

## Xpanded HereditaryCancer Panel A Targeted Test for Genetic Causes of Cancer

### **Overview:**

Cancer is a common disease affecting approximately 1 in 3 individuals in the U.S.<sup>1</sup> Hereditary cancer syndromes are a clinically and genetically heterogeneous group of genetic disorders; therefore, it can be challenging to predict the disease-causing gene in a patient with cancer based on clinical features alone. Features suggestive of a hereditary cancer predisposition include: young ages at diagnosis, multiple primary cancers in a single individual, and several relatives affected with the same type of cancer or related cancers spanning multiple generations.

It is often necessary to perform testing of multiple genes (concurrently or as reflex tests) to identify the underlying genetic cause of cancer in an individual or family. Moreover, new genes are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels updated.

In contrast to other Xpanded panels, Xpanded HereditaryCancer is typically performed on a singleton (proband only). When submitted, the Xpanded HereditaryCancer Panel will use a trio approach that includes concurrent analysis of the affected proband and both parents, which increased the likelihood of identifying a definitive genetic explanation for cancer. Depending on the family structure, family history of cancer, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx to inquire about submitting family members in addition to the proband for the Xpanded HereditaryCancer Panel. The Xpanded HereditaryCancer Panel is based on whole exome capture (WEC), Next Generation sequencing (NGS), and targeted analysis of a comprehensive list of 600+ genes currently associated with cancer. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with cancer.

### **Inheritance Pattern/Genetics:**

The etiology of cancer is complex, including multiple genetic, epigenetic, and environmental factors. While the majority of cancers are sporadic in nature, some families have hereditary forms of cancer that are associated with increased cancer risks compared with the general population. Approximately 5-10% of cancer cases are thought to be due to a hereditary predisposition. The inheritance pattern can be autosomal dominant, autosomal recessive, or X-linked. Recent studies have demonstrated that exome sequencing (ES) has even higher diagnostic yields for these affected individuals.<sup>2</sup> Pathogenic variants in a single gene may be

associated with different types of cancer (clinical heterogeneity), and conversely, pathogenic variants in different genes can cause the same cancer phenotype (genetic heterogeneity). In addition, a single individual may have multiple pathogenic variants (multigenic inheritance). In some cases, confirmation of the molecular genetic cause of cancer may have implications for treatment and management.

## Test Methods:

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions are enriched for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Using a custom-developed analysis tool (XomeAnalyzer), data are filtered and analyzed to identify sequence variants and most deletions and duplications involving three or more coding exons (Retterer et al., 2015). Smaller deletions or duplications may not be reliably identified. Reported clinically significant variants are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected relatives as necessary. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Reportable variants include pathogenic variants and likely pathogenic variants. Variants of uncertain significance, likely benign and benign variants, if present, are not routinely reported. A list of additional variants not included in the report is available upon request.

Please note that while the Xpanded HereditaryCancer panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for the Xpanded HereditaryCancer panel. The Xpanded HereditaryCancer Panel gene list includes more than 600 genes. The list was developed by searching for genes associated with cancer in multiple sources, including all genes offered on clinical cancer panels at GeneDx and other laboratories, NHGRI database of Oncologic Manifestations, review of cancer exome/genome literature, internal GeneDx data, and genes with a role in known cancer pathways. GeneDx data from clinical whole exome sequencing done on patients with cancer was also used to inform the gene list. The gene list is stratified by the degree of evidence linking the gene to germline cancer risk – clinical cancer, limited evidence, and candidate genes. Genes may be removed from the gene list if they are found to have no association with cancer. In rare situations, genes are removed from the panel if they are expected to be low yield for this phenotype but contain an inherent high risk for incidental findings. The current gene list is available on our website. Xpanded panel gene lists are regularly updated/improved using evidence from the literature and from GeneDx data from clinical exome sequencing done on

patients with cancer. Rarely, during this internal evaluation, a positive finding in a gene not on this Xpanded gene list may be uncovered. If this happens, an updated report will be issued.

## **Result Reporting:**

The Xpanded HereditaryCancer Panel is performed on an affected proband. When submitted concurrently, parental (and/or additional relatives) samples may be included for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for parents or other relatives who may have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If reports are requested for other affected family members, additional fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with cancer in the published or emerging literature.

Pathogenic/likely pathogenic variants in genes responsible for the phenotype of the patient will be reported; however, because this is a phenotype-driven panel of a large number of genes, variants of uncertain significance (VUS) are only reported in some genes, at our discretion.

Variants that are considered to be benign or likely benign will not be reported. As the Xpanded HereditaryCancer Panel includes over 600 genes, the report will not include a comprehensive list of all observed variants.

- **Clinical Cancer genes**

Clinical cancer genes are those with sufficient evidence to be definitively associated with cancer risk. These 87 genes are offered on the GeneDx Hereditary Cancer panels. All variants in these genes with a classification of pathogenic, likely pathogenic, and variant of uncertain significance will be reported in the main body of the report.

- **Limited Evidence genes**

The Xpanded HereditaryCancer Panel includes limited evidence genes for which there is some evidence of an association with cancer risk or a cancer-related phenotype. The evidence for these genes does not warrant inclusion on OncoGeneDx Hereditary Cancer panels at this time. This group of genes includes emerging cancer genes, such as *MSH3*, as well as genes with a defined phenotype that may be related to cancer or include cancer as a secondary manifestation such as, *RPS10* with Diamond-Blackfan Anemia. GeneDx clinical expertise and internal exome data will be leveraged to determine which variants in these genes are included in the main body of the report. Any non-benign/likely benign variants in the limited evidence genes that are not selected for the main body of the report will be included in a report addendum (except benign variants based on population frequency).

- **Candidate genes**

A separate file containing a list of identified variants in candidate genes that were not selected for the main body of the report will be provided as an addendum. The Xpanded

HereditaryCancer report will include the addendum unless the proband is opted-out. Candidate genes are genes hypothesized to be related to the cause of the disease, based upon the function, tissue of expression, and phenotype of model organisms with alterations in the gene. Variants in candidate genes may also be reported based on internal data, such as observations of previous XomeDx cases with similar phenotypes and types of variations in the same gene.

In rare instances, this test may reveal a pathogenic variant that is not directly related to the patient's clinical phenotype or test indication. In the event that an incidental finding is identified, this information will be disclosed to the ordering health care provider if it is likely to impact medical care. The absence of reportable incidental findings for any particular gene does not rule out the possibility of pathogenic variants in that gene.

### **Test Sensitivity:**

The clinical sensitivity of the Xpanded HereditaryCancer Panel depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of a hereditary predisposition to cancer as outlined above. It has been demonstrated that the yield of WES testing is higher with a Trio approach compared to a Proband-only approach.<sup>3</sup> The sensitivity of this test is expected to be comparable to trio-based exome sequencing when a trio is submitted for testing. The clinical sensitivity is expected to be lower for singleton testing when only the affected proband is tested.

The average coverage of all genes on the panel is greater than 99% at 10X (with a depth of 10 or more reads). The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included on the final report.

### **Limitations:**

Gene fusions, nucleotide repeat expansion/contraction, abnormal DNA methylation, and other disease mechanisms may not be detectable with this test. There may be some genes or portions of genes that are not amenable to capture, sequencing, and alignment. Additionally, certain types of sequence variations are difficult to identify using WES, including repeat expansions and copy number variants. Small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be reliably detected. This test only analyzes genes included on the gene list below; therefore, the ability to detect large contiguous gene deletions/duplications is limited. APC, CHEK2, EPCAM, MSH2 inversion of exons 1-7, PMS2, SCG5/GREM1, and TERC analysis will not be as sensitive or comprehensive as for a Hereditary Cancer Panel. If clinical suspicion is high for a condition related to one of these genes, order a Hereditary Cancer Panel.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the Xpanded HereditaryCancer Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not

recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the Xpanded HereditaryCancer Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

## References:

1. Probability of Developing or Dying of Cancer - Surveillance Research Program. Available at: <https://surveillance.cancer.gov/devcan/>. (Accessed: 18th September 2017)
2. Foley SB et al. Use of Whole Genome Sequencing for Diagnosis and Discovery in the Cancer Genetics Clinic. *E Bio Medicine*. 2(1):74-81 (2015).
3. Retterer K et al. Clinical application of whole-exome sequencing across clinical indications. *Genetics In Medicine : Official Journal Of The American College Of Medical Genetics*. 18(7):696-704 (2016).