This is a next generation sequencing (NGS) test to identify molecular abnormalities in 177 genes implicated in hematologic cells. The assay is designed to detect genomic abnormalities in hematologic cells. The presence of genomic abnormalities indicates clonal hematopoiesis. Clonal hematopoiesis has implication on the development of hematologic neoplasms as well as cardiovascular diseases.

### Clonal Hematopoiesis Detected

#### Mutated Genes

| TET2 | ASXL1 |

#### Diagnostic Implications

| Clonal hematopoiesis of Indeterminate Potential (CHIP) | Yes |
| Clonal Cytopenia of Unknown Significance (CCUS) | No |
| Risk of Cardio Vascular Disease | Yes |
| MDS | NO |
| Other | Non |

#### Prognostic Implications

| Clonal hematopoiesis of Indeterminate Potential (CHIP) | May evolve into CCUS or MDS |
| Clonal Cytopenia of Unknown Significance (CCUS) | N/A |
Cardio Vascular Disease | 4 times greater | NA | Neutral
---|---|---|---
MDS | | | |
Other | | | |

#### Therapeutic Implications

<table>
<thead>
<tr>
<th>Gene</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2</td>
<td>Monitor Hematologic indices/Check other CVD risk factors</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Monitor Hematologic indices/Check other CVD risk factors</td>
</tr>
</tbody>
</table>

### Results Summary

- Mutations in TET2 and ASXL1 genes are detected at very low levels.
- The presence of these mutations suggests the presence of abnormal clonal hematopoiesis. However, since no hematologic abnormality is detected, the findings are consistent with CHIP (Clonal Hematopoiesis of indeterminate potential).
- The presence of mutations in two genes suggests higher possibility of transformation into MDS or other hematologic neoplasm at perhaps earlier age as compared with the presence of a mutation in one gene.
- The presence of these abnormalities is also consistent with higher risk of developing cardiovascular disease and myocardial infarction.

### Clinical Background:

Random somatic mutations occur in normal cells, but rarely these cells evolve into viable clone, but with aging, the possibility of a clone to accumulate increases. Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the presence of low-level mutations in the peripheral blood in clinically normal individuals. CHIP is detected in 3-5% of normal individuals above the age of 50 and in approximately 10% of people aged 70 to 80. The most common mutation is on the DNMT3A gene, followed by TET2 and ASXL1. The rate of transformation to a hematological neoplasia is 0.5–1% per year. Clonal cytopenias of undetermined significance (CCUS) is defined by the presence of cytopenia (anemia, low platelets or white cells) along with low level mutations but does not meet World Health Organization (WHO)-defined criteria for MDS and the mutations are detected in <40% of cells. Approximately 25% to 65% of patients with cytopenia will have mutation in one or more genes. These patients with mutations have significantly higher probability of developing MDS or other hematopoietic neoplasms (AML, MPN, lymphoma,...) within 5 years. Recent studies demonstrated that patients with acute myeloid leukemia (AML) had significantly high incidence of clonal hematopoiesis multiple years prior to developing AML. All patients with mutations in specific genes such as IDH1, IDH2, and TP53 developed AML within few years.

In addition, recent studies linked mutations in peripheral blood to cardiovascular disease (CVD). Recent data show that patients with CHIP have 4.0 times greater risk of myocardial infarction as compared to individuals without such clone. The prevalence of CHIP in patients with coronary artery disease is reported to be at 18.2%. In contrast, the prevalence of CHIP in centenarians is only at 2.5%. It has been shown that mutations in TET2 gene, which is one of the commonly mutated genes in CHIP, are pro-inflammatory and lead to the development of atherosclerotic plaques. Based on that it has been suggested that anti-inflammatory agents might slow the progression of cardiovascular disease in patients with low level mutations in peripheral blood. Additional studies are needed to determine the clinical relevance of anti-inflammatory agents in reducing CVD or to determine the relationship between duration of the presence of CHIP or level of the mutated clone with progression of CVD or hematologic disease.
neoplasms. In addition, studies are needed to determine if early therapy especially using targeted therapy (IDH1/IDH2 inhibitors) would prevent or delay the onset of hematologic neoplasms.

**Biological Relevance of Detected Alterations**

- **TET2** (Tet Methylcytosine Dioxygenase 2) gene encodes a methylcytosine dioxygenase that catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine. The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders. In addition to its role in DNA demethylation, also involved in the recruitment of the O-GlcNAc transferase OGT to CpG-rich transcription start sites of active genes, thereby promoting histone H2B GlcNAcylation by OGT. No targeted therapy is available for this gene. However hypomethylation agents are considered to be relevant in treatment of diseases with abnormalities in this gene.

- **ASXL1** (Additional Sex Combs Like 1, Transcriptional Regulator) gene encodes a protein that disrupt chromatin in localized areas, enhancing transcription of certain genes while repressing the transcription of other genes. The protein encoded by this gene functions as a ligand-dependent co-activator for retinoic acid receptor in cooperation with nuclear receptor coactivator 1. Mutations in this gene are associated with myelodysplastic syndromes and chronic myelomonocytic leukemia, but also reported in colorectal and other types of cancers.

**Drug Information:**

None

**Potential Clinical Trials**

None

**Detailed Results**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Hgvsp</th>
<th>Hgvsc</th>
<th>Amino acids</th>
<th>Codons</th>
<th>Consequence</th>
<th>Allele frequency</th>
<th>Read depth</th>
<th>Predicted effect on protein</th>
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<tbody>
<tr>
<td>TET2</td>
<td>NP_001120680.1</td>
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<td>Q/*</td>
<td>Caa/Taa</td>
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<td>NM_015338.5</td>
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<td>*/G</td>
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**Methodology and Test Background**

This is next generation sequencing (NGS) test that analyzes DNA for abnormalities in 65 genes that are reported to be altered in hematopoietic cells. The assay is designed to detect single nucleotide variations (SNV) and indels. Our sequencing method has a typical sensitivity of 1% to 3% for detecting common specific mutations in hematopoietic cells. Performance of the assay may vary dependent on the quantity and quality of nucleic acid, sample preparation and sample age.

**Tested genes**

The professional and technical components of this assay were performed at Genomic Testing Cooperative, LCA, 27 Technology Drive, Suite 100, Irvine, CA 92618 (CLIA ID: 05D2111917). The assay is not FDA cleared and the performance characteristics were established at this location.
## Genes Tested for abnormalities

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<thead>
<tr>
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<th>BTK</th>
<th>CBLC</th>
<th>CIC</th>
<th>DNMT3A</th>
<th>FBXW7</th>
<th>H3F3A</th>
<th>KIT</th>
<th>MYC</th>
<th>PBRM1</th>
<th>PTNP11</th>
<th>SETD2</th>
<th>TP53</th>
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<tbody>
<tr>
<td>ASXL1</td>
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<td>CD274</td>
<td>CSF1R</td>
<td>EP300</td>
<td>FLT3</td>
<td>IDH1</td>
<td>KMT2A</td>
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<td>RHOA</td>
<td>SRSF2</td>
<td>TSC1</td>
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<td>CSF3R</td>
<td>ERG</td>
<td>GATA1</td>
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<td>JAK2</td>
<td>KRAS</td>
<td>NRAS</td>
<td>PIM1</td>
<td>SDHB</td>
<td>STAT3</td>
<td>WT1</td>
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<tr>
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<td>CEBPA</td>
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References


Electronic Signature
Maher Albitar, M.D., Pathologist - GTC Laboratories
The Technical Component Processing, Analysis and Professional Component of this test was completed at GTC Laboratories, 21 Technology Dr. #100, Irvine, CA / 92618/ Medical Director: Maher Albitar, M.D.

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