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Therapy-related leukemia: clinical characteristics and analysis of new molecular risk factors in 114 adult patients

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We studied the clinical characteristics of 114 patients with therapy-related leukemia or therapy related myelodysplastic syndrome (t-Leuk/MDS).

The mean age of the patients was 48 years. The Hematological malignancies were the most common primary (53%), followed by breast and ovarian cancer (30% combined). The mean latency until the development of t-Leuk/MDS was 45.5 months. Median survival was 10 months. Cytogenetics was abnormal in 89% of the patients. FLT3 internal tandem duplications were found in six of 41 (14.6%) patients, of whom four had an abnormal karyotype

We also analyzed genetic factors that could predispose to t-leuk/MDS. These genetic factors can be subdivided into two groups: (1) Genes involved in drug disposition and metabolism. These genes may prevent DNA damage by minimizing toxic exposure through removal and/or metabolism of harmful agents. (2) Genes involved in DNA repair, which directly prevent or reduce hereditable DNA damage which may be incurred during the administration of genotoxic treatments.

Analysis of drug metabolism and disposition genes showed a protective effect of the CYP3A4-V genotype against the development of t-leuk/MDS, whereas the CC genotype of MDR1 C3435T and the NAD(P)H:quinone oxidoreductase1 codon 187 polymorphism were both noncontributory.

More specifically:

1. CYP3A4-V: The A to G polymorphism was found in only 1/44 (2.2%) t-leuk/MDS patients analyzed. In comparison, we found heterozygosity or homozygosity for this polymorphism in 15% of the healthy Israeli population (Arabs and Jews, 20/134 individuals tested) ($\chi 2$: $p<0.025$).

2. MDR1 C3435T polymorphism: A total of 36 patients were analyzed for this polymorphism in exon 26. Four out of the 36 (11%) of t-leuk/MDS patients were found to be homozygous for the putative protective C allele. In comparison, 14% of the normal Israeli population is homozygous for this allele. There was no difference in the number of chromosomes carrying the T allele (either in the heterozygous or homozygous state) between patients and 87 ethnic matched controls (Fisher's exact test, $p=0.295$ for TT and $p=0.559$ for CT). The controls included DNA samples from 33 Arabs and 54 Jews.

3. NQO1 polymorphism: In all, 46 patients were analyzed for the point mutation in codon 187, which has previously been reported to be associated with an increased tendency to develop AML and t-leuk. We found that, of 46 patients, 15 were heterozygous and one was homozygous for the mutant allele (35% hetero- or homozygotes). This was identical to the frequency of hetero- or homozygosity for the mutant allele seen in ethnic matched controls (150 Jews and 170 Arabs tested).

We also studied the mismatch repair system (MMR) which is crucial for faithful replication of DNA during cell division. The MMR consists of a number of genes, six of which have thus far been cloned and characterized. Defects in these genes result in the so-called *mutator phenotype*, which leads to the accumulation of mutations which may contribute to the development of t-leuk/MDS upon exposure to genotoxic stress. A number of studies, including our own, have demonstrated that defects in DNA repair play a role in the predisposition to develop t-leuk/MDS.

Microsatellite instability (MSI) is the hallmark of impaired MMR system.

In all, 82 samples from 22 patients were analyzed for MSI using a panel of 10 microsatellite loci, which included six mononucleotide and four dinucleotide loci. Three loci, BAT26, BAT40, and BAT25, had been previously recommended for detecting MSI in colon cancer and have been reported to show clear-cut results. The other three were selected because of their location in the genes considered to play a role in the etiology of MSI. Thus, BAT13, a 13-thymidine mononucleotide repeat, is a monomorphic marker without any known allelic size variation, located upstream of the AG consensus sequence of the donor splice site of the first intron of the hMSH2 gene. BAT34C is a quasimonomorphic mononucleotide marker located in the 30 untranslated part of the exon 11 of the p53 gene, which is known to play an important role in apoptosis. BAT16 is a mononucleotide quasimonomorphic marker consisting of 16 thymidine repeats in the sixth intron of the hPMS2 gene, one of the MMR system's genes. Microsatellite markers BAT26 (26 adenine repeats located within donor–acceptor sequence in the fifth intron of the hMSH2 another MMR gene) and BAT25 (25 thymidine repeats located within the 16th intron of the c-kit gene) were described as quasimonomorphic loci with allelic variation not exceeding two nt's, with one major allele for BAT26. Analysis using fluoresceinated PCR with ABI sequence analyzer demonstrated that 41% of patients with t-Leuk/MDS had high levels of MSI in four or more of 10 microsatellite loci. Immunohistochemistry demonstrated reduced expression of the two MMR genes, MSH2 and MLH1 in 6/10 patients with MSI as compared to 0/5 of pts without MSI.

In conclusion, genetic predisposition as well as epigenetic events, contribute to the etiology of t-Leuk/MDS.

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