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Patient Name:	Janis Johnson	**Patient DOB:**	4/24/1972
Date Collected:	8/24/21	**Referring Physician:**	Michael Smith
Biopsy Site:	Bone Marrow	**Primary Diagnosis:**	Acute Myeloid Leukemia
Sample ID:	10323-AJ54BX97-1	**MoCha ID:**	OCA-15003
Telephone:	202-555-6789	**Fax:**	202-555-9876
Tumor Content (%):	50%		

Tumor content assessment performed by:

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Report Highlights

4 Relevant Biomarkers
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Relevant Acute Myeloid Leukemia Findings

Gene	Finding	Gene	Finding
ABL1	None detected	MECOM	None detected
ASXL1	None detected	MLLT3	None detected
CEBPA	None detected	MYH11	None detected
CREBBP	None detected	NPM1	***NPM1 W288Cfs*12***
FLT3	***FLT3 ITD mutation***	NUP214	None detected
IDH1	None detected	RARA	None detected
IDH2	None detected	RUNX1	None detected
KMT2A	None detected	TP53	None detected

Relevant Biomarkers

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IA	*FLT3 ITD mutation*	**gilteritinib** [1] **midostaurin + chemotherapy** [1] sorafenib + chemotherapy venetoclax + chemotherapy	None	45

Prognostic significance: ELN 2017: Favorable to Intermediate
Diagnostic significance: None

Public data sources included in relevant therapies: FDA[1], NCCN
Public data sources included in prognostic and diagnostic significance: NCCN
Tier Reference: Li et al. *Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists.* J Mol Diagn. 2017 Jan;19(1):4-23.

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Relevant Biomarkers (continued)

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IA	*NPM1 W288Cfs*12* **Prognostic significance:** ELN 2017: Favorable to Intermediate **Diagnostic significance:** None	None	None	8
IIC	*WT1 R375Gfs*6* **Prognostic significance:** None **Diagnostic significance:** None	None	None	3
IIC	*TET2 P1723S* **Prognostic significance:** None **Diagnostic significance:** None	None	None	2

Public data sources included in relevant therapies: FDA1, NCCN
Public data sources included in prognostic and diagnostic significance: NCCN
Tier Reference: Li et al. *Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists.* J Mol Diagn. 2017 Jan;19(1):4-23.

Prevalent cancer biomarkers without relevant evidence based on included data sources
*DNMT3A H677Qfs*26*

Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
DNMT3A	p.(H677Qfs*26)	c.2031_2037delCCAGGGG	.	chr2:25464475	45.64%	NM_022552.4	frameshift Deletion
TET2	p.(P1723S)	c.5167C>T	.	chr4:106196834	50.15%	NM_001127208.2	missense
NPM1	p.(W288Cfs*12)	c.863_864insTCTG	COSM17559	chr5:170837547	45.77%	NM_002520.6	frameshift Insertion
WT1	p.(R375Gfs*6)	c.1122_1122delAinsCGG	.	chr11:32417945	41.98%	NM_024426.6	frameshift Block Substitution
FLT3	p.(E604_F605insSNEYFYVDFREYEYDLKWE)	c.1812_1813insTCTAATGAGTACTTCTACGTTGATTTCAGAGAATATGAATATGATCTCAAATGGGAG	.	chr13:28608243	48.00%	NM_004119.2	nonframeshift Insertion

Comments:

Signed By: _____

Biomarker Descriptions

DNMT3A (DNA methyltransferase 3 alpha)

Background: The DNMT3A gene encodes the DNA methyltransferase 3 alpha which functions as a de novo methyltransferase (DNMT) with equal methylation efficiency for unmethylated and hemimethylated DNA[1]. Methylation of DNA occurs at CpG islands, a region of DNA consisting of sequential cytosine/guanine dinucleotide pairs. CpG island methylation plays an important role in development as well as stem cell regulation. Alterations to global DNA methylation patterns are dependent on DNMTs, which are associated with cancer initiation and progression[2,3].

Alterations and prevalence: DNMT3A mutations are observed in approximately 25% of all acute myeloid leukemia (AML) including 29-34% of AML with normal karyotype (NK-AML)[4,5,6,7,8,9,10]. Mutations in DNMT3A are also reported in 12-18% of myelodysplastic syndromes (MDS) as well as 4-6% of melanoma, lung adenocarcinoma, and uterine cancer[9,11]. The majority of mutations in DNMT3A are missense however, frameshift, nonsense, and splice site mutations have also been reported[4,9]. Missense mutations at R882 are most prevalent and are observed to coexist with NPM1 and FLT3 mutations[12,13]. The R882 mutations occur at the dimer/tetramer interface within the catalytic domain, which leads to disruption of DNMT3A tetramerization and loss of CpG methylation[14,15]. However, DNMT3A mutations observed in AML at positions other than R882 also contribute to pathogenesis by mechanisms that do not involve methyltransferase activity[16].

Potential relevance: DNMT3A mutations confer shorter overall survival (OS) in patients with AML including those with NK-AML[4,7,8,13]. DNMT3A mutations are a useful in the diagnosis of angioimmunoblastic T-cell lymphoma (AITCL) when trying to differentiate from other peripheral T-cell lymphomas (PTCL)[17].

FLT3 (fms related receptor tyrosine kinase 3)

Background: The FLT3 gene encodes the fms related tyrosine kinase 3, a tyrosine kinase receptor that is a member of the class III receptor tyrosine kinase family that also includes PDGFR, FMS, and KIT[18]. FLT3 is highly expressed in hematopoietic progenitor cells[19]. Genomic alterations in FLT3 activate downstream oncogenic pathways including PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways which promote cellular proliferation, survival, and inhibition of differentiation[18].

Alterations and prevalence: Somatic mutations occur in approximately 30% of acute myeloid leukemia (AML), 7-10% of melanoma, and up to 8% of uterine cancer[9,20,21,22]. The most common activating FLT3 mutations are internal tandem duplications (ITD) that range from 3 to 400 base pairs in length within exons 14 and 15 in the juxtamembrane (JM) domain[23]. The second most frequent mutations are point mutations in exon 20 within the tyrosine kinase domain (TKD)[24]. FLT3 is amplified in up to 8% of colorectal cancer, 3% of stomach cancer, and is commonly overexpressed in AML[9,22,25].

Potential relevance: The presence of FLT3-ITD confers poor prognosis in myelodysplastic syndrome (MDS) and AML[10,11]. Similarly, the FLT3 TKD mutation D835 confers poor prognosis in MDS[11]. Midostaurin[26] (2017) and gilteritinib[27] (2018) are kinase inhibitors approved for AML patients with FLT3-ITD and TKD mutations including D835 and I836 mutations. The FDA granted fast track designations in 2017 to crenolanib[28] for FLT3 mutation-positive relapsed or refractory AML and in 2018 to quizartinib[29] for AML with FLT3-ITD. A phase II trial testing crenolanib in 34 patients with FLT3-ITD and TKD mutated relapsed/refractory AML, reported that FLT3 inhibitor naïve patients demonstrated a longer overall survival (OS) and event free survival (EFS) in comparison to previously treated patients (median OS: 55 weeks vs 13 weeks; median EFS: 13 weeks vs 7 weeks)[30]. Another phase II trial of crenolanib with chemotherapy in newly diagnosed FLT3 mutated AML reported complete remission in 24/29 (83%) patients[31]. Several multi-targeted tyrosine kinase inhibitors such as sorafenib (2005), sunitinib (2006), cabozantinib (2012), and ponatinib (2012) are FDA approved and include FLT3 as a target. Sorafenib is recommended in combination with chemotherapy in FLT3-ITD mutated AML[10].

Biomarker Descriptions (continued)

NPM1 (nucleophosmin 1)

Background: The NPM1 gene encodes the nucleophosmin protein, a histone chaperone of the nucleophosmin/nucleoplasmin family, which also includes NPM2 and NPM3[32]. NPM1 functions as an oncogene and tumor suppressor, and is important in maintaining genomic stability, DNA repair, and apoptosis[32,33]. NPM1 has a highly conserved N-terminal region which constitutes the core domain responsible for oligomerization, an acidic domain, a nuclear localization signal, and a disorganized C-terminal region which is required for nucleolar localization[32]. Oligomerization of NPM1 localizes the protein in the nucleus of proliferating cells where it binds to Akt in response to growth factor stimulation and escapes proteolytic degradation by caspase activity, thereby promoting cell survival[32,33]. NPM1 is one of the most frequently altered genes in hematological cancers[34]. Most NPM1 mutations occur in the C-terminus, impacting protein folding or the nucleolar localization signal, and result in the localization of NPM1 to the cytoplasm (NPMc) instead of to the nucleus[32].

Alterations and prevalence: NPM1 mutations are observed in 45-60% of AML with a normal karyotype (NK-AML), 28-35% of de novo acute myeloid leukemia (AML) and are frequently co-mutated with DNMT3A and/or FLT3-ITD[10,35,36]. NPM1 fusions are associated with distinct partner genes in acute promyelocytic leukemia (APL), anaplastic large-cell lymphoma (ALCL), AML, and myelodysplasia[34]. Specifically, NPM1-ALK fusion is found in 30% of all ALCL and this specific fusion is observed in 85% of ALK-positive ALCL[32]. The t(5;17)(q35;q21) translocation that results in NPM1-RARA fusion is observed in APL[37].

Potential relevance: NPM1 mutated AML is recognized as a distinct diagnostic disease entity by the World Health Organization (WHO)[38]. NPM1 mutations are associated with better outcomes, increased complete remission, and improved overall survival in AML[10,36]. NPM1 without FLT3-ITD mutations or with <0.5 allelic ratio FLT3-ITD mutations are associated with favorable risk in AML, whereas wild-type NPM1 confers poor/adverse risk[10]. Concurrent NPM1 and with >0.5 allelic ratio FLT3-ITD mutations confer intermediate risk in AML[10]. The NPM1 frameshift mutation W288fs*12 is associated with poor prognosis in myelodysplastic syndrome (MDS)[11]. The ALK-NPM1 fusion, and translocation t(2;5)(p23;q35) which leads to an ALK-NPM1 fusion, is diagnostic of cutaneous and non-cutaneous anaplastic large cell lymphoma[17,39].

TET2 (tet methylcytosine dioxygenase 2)

Background: TET2 encodes the tet methylcytosine dioxygenase 2 protein and belongs to a family of ten-eleven translocation (TET) proteins that also includes TET1 and TET3[40]. TET2 is involved in DNA methylation, specifically in the conversion of 5-methylcytosine to 5-hydroxymethylcytosine[41,42]. The TET proteins contain a C-terminal core catalytic domain that contains a cysteine-rich domain and a double stranded ß-helix domain (DSBH)[43]. TET2 is a tumor suppressor gene. Loss of function mutations in TET2 are associated with loss of catalytic activity and transformation to hematological malignancies[40,41,42]

Alterations and prevalence: Somatic TET2 mutations, including nonsense, frameshift, splice site, and missense, are observed in 20-25% of myelodysplastic syndrome (MDS) associated diseases, including 40%-60% chronic myelomonocytic leukemia (CMML)[11]. TET2 mutations at H1881 and R1896 are frequently observed in myeloid malignancies[41,44]. TET2 mutations are also observed in 9% of uterine, 8% of melanoma and acute myeloid leukemia (AML), as well as 6% of diffuse large B-cell lymphoma (DLBCL).

Potential relevance: The presence of TET2 mutations may be used as one of the major diagnostic criteria in pre-primary myelofibrosis (pre-PMF) and overt PMF in the absence of JAK2/CALR/MPL mutations[38,45]. TET2 mutations are associated with poor prognosis in PMF and increased rate of transformation to leukemia[45,46]

WT1 (WT1 transcription factor)

Background: The WT1 gene encodes the Wilms tumor 1 homolog, a zinc-finger transcriptional regulator that plays an important role in cellular growth and metabolism[47,48]. WT1 is endogenously expressed in embryonic kidney cells as well as hematopoietic stem cells and regulates the process of filtration of blood through the kidneys[49]. WT1 protein contains N-terminal proline-glutamine rich regions that are involved in RNA and protein interaction while the C-terminal domain contains Kruppel link cysteine histidine zinc fingers that are involved in DNA binding[47]. WT1 interacts with various genes including TP53, STAT3, and epigenetic modifiers such as TET2 and TET3[47,50]. WT1 is primarily characterized as a tumor suppressor gene involved in the development of renal Wilm's tumor (WT), a rare pediatric kidney cancer[47,51]. Loss of function mutations observed in WT1, including large deletions and intragenic mutations, can impact the zinc finger domain, thereby decreasing the DNA binding activity[47]. WT1 overexpression is observed in acute myeloid leukemia (AML) and lymphoid cancers[47,52].

Biomarker Descriptions (continued)

Alterations and prevalence: Somatic mutations of WT1 occur in 7% of AML, 5% of melanoma, and 1% of mesothelioma[22]. WT1 overexpression is observed in AML, acute lymphoblastic lymphoma (ALL), and myelodysplastic syndrome (MDS)[47]

Potential relevance: Somatic mutations in WT1, including nonsense, frameshift, and splice-site mutations, are associated with poor prognosis in MDS[11]. Overexpression of WT1 in MDS is associated with a higher risk of progression to AML. WT1 overexpression is also associated with poor prognosis, resistance to chemotherapy, and poor overall survival in AML[50].

Relevant Therapy Summary

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ✖ No evidence

FLT3 ITD mutation

Relevant Therapy	FDA	NCCN	Clinical Trials*
gilteritinib	●	●	● (I/II)
midostaurin + cytarabine + daunorubicin	●	●	✖
midostaurin + cytarabine	✖	●	✖
sorafenib + azacitidine	✖	●	✖
sorafenib + decitabine	✖	●	✖
venetoclax + azacitidine	✖	●	✖
venetoclax + cytarabine	✖	●	✖
venetoclax + decitabine	✖	●	✖
sorafenib	✖	✖	● (IV)
chemotherapy, midostaurin	✖	✖	● (III)
crenolanib, chemotherapy	✖	✖	● (III)
crenolanib, midostaurin, chemotherapy	✖	✖	● (III)
gemtuzumab ozogamicin, chemotherapy, gilteritinib	✖	✖	● (III)
gilteritinib, midostaurin, chemotherapy	✖	✖	● (III)
chemotherapy	✖	✖	● (II/III)
ibrutinib, sorafenib, chemotherapy	✖	✖	● (II/III)
alemtuzumab, chemotherapy	✖	✖	● (II)
allogeneic stem cells, chemotherapy	✖	✖	● (II)
chemotherapy, radiation therapy	✖	✖	● (II)

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

Relevant Therapy Summary (continued)

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ✖ No evidence

FLT3 ITD mutation (continued)

Relevant Therapy	FDA	NCCN	Clinical Trials*
chemotherapy, stem cell therapy, radiation therapy	✖	✖	● (II)
gilteritinib, chemotherapy	✖	✖	● (II)
natural killer cell therapy, stem cell therapy	✖	✖	● (II)
ponatinib	✖	✖	● (II)
quizartinib, chemotherapy	✖	✖	● (II)
SKLB1028	✖	✖	● (II)
sorafenib, chemotherapy, cytokine, stem cell therapy	✖	✖	● (II)
venetoclax, chemotherapy	✖	✖	● (II)
CA 4948	✖	✖	● (I/II)
CART-FLT3	✖	✖	● (I/II)
CC-90009, gilteritinib	✖	✖	● (I/II)
chemotherapy, sorafenib	✖	✖	● (I/II)
dubermatinib, chemotherapy	✖	✖	● (I/II)
KRT-232, TL-895	✖	✖	● (I/II)
NMS-P948	✖	✖	● (I/II)
ponatinib, chemotherapy	✖	✖	● (I/II)
stem cell therapy	✖	✖	● (I/II)
venetoclax, gilteritinib, chemotherapy	✖	✖	● (I/II)
venetoclax, quizartinib	✖	✖	● (I/II)
allogeneic double negative T cells	✖	✖	● (I)
clifutinib	✖	✖	● (I)
LNK01002	✖	✖	● (I)
nintedanib, chemotherapy	✖	✖	● (I)
PHI-101	✖	✖	● (I)

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

Relevant Therapy Summary (continued)

● In this cancer type ○ In other cancer type ◑ In this cancer type and other cancer types ✖ No evidence

NPM1 W288Cfs*12

Relevant Therapy	FDA	NCCN	Clinical Trials*
chemotherapy	✖	✖	● (II)
pembrolizumab, chemotherapy	✖	✖	● (II)
chemotherapy, supplement	✖	✖	● (I/II)
KO-539	✖	✖	● (I/II)
SNDX-5613	✖	✖	● (I/II)
venetoclax, pevonedistat, chemotherapy	✖	✖	● (I/II)
JNJ-75276617	✖	✖	● (I)

WT1 R375Gfs*6

Relevant Therapy	FDA	NCCN	Clinical Trials*
chemotherapy	✖	✖	● (II)
sorafenib, chemotherapy, cytokine, stem cell therapy	✖	✖	● (II)
BI-836858 + chemotherapy	✖	✖	● (I/II)

TET2 P1723S

Relevant Therapy	FDA	NCCN	Clinical Trials*
BI-836858 + chemotherapy	✖	✖	● (I/II)
nonengraftment donor lymphocyte infusion	✖	✖	● (I/II)

* Most advanced phase (IV, III, II/III, II, I/II, I) is shown and multiple clinical trials may be available.

Clinical Trials Summary

FLT3 ITD mutation + WT1 R375Gfs*6

NCT ID	Title	Phase
NCT03164057	A Phase II Trial of Epigenetic Priming in Patients With Newly Diagnosed Acute Myeloid Leukemia	II

Clinical Trials Summary (continued)

FLT3 ITD mutation

NCT ID	Title	Phase
NCT02156297	Sorafenib to Treat AML Patients With FLT3-ITD Mutation: a Non-interventional Cohort Study	IV
NCT04174612	A Phase III, Prospective, Randomized Multi-center Intervention Trial of Early Intensification in AML Patients Bearing FLT3 Mutations Based on Peripheral Blast Clearance: A MYNERVA-GIMEMA Study	III
NCT03250338	Phase III Randomized, Double-blind, Placebo-controlled Study Investigating the Efficacy of the Addition of Crenolanib to Salvage Chemotherapy Versus Salvage Chemotherapy Alone in Subjects < or = 75 Years of Age With Relapsed/Refractory FLT3 Mutated Acute Myeloid Leukemia	III
NCT03258931	Phase III Randomized Study of Crenolanib Versus Midostaurin Administered Following Induction Chemotherapy and Consolidation Therapy in Newly Diagnosed Subjects With FLT3 Mutated Acute Myeloid Leukemia	III
NCT04293562	A Phase III Randomized Trial for Patients With De Novo AML Comparing Standard Therapy Including Gemtuzumab Ozogamicin (GO) to CPX-351 With GO, and the Addition of the FLT3 Inhibitor Gilteritinib for Patients With FLT3 Mutations	III
NCT04027309	A Phase III, Multicenter, Open-label, Randomized, Study of Gilteritinib Versus Midostaurin in Combination With Induction and Consolidation Therapy Followed by One-year Maintenance in Patients With Newly Diagnosed Acute Myeloid Leukemia (AML) or Myelodysplastic Syndromes With Excess Blasts-2 (MDS-EB2) With FLT3 Mutations Eligible for Intensive Chemotherapy (HOVON 156 AML / AMLSG 28-18)	III
NCT03256071	Low Dose Decitabine + Modified BUCY Conditioning Regimen for High Risk Acute Myeloid Leukemia Undergoing Allo-HSCT	II/III
NCT03642236	Combination of Brutons Tyrosine Kinase (BTK) Inhibitor Overcomes Drug-resistance in Refractory/ Relapsed FLT3 Mutant Acute Myeloid Leukemia (AML)	II/III
NCT02626715	A Phase II Study of Myeloablative and Reduced-Intensity Conditioning Regimens for Children and Young Adults With Acute Myeloid Leukemia or Myelodysplastic Syndrome Undergoing Allogeneic Hematopoietic Stem Cell Transplantation.	II
NCT01760655	A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for High Risk Hematologic Malignancies	II
NCT03121014	A Phase II Study of Intensity Modulated Total Marrow Irradiation (IM-TMI) in Addition to Fludarabine/ Busulfan Conditioning for Allogeneic Transplantation in High Risk AML and Myelodysplastic Syndromes	II
NCT03333486	A Phase II Trial of Haploidentical Allogeneic Stem Cell Transplantation Utilizing Mobilized Peripheral Blood Stem Cells	II
NCT03836209	Randomized Trial of Gilteritinib vs Midostaurin in FLT3 Mutated Acute Myeloid Leukemia (AML)	II
NCT03690115	Phase II Study of Ponatinib (Iclusig) for Prevention of Relapse After Allogeneic Stem Cell Transplantation (Allo-SCT) in FLT3-ITD AML Patients: the PONALLO Trial."	II
NCT03989713	Quizartinib and High-dose Ara-C Plus Mitoxantrone in Relapsed/Refractory AML With FLT3-ITD	II
NCT04278768	A Phase I/IIA, Open Label Dose Escalation and Expansion Study of Orally Administered CA-4948 as a Monotherapy in Patients With Acute Myelogenous Leukemia or Myelodysplastic Syndrome and in Combination With Azacitidine or Venetoclax	I/II

Clinical Trials Summary (continued)

FLT3 ITD mutation (continued)

NCT ID	Title	Phase
NCT04336982	An Exploratory Phase I/II Open-Label Multi-Arm Trial to Evaluate the Safety and Efficacy of CC-90009 Combinations in Subjects With Acute Myeloid Leukemia	I/II
NCT04518345	A Phase Ib/II Study of TP-0903 in Patients With Acute Myeloid Leukemia and FLT3 Mutations	I/II
NCT03013998	A Master Protocol for Biomarker-Based Treatment of AML (The Beat AML Trial)	I/II
NCT04240002	A Phase I/II, Multicenter, Open-Label, Single Arm, Dose Escalation and Expansion Study of Gilteritinib (ASP2215) Combined With Chemotherapy in Children, Adolescents and Young Adults With FMS-like Tyrosine Kinase 3 (FLT3)/Internal Tandem Duplication (ITD) Positive Relapsed or Refractory Acute Myeloid Leukemia (AML)	I/II
NCT04669067	An Open-Label, Multicenter, Phase Ib/II Study of the Safety and Efficacy of TL-895 Combined With KRT-232 in Patients With Relapsed/Refractory (R/R) FLT3+ Acute Myeloid Leukemia (AML)	I/II
NCT03922100	A Phase I/II Study of NMS-03592088, a FLT3, KIT and CSF1R Inhibitor, in Patients With Relapsed or Refractory AML or CMML	I/II
NCT02829840	Phase I/II, Dose-Escalation Study of Ponatinib, a FLT3 Inhibitor, With and Without Combination of 5-Azacytidine, in Patients With FLT3-Mutated Acute Myeloid Leukemia (AML)	I/II
NCT01892371	Phase I/II Study of the Combination of Quizartinib (AC220) With 5-Azacytidine or Low-Dose Cytarabine for the Treatment of Patients With Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)	I/II
NCT03793478	A Phase I/II, Multicenter, Dose-Escalating Study To Evaluate the Safety, Pharmacokinetics, Pharmacodynamics, and Efficacy Of Quizartinib Administered in Combination with Re-Induction Chemotherapy, and as a Single-Agent Continuation Therapy, in Pediatric Relapsed/Refractory AML Subjects Aged 1 Month to <18 Years (and Young Adults Aged up to 21 Years) with FLT3-ITD mutations.	I/II
NCT01203722	Reduced Intensity, Partially HLA Mismatched Allogeneic BMT for Hematologic Malignancies Using Donors Other Than First-degree Relatives	I/II
NCT04140487	A Phase I/II Study of Azacitidine, Venetoclax, and Gilteritinib for Patients With Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome With an Activating FLT3 Mutation	I/II
NCT05010122	A Phase I/II Study of ASTX727, Venetoclax, and Gilteritinib for Patients With Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome With an Activating FLT3 Mutation	I/II
NCT03735875	A Phase Ib/II Study of Venetoclax in Combination With Quizartinib in FLT3-Mutated Acute Myelogenous Leukemia (AML)	I/II
No NCT ID	Safety and Effective of Prophylactic Infusion of DNT cells in Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Myeloid Malignancies: A Single Center Clinical Research	I
No NCT ID	A Phase I Study to Assess the Safety of Micro-dose Lenalidomide as Maintenance Therapy Post-allogeneic Haematopoietic Cell Transplantation for Patients with Acute Myeloid Leukaemia or Myelodysplastic Syndromes, at High Risk of Relapse	I
NCT04827069	A Phase I, Multi-center, Open,Single Arm, Dose-escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Efficacy of Clifutinib Besylate(HEC73543) in Relapsed or Refractory Acute Myeloid Leukemia (AML)	I

Clinical Trials Summary (continued)

FLT3 ITD mutation (continued)

NCT ID	Title	Phase
NCT05024552	A Phase I Study of Vyxeos Plus Gilteritinib in Relapsed or Refractory, FLT3-Mutated Acute Myeloid Leukemia	I
NCT04896112	An Open-Label, Multicenter, Phase I Study to Evaluate the Safety, Pharmacokinetics and Preliminary Efficacy of LNK01002 in Patients With Malignant Myeloid Hematologic Neoplasms	I
NCT03513484	Phase Ib, Open Label, Combination Study of Nintedanib With 5-Azacitidine in Acute Myeloid Leukemia Characterized by HOX Gene Overexpression, That Are Not Candidates of Intensive Chemotherapy	I
NCT04842370	A Prospective, Phase Ia/Ib, Multicenter Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of the FLT3 Inhibitor, PHI 101, Alone in Subjects With Relapsed or Refractory Acute Myeloid Leukemia (AML)	I
NCT02126553	Phase II Study of Lenalidomide Maintenance in Patients With High Risk AML in Remission	II
No NCT ID	Open-Label, Multicenter, Prospective Intervention Trial Of Sequential MEC (Mitoxantrone / Etoposide / Cytarabine) And Gilteritinib In Patients With Relapsed Or Refractory FLT3 Mutation-Positive Acute Myeloid Leukemia	II
NCT02727803	Personalized NK Cell Therapy in Cord Blood Transplantation	II
NCT04015024	Phase IIa Clinical Study of SKLB1028 Capsule in the Treatment of FLT3 Mutation Recurrence / Refractory AML Patients	II
NCT04062266	Phase II Study of 5-Azacytidine (AZA) + Venetoclax as Maintenance Therapy in Patients With High Risk AML in Remission	II
NCT05023707	Pilot Study of the Safety and Efficacy of Anti-FLT3 Chimeric Antigen Receptor Engineered T-Cells in the Treatment of Relapsed or Refractory Acute Myeloid Leukemia (AML)	I/II
NCT03247088	Phase I/II Study Of Sorafenib Added To Busulfan And Fludarabine Conditioning Regimen In Patients With Relapsed/Refractory AML Undergoing Stem Cell Transplantation	I/II
NCT02272998	Phase II Study Of Ponatinib For Advanced Cancers With Genomic Alterations In Fibroblastic Growth Factor Receptor (FGFR) And Other Genomic Targets (KIT, Pdgfra, RET FLT3, ABL1)	II

NPM1 W288Cfs*12

NCT ID	Title	Phase
No NCT ID	International Multicenter, Open-Label, Phase II Study to Treat Molecular Relapse of Pediatric Acute Myeloid Leukemia With Azacitidine	II
NCT04689815	Measurable-residual Disease (MRD) Monitoring of Nucleophosmin 1 (NPM1)-Mutated Acute Myeloid Leukaemia (AML) and Pre-emptive Therapy With Oral Arsenic Trioxide-based Regimen	II
NCT03769532	MRD-guided Treatment With Pembrolizumab and Azacitidine in NPM1mut AML Patients With an Imminent Hematological Relapse	II
NCT03031249	Efficacy and Safety of ATO Plus ATRA in Nucleophosmin-1 Mutated Acute Myeloid Leukemia	I/II
NCT04067336	A Phase I/IIA First in Human Study of the Menin-MLL(KMT2A) Inhibitor KO 539 in Patients With Relapsed or Refractory Acute Myeloid Leukemia.	I/II

Clinical Trials Summary (continued)

NPM1 W288Cfs*12 (continued)

NCT ID	Title	Phase
NCT04065399	AUGMENT-101: A Phase I/II, Open-label, Dose-Escalation and Dose-Expansion Cohort Study of SNDX 5613 in Patients With Relapsed/Refractory Leukemias, Including Those Harboring an MLL/KMT2A Gene Rearrangement or Nucleophosmin 1 (NPM1) Mutation	I/II
NCT03862157	A Phase I/II Study of Azacitidine, Venetoclax and Pevonedistat in Adults With Newly Diagnosed Secondary or Therapy-Related AML	I/II
NCT04811560	A First in Human Study of the Menin-KMT2A (MLL1) Inhibitor JNJ-75276617 in Participants With Acute Leukemia	I

WT1 R375Gfs*6

NCT ID	Title	Phase
NCT03013998	A Master Protocol for Biomarker-Based Treatment of AML (The Beat AML Trial)	I/II
No NCT ID	International Multicenter, Open-Label, Phase II Study to Treat Molecular Relapse of Pediatric Acute Myeloid Leukemia With Azacitidine	II

TET2 P1723S

NCT ID	Title	Phase
NCT03013998	A Master Protocol for Biomarker-Based Treatment of AML (The Beat AML Trial)	I/II
NCT04620681	CD8 Depleted, Non-Engrafting, HLA Mismatched Unrelated Donor Lymphocyte Infusion in Patients With MDA and Secondary AML	I/II

Assay Information

Methodology, Scope, and Application:The NCI Myeloid Assay (NMA) is a next-generation sequencing (NGS) assay which identifies pre-defined and novel genomic variants covering 40 DNA genes and 29 fusion drivers that are categorized into 4 mutation types: single nucleotide variants (SNVs), small insertions/deletions (Indels), large (>3 bases) insertions/deletions (Large Indels) including FLT3 internal tandem duplications (ITDs), and gene fusions. The assay utilizes Thermo Fisher Scientific's Oncomine® Myeloid Assay GX, a next-generation sequencing (NGS) assay that utilizes a multiplex polymerase chain reaction (PCR) with DNA and RNA extracted from blood and bone marrow mononuclear cells for sequencing on the Ion Torrent Genexus Integrated Sequencer and analyzed by the current version of the Ion Torrent Genexus pipeline. The NMA currently can reliably identify the presence or absence of >1600 known mutations and polymorphisms compared to the Human Reference Genome hg19, including the genes listed below. The NMA is a laboratory developed test designed to find gene mutations for major myeloid disorders.

Analytical Sensitivity and Specificity:The NMA has been determined to be suitably analytically sensitive and specific for the various types of abnormalities within its reportable range, demonstrating XX.XX% sensitivity compared with orthogonal assay results and 100.00% specificity for the 4 mutation types reported. XX.XX% or greater reproducibility has been demonstrated. All quality measures for this assay were within defined assay parameters.

Hereditary and Germline Mutations:This assay examines cancer cells only and does not examine normal (non-cancer) cells. Mutations detected by the assay may be present only in the cancer cells, or in every cell of the body (including non-cancer cells). This test also cannot tell whether a potential germline mutation causes or will cause a hereditary cancer syndrome. If the patient's history includes

any one or combination of features suggestive of a possible hereditary cancer predisposition (such as, cancer arising at a young age, especially a cancer of a type unusual in younger patients; a personal history of multiple different types of cancers; or a significant cancer history among blood relatives, especially but not exclusively of the same type as the patient's) it is recommended that the physician arrange for the patient to meet with a genetic counselor and, if warranted, undergo the appropriate genetic test on normal (i.e. non-cancer) tissue (blood or cells brushed from the oral surface of the cheek) to check for a germline abnormality, regardless of the results of this research study.

Report Content:Thermo Fisher Scientific's Ion Torrent Oncomine Reporter software was used in the generation of this report. Software was developed and designed internally by Thermo Fisher Scientific. The analysis was based on the current version of Oncomine Reporter. The data presented here is from a curated knowledgebase of publicly available information, but may not be exhaustive. FDA information was sourced from www.fda.gov and is current as of this version. NCCN information was sourced from www.nccn.org and reflects its current version. Clinical Trials information reflects the current version. For the most up-to-date information regarding a particular trials, search www.clinicaltrials.gov by NCT ID or search local clinical trials authority website by local identifier listed in 'Other Identifiers.' Variants are reported according to HGVS nomenclature and classified following AMP/ASCO/CAP guidelines (Li et al. 2017). Based on the data sources selected, variants, therapies, and trials identified in this report are listed in order of potential clinical significance but not for predicted efficacy of the therapies.

Genes assayed for hotspot mutations:
ABL1, BRAF, CBL, CSF3R, DNMT3A, FLT3, GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PTPN11, SETBP1, SF3B1, SRSF2, U2AF1, WT1

Genes assayed with full exon coverage:
ASXL1, BCOR, CALR, CEBPA, ETV6, EZH2, IKZF1, NF1, PHF6, PRPF8, RB1, RUNX1, SH2B3, STAG2, TET2, TP53, ZRSR2

Genes assayed for detection of fusions:
ABL1, ALK, BCL2, BRAF, CCND1, CREBBP, EGFR, ETV6, FGFR1, FGFR2, FUS, HMGA2, JAK2, KMT2A (MLL), MECOM, MET, MLLT10, MLLT3, MYBL1, MYH11, NTRK3, NUP214, PDGFRA, PDGFRB, RARA, RBM15, RUNX1, TCF3, TFE3

DISCLAIMER

This assay can detect indels, including FLT3-ITDs up to 120bps. Sensitivity for larger indels may be reduced and variant allele frequencies may be reported lower than expected.
This assay is not sensitive enough to detect mutations in the CEBPA gene
This assay is considered a Laboratory Developed Test (LDT). Its performance characteristics have been determined through extensive testing although it has not been cleared by the US Food and Drug Administration and such approval is not required for clinical implementation. Furthermore, any comments included in the report are strictly interpretive and the opinion of the reviewer. This laboratory is certified under the Clinical Laboratory Improvements Amendments (CLIA) for the performance of high-complexity testing for clinical purposes. The correlative data presented is a result of the curation of published data sources, but may not be exhaustive. The content of this report has not been evaluated or approved by the FDA or other regulatory agencies.

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