GENOMIC TESTING COOPERATIVE

175 Technology Drive, Suite 100, Irvine, CA 92618
Tel: 1-866-484-8870 genomictestingcooperative.com
CLIA #: 05D2111917 CAP #: 9441547

Hematology Profile Plus

Patient Name:		Ordering Physician:	
Date of Birth:	10/11/1960	Physician ID:	
Gender (M/F):	M	Accession #:	100712
Client:		Specimen Type:	Tissue
Case #:		Specimen ID:	
Body Site:	Lymph node		

MRN:				Indication for Testing:	C83.90 Non-follicular (diffuse) lymphoma unspecified, unspecified site
Collected Date:	02/18/2019	Time:	12:00 AM		
Received Date:	08/07/2019	Time:	11:50 AM		
Reported Date:	08/20/2019	Time:	08:13 AM		

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS), fragment length analysis and Sanger Sequencing testing to identify molecular abnormalities in DNA of 177 genes and RNA in 68 genes implicated in hematologic neoplasms, including leukemia, lymphoma melanoma and MDS. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Detected Genomic Alterations				
PRDM1	CD79B (Double)	CDK12	KDM6A	HUWE1
ARHGEF12	SPOP			

Heterogeneity
There is a dominant abnormal clone with PRDM1 mutation. The CD79B, CDK12, KDM6A, HUWE1, ARHGEF12, and SPOP mutations are detected in subclones.

Expression	
Expression of B-cell markers (CD19, CD22, CD79A, PAX5). However, CD79B mRNA is relatively low.	Significantly high expression of PD-L1 and CTLA4 mRNA
High expression of Ki67 mRNA	Expression profile consistent with ABC type 1

Diagnostic Implications	
PRDM1, CD79B, CDK12, KDM6A, HUWE1, ARHGEF12, SPOP	These abnormalities are consistent with aggressive neoplasm.

Therapeutic Implications	
CD79B	Antibody-drug conjugates targeting CD79B (polatuzumab vedotin)

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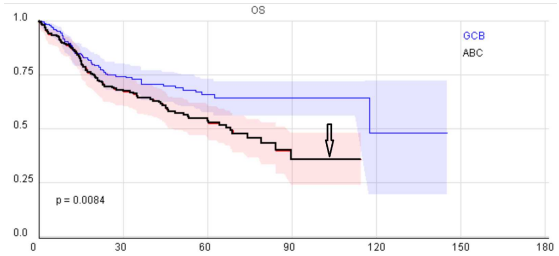
Prognostic Implications	
PRDM1	Poor
CD79B	Poor
CDK12	Poor
KDM6A	Poor
HUWE1	Unknown
ARHGEF12	Unknown
SPOP	Unknown

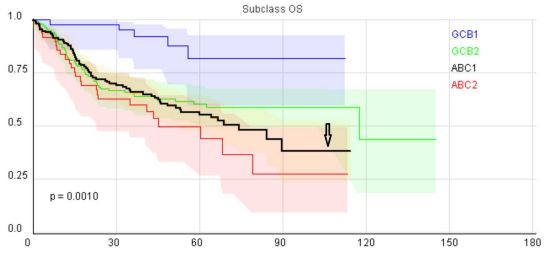
Relevant Genes with NO Alteration
No evidence of mutation in: FLT3, NPM1, IDH1 and IDH2

Results Summary

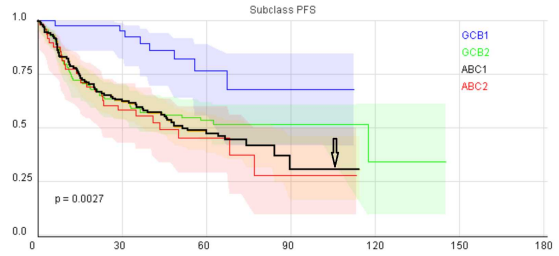
- -MUTATIONS IN PRDM1, CD79B, CDK12, KDM6A, HUWE1, ARHGEF12, AND SPOP GENES.
  -HIGH EXPRESSION OF PD-L1 mRNA.
  -EXPRESSION PROFILE CONSISTENT WITH B-CELL LYMPHOMA OF ABC TYPE, BUT RELATIVELY LESS AGGRESSIVE (ABC1)
  -The findings are consistent with diffuse large B-cell lymphoma of ABC subtype.
  -The presence of CD79B mutation raises the possibility of response to antibody-drug conjugates targeting CD79B (polatuzumab vedotin).



Overall survival of patients with expression profile consistent with ABC



Overall survival of patients with expression profile consistent with ABC1 subtype



Progression free survival of patients with expression profile consistent with ABC1 subtype

Biological relevance of detected Alterations

- PRDM1 encodes a transcriptional repressor involved in the cellular response to viral infection and B-cell differentiation. Deletions of PRDM1 are found in diffuse large B-cell lymphomas and prostate cancer.
BACKGROUND
PRDM1, also known as BLIMP-1 and PRDI-BF1, encodes PR domain zinc finger 1 (PRDM1), a DNA-binding protein with five zinc fingers (PMID: 1851123). PRDM1 was initially described as a transcriptional repressor of interferon ß, and as an essential component of B cell differentiation, wherein it represses MYC (PMID: 1851123, 9887105, 8168136, 9110979. It has been shown to function by interaction with the Groucho family of transcriptional co-repressors as well histone-modifying factors (PMID: 9887105, 14985713, 19124609). More recent results from animal studies indicate PRDM1 as an important factor in establishment in the germ cell lineage, T cell differentiation and homeostasis and heart function (PMID: 15937476, 16565720, 24821700). Two single-nucleotide polymorphisms (SNPs) located intergenic to PRDM1 and ATG5 have been associated with increased risk for radiation therapyinduced second malignant neoplasms after radiotherapy for pediatric Hodgkin's lymphoma (PMID: 21785431).

- CD79B, a component of the B-cell antigen receptor, is recurrently altered in diffuse large B-cell lymphoma.
BACKGROUND
CD79B is a surface immunoglobulin that forms a complex with CD79A to form a component of the B-cell receptor (BCR) (PMID:1439759). The CD79 (Iga/Igb) complex is important for signaling from the BCR to support maturation and survival of B-cells throughout development (PMID:15186779, 8602530). The cytoplasmic portion of CD79B contains immunoreceptor tyrosine-based activation motif (ITAM) domains that become phosphorylated by SRC proto-oncogene family protein kinases. These regions serve as docking sites for signaling complex formation with kinases such as SYK (spleen tyrosine kinase) and for signal regulation (PMID: 17114463, 20940318, 22078222). Activating mutations of CD79B have been identified in diffuse large B-cell lymphoma, particularly the activated B-cell-like subtype (PMID: 20054396) suggesting that CD79B acts as an oncogene. CD79B mutations result in increased surface BCR expression, reduced BCR internalization and dampening of LYN (Lyn proto-oncogene) kinase feedback inhibition, thus resulting in increased BCR signaling. Somatic mutation in the protein's ITAM domain have also been identified and shown to affect signaling (PMID: 20054396). Germline CD79B mutation leads to agammaglobulinemia, a severe immunodeficiency syndrome with B-cell dysfunction (PMID: 17709424). Antibody-drug conjugates targeting CD79B and inhibitors targeting SRC activity, such as dasatinib, can reduce BCR signaling (PMID: 17374736, 20054396, 25925619, 28009435).

- CDK12 (Cyclin-dependent kinase 12) is a kinase involved in the regulation of the cell cycle and the regulation of transcriptional elongation of many DNA-damage-response genes (PMID: 11683387, 22012619). Recurrent inactivating CDK12 mutations in metastatic prostate and serous ovarian cancers have been observed (PMID: 28843286, PMID: 26787835). CDK12 interacts with and phosphorylates the C-terminal domain (CTD) of RNA polymerase II (RNAP) in vitro (PMID: 11683387) and complexes with Cyclin K to maintain genomic stability via regulation of the expression of DNA damage response genes, such as BRCA1, ATR, FANCI and FANCD2. Loss of the CDK12/cyclin K complex renders HEK293 cells sensitive to various DNA damaging agents, including camptothecin, etoposide and mitomycin C (PMID: 22012619, 24662513). CDK12 is one of the most frequently somatically mutated genes in ovarian cancer (PMID: 21720365), which is consistent with its role in the maintenance of genomic stability. The genomic location of CDK12, is adjacent to that of ERBB2, and while there is some laboratory data that associates an amplification of CDK12 to a tumorigenic phenotype (PMID: 28187285, 27880910, 28334900), it is likely that CDK12 amplification is a passenger event in this context, co-occurring with ERBB2 amplification in breast and gastric cancers (PMID: 20932292, 21097718, 26658019).
SUMMARY
CDK12, a cyclin dependent kinase, is recurrently mutated in metastatic prostate and serous ovarian cancers.

- HUWE1 (HECT, UBA And WWE Domain Containing E3 Ubiquitin Protein Ligase 1) gene is located at Xp11.22 and encodes for a protein containing a C-terminal HECT (E6AP type E3 ubiquitin protein ligase) domain that functions as an E3 ubiquitin ligase. The encoded protein is required for the ubiquitination and subsequent degradation of the anti-apoptotic protein Mcl1 (myeloid cell leukemia sequence 1 (BCL2-related)). This protein also ubiquitinates the p53 tumor suppressor, core histones, and DNA polymerase beta. Mutations in this gene are associated with Turner type X-linked syndromic cognitive disability.

- ARHGEF12 (Rho Guanine Nucleotide Exchange Factor 12) gene is located at 11q23.3 and encodes for Rho GTPases, which play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli working through G protein-coupled receptors. The encoded protein may form a complex with G proteins and stimulate Rho-dependent signals. This protein has been observed to form a myeloid/lymphoid fusion partner in acute myeloid leukemia.

- SPOP encodes an adaptor protein involved in targeting proteins for degradation. SPOP mutations are predominantly found in prostate and endometrial cancers; however, the full functional consequence of these mutations remains under investigation.
BACKGROUND

SPOP (Speckle-type POZ protein) is an adaptor protein in the CUL3 ubiquitin ligase complex that recognizes substrates for ubiquitination and subsequent degradation via the proteasome (PMID: 19818708). The repertoire of SPOP substrates is not well characterized; however, notable proteins include SRC3, DAXX, H2AFY, AR, BMI1, DEK, ESR1 and TRIM24 (PMID: 25278611, 15897469, 25274033, 21577200, 25766326). The CUL3-SPOP complex negatively regulates the transcriptional repressor DAXX, hence impacting the expression of endothelial pathway genes that are regulated by DAXX (PMID: 28216678). Somatic mutations in SPOP are reported in approximately 5% of endometrial cancers (PMID: 23104009) and 10% of prostate cancers (PMID: 21307934, 22610119). SPOP-mediated degradation has also been implicated in the regulation of PD-L1, a key regulatory immune ligand (PMID: 29160310). SPOP mutations in both endometrial and prostate cancer cluster in conserved residues of the MATH domain important for substrate recognition, suggesting that the mutations either alter substrate recognition or act as a dominant negative to prevent substrate degradation (PMID: 21307934, 22610119). There is emerging evidence that SPOP may be a more general tumor suppressor in glioblastoma, gastric and colorectal cancers, as SPOP expression is decreased through tumor progression (PMID: 25351530, 23216165).

- KDM6A, a histone demethylase, is altered in various cancer types, most frequently in bladder cancer.
BACKGROUND
KDM6A (lysine-specific demethylase 6A) encodes a chromatin-modifying enzyme that mediates transcriptional co-activation by functioning as a di- and tri-methylated histone H3 lysine 27 (H3K27) demethylase. KDM6A is part of the larger ASC-2 complex (ASCOM) that also contains lysine-specific methyltransferase 2D (KMT2D) and lysine-specific methyltransferase 2C (KMT2C). KDM6A is located on Xp11.2, but it escapes X inactivation, resulting in bi-allelic expression in females (PMID: 9499428). Association of KDM6A with KMT2D and KMT2C couples H3K27 demethylation to H3K4 methylation (PMID: 17761849). Germline deletions and point mutations in KDM6A cause Kabuki syndrome, which is characterized by typical facial features, skeletal anomalies, dermatoglyphic abnormalities, mild-to-moderate intellectual disability and postnatal short stature (PMID: 22197486, 22840376, 22901312, 23076834). Early sequencing efforts led to the discovery of inactivating KDM6A mutations in a number of human malignancies including multiple myeloma and esophageal squamous cell carcinoma (PMID: 19330029). Later studies found KDM6A mutations in clear cell renal cell carcinoma (PMID: 21248752), medulloblastoma (PMID: 22722829, 22832583), adenoid cystic carcinoma (PMID: 23685749), urothelial bladder cancer (PMID: 24476821, 21822268, 25092538, 25225064), aristolochic acid-associated upper tract urothelial carcinoma (PMID: 23926199), T-cell acute lymphoblastic leukemia (PMID: 25320243), and pancreatic cancer (PMID: 25719666). In prostate cancer, KDM6A mutations are seen in progression to lethal castration-resistant disease (PMID: 22722839). Loss of KDM6A may confer sensitivity to EZH2 inhibitors (PMID: 28228601).

Drug Information

POLATUZUMAB VEDOTIN

Polatuzumab vedotin is an antibody targeted to CD79b conjugated to the antineoplastic agent monomethyl auristatin E (MMAE). The antibody binds to CD79b on the surface of B cells, causing the conjugate to be endocytosed. Once inside the cell, lysosomal proteases cleave the link between MMAE and the antibody allowing MMAE to bind to microtubules, inhibit cell division, and induce apoptosis.

This medication is indicated to treat adults with relapsed or refractory diffuse large B-cell lymphoma in combination with bendamustine and rituximab that has returned of progressed after 2 or more previous therapies.

RITUXIMAB (RITUXAN)

Rituximab is a monoclonal antibody that targets the CD20 antigen, which is expressed on the surface of pre-B and mature B-lymphocytes. After binding to CD20, rituximab mediates B-cell lysis (or breakdown). The possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

Rituximab is indicated in the following conditions:
§ Non–Hodgkin's Lymphoma (NHL)
§ Chronic Lymphocytic Leukemia (CLL)
§ Rheumatoid Arthritis (RA) in combination with methotrexate in adult patients with moderately-to severely-active RA
§ Granulomatosis with Polyangiitis (GPA) (Wegener's Granulomatosis) and Microscopic Polyangiitis (MPA)
§ Moderate to severe Pemphigus Vulgaris (PV) in adult patients

ACALABRUTINIB (CALQUENCE)

Acalabrutinib is a small molecule inhibitor of BTK. Both acalabrutinib and its active metabolite, ACP-5862, act to form a covalent bond with a cysteine residue (Cys481) in the BTK active site, leading to inhibition of BTK enzymatic activity 2,3. As a result, acalabrutinib inhibits BTK-mediated activation of downstream signaling proteins CD86 and CD69, which ultimately inhibits malignant B-cell proliferation and survival.

Whereas ibrutinib is typically recognized as the first-in-class BTK inhibitor 2, acalabrutinib is considered a second generation BTK inhibitor primarily because it demonstrates highter selectivity and inhibition of the targeted activity of BTK while having a much greater IC50 or otherwise virtually no inhibition on the kinase activities of ITK, EGFR, ERBB2, ERBB4, JAK3, BLK, FGR, FYN, HCK, LCK, LYN, SRC, and YES1.

In effect, acalabrutinib was rationally designed to be more potent and selective than ibrutinib, all the while demonstrating fewer adverse effects - in theory - because of the drug's minimized bystander effects on targets other than BTK.

VENETOCLAX (VENCLEXTA)

A BCL-2 inhibitor indicated for the treatment of patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL), with or without 17p deletion, who have received at least one prior therapy. Venetoclax induces rapid and potent onset apoptosis of CLL cells, powerful enough to act within 24h and to lead to tumor lysis syndrome. Selective targeting of BCL2 with venetoclax has demonstrated a manageable safety profile and has been shown to induce significant response in patients with relapsed CLL (chronic lymphocytic leukemia) or SLL (small lymphocytic leukemia), including patients with poor prognostic features.

Bendeka (Bendamustine Hydrochloride)

Bendamustine is indicated for use in the treatment of chronic lymphocytic leukemia (CLL) and indolent B-cell non-Hodgkin lymphoma (NHL) that has progressed during or within six months of treatment with rituximab or a rituximab-containing regimen.

Bendamustine is a bifunctional mechlorethamine derivative capable of forming electrophilic alkyl groups that covalently bond to other molecules. Through this function as an alkylating agent, bendamustine causes intra- and inter-strand crosslinks between DNA bases resulting in cell death. It is active against both active and quiescent cells, although the exact mechanism of action is unknown.

Indication and associated conditions:

Chronic Lymphocytic Leukaemia (CLL)
Follicular Non-Hodgkin's Lymphoma Refractory
Refractory Hodgkin Lymphoma
Refractory Mantle Cell Lymphoma
Waldenström's Macroglobulinemia (WM)
Recurrent multiple myeloma
Refractory indolent B cell non-hodgkin lymphoma

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://ClinicalTrials.gov/show/NCT03450343	Recruiting	Oral Azacitidine Plus Salvage Chemotherapy in Relapsed/Refractory Diffuse Large B Cell Lymphoma	Large B-Cell Diffuse Lymphoma	Oral azacitidine R-ICE	Medical University of South Carolina, Charleston, South Carolina, United States
https://ClinicalTrials.gov/show/NCT03630159	Recruiting	Study of Tisagenlecleucel in Combination With Pembrolizumab in r/r Diffuse Large B-cell Lymphoma Patients	Diffuse Large B-cell Lymphoma	Tisagenlecleucel Pembrolizumab	Emory University School of Medicine SC CTL019, Atlanta, Georgia, United States University of Chicago Medical Center, Hematology & Oncology, Chicago, Illinois, United States University of Kansas Hospital and Medical Center U of Kansas Cancer Center, Kansas City, Kansas, United States (and 4 more sites)
https://ClinicalTrials.gov/show/NCT03876028	Recruiting	Study of Tisagenlecleucel in Combination With Ibrutinib in r/r Diffuse Large B-cell Lymphoma Patients	Diffuse Large B-cell Lymphoma	Tisagenlecleucel Ibrutinib	H Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, United States University of Pennsylvania, Abramson Cancer Center, Philadelphia, Pennsylvania, United States

https://ClinicalTrials.gov/show/NCT03688152	Recruiting	A Safety and Tolerability Study of INCB053914 in Combination With INCB050465 in Diffuse Large B-Cell Lymphoma	Relapsed Diffuse Large B-Cell Lymphoma	INCB053914 INCB050465	University of Arizona Cancer Center, Tucson, Arizona, United States UCLA Healthcare Hematology-Oncology, Santa Monica, California, United States Clinical Research Alliance, Lake Success, New York, United States
https://ClinicalTrials.gov/show/NCT03681535	Recruiting	Dose-Reduced Consolidation Radiation Therapy in Patients With Diffuse Large B-cell Lymphoma	Diffuse Large B Cell Lymphoma	Radiation Therapy	Mayo Clinic, Rochester, Minnesota, United States Duke University Medical Center, Durham, North Carolina, United States
https://ClinicalTrials.gov/show/NCT02763319	Recruiting	A Trial to Evaluate the Efficacy and Safety of MOR208 With Bendamustine (BEN) Versus Rituximab (RTX) With BEN in Adult Patients With Relapsed or Refractory Diffuse Large B-cell Lymphoma (DLBCL)	Diffuse Large B-cell Lymphoma	Rituximab (RTX) MOR208 Bendamustine (BEN)	MorphoSys Research Site, Anaheim, California, United States MorphoSys Research Site, Bakersfield, California, United States Morphosys Research Site, Burbank, California, United States (and 163 more sites)
https://ClinicalTrials.gov/show/NCT03589469	Recruiting	Study to Evaluate the Efficacy and Safety of Loncastuximab Tesirine in Patients With Relapsed or Refractory Diffuse Large B-Cell Lymphoma	Diffuse Large B-Cell Lymphoma Refractory	Loncastuximab tesirine	City of Hope (City of Hope National Medical Center, City of Hope Medical Center), Duarte, California, United States Compassionate Care Research Group, Inc., at Compassionate Care Medical Group, Inc., Fountain Valley, California, United States UC San Diego Moores Cancer Center, La Jolla, California, United States (and 34 more sites)
https://ClinicalTrials.gov/show/NCT03677154	Recruiting	A Trial of Mosunetuzumab (BTCT4465A) as Consolidation Therapy in Participants With Diffuse Large B-Cell Lymphoma Following First-Line Immunochemotherapy and as Therapy in Participants With	Diffuse Large B-cell Lymphoma	Mosunetuzumab Tocilizumab	University of California, Los Angeles (UCLA) - Hematology/Oncology Santa Monica, Santa Monica, California, United States University of Miami Sylvester Comprehensive Center, Miami, Florida,

		Previously Untreated Diffuse Large B-Cell Lymphoma Who Are Unable to Tolerate Full-Dose Chemotherapy			United States Fort Wayne Medical Institute, Fort Wayne, Indiana, United States (and 15 more sites)
https://ClinicalTrials.gov/show/NCT03274492	Recruiting	A Study Comparing the Efficacy and Safety of Polatuzumab Vedotin With Rituximab-Cyclophosphamide, Doxorubicin, and Prednisone (R-CHP) Versus Rituximab-Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (R-CHOP) in Participants With Diffuse Large B-Cell Lymphoma	Diffuse Large B-Cell Lymphoma	Polatuzumab Vedotin Rituximab Cyclophosphamide Doxorubicin Vincristine Vincristine Placebo Prednisone Polatuzumab vedotin Placebo	University of Alabama at Birmingham, Birmingham, Alabama, United States Southern Cancer Center, Daphne, Alabama, United States City of Hope, Duarte, California, United States (and 253 more sites)
https://ClinicalTrials.gov/show/NCT02405078	Recruiting	Tumor-Specific Clonotype, Metabolic Profile, and PET/CT in Predicting Chemotherapy Response in Patients With Relapsed or Refractory Diffuse Large B-cell Lymphoma	Recurrent Diffuse Large B-Cell Lymphoma	Chemotherapy Computed Tomography Fludeoxyglucose F-18 Positron Emission Tomography	M D Anderson Cancer Center, Houston, Texas, United States
https://ClinicalTrials.gov/show/NCT03391466	Recruiting	Efficacy of Axicabtagene Ciloleucel Compared to Standard of Care Therapy in Subjects With Relapsed/Refractory Diffuse Large B Cell Lymphoma	Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL)	Axicabtagene Ciloleucel Platinum-containing salvage chemotherapy (eg, R-ICE) followed by high dose therapy (eg, BEAM) and autologous stem cell transplant in responders. Cyclophosphamide Fludarabine	University of Alabama at Birmingham, Birmingham, Alabama, United States Banner MD Anderson Cancer Center, Gilbert, Arizona, United States Mayo Clinic Hospital, Phoenix, Arizona, United States (and 74 more sites)
https://ClinicalTrials.gov/show/NCT03939182	Recruiting	Abexinostat and Ibrutinib in Diffuse Large B-cell Lymphoma and Mantle Cell Lymphoma	Diffuse Large B-cell Lymphoma	Abexinostat Ibrutinib	Memoral Sloan Kettering Basking Ridge, Basking Ridge, New Jersey, United States Memoral Sloan Kettering Monmouth, Middletown, New Jersey, United States Memorial Sloan Kettering Commack, Commack, New York, United States (and 3 more sites)
https://ClinicalTrials.gov/show/NCT03684694	Recruiting	Safety and Antitumor Activity Study of Loncastuximab Tesirine + Ibrutinib in Diffuse Large B-Cell or Mantle Cell	Diffuse Large B-Cell Lymphoma	Loncastuximab Tesirine and Ibrutinib	Georgia Cancer Center at Augusta University, Augusta, Georgia, United States Norton Cancer Institute, St.

		Lymphoma			Matthews Campus, Louisville, Kentucky, United States The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, United States (and 2 more sites)
https://ClinicalTrials.gov/show/NCT03135262	Recruiting	A Study of Obinutuzumab in Combination With Idasanutlin and Venetoclax in Participants With Relapsed or Refractory (R/R) Follicular Lymphoma (FL) or Rituximab in Combination With Idasanutlin and Venetoclax in Participants With R/R Diffuse Large B-Cell Lymphoma (DLBCL)	Follicular Lymphoma	Idasanutlin Obinutuzumab Venetoclax Rituximab	University of Colorado, Aurora, Colorado, United States The University of Chicago Medical Center, Chicago, Illinois, United States Norton Cancer Institute - Dutchmans, Louisville, Kentucky, United States (and 22 more sites)
https://ClinicalTrials.gov/show/NCT02658968	Recruiting	Study of Betalutin for Treatment of Relapsed or Refractory Non-Hodgkin Lymphoma (LYMRIT-37-05)	Relapsed, Diffuse Large B-cell Lymphoma	Betalutin	University of California, San Diego (UCSD) - Moores Cancer Center, San Diego, California, United States University of California, San Francisco (UCSF) - Innovation, Technology & Alliances, San Francisco, California, United States Sylvester Comprehensive Cancer Centre, Miami, Florida, United States (and 7 more sites)
https://ClinicalTrials.gov/show/NCT03136497	Recruiting	A Study of ABT-199 Plus Ibrutinib and Rituximab in Patients With Relapsed/Refractory Diffuse Large B-cell Lymphoma	Relapsed Diffuse Large B-Cell Lymphoma	ABT-199 Ibrutinib Rituximab	John Theurer Cancer Center at Hackensack University Medical Center, Hackensack, New Jersey, United States
https://ClinicalTrials.gov/show/NCT03484819	Recruiting	Copanlisib Hydrochloride and Nivolumab in Treating Patients With Recurrent or Refractory Diffuse Large B-cell Lymphoma or Primary Mediastinal Large B-cell Lymphoma	Recurrent Diffuse Large B-Cell Lymphoma	Copanlisib Copanlisib Hydrochloride Nivolumab	University of Iowa/Holden Comprehensive Cancer Center, Iowa City, Iowa, United States University of Kentucky/Markey Cancer Center, Lexington, Kentucky, United States Mayo Clinic, Rochester, Minnesota,

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Detailed Results

Single Nucleotide Variant (SNV)

Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
PRDM1	NP_001189.2:p.Ala71ValfsTer15	NM_001198.3:c.172_211dupTGTACATACATTGTGAACGACCACCCCTGGGATTCTGGTG	K/KCTYIVNDHPWDSGX	aag/aaGTGTACATACATTGTGAACGACCACCCCTGGGATTCTGGTGg	frameshift_variant	43.85	187	0
CD79B	NP_001035022.1:p.Gly224ValfsTer14	NM_001039933.1:c.669delA	V/X	gtA/gt	frameshift_variant	26.92	78	0
CD79B	NP_001035022.1:p.Val223Leu	NM_001039933.1:c.667G>C	V/L	Gta/Cta	missense_variant	29.11	79	deleterious (0)
CDK12	NP_057591.2:p.Ala164Thr	NM_016507.2:c.490G>A	A/T	Gcg/Acg	missense_variant	26.59	94	0
HUWE1	NP_113584.3:p.Glu2124Ter	NM_031407.5:c.6370G>T	E/*	Gaa/Taa	stop_gained	18.75	32	0
ARHGEF12	NP_056128.1:p.Met552GlufsTer34	NM_015313.2:c.1654_1655delAT	Y/X	TAt/t	frameshift_variant	17.65	40	0
SPOP	NP_003554.1:p.Gln212Ter	NM_003563.3:c.634C>T	Q/*	Cag/Tag	stop_gained	5.81	86	0
KDM6A	NP_066963.2:p.Arg165Ter	NM_021140.2:c.493C>T	R/*	Cga/Tga	stop_gained	2.54	551	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 177 genes that are reported to be altered in various types of hematologic neoplasms. Nucleic acid is isolated from plasma, fresh cells peripheral blood cells or bone marrow), or paraffin-embedded tissue. Testing is performed using massive parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as 50 nucleotides at the 5' and 3' ends of each coding exon. Fragment length analysis is also performed for FLT3 to enhance the detection of large duplication. List of analyzed genes is provided separately. The DNA assay is optimized to be run using 50 ng from fresh cells, 100 ng from FFPE, and 20 ng from cfDNA. Extraction of DNA and RNA from various tissue type is automated. Library for targeted DNA sequencing is based on Single Primer Extension (SPE) chemistry. The DNA sequencing includes all coding exons of 177 genes. In addition to DNA analysis, targeted RNA NGS analysis was performed. This analyzes targeted RNA of 1408 genes for the purpose of quantifying the expression levels. RNA expression levels is performed on FFPE tissue of confirmed diffuse large B-cell lymphoma (DLBCL) and not validated for other types of tissue or diagnosis. The RNA assay is based on hybrid capture of targeted RNA. Duplicates are excluded for levels measurements. The Universal Human Reference (UHR) RNA is used as control. The list of analyzed genes is provided separately. The RNA expression testing for COO analysis in DLBCL is optimized be run using 200 ng. The GTC-Hematology PLUS assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded (FFPE), bone marrow cells, peripheral blood cells, and peripheral blood plasma cell-free DNA (cfDNA) from patients with hematologic neoplasms to detect genomic alterations using a multigene panel. The test is intended to provide information on somatic mutations (point mutations as well as small insertions and deletions) for use by qualified health care professionals in accordance with professional guidelines. This assay is not conclusive or prescriptive for labeled use of any specific therapeutic product. This Assay is a single-site assay performed at Genomic Testing Cooperative. Specifically, the test is indicated for: -Molecular profiling of genomic abnormalities (SNV and indels) in DNA from patients with hematologic neoplasms

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using bone marrow fresh cells, peripheral blood fresh cells, peripheral blood cfDNA and non-decalcified lymphoid tissue in formalin-fixed paraffin-embedded (FFPE). -cfDNA testing is to be used only for detecting abnormalities in myeloid neoplasms (AML, MDS, MPN and aplastic anemia) and not validated for non-myeloid neoplasms. -Determining cell of origin (COO) in tissue with confirmed diffuse large B-cell lymphoma (DLBCL) using algorithm combining NGS data for RNA expression and DNA mutations detected in FFPE. This test is for in vitro complementary diagnosis and classification. It should not be used as the primary diagnosis of hematologic neoplasm or for managing therapy in patients with hematologic neoplasms. Our sequencing method has a typical sensitivity of 3% for detecting hot-spots specific mutations and 5% for other mutations. Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 3% and higher. The FLT3-ITD fragment analysis assay has a sensitivity of 2%-5% for detecting FLT3-ITD in wildtype background. The assay is not designed to detect gene amplification. Based on our validation study, the following regions of the genes listed below are not covered appropriately (<100 X coverage) and sequencing by NGS may not be reliable in these regions. This poor coverage is due to high GC content with inherited problem in obtaining adequate coverage. Region; Transcript; Exon; AA Range; Promoter Range. TNFRSF14.8; NM_003820; 7; 232 to 242. MYCL.117; NM_001033082; 1; 1 to 27. AXIN1.1161; NM_003502; 1; NC. PIK3R2.1897; NM_005027; 6; 200 to 272. KMT2B.1928; NM_014727; 1; 1 to 121. CD79A.1981; NM_001783; 4; 167 to 189. ASXL1.2390; NM_015338; 1; 1 to 19. BCR.2530; NM_021574; 17; 981 to 1017. TERT.3105;;; -59 to -72. TERT.3106;;; -81 to -94. PMS2.3489; NM_001322008; 13; 710 to 757. RHEB.3700; NM_005614; 1; 1 to 18. Variant calling is based on DRAGEN somatic pipeline using tumor-only analysis against the GRCh37 reference genome. Variant calling is based on DRAGEN somatic pipeline using tumor-only analysis against the GRCh37 reference genome. The COO determination is bases on proprietary software using deep learning. Based on our validation data, the COO determination using our methodology showed accuracy and area under the ROC curve (AUC) of 92% and 95% respectively when compared with other expression methods.

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	BCL2	CBL	CDKN2C	DICER1	FAS	IDH2	KMT2A	MPL	PAX5	PTCH1	SMAD2	TGFBR2
AKT1	BCL2L1	CBLB	CEBPA	DNMT3A	FBXW7	IGF1R	KMT2B	MRE11A	PBRM1	PTEN	SMAD4	TP53
AKT2	BCL6	CBLC	CHEK1	EP300	FLT3	IKZF1	KMT2C	MTOR	PDGFRA	PTPN11	SMARCA4	TSC1
AKT3	BCOR	CCND1	CHEK2	ERG	GATA1	IKZF3	KMT2D	MUTYH	PDGFRB	RAD21	SMARCB1	TSC2
ALK	BCORL1	CCND3	CIC	ETV6	GATA2	IRF4	KRAS	MYC	PHF6	RAD50	SMC1A	TSHR
AMER1	BCR	CD274	CREBBP	EZH2	GATA3	JAK1	MAP2K1	MYD88	PIK3CA	RAD51	SMO	WT1
APC	BIRC3	CD79A	CRLF2	FAM175A	GEN1	JAK2	MAP2K2	NFKBIA	PIK3R1	RB1	SOCS1	ZNF217
ARID1A	BLM	CD79B	CSF1R	FAM46C	GNAQ	JAK3	MAP2K4	NOTCH1	PIK3R2	RHOA	SRC	ZRSR2
ARID1B	BRAF	CDH1	CSF3R	FANCA	GNAS	KAT6A	MAP3K1	NOTCH2	PIM1	RNF43	SRSF2	MEF2B
ARID2	BRCA1	CDK12	CTNNA1	FANCC	H3F3A	KDM5C	MAP3K14	NOTCH3	PLCG1	RUNX1	STAG2	
ASXL1	BRCA2	CDK4	CTNNB1	FANCD2	HNF1A	KDM6A	MAPK1	NPM1	POLD1	SDHB	STAT3	
ATM	BTK	CDK6	CUX1	FANCE	HOXB13	KDR	MCL1	NRAS	POLE	SETBP1	STK11	
ATRX	CALR	CDKN2A	CXCR4	FANCF	HSP90AA1	KEAP1	MDM2	NSD1	PPM1D	SETD2	TERT	
B2M	CARD11	CDKN2B	DDR2	FANCG	IDH1	KIT	MDM4	PALB2	PPP2R1A	SF3B1	TET2	

RNA Fusions/Expression

Fusion/Expression																
ABL1	ALK	BRAF	CREBBP	EPOR	ETV5	FGFR2	FOXO1	JAK2	MAP3K1	NOTCH1	NUP214	PCM1	PICALM	RET	RUNX1T1	TCF3
ABL2	BCL1	CBFB	CRLF2	ERG	ETV6	FGFR3	FUS	KMT2A	MECOM	NTRK1	NUP98	PDGFRA	PML	RHOA	SS18	TFG
AKT3	BCL2	CBL	CSF1R	ETV1	EWSR1	FIP1L1	GLI1	KRT18P6	MYC	NTRK2	P2RY8	PDGFRB	PTK2B	ROS2	STAT6	TYK2
ALK	BCL6	CIC	EGFR	ETV4	FGFR1	FLT3	IKZF3	LYN	MYH9	NTRK3	PBX1	PD-L1	RARA	RUNX1	TAL1	

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Electronic Signature

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The performance characteristics of this test have been determined by GTC Laboratories. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.