Poster #1689

Interclonal and Intraclonal Heterogeneity in Patients with IDH1/2 Mutation

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INTRODUCTION

DNA methylation in AML/MDS plays a major role in the pathogenesis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The major genes involved in DNA methylation in AML/MDS are IDH1 and IDH2, TET2 and DNMT3A. Mutations in IDH1/2 result in the production of an aberrant metabolite, 2-hydroxyglutarate, which acts as a competitive inhibitor of alpha-ketoglutarate and inhibits TET2 oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC). Mutations in TET2 or IDH1/2 are associated with reduced levels of 5hmC and genomic hypermethylation. TET2 mutations and IDH1/2 mutations are believed to be mutually exclusive. In addition, DNMT3A as a DNA methyltransferase enzyme is commonly mutated in AML/MDS and its mutation is believed to lead to hypomethylation. Understanding the interaction between these genes may influence therapy with IDH1/2 inhibitors.

OBJECTIVE

Toward better understanding of interaction between these genes, the mutation profile of these genes was analyzed in patients with AML/MDS.

SAMPLES AND METHODS

Samples

1182 bone marrow aspirates

DNA extraction

DNA was extracted from bone marrow aspirate using the QIAamp DNA Mini Kit

Sequencing

TruSight Myeloid Next Generation Sequencing Panel (Illumina, San Diego, CA) covering hot spot mutations in 54 genes

Average depth of sequencing of 10,000X

RESULTS

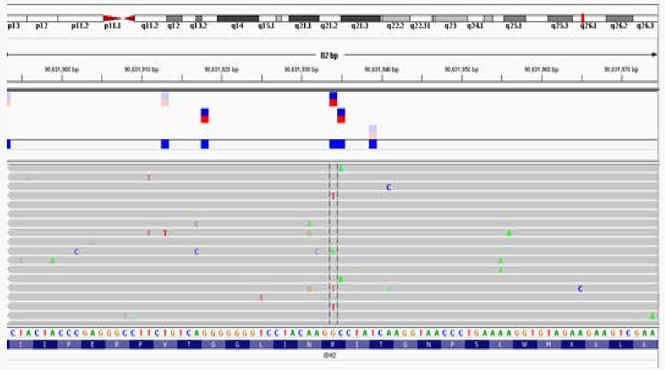
1. Co-Occurrence of IHD1 & IDH2 Mutations

- IDH1/2 mutations were detected in 201 of the 1182 (17%).
- IDH1 was detected in 87 (7.4%).
- IDH2 was detected in 120 (10.1%), including 6 patients with mutations in both IDH1 and IDH2.

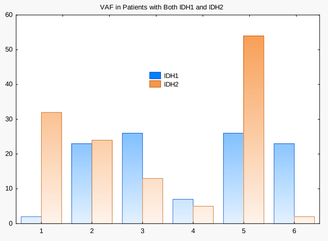
1a: Unique Mutations in IDH1 & IDH2

Hgvsp	Hgvsc
IDH1	
NP_005887.2:p.Arg132His	NM_005896.2:c.395G>A
NP_005887.2:p.Arg132Cys	NM_005896.2:c.394C>T
NP_005887.2:p.Arg132Gly	NM_005896.2:c.394C>G
NP_005887.2:p.Arg132Ser	NM_005896.2:c.394C>A
NP_005887.2:p.Arg132Leu	NM_005896.2:c.395G>T
IDH2	
NP_002159.2:p.Arg140Gln	NM_002168.2:c.419G>A
NP_002159.2:p.Arg172Lys	NM_002168.2:c.515G>A
NP_002159.2:p.Arg140Trp	NM_002168.2:c.418C>T
NP_002159.2:p.Arg140Gly	NM_002168.2:c.418C>G
NP_002159.2:p.Arg172Thr	NM_002168.2:c.515G>C

1b: Example of Bi-Allelic Mutations in IDH2: Arg140Gln and Arg140Trp

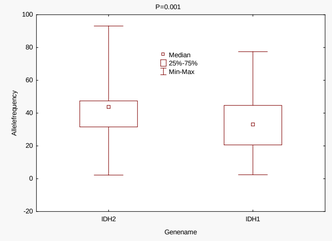


1c: Variant Allele Frequency in Patients with Both IDH1 & IHD2



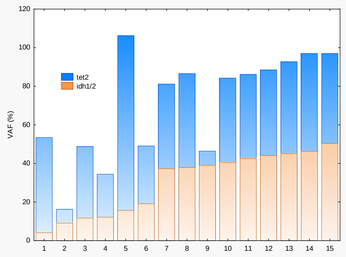
- **Six patients had mutations in IDH1 and IDH2**
- **Two of the 6 patients with both IDH1 and IDH2 mutations had VAF <20%, raising the possibility of two independent clones.**

1d: Comparison of VAF for IHD1 & IDH2



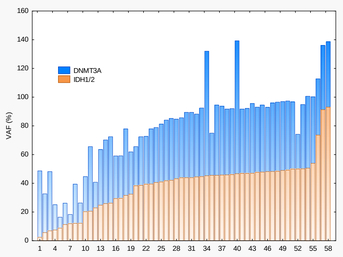
- **Variant (mutant) allele frequency (VAF) was significantly higher (P=0.001) in IDH2 as compared to IDH1 (median of 43.35% vs 35.0%, respectively).**
- **Thirteen patients (6.5%) had mutant VAF >50% suggesting homozygosity, 11 with IDH2 mutation.**

2. Co-Occurrence of TET2 & IDH1/2 Mutations



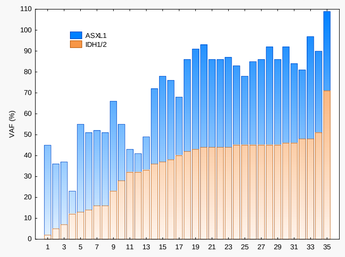
- **TET2 mutations were detected in 15 (7.5%) of the patients with IDH1/2 mutations.**
- **There was significant difference (P=0.03) in VAF between IDH1/2 and TET2.**
- **Nine patients showed comparable VAF while 6 patients showed completely different VAF, suggesting subclonal heterogeneity.**

3. Co-Occurrence of DNMT3A & IDH1/2 Mutations



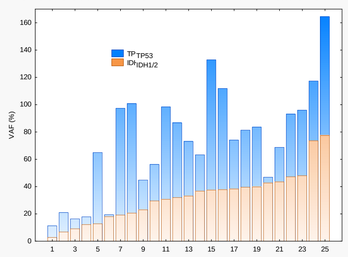
- **58 (29%) patients showed mutations in IDH1/2 and DNMT3A.**
- **While there was no significant difference in VAF between IDH1/2 and DNMT3A, VAF in IDH1/2 was >50% in 6 of these patients and in DNMT3A in 3 patients.**

4. Co-Occurrence of ASX1 & IDH1/2 Mutations



- **High Frequency of IDH1/2 and ASXL1 mutations**
- **ASXL1 mutation was detected in 35 patients with IDH1/2 mutations (17%). The VAFs of the two mutations were overall similar without statistical difference.**

5. Co-Occurrence of TP53 & IDH1/2 Mutations



- **Twenty four patients had TP53 mutation, of which 16 had IDH1 mutation and 8 had IDH2 mutation, which is disproportional with the prevalence of IDH1 mutation.**
- **There was no statistically significant difference in VAF between TP53 and IDH1/2, but 4 of these patients had both DNMT3 and IDH2 mutations and one had both IDH1 and IDH2 mutations.**
- **None of the patients with TP53 mutation had TET2 mutation.**

CONCLUSIONS

- IDH2 mutations may coexist with IDH1 and TET2 mutations. This co-mutation appears to be in the same clone in some patients and in a separate clone in others.
- The presence of VAF>50% in 6.5% of patients, suggesting homozygosity, along with co-presence of IDH1 and IDH2 and TET2 mutations suggests possible dosage effects in the biology of MDS/AML.
- The high rate (29%) of co-presence of DNMT3A with IDH/1/2 mutations suggests cooperation between the two mechanisms in influencing DNA methylation and leukemogenesis.
- The relatively high incidence of TP53 mutation in IDH1 patients suggests that IDH1 mutation might be associated with more aggressive disease than IDH2.
- This data suggests that there is interaction and significant interclonal and intraclonal heterogeneity in DNA methylation genes in AML/MDS.
- Complete profiling of these genes is necessary for better understanding and proper prediction of clinical behavior particularly when patients are treated with DNA methylation inhibitors.