GEM ExTra® Report

ASHION
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Report Date:

Patient:	Ordering Client:
Gender:	Specimen Type:
DOB:	Specimen Site:
Medical Record #:	Tumor Collection Date:
Client Accession #:	Normal Collection Date:
Ordering Physician:	Received Date:

Genomic Snapshot
- Analytes sequenced: DNA+RNA
- Actionable Targets: 8
- TMB: Low
- MSI: Stable
- Clinical Trials: Yes

Diagnosis: **AML**

TUMOR GENOMIC ALTERATIONS[1]

DNMT3A	FLT3	SF3B1	WT1	
GENOMIC TARGETS	FDA-APPROVED DRUGS -for patient's cancer	FDA-APPROVED DRUGS -for another cancer	DRUGS PREDICTED NON-BENEFICIAL	POTENTIAL CLINICAL TRIALS
8	2	3	0	Yes
DNMT3A (R736S)		azacitidine, decitabine		Yes
FLT3 (D835Y)	gilteritinib, midostaurin	sunitinib		Yes
SF3B1 (T663I)				Yes
WT1 (A237fs)				Yes
WT1 (S243*)				Yes

TUMOR MUTATION BURDEN (TMB)

LOW (1 mut/Mb)	No

MICROSATELLITE STATUS (MSI)

STABLE	No

ADDITIONAL SIGNIFICANT ALTERATIONS

CCND3 (S274fs)	No
EZH2 (R64fs)	No
NPM1 (W288fs)	No

[1]Alterations with predictive value according to Ashion's database and/or clinical trials identified by Ashion. For a complete list of alterations, please see the VUS section near the end of the report.

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Genomic Alterations Detail

Genomic Alteration		Therapeutic Implication	
Alteration:	CCND3 (S274fs)	**Drug**	**Status**
Alteration Type:	Frameshift	See Clinical Trials Section	
Coordinate:	chr6:41903731		
Allele Frequency:	34%		
Transcript ID:	ENST00000372991		
Origin:	DNA		

Biomarker Summary

CCND3 codes for a highly conserved member of the cyclin family, which are characterized by a periodicity in protein abundance through the cell cycle. CCND3 forms a complex with, and functions as a regulatory subunit of, CDK4 or CDK6, which is required for G1/S transition in the cell cycle. CCND3 has also been shown to interact with and be involved in the phosphorylation of tumor suppressor protein Rb (refSeq). CCND3 is reported to play a critical role in T-cell development and is also required for early B lymphopoiesis (Cooper AB et al., 2006; PMID: 16582912). Mutations in this gene have been reported in both pediatric and adult AML patients. The mutations reported in AML were predominantly of frameshift nature, and clustered in the PEST domain of the protein, which is critical for the degradation of the protein (Matsuo H et al., 2018; PMID: 30381403). According to one study, CCND3 is one of the most frequently mutated genes observed exclusively in non-responding AML patients. The majority of the mutations reported in the study were in the PEST domain and were non-sense or frameshift mutations. CCND3 mutation in AML are also a cause of clinical primary resistance to FLT3 inhibitors. These mutations were reported to result in a more stable isoform of cyclin D3 and confer resistance to apoptosis induced by FLT3 inhibitors (quizartinib and crenolanib) (Schmitz R et al., 2012; PMID: 22885699, Smith CC et al., Blood 2015 126:677). According to an earlier report, increased CCND3 expression maybe caused by mutations, and CDK4/6 inhibitors maybe effective in inhibiting the growth of CCND3-mutated cell line (Gong X et al., 2017; PMID: 29232554, Matsuo H et al., 2018; PMID: 30381403).

Molecular Function

CCND3 (S274fs) is a frameshift mutation which is predicted to result in a premature stop-gain and truncation of the protein, leading to loss of the PEST domain. It lies in the PEST domain of the CCND3 protein (Matsuo H et al., 2018; PMID: 30381403), and loss of PEST domain can result in a more stable isoform of cyclin D3, leading to constitutive CDK4/6 signaling and unchecked cell proliferation (Schmitz R et al., 2012; PMID: 22885699). Currently, CDK4/6 inhibitors are being evaluated in clinical trials (eg., NCT03132454, NCT03878524).

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Genomic Alteration		Therapeutic Implication	
Alteration:	DNMT3A (R736S)	**Drug**	**Status**
Alteration Type:	Missense	azacitidine (Vidaza)	PREDICTED BENEFICIAL
Coordinate:	chr2:25463287	decitabine (Dacogen)	PREDICTED BENEFICIAL
Allele Frequency:	49%		
Transcript ID:	ENST00000264709		
Origin:	DNA		

Biomarker Summary

DNMT3A codes for a member of the DNA methyltransferase enzymes that catalyzes de novo DNA methylation at CpG dinucleotides (Gowher H and Jeltsch A, 2001; PMID: 11399089). DNMT3A mutations are frequent in AML with a normal karyotype (NK-AML), and associated with French-American-British (FAB) M5 morphology (Ley TJ et al., 2010; PMID: 21067377), and an important factor for risk stratification of the disease. Patients with DNMT3A-mutant AML had significantly higher age, higher white blood cell (WBC) counts, and higher platelet counts, and significantly associated with FLT3-ITD and NPM1 mutation and an unfavorable prognosis in AML (Ribeiro AF et al., 2012; PMID: 22490330). DNMT3A mutations are thought to contribute to leukemogenesis via dramatically altered enzymatic activity resulting in altered patterns of hyper- and hypo- methylated genes (Sandoval JE et al., 2019; PMID: 30705090). In this context, hypomethylating agents (HMAs) have shown clinical response in the treatment of AML patients with DNMT3A mutations. Studies have reported a significantly improved CR rate in AML patients with DNMT3A/NPM1 co-mutants treated with HMAs like azacitidine or decitabine (Coombs CC et al., 2016; PMID: 27418649). Pre-clinical studies have also shown overexpression of the histone 3 lysine 79 (H3K79) methyltransferase, Dot1l, in DNMT3A-deficient cells. Consistently, DOT1L inhibition resulted in decreased expression of oncogenic genes and inhibition of proliferation, induction of apoptosis, cell-cycle arrest, and terminal differentiation in DNMT3A-mutant cell lines, in vitro. DOT1L efficacy was also observed in xenograft models of DNMT3A-mutant AML (Rau RE et al., 2016; PMMID: 27335278). Patients with DNMT3A-mutant AML also benefited from high dose of daunorubicin, a topoisomerase inhibitor (Luskin MR et al., 2016; PMID: 26755712, NCCN Guidelines v 3.2019, AML). DNMT3A mutations have been reported in 18-22% of AML patients, but data concerning the prognostic significance has been conflicting. A decreased OS has been reported for patients with DNMT3A mutation co-occurring with NPM1 mutation in the background of FLT3 ITD, but not in the favorable subgroup with NPM1 mutation with wild-type FLT3. Also, in patients <60 y of age with NK-AML, non-R882 DNMT3A mutation was associated with significantly poor outcome, suggesting that prognostic relevance of DNMT3A maybe age and mutation dependent (NCCN Guidelines v 3.2019, AML).

Molecular Function

DNMT3A (R736S) lies in the exon 19 of the gene and maps to the DNA-methylase domain of the protein. This mutation has not been characterized but a different substitution at this site revealed that the mutant protein led to at least a 2-fold decrease in activity relative to wild type DNMT3A, suggesting highly variable changes in its ability to methylate regions of high and low density CpG sites. The R736 mutant displayed a 9-fold increase in non-CpG methylation, compared to the wild enzyme. In terms of substrate sensitivity, in vitro assays revealed that the R736 mutant protein methylated DNA even at non-CpG sites as substrate (Sandoval JE et al., 2019; PMID: 30705090). The R736 is one of the non-R882 mutational hotspot in AML (COSMIC) observed in 2% of cases (Sandoval JE et al., 2019; PMID: 30705090). Mutations in DNMT3A with altered non-CpG methylation activity may have detrimental consequences in vivo (Sandoval JE et al., 2019; PMID: 30705090).

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Genomic Alteration		Therapeutic Implication	
Alteration:	EZH2 (R64fs)	**Drug**	**Status**
Alteration Type:	Frameshift	See Clinical Trials Section	
Coordinate:	chr7:148543619		
Allele Frequency:	45%		
Transcript ID:	ENST00000320356		
Origin:	DNA		

Biomarker Summary

EZH2, or the Enhancer of Zeste Homologue 2, codes for a member of the Polycomb Repressive Complex 2 (PRC2), which mediates transcriptional silencing through di- and trimethylation of lysine 27 of histone H3 (H3K27me2/3) (Margueron R and Reinberg D„ 2011; PMID: 21248841). EZH2 is thought to act as oncogene or as tumor suppressor in the context of the tumor-type. In certain solid tumors, EZH2 was found to act as an oncogene. However, EZH2 inactivation in myeloid malignancies, such as MDS and MDS/MPN, was associated with a poor prognosis (Ernst T et al., 2010; PMID: 20601953, Nikoloski G et al., 2010; PMID: 20601954), and contributed to disease pathogenesis (Sashida G et al., 2014; PMID: 24953053). In AML, loss of EZH2 was observed during disease progression, and EZH2 deficiency in AML cell lines was seen to consistently induce AraC resistance, with an average 5-fold increase in IC50 values compared to control cells, in vitro. Thus, loss of EZH2 induced chemo-resistance towards multiple drugs, and, in addition, EZH2 suppression occurred frequently in relapsed AML (Göllner S et al., 2017; PMID: 27941792). In matched diagnosis-relapse AML specimens, a reduction of EZH2 protein and H3K27me3 levels was observed in 45% of the relapse samples. Lastly, EZH2 loss induced overexpression of HOXB7 and HOXA9 proteins, suggesting their regulation by EZH2 in the leukemic cells. It has been suggested that a combination of agents that prevent EZH2 degradation along with chemotherapy and/or TKIs may be a viable treatment option in drug-resistant AML. In this context, loss of EZH2 by proteasomal degradation was found to promote chemoresistance, suggesting that proteasome inhibitors may improve AML chemotherapy in the relapse situation. Consistently, treatment with proteasome inhibitors, such as bortezomib, partially restored EZH2 protein levels and drug sensitivity in resistant AML cells (Göllner S et al., 2017; PMID: 27941792, Göllner S and Müller-Tidow C, 2017; PMID: 28401191). Prognostically, as in MDS, EZH2 inactivating mutations were significantly associated with shorter OS (median, 13 vs 26 months, p = 0.02) and RFS (median, 5 vs 15 months, p = 0.038) in AML (Saygin C et al., 2018; PMID: 29321554). EZH2 is also frequently mutated in MDS and MDS/MPN sub-type CMML, and associated with poor prognosis (NCCN Guideline v 2.2019, MDS).

Molecular Function

EZH2 (R64fs) is a frameshift mutation predicted to result in premature truncation, resulting in loss of functional domains if translated. The SET domain, which has the methyltransferase activity, lies at the C-terminus of EZH2 protein (503-725 amino acids), and the premature truncation is expected to result in loss of a functional protein. EZH2 mutations are reported to be frequent in de novo AML and associated with lower blast percentage in bone marrow (Wang X et al., 2013; PMID: 23613835).

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Genomic Alteration		Therapeutic Implication	
Alteration:	FLT3 (D835Y)	**Drug**	**Status**
Alteration Type:	Missense	gilteritinib (Xospata)	PREDICTED BENEFICIAL
Coordinate:	chr13:28592642	midostaurin (Rydapt)	PREDICTED BENEFICIAL
Allele Frequency:	97%	sunitinib (Sutent)	PREDICTED BENEFICIAL
Transcript ID:	ENST00000241453		
Origin:	DNA		

Biomarker Summary

FLT3 (fms-related tyrosine kinase 3) gene encodes a protein member of the type III platelet-derived growth factor family of receptor tyrosine kinases that play a critical role in normal hematopoiesis (Parcells BW et al., 2006; PMID: 16410383). Activating FLT3 mutations have been reported to promote proliferation and prevent apoptosis (Small et al., 1994; PMID:7507245, Brandts et al., 2005; PMID:16266983). Activating mutations in FLT3 may predict sensitivity to small molecule multi-tyrosine kinase inhibitors. Several kinase inhibitors have been approved by the FDA in certain indications; others are under investigation in clinical trials (Schittenhelm et al., 2006; PMID:16990784, Ravandi et al., 2010; PMID:20212254, von Bubnoff et al., 2009; PMID:19318574). Second generation Flt3 inhibitors with greater specificity for Flt3, such as quizartinib (AC-220), crenolanib (CP-868596), and PLX3397, are also in clinical development (Wander et al., 2014; PMID:24883179). Recently, gilteritinib was approved for the treatment of AML patients with FLT3 mutations (FDA). FLT3 mutations are considered high-risk unless they occur concurrently with an NPM1 mutation, in which case it is intermediate risk (NCCN Guidelines v 3.2019, AML). Further, NCCN guidelines recommend that FLT3 mutation status be evaluated rapidly to allow for addition of FLT3 inhibitor on day 8 of upfront intensive chemotherapy.

Molecular Function

FLT3 (D835Y) is one of the most frequent alterations associated with adult cytogenetically normal acute myeloid leukemia (CN-AML), and implicated in clinical resistance to FLT3 TKI therapy (Baker SD et al., 2013; PMID: 23969938). It lies in the TK domain of the protein, and commonly referred to as FLT3-TKD, and present in ~7% of CN-AML patients (Mrózek K et al., 2007; PMID: 16960150). The FLT3-TKD mutations exhibit ligand–independent FLT3 dimerization and constitutive activation through autophosphorylation (Grundler R et al., 2003; PMID: 12663439). Pre-clinical and clinical studies have also revealed that the FLT3 (D835Y) alteration, either alone or in combination with FLT3-ITD, results in reduced sensitivity to FLT3 tyrosine kinase inhibitors (TKIs), including quizartinib, sorafenib, cabozantinib, and ponatinib; however, FLT3 (D835Y) has been reported to retain sensitivity to sunitinib, crenolanib, and midostaurin (Moore A et al, 2012: PMID: 22354205; O'Farrell A et al, 2003: PMID: 12531805; Smith C et al, 2013: PMID: 23430109; Zimmerman E et al, 2013: PMID: 24046014; Smith C et al, 2015: PMID: 26108694; Baker S et al, 2013: PMID: 23969938; Fiedler W et al, 2015: PMID: 25818407; Fathi AT et al., 2018; PMID: 28960265). Contradictory results have also been reported for sorafenib and sunitinib in FLT3 (D835Y) mutants. Sorafenib monotherapy was found to induce complete remission in a FLT3 (D835Y)-positive patient with refractory AML (Yue Y et al., 2016; PMID: 27408351). The D835Y substitution constitutes approximately 50% of FLT3-TKD mutations (Whitman SP et al., 2008; PMID: 17940205).

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Genomic Alteration		Therapeutic Implication	
Alteration:	NPM1 (W288fs)	**Drug**	**Status**
Alteration Type:	Frameshift	See Clinical Trials Section	
Coordinate:	chr5:170837543		
Allele Frequency:	35%		
Transcript ID:	ENST00000296930		
Origin:	DNA		

Biomarker Summary

NPM1 (nucleophosmin) gene, also called B23 or numatrin, is a nucleocytoplasmic shuttling protein (Borer RA et al., 1989; PMID: 2914325). NPM1 protein regulates many cellular processes, including ribogenesis, regulation of centrosome duplication, and apoptosis via its interaction with p53 and proteins associated with it functionally (Brodská B et al., 2017; PMID: PMID: 28384310). NPM1 is thought to be a major stress-induced regulator of p53 function in response to hypoxia, UV irradiation or cytotoxic drugs. In AML, mutations in exon 11 (or exon 12, based on numbering) of NPM1, leading to frameshift and an elongated protein, results in its retention in the cytoplasm. NPM1 mutations have been associated with specific clinical features, including a normal karyotype (NK), low or absent CD34+ expression, and FLT3-ITD mutations (Thiede C et al., 2006; PMID: 16455956). Per NCCN guidelines, presence of NPM1 mutation without FLT3-ITD, or NPM1 mutation co-occurring with low allelic ratio (<0.5) of FLT3-ITD is classified as favorable risk, while NPM1 mutation co-occurring with high allelic ratio (>0.5) of FLT3-ITD is considered intermediate risk, and AML with wild-type NPM1 and high allelic ratio (>0.5) of FLT3-ITD is classified under poor/adverse risk category. NPM1 mutations have been reported in 28-35% of AML cases and associated with NK-AML. Further, isolated NPM1 mutants which localized to cytoplasm conferred a higher complete response (CR) rate to induction therapy, and improved event-free survival (EFS) and OS, compared with patients who are NK-AML and wild-type NPM1 (NCCN Guidelines v 3.2019, AML). AML patients with NPM1 mutation also benefited from high-dose daunorubicin (Luskin MR et al., 2016; PMID: 26755712, NCCN Guidelines v 3.2019, AML). Other treatment options at experimental stages suggest targeting the enforced expression of HOXA and MEIS1 in NPM1mut leukemic cells. Overexpression of HOX1 and MEIS1 in NPM1mut leukemic cells were associated with H3K79 di/tri-methylation. In this study, DOT1L inhibitor, EPZ4777, was used to treat NPM1mut model in vivo, which resulted in a survival advantage accompanied by myeloid differentiation in the treated models. Further, a combination of the menin-MLL1 inhibitor (MI-503) and DOT1L inhibitor (EPZ4777) led to synergistic suppression of HOXA/MEIS1 expression and growth, accompanied by monocytic and myeloid differentiation of NPM1mut cells (Kühn MW et al., 2016; PMID: 27535106), suggesting that targeting the MLL1 and DOT1L histone modification complexes, both alone and in combination, may show activity against AML driven by an NPM1-mutant protein (Hourigan CS and Aplan PD, 2016; PMID: 27698101).

Molecular Function

NPM1 (W288fs) is the most frequently (>3000 samples) reported mutation in AML (COSMIC). This frameshift mutation is located in the last exon of the gene, short of 6 residues to the stop-codon, and maps to the C-terminus. It is predicted to result in stop-loss and an elongated protein. The frameshift mutations at this site highly compromise the nucleolar localization signal (NLS), and the protein acquires an extra nuclear export signal (NES) in addition to two NESes already present in its N-terminal domain (Federici L and Falini B., 2013; PMID: 23436734). The resulting mutated protein (NPMmutA) lacks both tryptophans W288 and W290 and gains the most frequent NES motif L-xxx-V-xx-V-x-L (Falini B et al., 2005; PMID: 15659725). Of the frameshift mutants at this site, six different NES motifs with varying strength and exporting efficiency have been described. The impact of the mutation type on survival characteristics has been well examined and the results of individual studies were highly varied. Further, the localization of each NPMmut type was strongly affected by the co-expression of NPMwt, potentially due to hetero-oligomer formation (Bolli N et al., 2009; PMID: 19005479, Brodská B et al., 2017; PMID: PMID: 28384310).

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Genomic Alteration		Therapeutic Implication	
Alteration:	SF3B1 (T663I)	**Drug**	**Status**
Alteration Type:	Missense	See Clinical Trials Section	
Coordinate:	chr2:198267369		
Allele Frequency:	47%		
Transcript ID:	ENST00000335508		
Origin:	DNA		

Biomarker Summary

SF3B1 encodes a Splicing factor 3B subunit 1 (SF3b155), a protein that forms part of multiple snRNP complexes involved in pre-mRNA splicing (Golas MM et al, 2003; PMID: 12738865,Wang C et al, 1998; PMID: 9585501, Will C et al, 2001; PMID: 11500380, Gozani O et al, 1998; PMID: 9671485). SF3B1 protein regulates the pre-mRNA splicing step that determines the ratio of pro-apoptotic and anti-apoptotic isoforms of the Bcl-2 family of proteins (Moore M et al, 2010; PMID: 20705336, Massiello A et al, 2006; PMID: 16790528). Thus, tumors harboring SF3B1 activating mutations, which may promote the anti-apoptotic isoforms of Bcl-2 proteins, may be sensitive to SF3b155 inhibitors, like Meayamycin B, which are in clinical development (Gao Y et al, 2013; PMID: 23172726, Kaida D et al, 2007; PMID: 17643111, Gao Y et al, 2013; PMID: 23485022). SF3B1 is a major component of SF3b complex which mediates exon splicing as part of the U2 snRNP spliceosome complex. In a different context, SF3B1 alterations, including missense and partial loss, may be neither gain- or loss-of-function, but change-of-function mutations, resulting in consequent aberrant splicing and potential tumorigenesis (Alsafadi S et al., 2016; PMID: 26842708). SF3B1 mutant cells have been shown to be sensitive to SF3B1 inhibitors that perturb U2 spliceosome function and upregulate wild type p53 (Folco EG et al., 2011; PMID: 21363962, Kaida D et al., 2007; PMID: 17643111, Hepburn LA et al., 2018; PMID: 29796170).

Molecular Function

SF3B1 (T663I) is a frequently reported MDS-associated mutation, while the incidence of SF3B1 mutations in de novo AML is low (Malcovati L et al., 2011; PMID: 21998214). SF3B1 mutations are associated with ring sideroblasts (RS), most frequent in MDS-RS, and with a favorable prognosis (NCCN Guidelines v 2.2019, MDS). Ring sideroblasts (RS) are a distinct morphological feature present in myelodysplastic syndromes (MDS), MDS/MPN, and AML. According to one study, NPM1 mutations, which are associated with de novo AML, did not exclude mutations in SF3B1 (Martin-Cabrera P et al., 2017; PMID: 28057736). It has been proposed that RS formation associated with SF3B1 mutations might be due to the reduced expression of the gene ABCB7 required for normal hematopoiesis; the reduced expression of ABCB7 is thought to result from reduced transcription and abnormal splicing of ABCB7 in patients with somatic SF3B1 mutations (Cazzolla M et al., 2013; PMID: 23160465).

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Genomic Alteration		Therapeutic Implication	
Alteration:	WT1 (A237fs)	**Drug**	**Status**
Alteration Type:	Frameshift	See Clinical Trials Section	
Coordinate:	chr11:32450104		
Allele Frequency:	48%		
Transcript ID:	ENST00000332351		
Origin:	DNA		

Biomarker Summary

WT1 codes for a zinc-finger protein that functions as a transcriptional regulator, with the capability of either activating or repressing transcription, depending on the cellular context, target promoter, and the type of WT1 isoform (King-Underwood L, Pritchard-Jones K, 1998; PMID: 9531607, Huff V, 2011; PMID: 21248786, Sugiyama H, 2010; PMID: 20395243). WT1 is overexpressed in a majority of AML (NCCN Guidelines v 3.2019, AML) and mutated in a proportion of AML cases with normal karyotype (Rampal R and Figueroa ME, 2016; PMID: 27252512). The fact that WT1 is overexpressed in many patients with AML, which can drive leukemogenesis in murine models, yet it is also mutated (with presumed loss-of-function) in a significant proportion of AML cases presents a paradox as to the role of this protein in AML (Rampal R and Figueroa ME, 2016; PMID: 27252512). There are no approved targeted therapies directly addressing WT1 mutations or expression. Wt1 vaccines are in clinical trials for WT1-over-expressing hematologic and solid tumors (Sugiyama H, 2010: PMID: 20395243; Okusaka T et al, 2012: PMID: 22273718; Takakura K et al, 2015: PMID: 25550602; Takahashi H et al, 2013: PMID: 23245331). An analysis of both global 5-hydroxymethylcytosine (5-hmC) levels in primary AML patient samples, as well as genome-wide distribution of 5-hmC by NGS, demonstrated that WT1-mutant AMLs presented a global reduction in 5-hmC levels comparable to that seen in TET2- and IDH1/2-mutant AMLs (Rampal R and Figueroa ME, 2016; PMID: 27252512). However, DNA methyltransferase (DNMT) inhibitors, such as azacitidine and decitabine, have been suggested as potential therapies for patients with WT1 mutations (Kasi PM et al., 2016; PMID: 26904404). Another therapeutic approach for WT1-mutated AML involves the use of EZH2 inhibitors based on the role of EZH2 in suppressing myeloid differentiation (Sinha S et al., 2015; PMID: 25398938). WT1 mutation is associated with aberrant repression of H3K27me3-marked genes in AML, and upregulation of EZH2 further contributes to suppression of myeloid differentiation. Pharmacologic inhibition of EZH2 in WT1-mutated AML promoted myeloid differentiation, providing a therapeutic potential (Sinha S et al., 2015; PMID: 25398938). WT1 mutation has also been associated with resistance to induction chemotherapy, and poor overall survival (OS) and relapse-free survival (RFS) (Hou HA et al., 2010; PMID: 20368469).

Molecular Function

WT1 (A237fs) is a frameshift mutation expected to prematurely truncate the WT1 protein in exon 2, resulting in loss of functinal domains, including the four C2H2 zinc finger domains (UniProt) (Bansal H et al, 2010; PMID: 20651072). The zinc finger domains have been reported to be important for proper nuclear localization of WT1, as well as DNA and RNA binding (Bruening W et al, 1996; PMID: 8804420; Bardeesy N et al, 1998; PMID: 9512553). This mutation may result in loss of DNA binding ability of the WT1 protein (Rampal R and Figueroa ME, 2016; PMID: 27252512). A similar truncating mutation that eliminates all four Zn finger domains was observed to result in altered subnuclear localization of Wt1 and dominant-negative inhibition of wild-type Wt1 transactivation activity (Englert C et al, 1995; PMID: 8618823). The mechanism by which WT1 mutations might contribute to leukemogenesis is not known (Rampal R and Figueroa ME, 2016; PMID: 27252512). According to one study, WT1-mutated samples had frameshift or point mutations in either exon 1, 7 or exon 9 of WT1 that disrupted the DNA binding domains, and, in addition, exhibited increased methylation of the WT1 hypermethylated CpG sites (Sinha S et al., 2015; PMID: 25398938).

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Genomic Alteration		Therapeutic Implication	
Alteration:	WT1 (S243*)	**Drug**	**Status**
Alteration Type:	Stop Gain	See Clinical Trials Section	
Coordinate:	chr11:32450084		
Allele Frequency:	47%		
Transcript ID:	ENST00000332351		
Origin:	DNA		

Biomarker Summary

WT1 codes for a zinc-finger protein that functions as a transcriptional regulator, with the capability of either activating or repressing transcription, depending on the cellular context, target promoter, and the type of WT1 isoform (King-Underwood L, Pritchard-Jones K, 1998; PMID: 9531607, Huff V, 2011; PMID: 21248786, Sugiyama H, 2010; PMID: 20395243). WT1 is overexpressed in a majority of AML (NCCN Guidelines v 3.2019, AML) and mutated in a proportion of AML cases with normal karyotype (Rampal R and Figueroa ME, 2016; PMID: 27252512). The fact that WT1 is overexpressed in many patients with AML, which can drive leukemogenesis in murine models, yet it is also mutated (with presumed loss-of-function) in a significant proportion of AML cases presents a paradox as to the role of this protein in AML (Rampal R and Figueroa ME, 2016; PMID: 27252512). There are no approved targeted therapies directly addressing WT1 mutations or expression. Wt1 vaccines are in clinical trials for WT1 over-expressing hematologic and solid tumors (Sugiyama H, 2010: PMID: 20395243; Okusaka T et al, 2012: PMID: 22273718; Takakura K et al, 2015: PMID: 25550602; Takahashi H et al, 2013: PMID: 23245331). An analysis of both global 5-hydroxymethylcytosine (5-hmC) levels in primary AML patient samples, as well as genome-wide distribution of 5-hmC by NGS, demonstrated that WT1-mutant AMLs presented a global reduction in 5-hmC levels comparable to that seen in TET2- and IDH1/2-mutant AMLs (Rampal R and Figueroa ME, 2016; PMID: 27252512). However, DNA methyltransferase (DNMT) inhibitors, such as azacitidine and decitabine, have been suggested as potential therapies for patients with WT1 mutations (Kasi PM et al., 2016; PMID: 26904404). Another therapeutic approach for WT1-mutated AML involves the use of EZH2 inhibitors, based on the role of EZH2 in suppressing myeloid differentiation (Sinha S et al., 2015; PMID: 25398938). WT1 mutation is associated with aberrant repression of H3K27me3-marked genes in AML, and upregulation of EZH2 further contributes to suppression of myeloid differentiation. Pharmacologic inhibition of EZH2 in WT1-mutated AML promoted myeloid differentiation, providing a therapeutic potential (Sinha S et al., 2015; PMID: 25398938). WT1 mutation has also been associated with resistance to induction chemotherapy, and poor overall survival (OS) and relapse-free survival (RFS) (Hou HA et al., 2010; PMID: 20368469).

Molecular Function

WT1 (S243*) is a stop-gain mutation expected to prematurely truncate the WT1 protein in exon 2, resulting in loss of functional domains, including all four C2H2 zinc finger domains (UniProt) (Bansal H et al, 2010; PMID: 20651072). The zinc finger domains have been reported to be important for proper nuclear localization of WT1, as well as DNA and RNA binding (Bruening W et al, 1996; PMID: 8804420; Bardeesy N et al, 1998; PMID: 9512553). This mutation may result in loss of DNA binding ability of the WT1 protein (Rampal R and Figueroa ME, 2016; PMID: 27252512). A similar truncating mutation that eliminates all four Zn finger domains was observed to result in altered subnuclear localization of Wt1 and dominant-negative inhibition of wild-type Wt1 transactivation activity (Englert C et al, 1995; PMID: 8618823). The mechanism by which WT1 mutations might contribute to leukemogenesis is not known (Rampal R and Figueroa ME, 2016; PMID: 27252512). According to one study, WT1-mutated samples with frameshift or point mutations in either exon 1, 7 or exon 9 of WT1, disrupted the DNA binding domains and exhibited increased methylation of the WT1 hypermethylated CpG sites (Sinha S et al., 2015; PMID: 25398938).

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Drug Evidence Detail

Literature Supporting Therapeutic Implication

Drug	Gene	Therapeutic Implication
azacitidine (Vidaza)	DNMT3A (R736S)	PREDICTED BENEFICIAL

In a meta-analysis study investigating the relationship between somatic mutations affecting DNA methylation and hypo-methylating agents (HMA) azacytidine or decitabine response in an expanded AML patient cohort, DNMT3A mutations predicted response to HMAs in patients treated in the frontline setting (odds ratio (OR), 3.12; P=0.001), but not in the total cohort when including relapsed/refractory patients (OR 1.72; P=0.23). A systematic search was conducted to identify studies examining response to HMAs in patients with AML in relation to presence/absence of mutations in DNMT3A, IDH1/2, and/or TET2. Studies analyzing HMAs in combination with intensive induction chemotherapy were excluded. Among patients treated with HMAs in the frontline setting (n=45), a 60% CR rate [including CR and incomplete blood count recovery (CRi)] was noted in DNMT3A mutants compared to 33% of those with wild-type DNMT3A, although numbers were insufficient to allow statistical comparisons. In a different cohort, in the frontline setting only, a statistically significant association was observed between presence of DNMT3A mutation and attainment of CR [57% vs. 29%, OR 3.12 (1.63–5.94) with P=0.001]. Presence of mutation in both DNMT3A and NPM1 demonstrated a CR rate of 73% compared to 21% in patients without co-mutation of these genes [OR 2.82 (1.33–6.00) with P=0.007]. Study concluded that a statistically significant improved CR existed in DNMT3A/NPM1 co-mutants who were treated in both frontline and relapsed/refractory settings.

https://www.ncbi.nlm.nih.gov/pubmed/27418649 *(Coombs CC et al., Haematologica. 2016 Nov;101(11):e457-e460.)*

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Drug	Gene	Therapeutic Implication
decitabine (Dacogen)	DNMT3A (R736S)	PREDICTED BENEFICIAL

A total of 46 AML patients were treated with decitabine either as single agent or in combination with bortezomib. DNMT3A mutations were found in 8/46(17%) patients. Six were missense mutations affecting the mutational hot spot codon R882 [3 patients with c.2645G>A; p. (R882H), and 3 with c.2644C>T; p.(R882C)]. One patient had a nonsense mutation [c. 1729A>T; p.(K577)], and one had a splice-site mutation [c.2322+1G>A; p.0?]. Overall, the CR rate in this cohort was 41% (19/46). Six of eight DNMT3A-mutated patients (75%) achieved CR, compared to 13 of 38 with wild type DNMT3A (34%, P=.05). Notably, all five patients with mutated DNMT3A and mutated NPM1 achieved CR, a significantly better response rate than among the remaining patients with other genotypes [14/41 (34%); P=.008]. The median OS of patients with DNMT3A mutations was 15.2 months, compared to 11.0 months for patients with DNMT3A-wild type.*

https://www.ncbi.nlm.nih.gov/pubmed/22124213 *(Metzeler KL et al., Leukemia. 2012 May;26(5):1106-7)*

In a meta-analysis study investigating the relationship between somatic mutations affecting DNA methylation and hypo-methylating agents (HMA) azacytidine or decitabine response in an expanded AML patient cohort, DNMT3A mutations predicted response to HMAs in patients treated in the frontline setting (odds ratio (OR), 3.12; P=0.001), but not in the total cohort when including relapsed/refractory patients (OR 1.72; P=0.23). A systematic search was conducted to identify studies examining response to HMAs in patients with AML in relation to presence/absence of mutations in DNMT3A, IDH1/2, and/or TET2. Studies analyzing HMAs in combination with intensive induction chemotherapy were excluded. Among patients treated with HMAs in the frontline setting (n=45), a 60% CR rate [including CR and incomplete blood count recovery (CRi)] was noted in DNMT3A mutants compared to 33% of those with wild-type DNMT3A, although numbers were insufficient to allow statistical comparisons. In a different cohort, in the frontline setting only, a statistically significant association was observed between presence of DNMT3A mutation and attainment of CR [57% vs. 29%, OR 3.12 (1.63–5.94) with P=0.001]. Presence of mutation in both DNMT3A and NPM1 demonstrated a CR rate of 73% compared to 21% in patients without co-mutation of these genes [OR 2.82 (1.33–6.00) with P=0.007]. Study concluded that a statistically significant improved CR existed in DNMT3A/NPM1 co-mutants who were treated in both frontline and relapsed/refractory settings.

https://www.ncbi.nlm.nih.gov/pubmed/27418649 *(Coombs CC et al.,Haematologica. 2016 Nov;101(11):e457-e460)*

Drug	Gene	Therapeutic Implication
gilteritinib (Xospata)	FLT3 (D835Y)	PREDICTED BENEFICIAL

In a multicenter, open-label, phase 1-2 study of patients with acute myeloid leukemia (AML) who either were refractory to induction therapy or had relapsed after achieving remission with previous treatment were treated with gilteritinib. Trial required ten or more patients with locally confirmed FLT3 mutations (FLT3mut+) to be enrolled in expansion cohorts at each dose level. 252 adults with relapsed or refractory acute myeloid leukemia received oral gilteritinib in one of seven dose-escalation (n=23) or dose-expansion (n=229) cohorts. Gilteritinib was well tolerated; An exposure-related increase in inhibition of FLT3 phosphorylation was noted with increasing concentrations in plasma of gilteritinib. In-vivo inhibition of FLT3 phosphorylation occurred at all dose levels. At least 90% of FLT3phosphorylation inhibition was seen by day 8 in most patients receiving a daily dose of 80 mg or higher. 100 (40%) of 249 patients in the full analysis set achieved a response, with 19 (8%) achieving complete remission, ten (4%) complete remission with incomplete platelet recovery, 46 (18%) complete remission with incomplete hematological recovery, and 25 (10%) partial remission. It was concluded that gilteritinib had a favorable safety profile and showed consistent FLT3 inhibition in patients with relapsed or refractory acute myeloid leukemia.

https://www.ncbi.nlm.nih.gov/pubmed/28645776 *(Perl AE et al., Lancet Oncol. 2017 Aug;18(8):1061-1075)*

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Drug	Gene	Therapeutic Implication
midostaurin (Rydapt)	FLT3 (D835Y)	PREDICTED BENEFICIAL

The Phase 3 RATIFY trial in 717 FLT3-mutant AML patients has reported that the addition of midostaurin, as compared with placebo, to induction and consolidation therapy followed by one year of maintenance therapy was associated with an improved median overall survival (74.7 months versus 26.0 months) and event-free survival (eight months versus three months). The FLT3 mutation type (ITD or TKD) and allelic fraction were not associated with clinical outcome in the study.
https://www.ncbi.nlm.nih.gov/pubmed/28644114 *(Stone R et al, The New England journal of medicine, 2017; 5:454-464)*

A Phase 2b trial evaluating midostaurin in 92 evaluable patients with either AML or myelodysplastic syndrome (MDS) noted greater than 50% reductions in peripheral or bone marrow blasts in 71% (25/35) of cases with FLT3 mutation and 42% (24/57) of cases with wild-type FLT3; partial remission was noted in another FLT3 mutant case, and treatment was well tolerated.
https://www.ncbi.nlm.nih.gov/pubmed/20733134 *(Fischer T et al, Journal of clinical oncology, 2010; 28:4339-45)*

A Phase 1/2 trial of midostaurin in combination with azacitidine in 54 patients with either AML or high-risk MDS reported an overall response rate of 26%. The median remission duration was 20 weeks and was significantly increased in both patients harboring FLT3 mutation without previous Flt3 inhibitor therapy and patients who had not received a transplant. Additionally, grade 3-4 adverse events were reported in 70% of patients.
https://www.ncbi.nlm.nih.gov/pubmed/25530214 *(Strati P et al, American journal of hematology, 2015; 4:276-81)*

Drug	Gene	Therapeutic Implication
sunitinib (Sutent)	FLT3 (D835Y)	PREDICTED BENEFICIAL

A Phase 1/2 study of sunitinib treatment and chemotherapy in elderly AML patients (median age of 70) with FLT3 mutations has reported that 59% (13/22) of patients achieved complete remission, with 53% (8/15) and 71% (5/7) of patients harboring FLT3-ITD and FLT3-TKD mutations, respectively. In addition, partial remission was reported in one patient (4.5%), refractory disease in five patients (23%), and death occurred in three patients (13.5%). Twelve patients achieving complete remission subsequently died, resulting in a median relapse-free survival time of 11 months, a median survival time of 18.8 months, and a two year survival rate of 36%.
https://www.ncbi.nlm.nih.gov/pubmed/25818407 *(Fiedler W et al, British journal of haematology, 2015; 5:694-700)*

A Phase 1 study has reported that sunitinib treatment was associated with partial remissions of short duration in AML patients, especially those with FLT3 mutations; sunitinib was reported to decrease the number of phospho-Kit, phospho-VEGFR-2, phospho-Akt, and phospho-Stat5 cells in the bone marrow.
https://www.ncbi.nlm.nih.gov/pubmed/15459012 *(Fiedler W et al, Blood, 2005; 3:986-93)*

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Clinical Trials Report

Potential trials based on genomic targets indicated in the GEM ExTra Report

Genomic Alterations	Targeted Investigational Agents	Trial IDs
DNMT3A (R736S)	Hypomethylating agents: (Azacitidine, Decitabine)	NCT03013998 NCT03066648 NCT02684162 NCT02935361 NCT02551718 NCT02953561 NCT03397173
FLT3 (D835Y)	FLT3 inhibitors: (Midostaurin, Sunitinib, Crenolanib, Gilteritinib, FF-10101-01)	NCT03013998 NCT02551718 NCT03070093 NCT02624570 NCT03258931 NCT03250338 NCT02752035
SF3B1 (T663I)	SF3B1 inhibitor: (H3B-8800)	NCT02841540
WT1 (A237fs)	Hypomethylating agents: (Azacitidine, Decitabine), EZH2 inhibitor: (Tazemetostat, SHR2554, CPI-1205), EZH1/2 dual inhibitor: (DS-3201b)	NCT03013998 NCT02935361 NCT02953561 NCT03397173 NCT02891278
WT1 (S243*)	Hypomethylating agents: (Azacitidine, Decitabine), EZH2 inhibitor: (Tazemetostat, SHR2554, CPI-1205), EZH1/2 dual inhibitor: (DS-3201b)	NCT03013998 NCT02935361 NCT02953561 NCT03397173 NCT02891278

Disclaimer:

These clinical trial results were procured by keyword search on www.ClinicalTrials.gov, last updated on 05/21/2019. The information contained in this site changes frequently and may be out of date. Search terms were based on alterations identified in the GEM ExTra report, drugs indicated in the GEM ExTra Report, and the reported cancer type of the patient. The search strategy was not exhaustive and may not have retrieved every relevant trial for this patient. Healthcare professionals are encouraged to investigate other possibilities through additional searches at this site. The identified trials may have specific inclusion or exclusion criteria that would make a trial inappropriate for the patient. Consideration of any listed option should be made in the context of the patient's complete medical history.

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Variants of Unknown Significance

Alteration	Alteration Type	Allele Freq
ADAMTS15 (R911Q)	Missense	48
CDC42BPB (R764*)	Stop Gain	44
CHL1 (Q474K)	Missense	9
CUX1 (K1283E)	Missense	54
EMILIN3 (R549H)	Missense	48
FRAS1 (R1167H)	Missense	42
GTF3C2 (V190M)	Missense	45
GTSE1 (PD481LE)	Missense	41
HHIP (P117L)	Missense	44
KIAA0355 (V935I)	Missense	48
KIAA1217 (S851N)	Missense	50
KRTAP26-1 (G121S)	Missense	47
LATS2/ZMYM2	Fused Genes (RNA)	.
LRRC14B (S401L)	Missense	47
MAFF/CSNK1E	Fused Genes (RNA)	.
MICAL3 (P1779S)	Missense	43
NCAPD3 (A1361V)	Missense	49
NOX4 (R380*)	Stop Gain	47
PSPC1/ZMYM2	Fused Genes (RNA)	.
RANBP3 (R330*)	Stop Gain	47
RD3L (S144N)	Missense	46
SLC43A1 (L552R)	Missense	43
TNFRSF10B/SGK223	Fused Genes (RNA)	.
XPC (G442D)	Missense	45
ZNF433 (T445M)	Missense	48

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General Information

Methodology:
GEM ExTra™, a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay, provides for the detection of SNVs, indels, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, and EGFRvIII variants are also detected in RNA. Genomic DNA is extracted from the patient's normal and tumor samples. The isolated DNA is then prepared using a custom xGen target capture (IDT). This library preparation includes shearing, purification, adaptor ligation and PCR amplification. Total RNA is extracted from the patient's tumor sample. The isolated RNA is then prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems). Libraries are then clustered on a flow cell and sequenced using the Illumina HiSeq 2500 or NovaSeq 6000.
Sequence data are analyzed using various validated bioinformatics tools (GEM ExTra™ pipeline 3.0). The reference genome assembly used for alignment is NCBI GRCh37. Each tumor's cancer-specific mutations are then queried against a proprietary gene-drug database based on peer-reviewed literature to identify potential therapeutic associations. Additional analysis and annotation may be provided by N-of-One, Inc.
Copy number events (amplifications/deletions) reported are focal in nature (<25mb).
Allele frequency is dependent on tumor purity. Tumor purity is not taken into account when reporting allele frequencies.
Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the amino acid sequence of the impacted protein. TMB results are rounded to the nearest integer and are classified as follows: TMB-High: >= 20 mutations per megabase (mut/Mb); TMB-Intermediate: 6-19 mut/Mb inclusive; TMB-Low: <= 5 mut/Mb. "Indeterminate" results may be due to poor sample quality or sequencing coverage.
Mean target coverage for tumor sample DNA averages 440x (unique reads). Tumor sample RNA averages 121 million reads.

Limitations:
Samples with a tumor content of less than 30% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection (5% allele frequency), or in a region not included in our assay.
Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting amplifications/deletions.
The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection.
Given the nature of RNA isolated from FFPE, sequencing failures may be seen with highly degraded samples, as they may produce sequence reads too short to align informatically.
Previously unspecified fusions cannot be called by the informatics pipeline if the partner genes occur between two closely adjacent genes on the same strand of the same chromosome.

Disclaimer:
This test was developed, and performance characteristics determined by Ashion Analytics. This test has not been approved by the U.S. FDA. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. Ashion Analytics is certified under the Clinical Laboratory Improvement Amendments (#03D2048606) as qualified to perform high complexity clinical laboratory testing.
This test is used for screening purposes and is not intended to be a stand-alone diagnostic tool. It should also be noted that the data interpretations are based on our current understanding of genes and variants and are current as of the report date. Alterations are listed alphabetically, and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating physician.
General genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Consideration of germline evaluation testing in light of such information is at the discretion of the ordering physician.
Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Ashion does not update reports or send notification regarding reclassification of these alterations.
Standard lab limitations caused by human error, such as sample contamination or sample mix-up, may occur but are unlikely.

Electronically Signed By

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