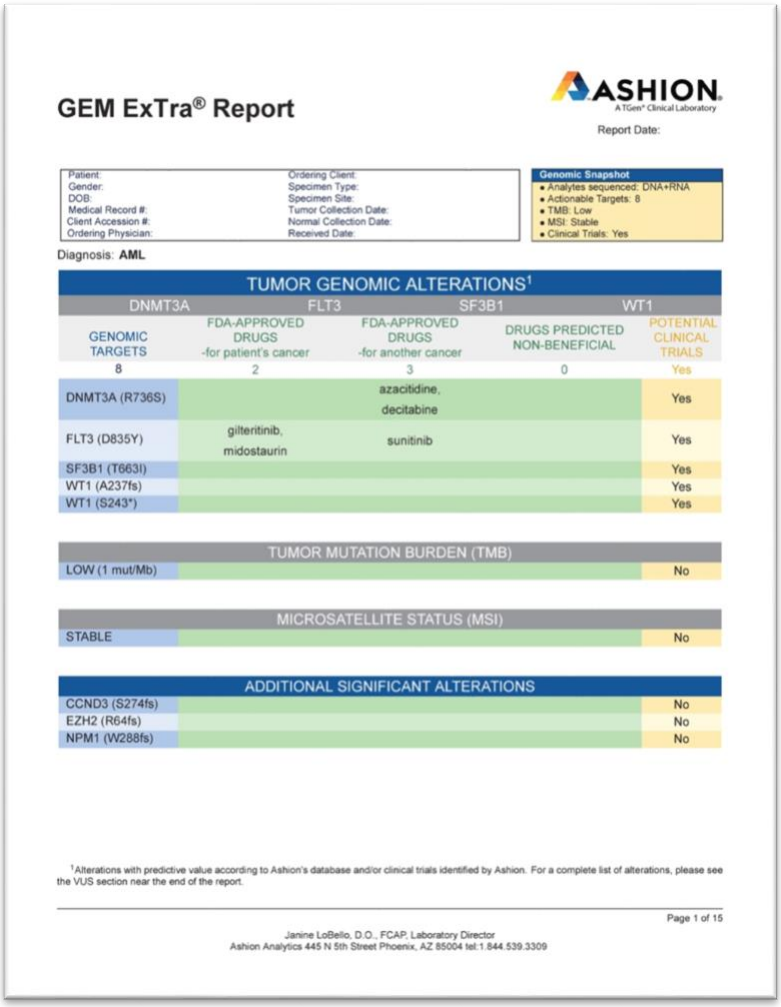
Guide to the Biomarker Report for Blood Cancers

The Ashion biomarker test looks at your blood or bone marrow to compare the genetic information in your cancer to the genetic information you were born with in order to find the gene alterations (or variants) specific to your cancer. If genomic alterations are found in the DNA or RNA of your cancer biospecimen they'll be reported under the Tumor Genomic Alterations section below. Included in this section are alterations that have been matched with Food and Drug and Administration (FDA)-approved treatments. Your doctor can use this information to help make treatment decisions.



GEM ExTra® Report

ASHION
A TGen* Clinical Laboratory

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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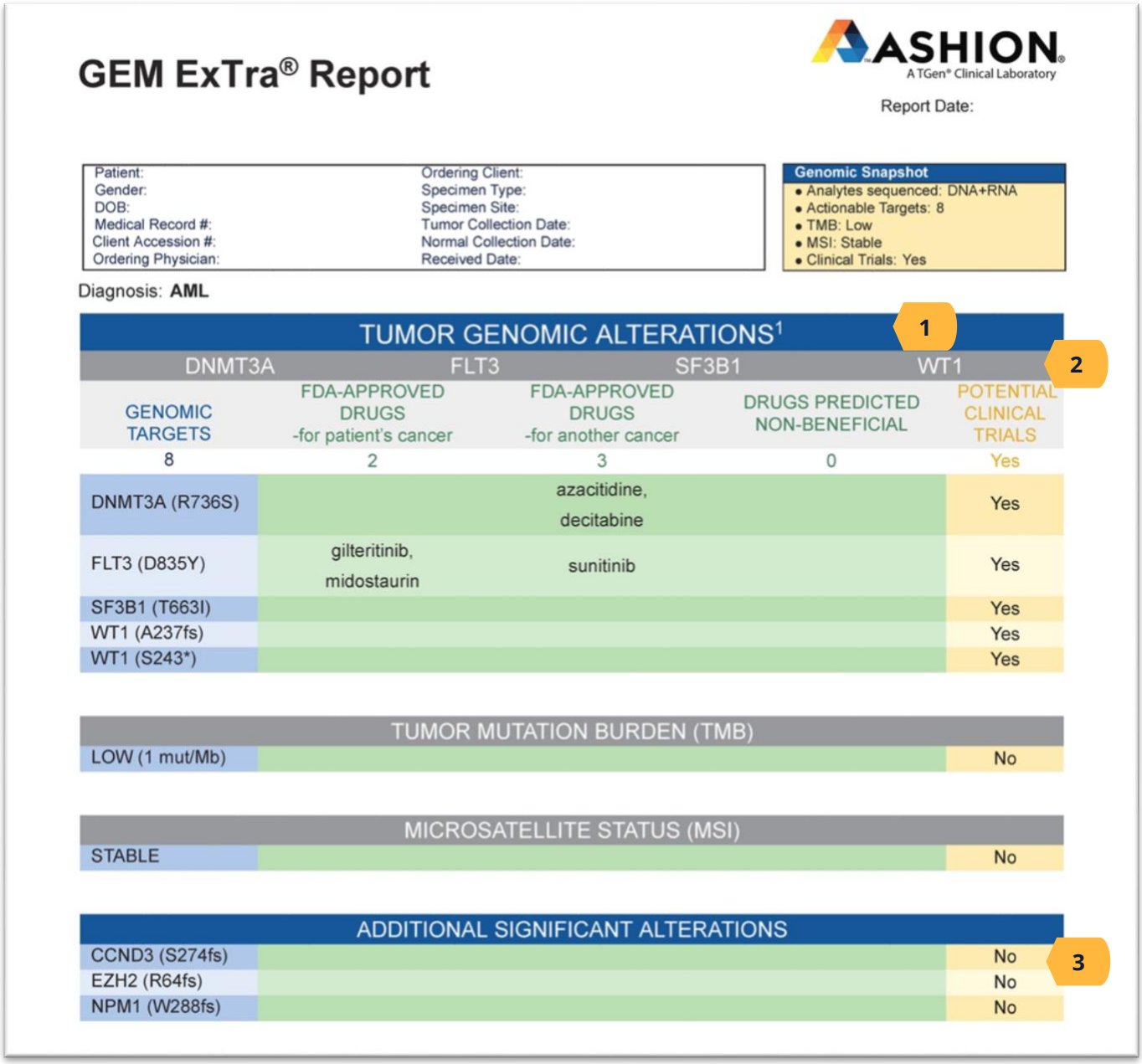

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TUMOR GENOMIC ALTERATIONS[1] — 1

DNMT3A FLT3 SF3B1 WT1 — 2

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
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1. Tumor Genomic Alterations

This section may have important information about gene alterations found in your tumor and relevant approved drug treatments that may be beneficial (or may not be beneficial) for your treatment. Cancers can have many types of gene alterations.

The section lists several of these types of gene alterations, only if they are found in your cancer:

1. Single Nucleotide Variants–variations of a gene
2. Copy Number Variants–the number of times a gene is repeated
3. Gene fusions–when two separate genes combine together
4. Tumor Mutation Burden (TMB)-total number of mutations (changes) found in the tumor cancer cells
5. Microsatellite Instability (MSI) status–mistakes made when DNA was copied in a cell

Visit cancer.gov for more information on the genetics of cancer.

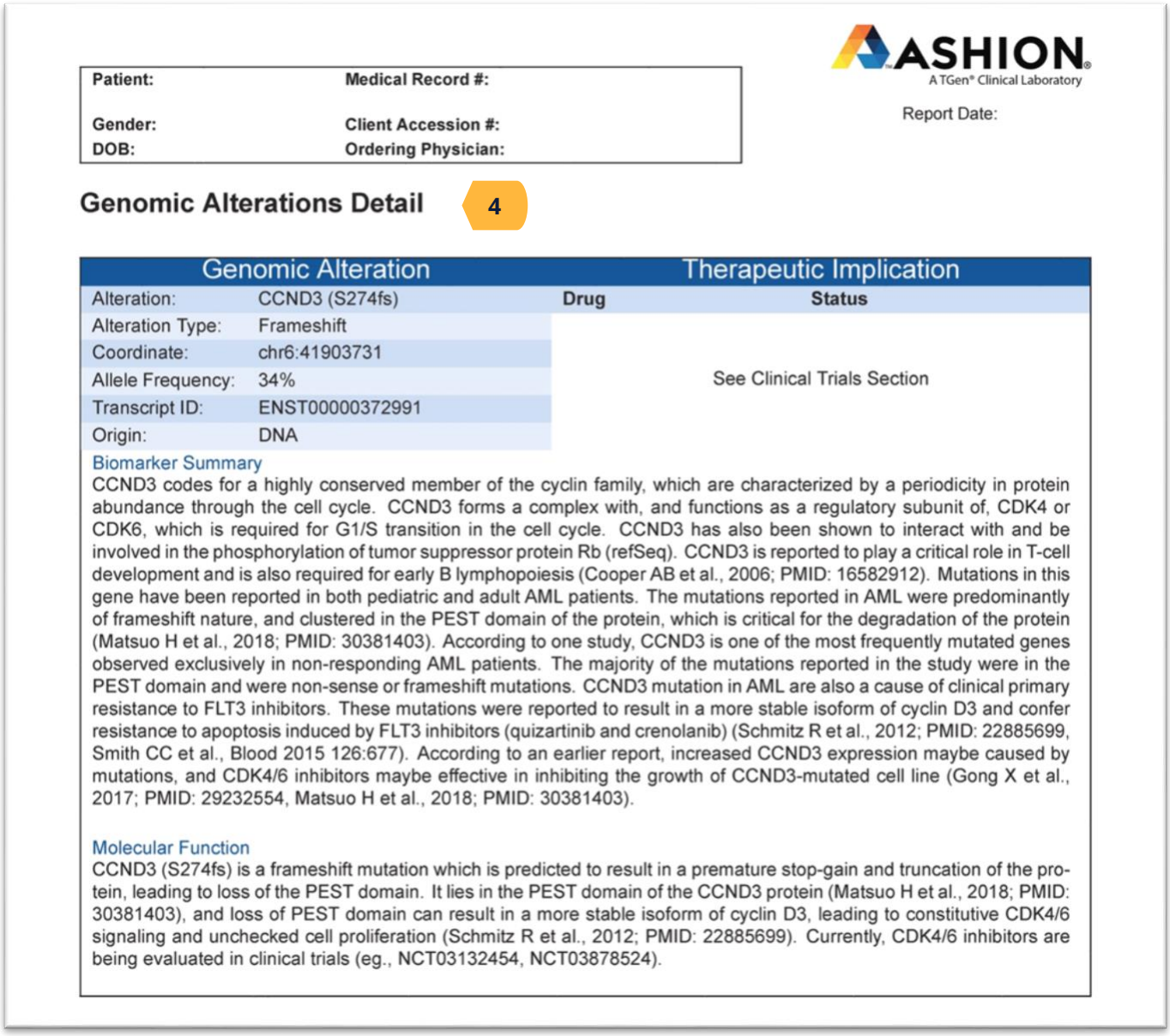
The FDA publishes therapy guidelines for specific cancer types. This table includes FDA approved treatments based on the cancer gene alterations found in the tumor. It also includes drugs predicted to not be of benefit. The list includes therapies for the patient's specific cancer type as well as for other types of cancers that have the same biomarkers. Different cancer types that share biomarkers may respond to the same biomarker-targeted therapies.

2. Clinical Trials

Potential clinical trials related to the tumor genetic alterations detected are listed here. A clinical trial is a type of research study that tests potential new therapies. You may wish to talk with your doctor about clinical trials.

3. Additional Significant Alterations

This section may be present in your report. Additional significant gene alterations found in your tumor are listed here and described in further detail below. Notes may be provided for other cancer gene alterations, including the addition or loss of chromosomal material.



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Genomic Alterations Detail 4

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

Biomarker Summary

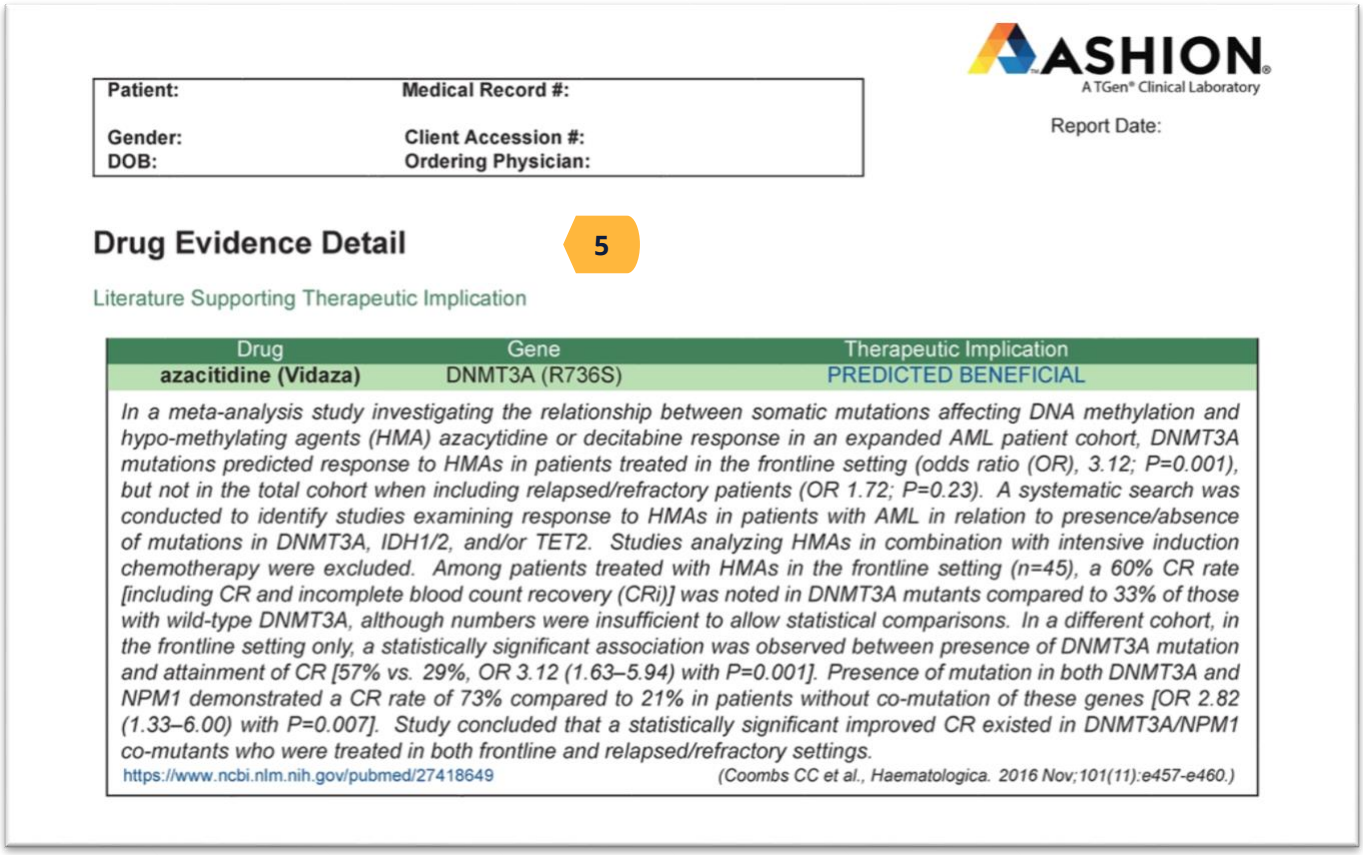
CCND3 codes for a highly conserved member of the cyclin family, which are characterized by a periodicity in protein abundance through the cell cycle. CCND3 forms a complex with, and functions as a regulatory subunit of, CDK4 or CDK6, which is required for G1/S transition in the cell cycle. CCND3 has 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).

4. Genomic Alterations Detail

This section provides further details about the specific genes found to have gene alterations in the patient's tumor. Doctors may find this information useful when recommending treatment. Each gene alteration is summarized based on published research with relevant information about potential treatments. The specific action (molecular function), when known, is described based on published research.



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Drug Evidence Detail 5

Literature Supporting Therapeutic Implication

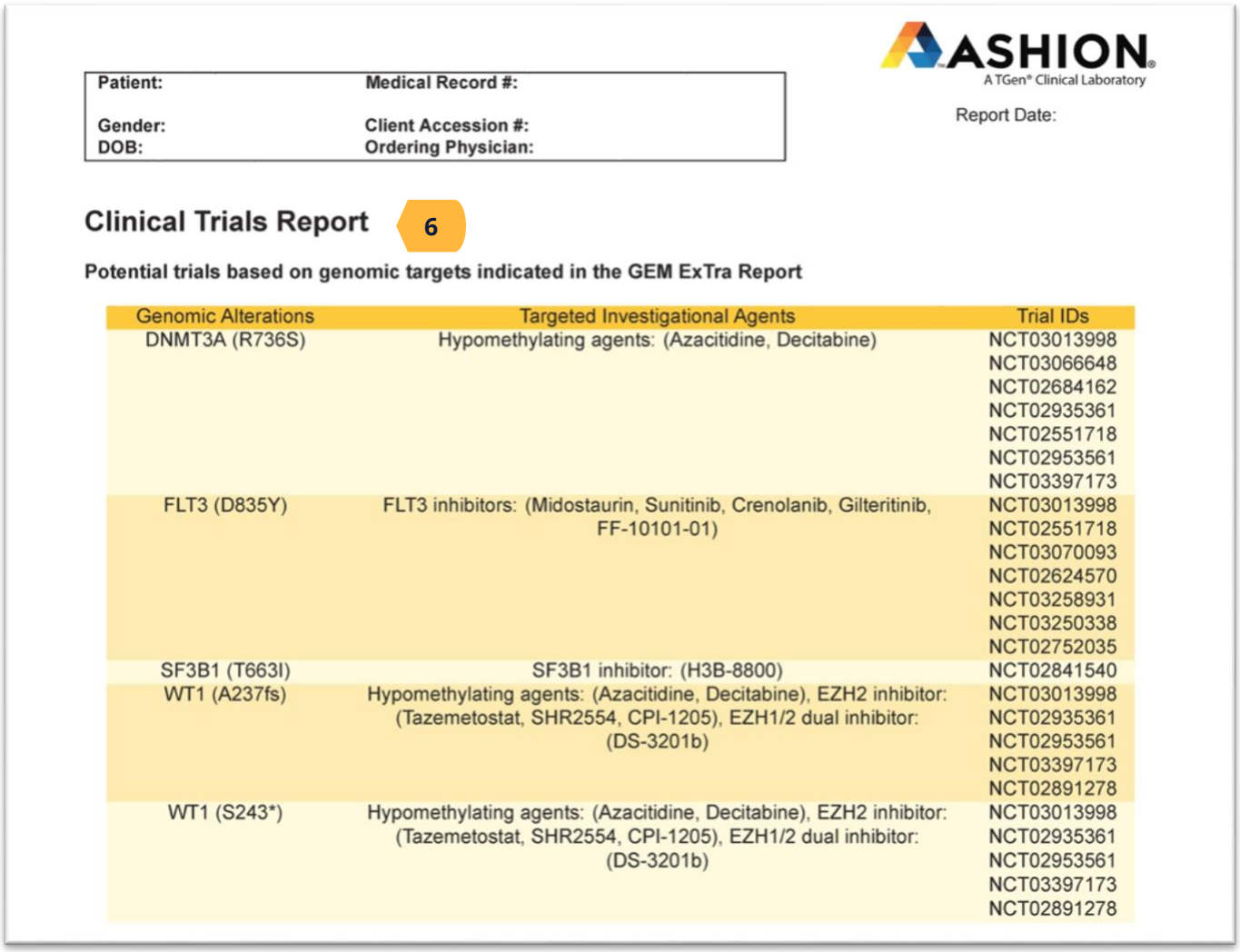
Drug	Gene	Therapeutic Implication
azacitidine (Vidaza)	DNMT3A (R736S)	PREDICTED BENEFICIAL

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.)*

5. Drug Evidence Detail

This section provides further information regarding the list of therapies predicted to be beneficial (or ones that are not beneficial) to the patient, based on the specific gene alterations found in the tumor.



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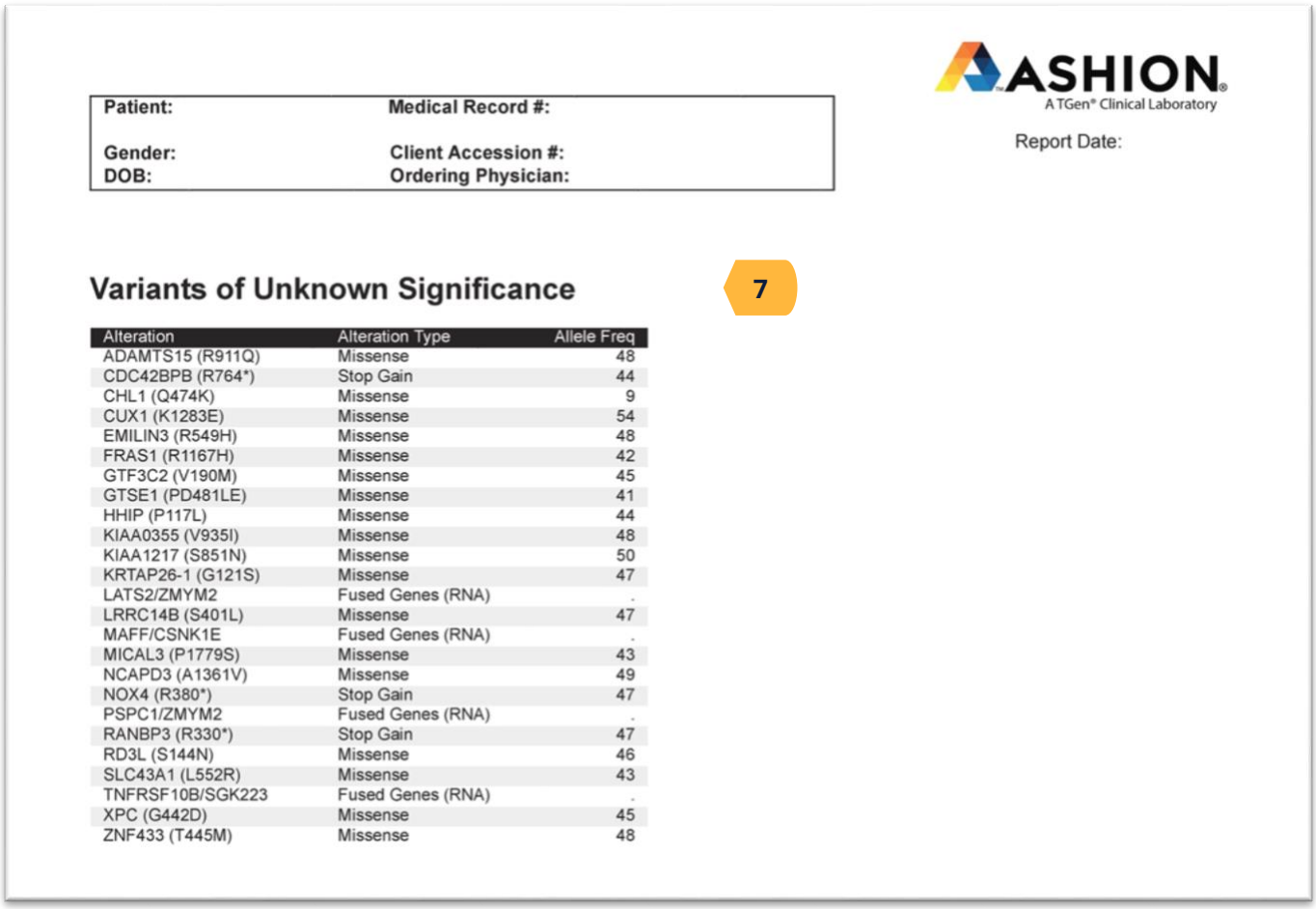
Clinical Trials Report 6

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

6. Clinical Trials Report

This table lists some clinical trials that may be available for a specific type of cancer, based on gene alterations found in the tumor. Investigational drugs (agents) being tested in clinical trials are also included. A clinical trial is a type of research study that tests potential new therapies. More information on each clinical trial, such as where the clinical trial is being done, can be found at ClinicalTrials.gov. You may wish to talk with your doctor about clinical trials.



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Variants of Unknown Significance 7

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

7. Variants of Unknown Significance

There are some alterations in cancer genes that are not yet understood. They may be included here, if they were detected, since they have been found in many cancers. This is another reason why research on these biomarkers is important.