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Proteogenomic Testing of Individuals with Cancer

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Line(s) of Business: HMO; PPO; QUEST; Medicare; FEP	**Precertification:** Refer to the GTM Utilization Review Matrix

I. Policy Description

Proteogenomic testing (encompassing analysis of both the genome and the proteome) is emerging as a new discipline in clinical settings. Until recently, genomic and proteomic analyses have remained in relative isolation. However, as techniques continue to improve, integrated analysis of both large-scale items has become more and more feasible. With precise and personalized medicine continuing to be a point of emphasis in clinical evaluation, proteogenomic analysis has received significant attention in treating cancer.[1]

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with cancer, integrated comprehensive tumor profiling to calculate a tumor mutational burden score using genomic and/or transcriptomic data in conjunction with immunohistochemistry analysis (e.g., OncoExTra™, Caris MI Tumor Seek™, Caris MI Profile™ Comprehensive Testing) **MEETS COVERAGE CRITERIA** as defined within AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For individuals with cancer, integrated analysis of proteomic, genomic, **and/or** transcriptomic testing with or without algorithmic analysis (e.g., GPS Cancer®, DarwinOncoTarget™/DarwinOncoTreat™) **DOES NOT MEET COVERAGE CRITERIA**.

3) Tumor gene expression profiling with algorithmic analysis providing gene pathway activity scores **DOES NOT MEET COVERAGE CRITERIA**.

4) Optical genome mapping with or without whole genome sequencing and transcriptome analysis **DOES NOT MEET COVERAGE CRITERIA**

III. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics
AKT1	Protein kinase B
ALK	Anaplastic lymphoma kinase
AML	Acute myeloid leukemia
AMP	Adenosine monophosphate
APC	*Adenomatous polyposis coli*
APOLLO	Applied Proteogenomics Organization Learning and Outcomes
ASCO	American Society of Clinical Oncology
AURKB	Aurora kinase b
BCCs	Basal cell carcinomas
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
BRDs	Bromodomain
CAP	College of American Pathologists
CAPN2	*Calpain 2*
CDK4/6	Cyclin-dependent kinases 4 and 6
CEBPA	*CCAAT Enhancer Binding Protein Alpha*
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CPTAC	Clinical Proteomic Tumor Analysis Consortium
CREBBP	cAMP response element-binding protein
CRISPR	*Clustered regularly interspaced short palindromic repeats*
CTDSP2	*Carboxy-terminal domain, RNA polymerase II, polypeptide A*
DBNL	Drebrin like
dMMR	Mismatch repair deficiency
DNA	Deoxyribose nucleic acid
DoD	Department of Defense
4E-BP1	*Eukaryotic translation initiation factor 4E-binding protein 1*
EGFR	Epidermal growth factor receptor
EP300	E1A binding protein P300
ER+	Estrogen receptor-positive
ERBB2	Erb-B2 receptor tyrosine kinase 2
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FLT3	*Fms related receptor tyrosine kinase 3*
GBMs	Glioblastomas

GEM	Georgia Estoteric and Molecular Laboratory
GRP	Glucose related protein
GRP78	Glucose regulated protein 78
H2B	Histone H2b
HCC	Hepatocellular carcinoma
HER2	Human epidermal growth factor receptor 2
HSP90β	Heat shock protein HSP 90-beta
HSPB1	Heat shock protein beta member 1
ICIs	Immune checkpoint inhibitors
INTS7	*Integrator complex subunit 7*
ISR	Illumina short read
KMT2A-PTD	*Lysine (K)-specific methyltransferase 2A- partial tandem duplication*
KRAS	*Kirsten rat sarcoma viral oncogene homolog*
LCDs	Local coverage determinations
LDTs	Laboratory developed tests
LNCaP	Lymph node carcinoma of the prostate
MB	Mutational burden
METABRIC	Molecular Taxonomy of Breast Cancer International Consortium
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
NCCN	National Comprehensive Cancer Network
NCDs	National coverage determinations
NFKBIZ	*NF-kappa-B inhibitor zeta*
NGS	Next generation sequencing
NICE	The National Institute for Health and Care Excellence
NPM1	*Nucleophosmin 1*
NRAS	*Neuroblastoma RAS viral oncogene homolog*
NTRK	Neurotrophic tropomyosin kinase receptors
OGM	Optical genome mapping
PC-3	Prostate cancer cell line 3
PC-3M	Prostate cancer cell 3M
PCOs	Provisional clinical opinions
PD-L1	Programmed death ligand 1
PG	Proteogenomic
PI3K	Phosphoinositide 3-kinases
postM	Post-menopausal
preM	Pre-menopausal
pre-mRNA	Messenger ribonucleic acid precursors
Rb	Retinoblastoma
RB1	*Retinoblastoma transcriptional corepressor 1*

RNA	Ribonucleic acid
rRNAs	Ribosomal ribonucleic acids
RUNX1	*Runt related transcription factor 1*
SeC	Sebaceous carcinomas
SCCs	Squamous cell carcinomas
SH3BGRL	*SH3 domain binding glutamate rich protein like*
siRNAs	Short-interfering ribonucleic acids
SNPs	Single nucleotide polymorphisms
S6K	*S6 Kinase*
TCGA	The Cancer Genome Atlas
TGFβ	Transforming growth factor beta
TMB	Tumor mutational burden
TML	Tumor mutational load
TP53	*Tumor protein p53*
UV	Ultraviolet
VA	Department of Veterans Affairs
WGS	Whole genome sequencing
YBC	Young breast cancer

IV. Scientific Background

As newer and faster technology makes evaluating enormous amounts of molecular information possible, proteogenomic testing is on the rise. Techniques such as whole genome sequencing, transcriptome sequencing, and proteomic analysis, previously not clinically viable, are now within the clinical laboratory landscape. Information yielded from these tests may be used for a variety of purposes, including prognosis, diagnosis, identifying targeted treatments, and more. Proteogenomic testing typically revolves around three different sets of analytes: DNA, RNA, and proteins.[2]

DNA is the first stage in the genetic flow of information, represented by genes, including both exons and introns. All the protein-coding genes are represented in the exome, where at least 85% of pathogenic mutations are found. The exome represents a mere 1.5%-2% of the genome, thereby typically making it more cost-effective to sequence than the whole genome.[3] The entire exome includes approximately 30 megabases compared to the 3.3 gigabases of the genome. However, a pathogenic mutation may be in a noncoding region of the genome, such as mutations that result in dysfunction of gene regulation, resulting in situations where sequencing of the entire genome may be useful.[3]

RNA is the second stage in the genetic flow of information, as DNA is transcribed into RNA. Transcriptome sequencing refers to "digital counts" of each RNA molecule, or direct sequencing and quantification of RNA. The RNA transcript being quantified is not a perfect complement of the original DNA sequence; certain regulatory processes and post-transcriptional modifications, such as splicing, polyadenylation, and capping, alter the pre-mRNA sequence. Furthermore, additional regulatory RNA classes are not translated into a protein product, including but not limited to, ribosomal RNAs (rRNAs facilitate the translation of mRNA to protein) or short-interfering RNAs (siRNAs, capable of

downregulating the translation of mRNA to protein). Transcriptome sequencing identifies these regulatory RNA sequences that are not otherwise identified at the DNA or protein level.[4]

Proteins are the third stage in the genetic flow of information, as most RNA is translated into protein products. Proteomics is a qualitative and quantitative assessment of the protein constituents in each biological sample. Mass spectrometry is typically used to identify peptide sequences, and these sequences are used to infer protein identity and quantity. "Shotgun" proteomics is the most common method of identifying and labeling large amounts of proteins, analyzing both the "parent" ions eluting from the liquid chromatographer and the "daughter" ions, which are comprised of fragments of the parent ion. The apparatus then attempts to match the ions using several features, such as signal intensity and mass to charge ratio. From here, the peptide sequences (and therefore proteins) are inferred.[1]

Integration of all three disciplines may be termed "proteogenomic" testing. The drive for "precision" and "personalized" medicine has encouraged more in-depth research on the genetic landscape, particularly for heterogenous conditions such as cancer. Proteogenomic testing has been proposed to fill clinical gaps that exist with disciplines in isolation, including finding the connections from genotype to phenotype. Identifying targeted therapies, drug resistance mechanisms, and other potentially crucial clinical factors are all questions that may be answered with proteogenomic testing. Although the individual methodologies used to perform proteogenomic testing are well-validated in research settings (next generation sequencing [NGS], mass spectrometry), numerous challenges and limitations exist in translating them to the clinical realm. For example, there is currently no amplification technique available for proteins that would allow for smaller samples to be used. Additionally, reproducibility issues presented by the current techniques used in proteomic research can occur. Overall, validation of the enormous database of proteomics, as well as development of the bioinformatic infrastructure required to connect proteogenomics to the clinic, is still in progress.[1]

In 2016, the Clinical Proteomic Tumor Analysis Consortium (CPTAC) created the Applied Proteogenomics Organizational Learning and Outcomes (APOLLO) network. The APOLLO network was launched to incorporate proteogenomics into patient care and is a collaborative effort between the Department of Defense (DoD) and the Department of Veterans Affairs (VA). APOLLO is currently analyzing proteogenomic data from 8,000 human tissue samples; this data will be publicly available once curated.[5] As of 2022, the CPTAC has begun to release some individual genomic, proteomic, and imaging data sets from this ongoing research but combined proteogenomic analysis from this data is just beginning to emerge.[6-8]

Proprietary Testing

Several proteogenomic testing platforms are commercially available.

GPS Cancer®

The GPS Cancer® test from NantOmics, a member of the NantWorks family, utilizes quantitative proteomics through mass spectrometry, whole genome sequencing (over 20,000 genes across three billion base pairs), and whole transcriptome sequencing technologies (over 200,000 RNA transcripts). These three factors combined provide oncologists with a comprehensive molecular profile of a patient's cancer. The test is intended to provide information about targeted therapies, such as which therapies a

patient may benefit from or which therapies a patient may resist.[9] Finally, the third component of GPS Cancer® is the tumor "normal" sequencing. This component provides a comparison of a patient's healthy, unaffected genome to the genome affected by the tumor. It is intended to provide "pharmacogenomic analysis for potential drug toxicity and/or interactions" and to separate mutations caused by cancer from those that were present prior to cancer.[9]

DarwinOncoTarget™ and DarwinOncoTreat

DarwinOncoTarget™ and DarwinOncoTreat™ are synergistic proteogenomic tests offered by Columbia University. DarwinOncoTarget™ identifies 193 potentially targetable proteins while DarwinOncoTreat™ assesses the regulatory activity of 6293 proteins ("tumor-checkpoints"). DarwinOncoTreat™ then "prioritizes" drugs based on their ability to revert the activity of these checkpoints. DarwinOncoTarget™ is available for all malignancies whereas DarwinOncoTreat™ is only available for certain cancer subtypes.[10]

Caris Molecular Intelligence

Other proprietary proteogenomic platforms are offered by Caris Molecular Intelligence. The MI Cancer Seek utilizes an NGS technique to identify "Whole exome sequencing for DNA mutations, copy number alterations, insertions/deletions, genomic signatures MSI [microsatellite instability] and TMB [tumor mutational burden], and whole transcriptome sequencing for RNA fusions and variant transcripts."[11] The Caris Molecular Intelligence Comprehensive Tumor Profiling test uses precision medicine to assess DNA, RNA, and proteins to aid individualized treatment regimens.[12]

Praxis Genomics Optical Genome Mapping, Whole Genome Sequencing, and Transcriptome Analysis

Praxis Genomics offers a proteogenomic approach via combined testing with their Optical Genome Mapping, Whole Genome Sequencing, and Transcriptome Analysis. Optimal Genome Mapping (OGM), developed by Bionano Genomics LLC, evaluates DNA samples for large-scale changes such as chromosomal transfer of DNA fragments, chromosomal inversion or complex rearrangement, measurement of repetitive regions that control adjacent gene expression, and measurement of tandem repeat expansions. Whole Genome Sequencing obtains sequence from the entire genome (roughly 3 billion units). Praxis uses the Dragen alignment and variant calling pipeline to identify genomic changes. Highly repetitive DNA content and genomic rearrangements cannot be detected with whole genome sequencing. Transcriptome analysis allows for the evaluation of the functional consequences of DNA mutations found in Optical Genome Mapping or Whole Genome Sequencing. "Only by building on the diverse strength of OGM and ISR [Illumina Short Read] WGS and transcriptome sequencing can we increase the sensitivity and specificity of genetic diagnosis until novel technologies even more precise and faster come along."[13]

OncoSignal™

Philips' OncoSignal™ technology is used by Protean BioDiagnostics as an "innovative and unique test to expand the information that can be obtained from cancer tissue analysis," as part of their proprietary Oncology MAPS™ solution. OncoSignal™ uses advanced molecular and bioinformatic systems to measure mRNA expression patterns, calculating the specific activity of seven key oncogenic drive signal

pathways (Estrogen receptor, Androgen receptor, Phosphoinositide 3-kinase, Hedgehog pathway, Notch signal, transforming growth factor receptor beta, and Mitogen activated protean kinase). These pathways "measure key oncogenic drivers of numerous distinct cancer types including but not limited to breast cancer, prostate cancer, ovarian cancer, colon cancer, lymphoma and more." Each pathway is given a score based on molecular and bioinformatic findings and pathway activity is interpreted as low, normal, or high in comparison to the normal physiological range. The clinically-used Oncology MAPS™ report then provides targeted treatment recommendations.[14,15]

MosaicNeedle™

Nanomosaic Inc. developed MosaicNeedle™ technology for proteomic and multi-omic investigation and application.[16] The commercial launch of their Tessie™ platform for proteomics and multi-omics is slated to occur in 2022. According to Nanomosaic, their proteomics technology "bridges the gaps of functional information lost due to post-transcriptional and post-translational modifications in a genomic approach." Because the traditional proteogenomic approach typically requires multiple diverse assays and workflows, this novel technology simplifies by using "densely integrated nanoneedle sensors on a planar substrate that integrates proteogenomic analysis in one platform." Each nanoneedle binds a single molecule and detects both nucleic acids and proteins in a single assay process. The result is a full proteogenomic quantification in one single reaction, on one platform, with high sensitivity and lower cost.[17]

Clinical Utility and Validity

North, et al. (2018) used NantHealth's profile to characterize 32 cases of sebaceous carcinomas (SeC). The authors identified ultraviolet (UV) damage in ten samples and microsatellite instability in nine samples. UV cases of SeC were shown to have more severe histopathologic features, including poorer differentiation and an "infiltrative" growth pattern. The authors also noted that the transcriptomes of the UV SeC cases were like cutaneous squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs). Overall, three distinct classes of sebaceous carcinoma were identified based on mutation pattern and cell of origin.[18]

Liao, et al. (2015) used two genetic datasets (TCGA and METABRIC) to characterize over 2500 cases of breast cancer in pre-menopausal (preM) and post-menopausal individuals (postM) (defined as ≤45 years and ≥55 years respectively, individuals of ages 45-55 were not included). The following molecular features were examined: gene expression, somatic mutation, copy number, methylation, and protein expression. In preM tumors, the authors identified unique methylation patterns, copy numbers, and somatic mutations in estrogen receptor-positive (ER+) tumors. Further investigation of this subset revealed "elevated integrin/laminin and EGFR signaling, with enrichment for upstream TGFβ-regulation." The authors concluded that preM ER+ tumors have "distinct molecular characteristics" compared to postM ER+ tumors.[19]

Rabizadeh, et al. (2018) compared tumor-only DNA sequencing to tumor-normal DNA (containing controls for germline mutations) plus RNA sequencing. A total of 621 patients with 30 different cancer types were studied using a 35-gene sequencer and the precision of somatic variant calling was evaluated. Without the germline controls, 94% of the variants were single nucleotide polymorphisms (SNPs) and considered false positives. Removing these SNPs resulted in a 48% false-positive rate. Tumor-only

sequencing ultimately led to a 29% false-positive rate in "at least one of twelve genes with directly targetable drugs" and RNA analysis revealed that 18% of variants were not expressed.[20]

Sjostrom, et al. (2020) evaluated the utility of transcriptomic profiling in breast cancer and whether these results could safely allow patients to decline systemic therapy. A 141-gene signature was derived from a node-negative cohort previously untreated with chemotherapy. This signature was used to evaluate 454 node-negative, ER+, and systemically untreated cancer patients. The authors noted that this was a low-risk subgroup but did find that of the patients in the lowest 25th percentile of signature scores, 95% of patients were metastasis-free after 15 years despite lack of endocrine therapy. The authors concluded that "transcriptomic profiling identifies patients with an excellent outcome without any systemic adjuvant therapy in clinically low-risk patients of…two separate cohorts."[21]

Feng, et al. (2019) evaluated the proteomic profile of sorafenib resistance in hepatocellular carcinoma patients. Tumor samples from 60 patients were examined. The authors identified three proteins that were overexpressed in sorafenib-resistant cells: 78 kDa glucose related protein (GRP78), 14-3-3ε, and heat shock protein 90β (HSP90β). Approximately 73% of cells had high GRP78 expression, 18% had high 14-3-3ε expression, and 85% had high HSP90β expression. The authors also noted that elevated GRP78 was associated with the shortest progression-free survival of patients treated with sorafenib. The authors concluded that "GRP78 can be a predictive biomarker in HCC patients treated with sorafenib."[22]

Shiba, et al. (2019) evaluated the genetic landscape of pediatric acute myeloid leukemia. The authors performed a transcriptome analysis in 139 patients (of 369 in the total cohort). Fifty-four in-frame gene mutations and one *RUNX1* out-of-frame fusion were found in 53 of the 139 patients. Moreover, 258 gene fusions were found in the 369 total patients. Five novel gene fusions were found, and several fewer common gene rearrangements were identified. Out of the 111 remaining patients, "*KMT2A-PTD*, biallelic *CEBPA*, and *NPM1* gene mutations were found in 11, 23, and 17 patients, respectively." The authors noted these mutations were mutually exclusive compared with other gene fusions. The authors remark that risk stratification should be reconsidered.[23]

Yang, et al. (2019) evaluated the genomic landscape of rectal cancer patients who did not respond to chemotherapy. The authors performed whole exome sequencing on 28 paired tumors collected before and after chemotherapy. Several mutations were identified (*CTDSP2, APC, KRAS, TP53* and *NFKBIZ*) that appeared to confer selective advantages to cancer cells and the authors noted that chemotherapy altered the genomic landscape of these tumors and that high intratumoral heterogeneity in any stage of cancer contributed to poor survival in patients.[24]

Tredan, et al. (2019) evaluated the impact of broad molecular profiling on identifying targeted therapies. A "molecular tumor board," consisting of molecular biologists, medical oncologists, and pathologists, selected the genes to be included in the profile and a total of 69 genes were included on the final panel. A total of 1980 molecular profiles were constructed. Of these profiles, 948 had no actionable mutations (leaving 1032 with at least one actionable mutation) and a targeted therapy was recommended for 699 of these patients. A total of 182 targeted therapies were initiated, with only 23 patients experiencing an objective response (13% of patients receiving therapy, 0.9% of the total cohort of 2579 patients). The authors concluded that "molecular screening should not be used at present to guide decision-making in routine clinical practice outside of clinical trials."[25]

Kwon, et al. (2019) identified and analyzed mutant peptides in prostate cancer cell lines. The authors obtained four cell lines of varying aggression (LNCaP, LNCaP-LN3, PC-3, and PC-3M) and profiled the resulting mutant peptides. A total of 70 mutant peptides were identified. Expression of seven mutant peptides were found to be altered in tumors, with "CAPN2 D22E" as the most significantly up-regulated peptide. Increased levels of the *INTS7* gene and decreased levels of the *SH3BGRL* gene were found to be correlated with aggressiveness of prostate cancer.[26]

Wang, et al. (2019) constructed a "quantitative proteome and transcriptome abundance atlas" of 29 paired healthy human tissues. A total of 18,072 transcripts and 13,640 proteins (including 37 without "prior protein-level evidence") were represented. However, the authors concluded that proteogenomics remains challenging. The authors noted that out of 9848 amino acid variants found by exome sequencing, only 238 could be confidently detected at the protein level. The authors also remarked that many proteins could not be detected despite highly expressed mRNA, that very few proteins showed tissue-specific expression, and that strong differences existed between mRNA and protein quantities. Overall, the writers determined that proteogenomics "needs better computational methods and requires rigorous validation."[27]

Treue, et al. (2019) performed analysis on a model of a drug-resistant *EGFR*-mutated non-small cell lung cancer case. The authors integrated several proteogenomic techniques, including whole exome sequencing and "global time-course discovery phosphoproteomics" to identify molecular alterations. The writers remarked that this allowed them to reduce the complexity of the model down to 44 "predicted" phosphoproteins and 35 "topologically close" genetic alterations. From here, the authors found that targeting of HSPB1, DBNL, and AKT1 showed "potent antiproliferative effects overcoming resistance against EGFR-inhibitory therapy."[28]

Salem, et al. (2018) evaluated the correlation of tumor mutational load (TML; "high" defined as greater than or equal to 17 mutations/MB), PD-L1 expression, and mismatch repair deficiency (dMMR) status with response to immune checkpoint inhibitors (ICIs). A total of 4125 tumors from 14 different gastrointestinal sites were examined. A 592 gene panel was used to calculate the TML. Microsatellite instability (MSI), PD-L1 expression, and dMMR status were all evaluated. The authors found that high-TML was "strongly associated" with high MSI. Right-sided colon and small-bowel adenocarcinomas had the highest rates of high-TML tumors (14.6% and 10.2% respectively) whereas pancreatic neuroendocrine and gastrointestinal stromal tumors had the lowest (1.3%, 0%). The authors noted that high-TML rate varied "widely" among gastrointestinal cancers.[29]

Gillette, et al. (2020) utilized proteogenomics to reveal therapeutic vulnerabilities in lung adenocarcinoma. Comprehensive proteogenomic characterization was performed on 110 tumors. "Multi-omics clustering revealed four subgroups defined by key driver mutations, country, and gender."[30] Therapeutic vulnerabilities were identified in the *KRAS, EGFR,* and *ALK* genes and the authors note that "this proteogenomics dataset represents a unique public resource for researchers and clinicians seeking to better understand and treat lung adenocarcinomas."[30]

Krug, et al. (2020) integrated mass spectrometry-based proteomics and next generation DNA and RNA sequencing to create a proteogenomic profile of 122 treatment-naïve primary breast cancer tumors. They found that "proteogenomics challenged standard breast cancer diagnoses, provided detailed

analysis of the ERBB2 amplicon, defined tumor subsets that could benefit from immune checkpoint therapy, and allowed more accurate assessment of Rb status for prediction of CDK4/6 inhibitor responsiveness." The authors note that their results "underscore the potential of proteogenomics for clinical investigation of breast cancer through more accurate annotation of targetable pathways and biological features of this remarkably heterogeneous malignancy."[6]

Wang, et al. (2021) integrated genomic, proteomic, post-translational modification, and metabolomic data to examine 99 treatment-naïve glioblastomas (GBMs), where they identified key phosphorylation events as potential mediators of oncogenic pathway activation and potential targets for *EGFR*-, *TP53*-, and *RB1*-altered tumors. The identified "immune subtypes with distinct immune cell types are discovered using bulk omics methodologies" and note that "histone H2B acetylation in classical-like and immune-low GBM is driven largely by BRDs, CREBBP, and EP300." Their work highlights the important of an integrated proteogenomic approach in GBM and "highlights biological relationships that could contribute to stratification of GBM patients for more effective treatment."[7]

Joshi, et al. (2021) examined the stepwise evolution of gilteritinib resistance in *FLT3*-mutated acute myeloid leukemia (AML). To mechanistically define both early and late resistance in AML, they integrated whole exome sequencing, CRISPR-CAS9, metabolomics, proteomics, and pharmacologic approaches. They found that "early resistant cells undergo metabolic reprogramming, grow more slowly, and are dependent upon Aurora kinase B (AURKB). Late resistant cells are characterized by expansion of pre-existing NRAS mutant subclones and continued metabolic reprogramming," creating a model that closely mirrors the timing, and mutations of AML patients treated with gilteritinib. They also note that "pharmacological inhibition of AURKB resensitizes both early resistant cell cultures and primary leukemia cells from gilteritinib-treated AML patients." Their findings support a "combinatorial strategy to target early resistant AML cells with AURKB inhibitors and gilteritinib before the expansion of pre-existing resistance mutations occurs."[31]

Akcakanat, et al. (2021) integrated DNA, RNA, and functional proteogenomics from tumor samples of 52 patients with metastatic breast cancer (inclusive of ten patients with both primary and metastatic tumors to chart the evolution of the tumor's profile). The aim was to determine potentially actionable alterations in metastatic breast cancer and analyze the molecular evolution of the tumors. Regarding proteomic profiling, needle biopsy samples were evaluated via reverse phase protein arrays. The panel was composed of 295 antibodies and the PI3K pathway activity score was "defined as the sum of the normalized values of the 10phosphor-protein levels of Akt, 4E-BP1, S6K, and S6." Samples were considered PI3K activated if their PI3K scores were in the top quartile. In discussion, the authors noted: "Neither genomic alterations predicted gene or protein expression nor was there a strong correlation between proteomic and transcriptomic data. [However] we cannot exclude the possibility that the lack of concordance between DNA alterations and RNA and protein and protein phosphorylation are not merely the result of tumor heterogeneity and the tumor cellularity of the samples."[32]

Yoon, et al. (2024) performed an integrated proteogenomic analysis that combined genomic, transcriptomic, and proteomic data on 126 treatment-naïve primary tumor samples from Korean women aged 40 or younger diagnosed with early-onset breast cancer (YBC). Through this multi-omics approach, the authors identified five distinct functional subgroups that aligned closely with clinical traits and tumor behaviors. Notably, they demonstrated that "proteogenomic analysis integrating the

phosphorylation status of HER2 can be used to identify true HER2+ patients."[33] Moreover, the author noted that, "through proteomic profiling, not only quantitative expression but also posttranslational modification of HER2 could be used for a more precise classification." They also "identified the proteogenomic status of HER2 as a potential prognostic marker among patients with amplified HER2, as patients with HER2 PG+ status tended to have poorer clinical outcomes than PG- patients."[33] These findings highlight the potential of a proteogenomic to refine risk stratification and guide adjuvant treatment duration in young breast cancer (YBC) patients.

V. Guidelines and Recommendations

Since this is an emerging field, there is limited guidance from applicable professional societies. As of publication date, no specific guidance was found from professional medical societies, including NCCN, ACMG, NICE, AMP, and CAP.

National Comprehensive Cancer Network

Currently, the NCCN does not list proteogenomic testing as a recommended technique for any type of cancer. Furthermore, proprietary comprehensive genomic profiles have been submitted for inclusion in guidelines for several types of cancer, but they have never been included as a recommended technique as of June 23, 2022.[34]

American Society of Clinical Oncology

In 2020, the American Society of Clinical Oncology (ASCO) published a clinical oncology educational book which included an article on integrating genetic and genomic testing into oncology practice. Transcriptome and proteomic sequencing were not mentioned in the article. However, the authors note that "Examples of the integration of genomic information into the care of patients with cancer include germline testing for *BRCA1/2* in breast, ovarian, pancreatic, and prostate cancer; evaluation of mismatch repair in endometrial cancer; and somatic sequencing in lung cancer."[35] In 2022, they published a guideline on "Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer," where it was noted that "although tumor histology, grade, stage, and estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2) expression should routinely be used to guide clinical decisions, there is insufficient evidence to support the use of other markers or genomic profiles."[36]

In 2022, ASCO published a provisional clinical opinion titled, "Somatic Genomic Testing in Patients with Metastatic or Advanced Cancer." They note an increasing number of therapies have been approved to treat cancers harboring specific genomic biomarkers. The following represents their recommendations:[37]

- "Patients with metastatic or advanced cancer should undergo genomic sequencing in a certified laboratory if the presence of one or more specific genomic alterations has regulatory approval as biomarkers to guide the use of or exclusion from certain treatments for their disease."
- "Multigene panel–based assays should be used if more than one biomarker-linked therapy is approved for the patient's disease."

- "Site-agnostic approvals for any cancer with a high tumor mutation burden, mismatch repair deficiency, or neurotrophic tyrosine receptor kinase (NTRK) fusions provide a rationale for genomic testing for all solid tumors."
- "Multigene testing may also assist in treatment selection by identifying additional targets when there are few or no genotype-based therapy approvals for the patient's disease."
- "For treatment planning, the clinician should consider the functional impact of the targeted alteration and expected efficacy of genomic biomarker–linked options relative to other approved or investigational treatments."

Regarding proteogenomic or protein profiling, ASCO recommended:

- "Several hundred genes and their mutant protein products have been linked to increased cell signaling, proliferation, and survival in cell lines or mouse models and thus proposed to be cancer drivers. However, at this time, few genomic alterations have been clinically proven as therapeutic targets. Therefore, in addition to the functional effects of mutations on protein chemistry and signaling, evidence of clinical relevance should be considered."
- "Clinical decision making should incorporate (1) the known or predicted impact of a specific genomic alteration on protein expression or function and (2) clinical data on the efficacy of targeting that genomic alteration with a particular agent (strength of recommendation: strong)."[37,38]

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

Many labs have developed specific tests that they must validate and perform in house. These laboratory developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

VIII. Evidence-based Scientific References

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IX. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81354	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of structural and copy number variants, optical genome mapping (OGM)
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents Proprietary test: DarwinOncoTarget™/DarwinOncoTreat™ Lab/Manufacturer: Columbia University Department of Pathology and Cell Biology/Darwin Health

CPT	Code Description
0260U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Proprietary test: Augusta Optical Genome Mapping Lab/Manufacturer: Georgia Esoteric and Molecular (GEM) Laboratory, LLC/Bionano Genomics Inc
0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score Proprietary test: OncoSignal 7 Pathway Signal Lab/Manufacturer: Protean BioDiagnostics/Philips Electronics Nederland BV
0264U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Proprietary test: Praxis Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue-specific gene expression by whole-transcriptome and next-generation sequencing, blood, formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes Proprietary test: Praxis Transcriptome Lab/Manufacturer: Praxis Genomics LLC
0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing Proprietary test: Praxis Combined Whole Genome Sequencing and Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0298U	Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification Proprietary test: Praxis Somatic Transcriptome Lab/Manufacturer: Praxis Genomics LLC
0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification Proprietary test: Praxis Somatic Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification Proprietary test: Praxis Somatic Combined Whole Genome Sequencing and Optical

CPT	Code Description
	Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0436U	Oncology (lung), plasma analysis of 388 proteins, using aptamer-based proteomics technology, predictive algorithm reported as clinical benefit from immune checkpoint inhibitor therapy. Proprietary test: PROphet® NSCLC Test Lab/Manufacturer: OncoHost, Inc
0454U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Proprietary test: Chromosome Genome Mapping Lab/Manufacturer: UR Medicine Lab, Bionano Genomics, Inc
0485U	Oncology (solid tumor), cell-free DNA and RNA by next-generation sequencing, interpretative report for germline mutations, clonal hematopoiesis of indeterminate potential, and tumor-derived single-nucleotide variants, small insertions/deletions, copy number alterations, fusions, microsatellite instability, and tumor mutational burden Proprietary test: Caris Assure Lab/Manufacturer: Caris MPI, Inc d/b/a Caris Life Sciences
0597U	Oncology (breast), RNA expression profiling of 329 genes by targeted nextgeneration sequencing and 20 proteins by multiplex immunofluorescence, formalin-fixed paraffinembedded (FFPE) tissue, algorithmic analyses to determine tumor-recurrence risk score Proprietary test: AidaBreast™, PreludeDx™ Lab/Manufacturer: Prelude Corporation

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

X. Policy History

Action Date	Action
June 01, 2023	Policy created
Marhc 17, 2026	Policy approved by Medical Directors
March 27, 2026	Policy approved at UMC
August 1, 2026	Policy effective date following notification period