Control:

   See Traceability, Stability, Expected Values (Controls, Calibrators, or Methods) (section VI.A.5. below).

V Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – 3rd Edition

CLSI, EP09c, 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI, EP07, 3rd Edition, Interference Testing in Clinical Chemistry

Considerations for Design, Development, and Analytical Validation of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Intended to Aid in the Diagnosis of Suspected Germline Diseases; April 13, 2018

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; May 11, 2005

Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software; January 14, 2005

Off-the-Shelf Software Use in Medical Devices; September 9, 1999

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

A precision study was performed to assess precision of the POMC/PCSK1/LEPR CDx Panel across different sources of variability over five sequencing runs. The study used [(b)(4)] saliva samples collected into DNA Genotek ORAcollect Dx OCD-100 (K152464) and [(b)(4)] K_2EDTA whole blood samples from patients within the intended use population. The samples were tested over [(b)(4)] days using inputs of 200 ng DNA (one run), 300 ng DNA ([(b)(4)] runs), and 400 ng DNA (one run) and [(b)(4)] reagent lots, multiple operators, and [(b)(4)] instruments. Samples included representative SNVs and insertions and deletions < 50 base pairs in *POMC*, *PCSK1*, and *LEPR* genes, as well as a [(b)(4)] copy number variant (CNV) in the LEPR gene (a deletion of exons 6, 7, and 8). The POMC/PCSK1/LEPR CDx Panel detected a total of [(b)(4)] variants in each of the [(b)(4)] runs and a false positive CNV for one of the [(b)(4)] runs. It was later determined that the false positive CNV did not meet the threshold for a high confidence call and should not have been reported. The [(b)(4)] sequencing runs were compared at the variant level (where each variant detected was classified as pathogenic, likely pathogenic, VUS, benign, or likely benign) and the sample level (where samples were determined as eligible or not eligible for drug treatment) and there was [(b)(4)] concordance at the variant level and sample level ([(b)(4)] not eligible, [(b)(4)] eligible for treatment).

A [(b)(4)] precision study was performed to assess precision of the POMC/PCSK1/LEPR CDx Panel across different sources of variability over an additional [(b)(4)] sequencing runs. The study included a subset of the samples used in the [(b)(4)] precision study described above [(b)(4)] K_2EDTA whole blood samples and [(b)(4)] saliva samples that included representative single nucleotide variants (SNVs) and insertions and deletions [(b)(4)] base pairs in *POMC*, *PCSK1*, and *LEPR* genes). Samples were tested with inputs of 300 ng DNA and [(b)(4)] reagent lots, multiple operators, and [(b)(4)] instruments over [(b)(4)] non-consecutive days. An analysis was conducted for the [(b)(4)] K_2EDTA whole blood samples and [(b)(4)] saliva samples for these [(b)(4)] sequencing runs plus the [(b)(4)] sequencing runs included in the [(b)(4)] precision study described above for a total of [(b)(4)] sequencing runs. The positive percent agreement (PPA) for all variants detected, negative percent agreement (NPA) for all wild type (reference) base calls, and overall percent agreement (OPA) was calculated with 95% CI for whole blood and saliva

samples (see the table below). Wild type base calls are based on reference sequence Genome Reference Consortium Human Build 37 (GRCh37/hg19).

Sample Type	Metric	Total number of bases	Number of bases that agree	% Agreement	95% CI for % Agreement
Whole Blood	OPA	(b)(4)		100	(100.00, 100.00)
	PPA			100	(99.72, 100.00)
	NPA			100	(100.00, 100.00)
OCD-100 Saliva	OPA			100	(100.00, 100.00)
	PPA			100	(99.72, 100.00)
	NPA			100	(100.00, 100.00)

The samples used in the precision studies were also included in the method comparison study where (b)(4) concordance between the POMC/PCSK1/LEPR CDx Panel and the comparator methods was determined (see Method Comparison section below).

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Interference from substances in blood, saliva, and DNA was assessed for the POMC/PCSK1/LEPR CDx Panel. For each subject, the sequences of each test sample were compared to the sequence obtained for the control sample from that same subject. Percent agreement between the (b)(4) samples was calculated. For each subject, a total of (b)(4) bases was evaluated across *POMC*, *PCSK1*, and *LEPR* genes. There was (b)(4) sequence agreement between test sample and control sample for all subjects and spiked substances, demonstrating no interference from substances in blood, saliva, and the DNA extraction process. Further details of these studies are described below.

Interference from substances in blood

Interference from substances in K_2EDTA whole blood was assessed. K_2EDTA whole blood obtained from (b)(4) subjects was collected. Each subject's whole blood sample was aliquoted and spiked with conjugated bilirubin ((b)(4) mg/dL), unconjugated bilirubin ( ), albumin and γ-globulins ((b)(4)), triglycerides ((b)(4) mg/dL), cholesterol ((b)(4) K_2EDTA (b)(4) mg/dL; represents (b)(4) short draw into a blood collection device), or not spiked (control). The results of the study demonstrated no interference from substances in blood or up to (b)(4) short draw. Specimens received with greater than short draw will be rejected from further processing.

Interference from substances in saliva

Interference from substances introduced into saliva samples through various activities (eating, drinking, chewing gum, using mouthwash, smoking and brushing teeth) was assessed relative to no interferent activity (control). Each of subjects conducted each of the activities except for the smoking activity, which was conducted by (b)(4) of the (b)(4) subjects. The results of the study demonstrated no exogenous interference from saliva.

Interference from components used in the DNA extraction process

DNA extracted from K_2EDTA whole blood from (b)(4) subjects and DNA extracted from saliva obtained from (b)(4) subjects was spiked with elution buffer or ethanol to (b)(4) volume/volume (v/v) final concentrations, or not spiked (control). The results of the study demonstrated no interference from components used in the DNA extraction process.

Cross-contamination

Laboratory internal processes (e.g., sex chromosome checks, filters) and building/space barriers were developed and implemented to minimize cross-contamination.

A study was performed to assess potential carryover and co-mingling of two DNA specimens. Computationally, the equivalent of (b)(4), and (b)(4) of total reads from the HapMap sample GM18507 were randomly sampled and replaced with reads from FASTQ files from the HapMap sample NA12877 to create files that would mimic cross-contamination at these levels. In the laboratory, the same two HapMap samples (GM18507 and NA12877) were mixed at various concentrations (GM18507:NA12877 ratios of (b)(4) (b)(4), and (b)(4). The laboratory samples were independently prepared in triplicate for reproducibility. Contamination of an alternate genotype was consistently detected both computationally and in the laboratory-mixed cohort when present in a concentration of at least (b)(4) of the sample. The results behaved as predicted given the bioinformatics pipeline filtering criteria were designed to present a variant when at least 20% of the reads contained the alternate base.

Another study was conducted to assess sample cross-contamination within a sequencing run (intra-run) and carryover between successive sequencing runs (inter-run) using extracted whole blood and saliva genomic DNA from (b)(4) male subject and from (b)(4) female subject with unique variants. To evaluate intra-run cross-contamination, the male and female whole blood and saliva samples were set up in a checkerboard pattern on a plate and included the lower limit of the DNA range (200 ng) and the upper limit of the DNA range (400 ng). Negative non-target controls with no DNA were also interspersed amongst the samples. Samples were run on the same flow cell. To evaluate inter-run carryover, (b)(4) successive sequencing runs were performed using 400 ng of extracted genomic DNA from the same samples. Plates containing either male or female samples were run on (b)(flow cells. The male samples were processed in the (b)(4) sequencing run and the female samples were proceed in the (b)(4) sequencing run. (b)(4) percent of the samples included in the intra- and inter-run cross-contamination and carryover evaluation met the pre-established criteria. None of the negative controls in the cross-contamination evaluation showed DNA contamination.

Study	Specimen Type	Ratio of the average coverage of the SRY gene to the total average coverage in female samples	Mean read fraction of variants present in female and absent in male^	Arcsine Root Transformation (ART) of calculated contamination where < 0.4 corresponds to < 10% contamination
Intra-Run	Overall	0%	0%	0.119
	Whole Blood	0%	0%	0.125

Study	Specimen Type	Ratio of the average coverage of the SRY gene to the total average coverage in female samples	Mean read fraction of variants present in female and absent in male^	Arcsine Root Transformation (ART) of calculated contamination where < 0.4 corresponds to < 10% contamination
	Saliva	0%	0%	0.114
Inter-Run	Overall	0%	0%	0.158
	Whole Blood	0%	0%	0.189
	Saliva	0%	0%	0.127

^Mean values for female samples in every plate position when male samples were present in the same plate (intra-run) or following a male run (inter-run)

Cross-reactivity

An assessment was conducted to determine the degree of paralogy for the POMC, PCSK1, and LEPR genes. The assessment used a combination of in silico and empirical analyses including BLAT (BLAST-like alignment tool) score, segmental duplications, theoretical expected NGS read depth, observed NGS mapping quality, known pseudogenes, and literature review. None of the interrogated sequences in the POMC, PCSK1, and LEPR genes were identified to have either moderate or high degrees of paralogy, and no flagged regions (i.e., repeats, GC content) would impact testing of known or relevant variants.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The POMC/PCSK1/LEPR CDx Panel is not traceable to any known standard.

   Genome build hg19, GRCh37 is used as reference for this device.

   A minimum of [redacted] control samples (1 negative control and at least [redacted] positive controls) are run with each CDx library preparation. The negative control consists of a PCR reaction with no DNA that is run in a separate well from patient samples to ensure no reagent contamination or patient sample carry-over during the process. At least [redacted] positive controls with known variants (a mixture of male and female human samples), a control of established DNA quantity range, and an internal DNA control library are also processed along with the patient samples. A routine check for the presence of Y chromosome DNA in female samples is also routinely performed.

6. Detection Limit:

   The DNA input range for the POMC/PCSK1/LEPR CDx Panel is 200 ng to 400 ng DNA. If a sample contains less than [redacted] of DNA, a new sample will be requested. The DNA input range was established based on the precision studies described above. The precision studies

demonstrated acceptable precision performance of the POMC/PCSK1/LEPR CDx Panel at 200 ng, 300 ng, and 400 ng of input DNA.

7. Assay Cut-Off:

   Not applicable.

8. Accuracy (Instrument):

   See Method Comparison (section B.10. below)

9. Carry-Over:

   See Cross-contamination (section VI.A.3. above).

B Comparison Studies:

10. Method Comparison:

    A method comparison study was conducted to assess the analytical accuracy of the POMC/PCSK1/LEPR CDx Panel. The study included (b)(4) saliva samples collected into DNA Genotek ORAcollect Dx OCD-100 (K152464) and (b)(4) K_2EDTA whole blood samples from patients within the intended use population. (b)(4) variants in *POMC*, *PCSK1*, and *LEPR* genes were identified using the POMC/PCSK1/LEPR CDx Panel in these samples, which included representative SNVs and insertions and deletions < 50 base pairs in *POMC*, *PCSK1*, and *LEPR* genes, as well as a single copy number variant (CNV) in the LEPR gene (a deletion of exons 6, 7, and 8). The identified variants and the same exon of each variant plus adjacent intronic sequences were sequenced with the POMC/PCSK1/LEPR CDx Panel and validated orthogonal methods (i.e., Sanger sequencing for SNVs and insertions and deletions < 50 base pairs or high-density gene-centric (HDGC) array comparative genomic hybridization (CGH) for larger CNVs). Variant and non-variant sequence comparisons were made between the POMC/PCSK1/LEPR CDx Panel and the orthogonal methods and OPA, PPA and NPA with corresponding 95% CIs were calculated (see table below).

Metric	Total number of bases	Number of bases that agree	% Agreement	95% CI for % Agreement
PPA	2,473	2,473	100%	(99.85, 100.00)
NPA	405,011	405,011	100%	(99.99, 100.00)
OPA	407,484	407,484	100%	(99.99, 100.00)

Sequencing results from the POMC/PCSK1/LEPR CDx Panel and orthogonal methods were also compared at the variant interpretation level (where each variant detected was classified as pathogenic, likely pathogenic, VUS, benign, or likely benign) and the sample interpretation level (where a positive or negative result for the POMC/PCSK1/LEPR CDx Panel was determined). There was (b)(4), concordance at the variant interpretation level and the sample interpretation level ((b)(4) negative, (b)(4) positive) across the three sequencing runs.

The study included all exons in *POMC*, *PCSK1*, and *LEPR* genes except for *PCSK1* exon 2 and *LEPR* exon 7, which did not have a variant in the studied samples and were not sequenced by comparator methods. An analysis of sequence characteristics of these regions revealed no problematic sequences (e.g., simple repeats, low complexity repeats, satellite repeats, simple tandem repeats) and %GC content for these regions was calculated as (b)(4) which falls within a range that is reliably analyzed by the NGS technology of the POMC/PCSK1/LEPR CDx Panel.

11. Matrix Comparison:

Not applicable.

C Clinical Studies:

12. Clinical Sensitivity:

Not applicable.

13. Clinical Specificity:

Not applicable.

14. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

IMCIVREE (setmelanotide) clinical studies

The safety and efficacy of IMCIVREE for chronic weight management in patients with obesity and certain variants in POMC, PCSK1, or LEPR genes were assessed in 2 identically designed, 1-year, open-label studies, each with an 8-week, double-blind withdrawal period. Study 1 enrolled patients aged 6 years and above with obesity and certain variants in POMC or PCSK1 genes, and Study 2 enrolled patients aged 6 years and above with obesity and certain variants in the LEPR gene. In both studies, the local genetic testing results used to select patients for the studies were centrally confirmed using Sanger sequencing. In both studies, adult patients had a body mass index (BMI) of $\geq$30 kg/m^2. Weight in pediatric patients was $\geq$ 95th percentile using growth chart assessments.

Dose titration occurred over a 2-to 12-week period, followed by a 10-week, open-label treatment period. Patients who achieved at least a 5-kilogram weight loss (or at least 5% weight loss if baseline body weight was <100 kg) at the end of the open-label treatment period continued into a double-blind withdrawal period lasting 8 weeks, including 4 weeks of IMCIVREE followed by 4 weeks of placebo (investigators and patients were blinded to this sequence). Following the withdrawal sequence, patients re-initiated active treatment with IMCIVREE at the therapeutic dose for up to (b)(4) weeks. The primary endpoint of the studies was the proportion of patients who demonstrated at least 10% weight reduction at one year compared to baseline.

Efficacy analyses were conducted in 21 patients (10 patients in Study 1 and 11 patients in Study 2) who had completed at least one year of treatment at the time of a prespecified data cutoff. Of the 21 patients included in the efficacy analysis in Studies 1 and 2, 62% were

adults and 38% were aged 16 years or younger. In Study 1, 50% of patients were female, 70% were White, and the median BMI was 40.0 kg/m^2 (range: 26.6-53.3) at baseline. In Study 2, 73% of patients were female, 91% were White, and the median BMI was 46.6 kg/m^2 (range: 35.8-64.6) at baseline.

In Study 1, 80% of enrolled patients who met the prespecified data cutoff met the primary endpoint, achieving a ≥ 10% weight loss after 1 year of treatment with IMCIVREE. In Study 2, 46% of enrolled patients who met the prespecified data cutoff achieved a ≥ 10% weight loss after 1 year of treatment with IMCIVREE.

Parameter	Statistic	Study 1 (N=10)	Study 2 (N=11)
Patients Achieving at Least 10% Weight Loss at Year 1	n (%)	8 (80.0%)	5 (45.5%)
	95% CI[1]	(44.4%, 97.5%)	(16.8%, 76.6%)
	P-value[2]	<0.0001	0.0002

Note: The analysis set includes patients who received at least 1 dose of study drug and had at least 1 baseline assessment.
[1] From the Clopper-Pearson (exact) method
[2] Testing the null hypothesis: Proportion =5%

Clinical bridging study

Due to the prevalence of the ultra-rare patient population, patients located across the world were selected for enrollment in the IMCIVREE clinical studies based on certain variants identified in POMC, PCSK1, or LEPR genes by local tests as part of a patient's standard of care. The IMCIVREE clinical studies enrolled a total of 30 subjects: 21 subjects who were included in the efficacy analyses (as described above) as well as 9 additional patients (5 patients in Study 1 and 4 patients in Study 2) who had not yet completed one year of treatment at the time of the cutoff and were not included the efficacy analyses. One patient in Study 1 was removed from the study before the primary efficacy endpoint could be determined because a clinical trial version of the CDx (identical to the POMC/PCSK1/LEPR CDx Panel with respect to the POMC, PCSK1, and LEPR genes) and Sanger sequencing detected no variants in POMC, PCSK1, or LEPR, indicating that the patient was ineligible for IMCIVREE treatment. The primary efficacy endpoint for 4 supplemental patients in Study 1 and 4 supplemental patients in Study 2 was assessed after the efficacy analysis cutoff (4 of the 4 patients in Study 1 achieved the primary endpoint and 3 of 4 patients in Study 2 achieved the primary endpoint).

The variants and variant interpretation determined by local tests as part of a patient's standard of care were confirmed by both the POMC/PCSK1/LEPR CDx Panel and orthogonal methods (Sanger sequencing or high-density gene-centric (HDGC) array comparative genomic hybridization (CGH)) for the 21 subjects included in efficacy analyses and 8 supplemental subjects not included in efficacy analyses. Considering only pivotal trial subjects, the PPA between local tests and the POMC/PCSK1/LEPR CDx Panel is 100% (21/21) with 95% confidence intervals (CI) of 84.5% - 100.0%. Considering both pivotal trial and supplemental subjects, the PPA between local test and the POMC/PCSK1/LEPR CDx Panel is 96.7% (29/30) with 95% CI of 83.3% – 99.4%. The single discordant result is the

supplemental patient in Study 1 who was removed from the study before the primary efficacy endpoint could be determined.

Given the rarity of the variants and how the clinical studies were designed, samples were not available to calculate the NPA between local tests and the POMC/PCSK1/LEPR CDx Panel. However, the clinical trial version of the CDx described above (which is identical to the POMC/PCSK1/LEPR CDx Panel with respect to the POMC, PCSK1, and LEPR genes), has previously identified 29 positives based on certain variants in POMC, PCSK1, and LEPR genes out of 30,000 patients who would fall into the POMC/PCSK1/LEPR CDx Panel intended use population. All 29 positives represent the 29 patients who were included in the clinical studies. Therefore, the efficacy of IMCIVREE (setmelanotide) determined in the clinical trials is representative of the efficacy of IMCIVREE (setmelanotide) in the POMC/PCSK1/LEPR CDx Panel intended use population.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

Not applicable.

F Other Supportive Performance Characteristics Data:

15. Specimen stability

Whole blood stability

K_2EDTA whole blood samples from 8 donors were collected into K_2EDTA blood collection devices. Each donor's whole blood was assessed fresh after collection (baseline sample) or stored in blood collection devices at room temperature (20 ± 0.2°C) for 3 days and 8 days, refrigerated (3.4 ± 0.7°C) for 8 days, frozen (-17.9 ± 1.2°C) for 1 month, or at elevated ambient (41°C) storage for 3 days. After storage under these conditions, DNA from each sample was extracted, processed, and sequenced. The complete sequence across *POMC*, *PCSK1*, and *LEPR* genes, including variants and reference sequence, was compared between each test condition and baseline. All samples passed quality control (QC) metrics and demonstrated 100% sequence agreement between each test condition and baseline for all conditions tested. Studies to assess the stability of samples stored frozen (-17.9 ± 1.2°C) for 12 months and 36 months are ongoing.

Saliva stability

Saliva samples from 8 donors were collected into DNA Genotek ORAcollect Dx OCD-100 (K152464). Each donor's saliva was assessed fresh after collection (baseline sample) or stored in the OCD-100 collection device at room temperature (19.9 ± 0.3°C) for 80 days, for 3 freeze/thaw cycles (each cycle consisted of a minimum of 3 h at ≤ -38.3 ± 0.3°C and a minimum of 3 h at 45.5 ± 0.2°C), and at elevated ambient temperature (41°C) for 3 days. After storage under these conditions, DNA from each sample was extracted, processed, and sequenced. The complete sequence across *POMC*, *PCSK1*, and *LEPR* genes, including variants and reference sequence, was compared between each test condition and baseline. All

samples passed QC metrics and demonstrated 100% sequence agreement between each test condition and baseline for all conditions tested.

Extracted DNA stability

DNA was extracted from K_2EDTA whole blood from 8 donors and from saliva from 8 donors. Each donor's DNA was assessed fresh after collection and extraction (baseline sample), after refrigerated storage (3.8 ± 0.8°C) 3 days and 1 month, and after 3 freeze/thaw cycles (each cycle consisted of a minimum of 3 h at -38.3 ± 0.3°C and a minimum of 3 h at 45.5 ± 0.2°C). The complete sequence across *POMC*, *PCSK1*, and *LEPR* genes, including variants and reference sequence, was compared between each test condition and baseline. All samples passed QC metrics and demonstrated 100% sequence agreement between each test condition and baseline for all conditions tested. Studies to assess the stability of extracted DNA stored frozen (-20°C) for 12 months and 36 months are ongoing.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Identified Risks to Health	Mitigation Measures
Incorrect performance of the test leading to false positive results (causing patients to receive drug treatment inappropriately) or false negative results (causing patients to miss an opportunity for drug treatment)	• Certain design verification and validation activities, including documentation of certain studies. • Certain labeling information, including certain limiting statements and performance information.
Incorrect interpretation of genetic data leading to false positive results (causing patients to receive drug treatment inappropriately) or false negative results (causing patients to miss an opportunity for drug treatment)	• Certain design verification and validation activities, including documentation of certain studies and variant interpretation and classification procedures. • Certain labeling information, including certain limiting statements and performance information.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The POMC/PCSK1/LEPR CDx Panel provides a companion diagnostic test to select patients who have obesity and certain variants in *POMC*, *PCSK1*, or *LEPR* genes for IMCIVREE (setmelanotide) treatment. IMCIVREE (setmelanotide) is currently the only approved drug treatment for this patient population.

B Summary of the Assessment of Risk:

Incorrect performance of the test and/or incorrect interpretation of genetic data could lead to false positive or false negative results. False positive results could lead to a patient not eligible

for treatment receiving drug treatment inappropriately. False negative results could lead to a missed opportunity for drug treatment.

The most common adverse reactions associated with IMCIVREE (setmelanotide) treatment in clinical studies were injection site reactions, skin hyperpigmentation, nausea, headache, diarrhea, abdominal pain, back pain, fatigue, vomiting, depression, upper respiratory tract infection, and spontaneous penile erection. Depression and suicidal ideation occurred in IMCIVREE (setmelanotide) clinical studies.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The risks of erroneous results are mitigated by the requirement of certain design verification and validation, including certain studies to ensure high analytical accuracy, precision, and specificity performance, and acceptable variant classification and interpretation procedures. Certain labeling information, including limiting statements in the test reports and labeling, serve to reduce the chances of false positives or negatives as a result of incorrect performance of the test and/or incorrect interpretation of genetic data.

Additionally, the IMCIVREE (setmelanotide) drug label includes stopping criteria to limit duration of drug exposure if a patient has not lost at least 5% of baseline body weight (or 5% of baseline body mass index for patients with continued growth potential) after 12-16 weeks of treatment, as well as periodic monitoring of treatment response. The drug label also includes monitoring for serious adverse effects, including new onset or worsening depression and discontinuation of the drug if a patient experiences suicidal thoughts or behaviors.

The probable benefits of this device outweigh the probable risks of this device, in light of the listed special controls and applicable general controls.

X Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): QRV
Device Type: Setmelanotide eligibility gene variant detection system
Class: II
Regulation: 21 CFR 862.1164