Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

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AMP ISV in Somatic Conditions Working Group

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Expertise that advances patient care through education, innovation, and advocacy.
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Working to address the challenges

• AMP Interpretation of Sequence Variants (ISV) in Somatic Conditions (Cancer) Working Group

• **Project goal:** Establish recommendations for standards in the interpretation of somatically acquired sequence variations (cancer)
  
  • Assemble collaborative multidisciplinary workgroup to review current practice and available literature
  
  • Gather input from AMP/ASCO/CAP/ACMG members regarding current practices for ISV in somatic conditions
  
  • Develop a best practice guidance manuscript to address analysis and reporting of somatic variants: SNV, Indel, CNV, Fusions
## Clinical and/or Experimental Evidence

<table>
<thead>
<tr>
<th>Category</th>
<th>Therapeutic</th>
<th>Diagnosis</th>
<th>Prognosis</th>
</tr>
</thead>
</table>
| **Level A** | 1. FDA-approved biomarkers that predict response or resistance to therapies for a specific type of tumor  
2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor | Biomarkers included in professional guidelines as diagnostic for a specific type of tumor | Biomarkers included in professional guidelines as prognostic for a specific type of tumor |
| **Level B** | Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field | Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field | Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field |
| **Level C** | 1. Biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different type of tumor  
2. Biomarkers that serve as inclusion criteria for clinical trials | Biomarkers of diagnostic significance based on the results of multiple small studies | Biomarkers of prognostic significance based on the results of multiple small studies |
| **Level D** | Biomarkers that show plausible therapeutic significance based on preclinical studies | Biomarkers that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports | Biomarkers that may assist disease prognosis themselves or along with other biomarkers based on small studies or a few case reports |
Clinical and/or Experimental Evidence

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</table>

**NSCLC**

**Fusions of ALK RET ROS1**

- G1269A
- G1123S
- F1174C

**ALK Mutations Predict RESISTANCE**

- Crizotinib
- Ceritinib
- Alectinib

**AMP**

*Association for Molecular Pathology*
## Clinical and/or Experimental Evidence

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</table>

**Promyelocytic Leukemia**

![RT-PCR PML-RARA bcr1](attachment:image1.png)

![PML-RARA](attachment:image2.png)

![Promyelocytic Leukemia](attachment:image3.png)
Clinical and/or Experimental Evidence

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<td>Biomarkers included in professional guidelines as prognostic for a specific type of tumor</td>
</tr>
<tr>
<td></td>
<td>2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AML

April 28, 2017, FDA approved the addition of the targeted therapy midostaurin (Rydapt®) for adults newly diagnosed AML with FLT3 ITD and certain mutations in its kinase domain.
### Clinical and/or Experimental Evidence

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<tr>
<td><strong>Level B</strong></td>
<td>Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field</td>
<td>Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field</td>
<td>Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field</td>
</tr>
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#### Hairy Cell Leukemia
- **BRAF V600E ~100%**
- **vemurafenib**
- **almost 100% response**

#### Pilocytic Astrocytoma
- **KIT D816V** found in the BM of ~100% SM pts
- **KIAA1548-BRAF**
- **Good Prognosis**
Clinical and/or Experimental Evidence

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<tr>
<td>Level C</td>
<td>1. Biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different type of tumor 2. Biomarkers that serve as inclusion criteria for clinical trials</td>
<td>Biomarkers of diagnostic significance based on the results of multiple small studies</td>
<td>Biomarkers of prognostic significance based on the results of multiple small studies</td>
</tr>
</tbody>
</table>

**Ph-like ALL**

Activates JAK-STAT Pathway

Clinical trial using JAK inhibitors

**Mutations in Spliceosome Machinery**

MDS-RS + SF3B1 mut

Good Prognosis
Clinical and/or Experimental Evidence

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<td>Level D</td>
<td>Biomarkers that show plausible</td>
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<tr>
<td></td>
<td>therapeutic significance based on</td>
<td>other biomarkers based on small studies or a few case reports</td>
<td>other biomarkers based on small studies or a few case reports</td>
</tr>
<tr>
<td></td>
<td>preclinical studies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

• Plausible preclinical data derived from cell lines (in vitro) and animal model (in vivo).
• Very early Phase 1 for safety/dosing

• TP53 mutations
• Deletion of TCR or IG loci
• Deletion of CDKN2A
Evidence-based Categorization

**Tier I: Variants of Strong Clinical Significance**
- *therapeutic, prognostic & diagnostic*
  - **Level A Evidence**
    - FDA-approved therapy
    - Included in professional guidelines
  - **Level B Evidence**
    - Well-powered studies with consensus from experts in the field

**Tier II: Variants of Potential Clinical Significance**
- *therapeutic, prognostic & diagnostic*
  - **Level C Evidence**
    - FDA approved therapies for different tumor types or investigational therapies
    - Multiple small published studies with some consensus
  - **Level D Evidence**
    - Pre-clinic trials or a few case reports without consensus

**Tier III: Variants of Unknown Clinical Significance**
  - Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases
  - No convincing published evidence of cancer association

**Tier IV: Benign or Likely Benign Variants**
  - Observed at a significant allele frequency in the general or specific subpopulation databases
  - No existing published evidence of cancer association
Example of Tier I Variants

<table>
<thead>
<tr>
<th>Tumor type: NSCLC</th>
<th>Variant: NM_005228.3(EGFR): c.2240_2257del, p.L747_P753delinsS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tier1/Actionable</strong></td>
<td>Therapeutic</td>
</tr>
<tr>
<td>FDA approved therapies</td>
<td>Yes</td>
</tr>
<tr>
<td>Guidelines</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Mutation type</strong></td>
<td>In-frame deletion, activating</td>
</tr>
<tr>
<td>Variant frequencies</td>
<td>32.5%</td>
</tr>
<tr>
<td>Potential Germline*</td>
<td>NA</td>
</tr>
<tr>
<td>Population database: ESP, dbSNP, 1000Genome, ExAC</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Database</strong></td>
<td><strong>Germline database:</strong> HGMD, ClinVar, etc.</td>
</tr>
<tr>
<td><strong>Somatic database:</strong> COSMIC, My Cancer Genome, etc.</td>
<td>Yes</td>
</tr>
<tr>
<td>Predictive software: SIFT, PolyPhen2, MutTaster, CADD</td>
<td>N/A</td>
</tr>
<tr>
<td>Pathway/Domain</td>
<td>Androgen Receptor Signaling pathway, EGFR1 Signaling Pathway, MAPK Signaling pathway, etc.</td>
</tr>
<tr>
<td>Publications:</td>
<td>Functional study, population study, other</td>
</tr>
<tr>
<td></td>
<td>25726043 25120814 25227801</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

18 bp inframe deletion
Example of Tier II Variants

- A 12 year old boy with high risk Pre B-cell acute lymphoblastic leukemia (ALL)
- AALL1131/VHR standard arm A Phase III Randomized Trial for Newly Diagnosed High Risk B-Lymphoblastic Leukemia (B-ALL)
- Day 8 MRD: 43.3%
- Day 29 MRD: 40%

Gene1: GOLGA5, JAK2
Example of Tier II Variants

<table>
<thead>
<tr>
<th>Tumor type: Tumor type: Ph Like ALL</th>
<th>Variant: GENE1-JAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potential Actionable</strong></td>
<td><strong>Therapeutic</strong></td>
</tr>
<tr>
<td>FDA approved therapies</td>
<td>For PV</td>
</tr>
<tr>
<td>Guidelines</td>
<td>None</td>
</tr>
<tr>
<td>Mutation type</td>
<td>Novel fusion gene</td>
</tr>
<tr>
<td>Variant frequencies</td>
<td>N/A</td>
</tr>
<tr>
<td>Potential Germline*</td>
<td>NO</td>
</tr>
<tr>
<td>Population database: ESP, dbSNP, 1000Genome, ExAC</td>
<td>N/A</td>
</tr>
<tr>
<td>Germline database: HGMD, ClinVar, etc.</td>
<td>NO</td>
</tr>
<tr>
<td>Somatic database: COSMIC, My Cancer Genome, etc.</td>
<td>NO</td>
</tr>
<tr>
<td>Predictive software: SIFT, PolyPhen2, MutTaster, CADD</td>
<td>N/A</td>
</tr>
<tr>
<td>Pathway analysis</td>
<td>JAK-STAT</td>
</tr>
<tr>
<td>Publications: Functional study, population study, other</td>
<td>25207766 25477088</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

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**GOLGA5-JAK2 Fusion**

**GOLGA5**

**JAK2 inhibitor Ruxolitinib**

**JAK2**

**Coiled coil**

**KINASE**

**FERM**

**PSUEDOKINASE**

**Ph like ALL: 19138582 25207766**

**Poor prognosis: 19138582**

**TT GACAA TGG TGAAG ATTA TGA ACTA TTA**

**300bp**
Example of Variants of Unknown Significance

**Variant Features**
- **gDNA:** Chr4(GRCh37):g.106164911A>G
- **cDNA:** NM_001127208.2(TET2):c.3779A>G
- **Location:** Exon 6
- **Type:** Substitution
- **Coding Effect:** Missense
- **Classification:** 5 Classes
- **Class:** Class 3-Unknown pathogenicity
- **Pathogenicity class is NOT automatically computed**

**Known Variations**
- **dbSNP:** rs367866583
- **Minor Allele:**
- **Freq:**
- **Count:**
- **Clin. signif.:**
- **ExAC:** ALL:G=0.0060%-AFR:0.039%-AMR:0%-EAS:0.039%-SAS:0.0044%-NF
- **ESP:**
- **GoNL:**
- **HGMD:**
- **Phenotype:**
- **ClinVar:**
- **PubMed Extracts**
- **LSDB List**
- **LOVD**

**Missense Predictions**
- **Invoke Manually**
  - Align GVGD...
  - SIFT...
  - Mutation Taster...
  - PolyPhen-2...
  - KD4v...
  - All...
- **Automatically computed**
  - Class C0 (GV: 228.51 - GD: 0.00)
  - Tolerated (score: 0.37)
  - Polymorphism (p-value: 0.965)
  - Possible damage
  - Decrease in size and increase in hydrophobicity
Benign/Likely Benign Variants

<table>
<thead>
<tr>
<th>Variant Features</th>
<th>Known Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA: Chr4(GRCh37):g.55593464A&gt;C</td>
<td>dbSNP: rs3822214</td>
</tr>
<tr>
<td>cDNA: NM_000222.2(KIT):c.1621A&gt;C</td>
<td>1000 Genomes</td>
</tr>
<tr>
<td>Location: Exon 10</td>
<td>Validated</td>
</tr>
<tr>
<td>Type: Substitution</td>
<td>Suspect</td>
</tr>
<tr>
<td>Coding Effect: Missense</td>
<td>Minor Allele: C</td>
</tr>
<tr>
<td>AA/AA p.Met541Leu</td>
<td>Freq: 1.064</td>
</tr>
<tr>
<td>Classification: 5 Classes</td>
<td>Count: 1/323</td>
</tr>
<tr>
<td>Class: Class 3-Unknown pathogenicity</td>
<td>Clin. signif.: benign</td>
</tr>
<tr>
<td>Pathogenicity class is NOT automatically computed</td>
<td></td>
</tr>
</tbody>
</table>

ExAC: ALL:C=7.72% - AFR:6.06% - AMR:4.89% - EAS:4.69% - SAS:7.63% - NFE:9.82% - FIN:4.18% - OTH:7.90%
ESP: EA: C=11.19% - AA: C=6.35%
GoNL: HGVD: CM088364
HGMD: Phenotype: Mastocytosis predisposition
ClinVar: RCV00026129.1 / RCV000370023.1 / RCV000422326.1 / RCV000121313.2 / RCV000034504.1 / RCV000315844.1
PubMed Extracts

<table>
<thead>
<tr>
<th>Missense Predictions</th>
<th>Automatically computed</th>
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</thead>
<tbody>
<tr>
<td>Invoking Manually</td>
<td></td>
</tr>
<tr>
<td>Alien GVGD...</td>
<td>Class C0 (GV: 142.37 - GD: 4.86)</td>
</tr>
<tr>
<td>SIFT...</td>
<td>Tolerated (score: 0.41)</td>
</tr>
<tr>
<td>Mutation Taster...</td>
<td>Polymorphism (p-value: 0.947)</td>
</tr>
<tr>
<td>PolyPhen-2...</td>
<td>Benign</td>
</tr>
<tr>
<td>KD4v...</td>
<td>Decrease in size; no other changes</td>
</tr>
<tr>
<td>All...</td>
<td></td>
</tr>
</tbody>
</table>
Germline Mutation in Tumor Testing

• Variant characteristics
  ▪ VAF - but taking CNVs (del, dup, LOH, Amp) into consideration
  ▪ Large indels
  ▪ Local sequence characteristics

• Gene characteristics
  ▪ Tumor suppressor vs. oncogene,
  ▪ known association with cancer predisposition syndromes

• Patient information
  ▪ Family history of cancer
  ▪ Early onset cancer
  ▪ Bilateral and/or multiple primary cancers
  ▪ Cancer of known association with cancer prone syndromes

• ACMG/AMP Standards and Guidelines for the Interpretation of Germline Variant
Germline Mutation in Tumor Testing

- 7 y/o male, no PMH
- One week vague symptoms of L-sided weakness
  - Day of admission with L facial droop and L foot drag
  - CT with R thalamic mass
- Biopsy of mass on Day 1 admission
- Pathology consistent with thalamic (WHO Grade III) anaplastic astrocytoma
- Spinal MRI with no metastatic disease
## Cancer Genomics: Fast Evolving Field

A child with thalamic anaplastic astrocytoma (WHO Grade III)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>VAF</th>
<th>Read depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>c.1906G&gt;C</td>
<td>p.A636P</td>
<td>0.905</td>
<td>876</td>
</tr>
<tr>
<td>TP53</td>
<td>c.847C&gt;T</td>
<td>p.R283C</td>
<td>0.872</td>
<td>836</td>
</tr>
<tr>
<td>BCOR</td>
<td>c.2256_2257del</td>
<td>p.R752fs*</td>
<td>0.754</td>
<td>1478</td>
</tr>
<tr>
<td>FLCN</td>
<td>c.1285del</td>
<td>p.H429fs*</td>
<td>0.706</td>
<td>690</td>
</tr>
<tr>
<td>ATM</td>
<td>c.1071del</td>
<td>p.F357fs*</td>
<td>0.383</td>
<td>149</td>
</tr>
<tr>
<td>NF1</td>
<td>c.4600C&gt;T</td>
<td>p.R1534*</td>
<td>0.373</td>
<td>1329</td>
</tr>
<tr>
<td>PPM1D</td>
<td>c.1349delT</td>
<td>p.L450*</td>
<td>0.367</td>
<td>1332</td>
</tr>
<tr>
<td>MSH6</td>
<td>c.3261dup</td>
<td>p.F1088fs*</td>
<td>0.36</td>
<td>1962</td>
</tr>
<tr>
<td>NF1</td>
<td>c.3739_3742del</td>
<td>p.F1247fs*</td>
<td>0.349</td>
<td>1105</td>
</tr>
<tr>
<td>ASXL1</td>
<td>c.1934del</td>
<td>p.G645fs*</td>
<td>0.348</td>
<td>943</td>
</tr>
<tr>
<td>H3F3A</td>
<td>c.83A&gt;T</td>
<td>p.K28M</td>
<td>0.243</td>
<td>1574</td>
</tr>
</tbody>
</table>
## Cancer Genomics: Fast Evolving Field

### 2016 new classification: Diffuse midline glioma, H3 K27M–mutant

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Tiered System</th>
<th>Germline Guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3F3A</td>
<td>c.83A&gt;T</td>
<td>p.K28M</td>
<td>Tier IA</td>
<td></td>
</tr>
<tr>
<td>MSH2 - Mat</td>
<td>c.1906G&gt;C</td>
<td>p.A636P</td>
<td>Tier II → Tier IB*</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>TP53 - Pat</td>
<td>c.847C&gt;T</td>
<td>p.R283C</td>
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<td>Likely Pathogenic</td>
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<tr>
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<td>p.R752fs*</td>
<td>Tier II</td>
<td></td>
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<tr>
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<td>p.R1534*</td>
<td>Tier II</td>
<td></td>
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<tr>
<td>PPM1D</td>
<td>c.1349delT</td>
<td>p.L450*</td>
<td>Tier II</td>
<td></td>
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<tr>
<td>MSH6</td>
<td>c.3261dup</td>
<td>p.F1088fs*</td>
<td>Tier II</td>
<td></td>
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<tr>
<td>NF1</td>
<td>c.3739_3742del</td>
<td>p.F1247fs*</td>
<td>Tier II</td>
<td></td>
</tr>
<tr>
<td>ASXL1</td>
<td>c.1934del</td>
<td>p.G645fs*</td>
<td>Tier II</td>
<td></td>
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</table>

*Recategorization after germline confirmation*
Reporting Recommendations
Tier-based Reporting

• Variant should be classified into the 4-tiered system
• Tiers I-III should be reported in descending order of clinical importance
• Suspected germline variants should be confirmed with normal tissue along with genetic counseling
• It is NOT recommended to include Tier IV or "benign/likely benign" variants/alterations in the report (should be available)
• Pertinent “negatives” should be reported, in a disease-specific manner
Variant Nomenclature

All detected genetic alterations should be annotated and reported as designated by the HUGO Gene Nomenclature Committee

- Gene name – official gene symbol, may include colloquial gene name in the interpretation (KMT2A/MLL)
- Transcript ID – transcript accession and version number (e.g. NM_004006.2)
- Nucleotide change – c.123G>A
- Amino acid change – p.Lys76Asn
- Variant allele frequency
- Genomic coordinates – at the discretion of laboratory director
- Exon number, protein domain, pathway involved - at the discretion of laboratory director
Reporting Recommendations

• Clinical significance of the variants should be clearly described in the interpretation
• Evidences used for variant categorization should be illustrated in the interpretation
• References such as publications and database should be appropriately cited for further information
• If confirmation of a variant is performed, method of confirmation and the result should be explained
Comprehensive Heme Panel

**CLINICAL INDICATION:** New Diagnosis Leukemia/B-ALL

**PATHOLOGY SPECIMEN ID:** BMR-17-2

**HISTOLOGIC DIAGNOSIS:** B lymphoblastic leukemia (99% blasts)

**PRELIMINARY RESULTS CALLED TO:** Preliminary report of fusion analysis issued on 1/10/2017

**SUMMARY OF FINDINGS**

Next generation sequencing (NGS) analysis of genes in the CHOP Comprehensive Heme NGS Panel performed on the DNA and RNA extracted from the tumor specimen of the patient showed the following results:

1. One Tier 2 fusion gene: **EPOR** (NM_000121.3) - **IGH** (NC_000014.9).
2. Tier 2 copy number variations (CNVs): Deletions of **IKZF1** on chromosome 7p12.2 and **PAX5** on chromosome 9p13.2.
3. Two variants of unknown significance as listed in the Tier 3 variant table.
4. No clinically significant (Tier 1 or Tier 2) single nucleotide variants or indels were identified within the detection limits of this assay.

The findings of an **EPOR-IGH** fusion along with **IKZF1** and **PAX5** deletions are characteristic of **EPOR-rearranged Ph-like ALL**. **IGH-EPOR** positive ALL is accompanied by high levels of **EPOR** expression and **EPOR-mediated JAK-STAT** activation (see interpretation for details). Clinical trials using JAK inhibitors are available for Ph-like ALL with JAK-STAT activation at clinicaltrial.gov.
INTERPRETATION

Tier 1. Variants With Strong Evidence of Clinical Significance

1A. Somatic (Tumor) Variants with Strong Evidence of Clinical Actionability

No Tier 1A variants were detected.

Tier 2. Variants With Potential Clinical Significance

The NGS analysis identified a **EPOR (NM_001211.3) - IGH (NC_000014.9)** fusion and two tier 2 copy number variants (CNVs).

**Fusion Analysis:**

**EPOR** (erythropoietin receptor) encodes the erythropoietin receptor which is a member of the cytokine receptor family. Upon erythropoietin binding, this receptor activates Jak2 tyrosine kinase which activates different intracellular pathways including: Ras/MAP kinase, phosphatidylinositol 3-kinase and STAT transcription factors. The stimulated erythropoietin receptor appears to have a role in erythroid cell survival. Defects in the erythropoietin receptor may produce erythrocytosis and familial erythrocytosis [Entrez Gene ID 2057].

**IGH** (immunoglobulin heavy locus) encodes the heavy chain of human antibodies. The locus contains numerous variable (IGHV), diversity (IGHD), joining (IGHJ) and constant (IGHC) gene segments. Recurrent translocations involving IGH have been reported to play significant roles in driving tumorogenesis in different cancers including ALL. More than 50 different genes have been identified as IGH fusion partners [PMID: 20042721; http://atlasgeneticsoncology.org//Genes/IGHMID40.html].

The IGH-EPOR fusion gene was first identified in acute lymphoblastic leukemia (ALL) with translocation t(14;19)(q32;p13). IGH-EPOR-positive ALL is accompanied by high levels of EPOR expression and EPOR-mediated JAK-STAT activation. Studies have reported that B-ALL associated with t(14;19)(q32;p13.1) / IGH-EPOR is a distinctive type of Ph-like ALL that is associated with younger age and an aggressive clinical course. Recent studies have shown effective results using JAK2 inhibitors to treat IGH-EPOR rearranged leukemic cells in a xenograft model, suggesting a therapeutic option for EPOR-rearranged Ph-like ALL [PMID: 27544511, 18819706, 26855458, 2403042, 22897847].

The identification of this fusion gene is consistent with the t(14;19)(q32;p13) translocation, involving IGH, identified by the fluorescence in situ hybridization (FISH) studies performed on this tumor (January 2017, reported separately). These results are characteristic of EPOR-rearranged Ph-like ALL. Clinical trials using JAK inhibitors are available for Ph-like ALL with JAK-STAT activation.

**Copy Number Analysis:**

NGS analysis performed on the DNA extracted from this tumor specimen identified copy number variants (CNVs) as listed in the table below. Clinically significant CNVs include partial loss of chromosome 7p12.2 including partial deletion of the IKZF1 gene (exon 1-3) and partial loss of chromosome 9p13.2 including partial deletion of the PAX5 gene (exon 2-6).

The loss of IKZF1 and PAX5 are characteristic of Ph-like ALL.

No Tier 2 single nucleotide variants or indel were detected.
FUSION GENE

<table>
<thead>
<tr>
<th>Fusion Gene</th>
<th>5' Transcript</th>
<th>3' Transcript</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFGR-IGH</td>
<td>EFGR (NM_001211.3) ex 8</td>
<td>IGH (NM_000012.) ex 1</td>
<td></td>
</tr>
</tbody>
</table>

CNV Table

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Band</th>
<th>Abnormality</th>
<th>CNV Start</th>
<th>CNV Stop</th>
<th>Gene</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>p12.2</td>
<td>LOSS</td>
<td></td>
<td></td>
<td>IKZF1</td>
<td>3/5 exons</td>
</tr>
<tr>
<td>9</td>
<td>p13.2</td>
<td>LOSS</td>
<td></td>
<td></td>
<td>PAX5</td>
<td>5/10 exons</td>
</tr>
</tbody>
</table>

Tier 3. Variants of Unknown Significance

NGS analysis on this tumor specimen identified two variants in this category as listed in the Variants of Unknown Significance table. Variants in this category have NOT been confirmed by a different method with a few exceptions. Confirmation of specific variants is available upon request. More information about specific genes can be found at [http://www.ncbi.nlm.nih.gov/gene](http://www.ncbi.nlm.nih.gov/gene). Pathway and protein domain information of these mutations can be found in the following databases: [http://cancer.sanger.ac.uk/cancergenome/projects/ cosmic/](http://cancer.sanger.ac.uk/cancergenome/projects/ cosmic/) and [http://www.kegg.jp/kegg/](http://www.kegg.jp/kegg/). Additional information regarding the specific variants detected through this analysis is available upon request.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic Position (hg19)</th>
<th>Reference / Isoform</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
<th>Žygosity</th>
<th>VAF</th>
<th>ExAC</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETV6</td>
<td>chr11: 11992223-11992223</td>
<td>NM_001987.4</td>
<td>c.313C&gt;G</td>
<td>p.Arg104Gly</td>
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<td>0.08</td>
<td></td>
<td>COSM306115</td>
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<tr>
<td>USH2A</td>
<td>chr11: 15801511-15801511</td>
<td>NM_206503.2</td>
<td>c.11627C&gt;T</td>
<td>p.Thr397Met</td>
<td></td>
<td>0.16</td>
<td>0.0005</td>
<td>COSM4782855; rs142381713; CM073404</td>
</tr>
</tbody>
</table>
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TEST BACKGROUND
The Comprehensive Hematological Cancer Panel V2.0 includes sequence and copy number analyses of 98 cancer genes and 566 known fusions and many more novel fusions associated with 106 cancer genes. The genes included in the panel are listed at [https://apps.chop.edu/service/laboratories/olsd.cell/division-genomic-diagnostics]. The mutation nomenclature is based on the convention recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).

METHODOLOGY
Next generation sequencing (NGS) and data analysis: Genomic DNA is extracted from the patient’s sample following standard DNA extraction protocols. Extracted DNA is fragmented and tagged using SureSelectXT target enrichment to generate adapter-tagged libraries. Bioin-labeled probes specific to the targeted regions are used for capture hybridization. Libraries are enriched for the desired regions using streptavidin beads. Enriched libraries are then indexed and pooled for sequencing. Libraries are sequenced on an Illumina MiSeq or HiSeq platform for 150 bp paired-end reads. All coding exons and the flanking intron sequences of targeted genes in the panel are sequenced, and select known intronic mutations are also evaluated. Sequence data are analyzed using the home brew software Concordant V2 and NextQEd V2 NGS Analysis Software. Sequence variants within exons and by flanking intron sequences are annotated. Copy number variation (CNV) analysis for gross deletions and duplications are evaluated using NGS data. Cancer gene fusions are analyzed using Archer technology and custom-designed CHOQ fusion panel primers with target-specific molecular barcodes. Sequencing data are analyzed using Archer™ Analysis for fusion genes. Clinically significant variants including single nucleotide variants (SNVs), indels, CNVs and fusion genes are confirmed by Sanger sequencing, MLPA, Real-Time PCR, or ddPCR only when necessary.

Variant categorization and reporting: Rare sequence variants, copy number variants, and gene fusions are evaluated based on the currently available information from relevant resources, such as professional guidelines, clinical and population variant databases, tumor specific databases, and the scientific literature. Sequence variants are reported according to HGVS nomenclature [Don Dunnen 2016, PMID: 26931103]. Somatic variants are classified using criteria consistent with those recommended by the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP) [Li 2017, PMID: 27689453], as described below. Tier 1-3 variants are reported. Tier 4 variants are not reported unless requested by the referring physician.

Tier 1: Variants with strong evidence of clinical significance. 1A. Somatic variants: a. Variants predict response to FDA approved therapy for the tumor type or investigational therapy with strong evidence from well powered clinical trials; b. Variants of diagnostic significance that are included in practice guidelines or reported with strong evidence and consensus; c. Variants of prognostic significance that are included in practice guidelines or reported with strong evidence and consensus. 1B. a. Constitutional variants that are known pathogenic based on ACMG Guideline [Richards 2015, PMID: 25741888]; b. Variants of pharmacogenomic significance.

Tier 2: Variants with potential clinical significance. a. Variants predict response to FDA approved therapy for a different tumor type or investigational therapy with some published evidence; b. Variants of diagnostic significance based on convincing published data but not yet included in practice guidelines; c. Variants of prognostic significance based on convincing published data but not yet included in practice guidelines.

Tier 3: Variants of uncertain significance.

Tier 4: Benign and likely benign variants.

LIMITATIONS
This analysis is based on the current understanding of the genomic alterations of pediatric hematologic malignancies. Clinical implications of some variants may be uncertain or unknown at the time of this report and may change over time. Interpretations are made with the assumption that any clinical information provided to the laboratory is accurate. Clinical interpretation of variants allele frequencies should take into consideration factors such as copy number variation, loss of heterozygosity, and tumor cellularity of the sample.

This test recommends a minimum of 30% tumor cells out of total cellularity in the sample. Single nucleotide variants and small indels have a limit of detection of 30% allele frequency, while copy number variants have a limit of detection of 30% mosaicism. Samples with tumor cellularity less than 30% may have compromised ability to detect subclonal variants, especially low-level mosaic indels, copy number variants, and gene fusions.

This test may or may not detect duplicated or inserted sequences greater than 100 base pairs, depending on the nature of the duplication or insertion. CNV analysis of this test is not quantitative. Specific mosaic levels of disease associated CNVs are not reported.

This test cannot detect variants, fusions, or copy number variations outside the regions of interest (ROI) defined by the test. This assay is at risk of false negatives when sequence coverage for a ROI is below 100X. In rare situations, some SNVs, indels, or gross rearrangements may not be detectable by this test due to local sequence characteristics or the presence of closely related pseudogenes.

Signed By:  
Marilyn M. Li M.D. FACMG  1/20/2017

Director - Cancer Genomic Diagnostics Laboratory
Summary

• We developed a tier-based somatic variant categorization and reporting system
• The manuscript “Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer” was published in JMD in January 2017
• Cancer genomics is a rapidly evolving field, the clinical significance of any variant should be re-evaluated on an ongoing basis
• Guideline dissemination & education is ongoing
• Implementation studies and surveys are being developed to inform future updates to the guidelines
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- Michael Datto (CAP representative)
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- Anas Younes (ASCO representative)
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Please Mark your Calendar for the Following AMP Events!!

AMP 2019
NOVEMBER 7-9, 2019
Corporate Workshop Day November 6
Baltimore Convention Center | Baltimore, MD

AMP EUROPE 2020
Clinical Genomics: Beyond the Somatic Mutation
Milan, Italy, May 11–13, 2020

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