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ABSTRACTS

GENOMIC DUPLICATION/AMPLIFICATION AT 6P21 IN SECONDARY MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA

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In secondary myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), 6p rearrangements account for less than 2% of cytogenetic abnormalities. Although mainly found in complex karyotypes which also bear –5/del(5)(q) and/or –7/del(7)(q), rare cases of isolated 6p rearrangements have been observed. Deletions, balanced and unbalanced translocations, insertions, and duplications have been described with a der(1)t(1;6) change as the only recurrent abnormality.

We used Fluorescence in Situ Hybridization (FISH) and Comparative Genomic Hybridization (CGH) to study 6p rearrangements in 9 MDS/AML arising after environmental/professional exposure or chemo-/radio-therapy: 3 patients had undergone chemotherapy, 1 chemo- and radiotherapy, two were farmers exposed to pesticides and insecticides, two were spray painters, and 1 was a plumber exposed to solvents and glues. We have also studied a patient with Fanconi Anemia who developed an MDS. In 7 patients the short arm of chromosome 6 was involved in unbalanced translocations while the other three patients showed full or partial trisomy of the 6p arm i.e. i(6)(p10) (1 patient) and dup(6)(p) (2 patients). Remarkably, in 5/7 patients with unbalanced translocations DNA sequences were over-represented at band 6p21 as either cryptic duplications of a genomic region contiguous to the translocation breakpoints (3 patients) or cryptic low-copy gains present on der(6) and/or inserted in other derivative chromosomes (2 patients). In the 8 patients with cytogenetic or cryptic 6p21 gains, we identified a 5-6 megabases common over-represented region where the MHC complex, NOTCH-4, BAK, FANCE, ETV-7, HMGIY and FKBP51 putative oncogenes/tumour suppressor genes have been mapped. Our observation of 6p21 gains as isolated karyotypic changes emphasizes the pathogenetic role of this event in secondary MDS/AML. In contrast with therapy-related MDS/AML with duplications/amplifications of MLL and AML1, neither TP53/17p13 nor putative suppressor/oncogenes (FHIT/3p14, CSF1R/5q33, D7S486/7q31, ETV6/12p13, D13S25/13q14, and AML1/ 21q22) are rearranged in secondary AML/MDS with 6p21 gains. In conclusion, we hypothesize that 6p gains in secondary AML/MDS characterize a new pathway common to MDS/AML after toxics or arising in congenital disorders.

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DEFECTS IN THE GENOME SURVEILLANCE PATHWAYS IN THERAPY-RELATED ACUTE LEUKAEMIAS AND MYELO-DISPLASTIC SYNDROMES (TAL/SMD)

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Background. Therapeutic cancer treatment promotes the development of Acute Leukaemia and Myelodysplastic Syndrome occurring after successful chemo- and radiotherapy (tAL/MDS) although the underlying mechanisms are unclear. We investigated whether defects in the genome surveillance pathways might influence the development of tAL/MDS.

Matherial and Methods. We collected DNA and RNA from leukaemic and normal cells of 15 tAL/SMD cases, mostly secondary to Hodgkin/ non-Hodgkin lymphoma or breast cancer. EBV-transformed lymphoblastoid cell lines (LCL) were also established in 5 cases. We analysed microsatellite instability (MSI) by automated sequencing and measured gene expression levels of a panel of DNA repair genes by quantitative real-time RT-PCR. In addition, post-translational modifications in key proteins of the FANC/BRCA pathway induced by ?-irradiation and crosslinking agents were investigated in tAL/SMD-derived LCL using western blotting and immunofluorescence microscopy.

Results. We confirmed a high frequency (>50%) of mismatch repair (MMR) defects in tAL/MDS, as measured by MSI, in comparison to 28 *de novo* cases (<3,6%). A significant reduction in the expression levels of several DNA repair genes involved in MMR and recombinational repair (*MLH1, MGMT, LIG4, BRCA1, BRCA2 and RAD51*) was identified in leukaemic cells in comparison to normal cells from one tAL case secondary to breast cancer. Interestingly, LCL from the same tAL patient showed spontaneous phosphorylation of the kinases ATM and CHK2 involved in DNA damage response. In addition, a high level of basal ?-H2AX foci was observed in LCL from this patient. It remains to be clarified whether this patient suffers of high levels of endogenous DNA damage or harbours a genetic defect in a care-taker function. This defect may be an alteration acquired as a consequence of treatment for the first tumor or a genetic change predisposing to both breast cancer and leukaemia.

Conclusions. Taken together, our data indicate that both defects in MMR and alterations in pathways that control the stability of the genome may underlie a significant fraction of tAL/MDS.

NOTCH3 AND THE NOTCH3-UPREGULATED RNA-BINDING PROTEIN HUD REGULATE IKAROS ALTERNATIVE SPLICING AND COOPERATE IN T CELL LEUKEMOGENESIS

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Background. The roles of Notch and Ikaros in human leukemogenesis are supported by several reports. Notch3 overexpression has been observed in virtually 100% of human T-cell acute lymphoblastic leukemia/lymphomas (T-ALL), including tumors from all major molecular and immunophenotypic subtypes, and activating mutations of Notch1 have been found in over 50% of these tumors. A high percentage of infant B- and T-ALLs also display the increased expression of short non DNA binding IK isoforms. Alternatively spliced transcripts of the *ikaros* gene encode at least nine protein isoforms (IK1 to IK9) with different DNA-binding capabilities. Isoforms IK1 and IK2 are characterized by at least three N-terminal zinc finger motifs that allow efficient DNA binding. Shorter isoforms, lacking one or more of these DNA-binding motifs form heterodimers with full length isoforms and exert dominant negative effects that can decrease or even suppress normal *Ikaros* activity. It has recently been suggested that increased expression of dominant negative Ikaros isoforms and constitutively activated Notch play cooperative roles in leukemogenesis, involving effects that converge in the transcriptional regulation of one or more key genes. However, the identity of these putative common targets is still obscure, and thus far there has been no demonstration of a direct link between aberrant Notch signaling and alterated IK isoform expression.

Materials and Methods. Notch3-IC transgenic and Notch3-IC/pT?-/- double mutant mice together with wild type littermates were sacrificed at different ages, before and after the leukemia onset. Pre-malignant thymocytes and lymphoma cells have been analyzed by immunoblotting and RT-PCR assay, to determine possible variation in the expression of Ikaros isoforms and HUD. Cultured cell lines have been used for luciferase assay in order to analyze the transcriptional regulation of the selected Notch target genes by different cloned Ikaros isoforms.

Results. We demonstrate the occurrence of cross-talk between Notch3 and Ikaros that results in transcriptional regulation of the gene encoding the pT? chain of the pre-TCR. We also show that, in the presence of a functional pre-TCR, constitutive activation of Notch3 causes increased expression of dominant negative non-DNA-binding Ikaros isoforms, which are able to restrain the Ikaros inhibition of Notch3's transcriptional activation of pT?. This effect appears to be mediated by Notch3's pre-TCR-dependent upregulation of the RNA-binding protein, HuD. Notch3 signalling thus appears to play a critical role in the diminished Ikaros activity described in several murine and human lymphoid leukemias. By exerting transcription-activating and –repressing effects on the pT promoter, Notch3 and Ikaros cooperate in the fine-tuning of pre-TCR expression and function, which has important implications for the regulation of thymocyte differentiation and proliferation.

Conclusions. The molecular model portrayed by our findings provides evidence for a novel non-redundant mechanism which may help in clarifying the regulatory mechanism of Ikaros alternative splicing and unveils, for the first time to our knowledge, a direct link between Notch3 signaling, pre-TCR and Ikaros splice variants in T cell leukemogenesis.

VEGFR-1 (FLT-1) EXPRESSION IS REGULATED BY CELL DIFFERENTIATION AND CHEMOTHERAPY DIFFERENTIATION-INDUCER AGENTS

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VEGF and its receptors are expressed not only in the vascular but also in the hematopoietic system, where they are known to play important roles in normal and pathological conditions. In both systems, the mechanisms involved in the regulation of VEGFR-1 (FLT-1) in particular are poorly understood. In this study, we hypothesized FLT-1 expression might de regulated during cell differentiation, and used the hematopoietic (lymphoid and myeloid subsets) system as a model. First, we studied FLT-1 expression levels (by RQ-PCR and FACS analysis) throughout B cell differentiation *in vitro*. In this model we observed a clear increase in FLT-1 expression at the latter stages of differentiation (at the large pre-B cell stage). In parallel we analyzed the effects of chemically-induced differentiation in different lymphoid and myeloid cell lines and primary samples. In detail, we studied the differentiation inducing effects of ATRA (used on AML), Taxol (used on erythroidleukemia cells) and Rituximab (used on Non-Hodgkings Lymphoma). Interestingly, regardless of the drug used or the cell line/primary sample studied, concomitant with cell differentiation, there was always a clear increase in FLT-1 mRNA (RQ-PCR) and protein levels (FACS), which in some patient samples appeared to correlate with response to treatment. Mechanistically, we verified that FLT-1 induction is not due to proteasome inhibition in the presence of the different drugs, neither is it regulated via the classical signaling pathways (ERK/MAP Kinase, Pi3Kinase). Based on our data, we propose two possible models to explain FLT-1 regulation during hematopoietic cell differentiation: 1) the activation of a general *stress signal* that results in membrane turnover and increased synthesis/export of pro-survival tyrosine kinase receptors; 2) modulation of the FLT-1 promoter activity during differentiation and consequent increase in FLT-1 translation. Increased FLT-1 may convey pro-survival signals or induce a more aggressive (i.e. invasive) disease phenotype. To our knowledge, this is one of the first studies focusing on the regulation of FLT-1 expression and hematopoietic (normal and malignant) cell differentiation.

METHYLATION PATTERN OF THERAPY-RELATED AML

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Aberrant pattern of DNA methylation is increasingly recognized as important pathogenetic event both in *de novo* and therapy-related AML. Global DNA hypomethylation and site-specific gene promoter hypermethylation represent two faces of the DNA methylation changes in cancer and are responsible of genomic instability and tumor suppressor gene inactivation. Experimental models have shown that radiation exposure causes global DNA hypomethylation, leading to chromosomal aberrations, while epigenetic changes in chromatin structure induced by cytotoxic drugs are less well understood. Moreover, recently, specific chromosomal translocations have been found to be associated with site specific promoter methylation in AML.

Global methylation analysis of human genome, studied by

two dimensional gel electrophoresis restriction landmark genomic scanning (RLGS), showed a non-random but tumor specific aberrant CpG-island methylation pattern in different types of cancer. Nevertheless, no comparative study has revealed clear differences in methylation between *de novo* and therapy related AML. Several studies have investigated promoter methylation of single genes involved in fundamental cellular pathways in AML. The list of methylation-repressed genes is expanding very rapidly, including genes essential for cell cycle control as p15, involved in cytokine signalling, as SHP1 and SOCS1, inducers of apoptosis, as DAP-kinase, transcription factors, as RARbeta, adhesion molecules, as E-cadherin, and finally genes codifying for detoxification and DNA repair enzymes, as GSTP1, hMLH1, MGMT, and BRCA-1. Different methylation frequencies for these genes have been reported in AML, but only few studies have analyzed their methylation status in t-AML/MDS.

Studying methylation status of multiple genes in t-AML patients, Uehara at al (Int J Oncol, 2003) identified methylation of at least one gene in 55% of patients. Moreover the average time to the development of t-AML after the treatment of the primary tumor was significantly shorter in methylated than unmethylated patients (49.3 months vs. 133.2 months, p=0.044). Aberrant p15 gene promoter methylation was found in 58-68% of t-MDS/AML patients and was unrelated to type of previous therapy. Nevertheless, p15 methylation has been significantly related to monosomy/deletion of chromosome 7q, suggesting that it could be a relevant event in the alkylating agents-induced leukemogenesis. We analyzed the methylation status of DAP-kinase and BRCA-1, in 160 and 133 AML patients, respectively. Both genes were found to be more frequently hypermethylated in t-AML than *de novo* AML (48.3% vs 22.9%, p=0.01, for DAP-kinase; and 76% vs 31%, p=0.0002, for BRCA1). The functional role of hypermethylation was confirmed by down-regulation of corresponding mRNAs in methylated samples, studied by semiquantitative RT-PCR or real time quantitative RT-PCR. Moreover, BRCA1 hypermethylation correlated to increased DNA methyltransferase DNMT3A (p=0.003) expression. These data suggest that promoter hypermethylation of genes involved in cell cycle control, apoptosis and DNA repair pathways is frequent in t-MDS/AML and could contribute to secondary leukemogenesis.

EVALUATION OF THE PROGNOSTIC RELEVANCE OF LECAM1 AND ICAM1 EXPRESSION IN MYELODYSPLASIA AND SECONDARY ACUTE LEUKEMIA

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Background. An aberrant pattern of expression of adhesion molecules (AM) may contribute to the pathogenesis of myelodysplastic syndromes (MDS) and secondary acute myeloid leukemia (sAML).

Methods. In a three-colour flow cytometric assay, we evaluated the expression of AM on $CD34^+$ progenitor cells from the bone marrow of 84 patients (66 MDS, 18 sAML) and 17 normal donors.

Results. The ratio of Lecam1/Icam1 expression was identified as a parameter correlated with poor-risk features such as a higher bone marrow (BM) blast infiltration and a shorter time to leukemic progression among MDS patients. In fact, the lowest values of Lecam1/ICAM1 ratio were associated with a BM blasts infiltration ≥20% (p=0.002). Furthermore, MDS patients with a baseline ratio <1 had a higher leukemic progression rate (41% vs. 19%, p=0.008). In univariate analysis, the actuarial risk of disease progression for this subgroup of MDS patients was also higher (64% vs. 11% at 2 year, p=0.002), this difference being confirmed in multivariate analysis. Furthermore, sequential monitoring showed that a decrease of the ratio preceded overt leukemic transformation; conversely, restoration of a normal ratio was observed in 2 patients after a chemotherapy-induced remission.

Conclusion. 1) Lecam1 is defective in the stem cell compartment of MDS and sAML, whereas ICAM1 is overexpressed; 2) the ratio of their expression has a prognostic role; 3) a ratio <1 significantly predicts progression to overt leukaemia in MDS patients.

GENE EXPRESSION PROFILING IN NORMAL PROMYELOCYTES AND IN ACUTE PROMYELOCYTIC LEUKAEMIAS *DE NOVO* AND OCCURRING AFTER A PRIMARY MALIGNANCY

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Background. PML-RARA, the molecular hallmark of Acute promyelocytic leukaemia (APL), is an aberrant transcription factor and expression profiling might represent a powerful tool in the comprehension of molecular alterations underlying the disease.

Material and Methods. Microarray technology was used to determine gene expression profiles of APL cells obtained from 16 patients in comparison with 8 samples of $CD34^+$-derived normal promyelocytes. To investigate the expression pathways underlying the development of APL occurring as a second malignancy (sAPL), we included in our study 8 cases of sAPL.

Results. Malignant promyelocytes showed widespread changes in transcription in comparison to their normal counterpart and 1020 differentially expressed genes were identified. Discriminating genes include transcriptional regulators (*FOS, JUN* and *HOX* genes) and genes involved in cell cycle and DNA repair. The strong up-regulation in APL of some transcripts (*FLT3, CD33, CD44* and *HGF*) was also confirmed at protein level. Interestingly, a trend towards a transcriptional repression of genes involved in DNA replication or a variety of DNA repair pathways was found in APL and confirmed by real-time PCR in a new set of 9 APLs. Expression profiling of a selection of DNA repair genes was also capable of successfully identifying sAPLs from *de novo* cases. In this case however unbalances in expression included both overexpression and downregulation and mostly involved mismatch repair and recombinational repair genes.

Conclusions. Although both secondary and *de novo* APL samples were characterized by a strong homogeneity in expression profiling, we were able to identify a small set of differentially expressed genes that discriminate sAPL from *de novo* cases. Our data suggest that deregulation of different DNA repair pathways plays a role in the development of both *de novo* and sAPL. The list of discriminating genes may offer several suggestions for future studies.

MITOXANTRONE THERAPY AND SECONDARY ACUTE PROMYELOCYTIC LEUKAEMIA IN PATIENTS WITH PROGRESSIVE MULTIPLE SCLEROSIS

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Mitoxantrone (MITO) a synthetic inhibitor of DNA-topoisomerase II is able to reduce neurologic disability and relapse frequency in multiple sclerosis (MS) patients either with secondary-progressive as well as progressive-relapsing or worsening relapsing-remitting disease.

In MS patients the use of MITO as single drug showed to be associated with an increased risk of treatment related acute leukemia (TRAL) whose reported incidence ranges from 0,07 to 0,25%. DNA-topoisomerase II inhibitors TRAL is usually characterized by a short latency and no previous myelodysplastic phase, while cytogenetic abnormalities and prognosis are comparable to these of *de novo* acute leukaemia (AL).

We report two cases of secondary acute promyelocytic leukemia (sAPL) occurred in patients treated with MITO for progressive MS.

Case 1. A 55 year-old man, with a previous MS diagnosis in March 1997, went at our Unit in November 2004 because of anemia, thrombocytopenia and leukocytosis (WBC count 20×10^9/l with 40% of atypical promyelocytes) and presence of a sub clinical DIC. Bone marrow aspirate showed a complete infiltration of leukemic promyelocytic with Auer Rods, thus the diagnosis of sAPL was made. Cytogenetic and molecular assays evidenced the presence of t(15;17) and PML/RAR alpha fusion gene. The patient died because of cerebral haemorrhage two days later before ATRA treatment start. As previous treatment, since in progressive MS, patient was given MITO (8 doses of 8 mg/m^2 every three months, total dose 80 mg) from May 2002 to September 2004. The interval between last MITO administration and leukemia was 2 months.

Case 2. In February 2006 a 27 year-old man was admitted in our Emergency Department because of cerebral haemorrhage. Peripheral blood (PB) count showed severe pancytopenia, while in PB smear atypical hypergranular promyelocytes were present, thus a diagnosis of sAPL was suggested. Patient died soon after the hospitalization. PML/RAR alpha fusion gene was successively found on PB sample. The patient had MS for 1996; he was treated from September 2004 to July 2005 with MITO (8mg/m2 monthly doses, total dose of 100 mg). The interval between last MITO dose and leukemia was 7 months.

Although the risk of TRAL in MS patients is reported to be low, it does seem to be higher than that of *de novo* acute leukaemia in general population (Brassat, 2003). In our cases it can argue that sAPL may be MITO treatment related, since MITO was the only cytotoxic drug used and leukaemia biologic features fulfil the diagnosis criteria for AL secondary to DNA-topoisomerase II inhibitors (Beaumont, 2003).

There is no agreement in the literature on the real TRAL incidence in MS patients underwent prolonged MITO treatment. It is not yet well stated if TRAL occurrence is directly related either to total MITO dosage administered, schedule timing (monthly vs every three month doses) and length of treatment.

To answer to these queries future larger studies on this subset of MS patients should be done.

BLAST CELL COUNT IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) OR SECONDARY ACUTE MYELOID LEUKEMIA (SAML): COMPARISON BETWEEN MORPHOLOGIC ASSESSMENT ON MARROW ASPIRATE (AS) AND IMMUNOHISTOCHEMISTRY ON BONE MARROW BIOPSY (BMB)

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Background. In the French-American-British (FAB) cooperative group and the WHO classification MDS are stratified accordingly to marrow blasts percentage. Blast cell count is also comprised within the international prognostic score system (IPSS), which enables to define four groups with distinct prognosis. Diagnosis of sAML is defined by marrow blast count >30% (FAB) or >20% (WHO), along with previous diagnosis of MDS or dysplastic features of marrow myeloid lineages. Accordingly, precise quantitation of marrow blasts is critical both for diagnosis and prognosis of pts with MDS. AS is currently retained the best tool to assess hematopoietic cellular morphology; actually, quantitation of blasts is afforded by BMB when AS is not availabile (e.g. dry tap).

Aim. To compare marrow blasts percentage quantified by morphology alone on AS and CD34$^+$ blasts by immunohistochemistry on BMB in MDS and sAML patients.

Methods. We reviewed the marrow aspirate and core biopsy reports of 169 pts with MDS or sAML at diagnosis, period 1997-2005. Marrow blasts have been morphologically quantified on May-Grunwald Giemsa stained AS and expressed as blast percentage over 500 nucleated marrow cells. Bouin's fixed, paraffin-embedded BMB have been evaluated for CD34+ immature cells counted over 1000 nucleated marrow cells. Diagnoses (FAB) according to morphology of marrow aspirate were: RA=106, RAEB=34, RAEB-T=12, CMML=5, sAML=12. According to FAB, WHO and IPSS, marrow blasts percentages have been grouped into four classes: A=0-4, B=5-9, C=10-19, D≥20. Each marrow AS and BMB from single patient has been assigned a class and compared.

Results. 50 cases (29.6%) showed a difference in blasts percentage, thus determining a class discordance; details are reported in the table. Most importantly a difference >1 class was observed in 10 cases (5.9%); in 5 cases blast cell count was higher and in the remaining 5 lower on BMB when compared to AS.

Conclusions. This large retrospective mono-institutional study highlights the necessity to perform blast cell count both in AS and BMB of patients with suspected MDS/sAML at diagnosis. Immunohistochemistry for CD34 on BMB is useful in those cases with low (< 10%) blastic marrow infiltration. Major differences in blast counts (difference >1 class) have been evidenced only in a strict minority of evaluated cases, thus allowing a reliable blast cell count also on BMB.

Aspirate	*Biopsy*	*N°*
A	B	22
A	C	4
A	D	0
B	A	2
B	C	3
B	D	1
C	A	5
C	B	4
C	D	7
D	A	0
D	B	0
D	C	2
tot		*50*

MOLECULAR GENETIC ALTERATIONS IN RADIATION-ASSOCIATED ACUTE MYELOID LEUKEMIA FOLLOWING THE CHERNOBYL ACCIDENT

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Background. A large body of epidemiological evidences has established the leukemogenic potential of ionizing radiation. However, little is known about the molecular mechanisms by which radiation induces the leukemia.

Material and Methods. The cohort of patients consisted of 154 unselected adult myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) patients, diagnosed between 1988 and 2006 in Ukraine. Of these patients, 84 had experienced radiation exposure due to the Chernobyl accident (radiation-associated cases), and 70 developed spontaneous AML and served as controls. Fifty-one and 59 AML samples were analyzed for the presence of *AML1* and *MLL* abnormalities respectively using fluorescent *in situ* hybridization and/or reverse transcription polymerase chain reaction (PCR). AML1 mutations were screened in 6 radiation-associated cases of MDS or AML following MDS by direct sequencing of genomic DNA. Using Affymetrix high-density single nucleotide polymorphism 10K mapping arrays, we performed a whole-genome loss of heterozygosity (LOH) and DNA copy number changes analysis in 19 radiation-associated AML cases. The conventional comparative genomic hybridization (CGH) was done on 25 radiation-associated and 12 spontaneous AML samples. One hundred and twenty four patients (71 radiation-associated and 53 spontaneous AML cases) were examined for the presence of FLT3 internal tandem duplications (ITD) by genomic PCR.

Results. AML1 translocations with unusual partners were not detected in AML patients exposed to ionizing radiation. The *AML1/ETO* translocation was found to be significantly less frequent in radiation-associated AML (1/24) than in spontaneous cases (9/29, p=0.02). No difference in *MLL* translocations frequency was found between radiation-associated and spontaneous AML cases (0/27 and 1/32 respectively). The *AML1* point mutation was detected in 1 out of 6 patients. The hexanucleotide duplication of CGGCAT in exon 8, inserted after base position 1502 was found in the patient who developed MDS following an acute radiation syndrome. The study demonstrated the notably high frequency of LOH at 5q and/or 7p, and 7q detected in 8 cases (42%) of radiation-associated AML. Combined SNP Chip and CGH data on DNA copy number changes revealed that DNA loss at 5q and/or 7q and 7 tended to be more frequent in radiation-associated AML cases (10/26 vs 1/12 in spontaneous cases, P=0.06). There was no significant difference in the prevalence of FLT3 ITD between patients with (8/71) and without history of radiation exposure (9/53, P = 0.4). Six out of 17 patients with ITD were found to harbor more than one FLT3 mutant alleles. Multiple duplications were found in 5 of the 8 FLT3 mutated radiation-associated AML, but in 1 of the 9 spontaneous cases (p=0.0498).

Conclusions. Chromosomal translocations of the *AML1* and *MLL* genes are not common among the AML patients exposed to ionizing radiation. Radiation may contribute to the development of leukemia through *AML1* gene point mutation. We hypothesize that LOH/DNA loss at 5q and/or 7q and 7 constitutes an important genetic mechanism involved in leukemogenesis following accidental radiation exposure. The higher prevalence of multiple ITD alleles in radiation-associated AML cases with FLT3 mutations may reflect an underlying radiation-induced genetic instability.

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THE RELATIONSHIP BETWEEN HEMATOTOXICITY AND THE DEVELOPMENT OF CHEMICALLY INDUCED ACUTE MYELOGENOUS LEUKEMIA

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The majority of acute myelogenous leukemia (AML) cases are *de novo*, having no readily identifiable cause. However, AML is also an established, albeit rare, consequence of high dose exposure to myelotoxic chemicals (including benzene), certain classes of cytotoxic chemotherapy or ionizing radiation. AML arising secondary to chemical or radiation exposure (s-AML) is believed to be a multi-step process involving both genetic as well as epigenetic events. The cell of origin for secondary and *de novo* AML has been shown to be a myeloid committed hematopoietic progenitor cell (HPC), historically found only in the bone marrow. Recent developments in experimental hematology have demonstrated that a small population of HPCs also freely circulates in the peripheral blood. The presence of HPC outside the bone marrow (extramedullary) has been used to support the hypothetical possibility that peripheral HPC could be the cell of origin in chemically induced leukemia. Further, it has been hypothesized that leukemogenic transformation could theoretically occur in the absence of bone marrow involvement/toxicity following exposure to exogenous chemicals that never reach the bone marrow (e.g. formaldehyde). For this to occur, a circulating HPC would undergo malignant transformation in the periphery, followed by migration back into the bone marrow, where the disease becomes manifest. Evidence accumulated over decades of study in both experimental animals and humans have consistently revealed that hematotoxicity and bone marrow damage are important, if not absolute requirements for chemically induced leukemia to develop. An increased understanding of stem cell biology has provided valuable insight into normal hematopoietic organization and leukemogenesis (including AML resulting from cytotoxic chemical exposure) and support the role that bone marrow involvement and hematotoxicity likely plays in the pathogenesis of AML development. Therefore, peripheral transformation in the absence of bone marrow toxicity is not currently supportable based on existing scientific evidence. In this review, the available scientific evidence supporting that bone marrow damage and hemotoxicity are necessary requirements for the induction of chemically induced AML will be discussed.

CHARACTERISTICS OF TREATMENT-RELATED ACUTE MYELOGENOUS LEUKEMIA AND MYELODYSPLASTIC SYNDROME AFTER NATIONAL CANCER INSTITUTE CANCER CLINICAL TRIALS

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Background. Since 1995, reports have been collected by the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) for treatment-related acute myelogenous leukemia (t-AML) and myelodysplastic syndrome (t-MDS) among subjects treated for cancer on NCI-sponsored clinical trials. We reviewed these reports to determine clinical and demographic characteristics of subjects with t-AML and t-MDS.

Materials and Methods. The NCI cooperative cancer clinical trials groups collect information about all diagnoses of t-AML/ MDS among patients treated for cancer in NCI-sponsored trials. Data include histopathologic reports and cytogenetic analyses; demographic information; cooperative group protocol number; clinical characteristics of t-AML/MDS; and further information on chemotherapy, radiation therapy, and growth hormone, including limited data on additional post-protocol or off-protocol treatments.

Results. Through August 15, 2006, there were 780 t-AML/MDS diagnoses reported among subjects on 360 clinical trials. Forty-one percent had the diagnosis of MDS. Twenty-six percent of all t-AML/MDS cases were diagnosed in children younger than age 21, and 63 percent occurred among female subjects. The age at t-AML diagnosis averaged 40.6 (median 46) years, and the age at MDS diagnosis averaged 49.6 (median 56) years. The most frequent t-AML subtypes were M5 (25 percent), M4 (22 percent), and M2 (21 percent). Based on 525 cases reported through March 25, 2002, the primary cancer before t-AML or t-MDS was breast (37 percent), leukemia (lymphocytic or other) (10 percent), non-Hodgkin lymphoma (8 percent), Hodgkin lymphoma (5 percent), Ewing's sarcoma/primitive neuroectodermal tumors (5 percent), multiple myeloma (4 percent), osteosarcomas (3 percent), colorectal cancers (3 percent), prostate cancer (3 percent), rhabdomyosarcomas (3 percent), others (14 percent) or unspecified (4 percent). Seventy-four percent of cases had been treated for the primary cancer with alkylating agents, 64 percent with anthracyclines, 23 percent with epipodophyllotoxins, and 15 percent with platinum drugs.

Conclusions. The NCI CTEP reporting mechanism indicates that patients treated for a wide variety of primary cancers are at risk for t-AML/MDS. Coordinating with clinical trials groups and linking data with biorepositories would provide an opportunity for further investigation of risk factors for t-AML/MDS, such as genetic polymorphisms in metabolic pathways.

ARE SUNLIGHT DEPRIVATION AND INFLUENZA EPIDEMICS ASSOCIATED WITH THE ONSET OF ACUTE LEUKEMIA?

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Background. Evidence on the seasonality of acute leukemia (AL) is weak. A previous study from Northern Finland (Ann Hematol 1999;78:408-14) found excess numbers of total AL and acute lymphoblastic (ALL) but not acute myeloblastic leukemia (AML) in the cold and dark period of the year. An explanation was suggested that sunlight deprivation leading to vitamin D deficiency might stimulate leukemic cell proliferation and block cell differentiation through dysregulation of growth factors, causing one mutation and an overt ALL in progenitor cells already damaged by influenza in current or previous winter. We have now re-tested this hypothesis using data from the entire population of Finland.

Material and Methods. All 7423 incident cases of AL during the period 1964-2003 were obtained from the Finnish Cancer Registry. Monthly data on mean solar radiation obtained from the Finnish Meteorological Institute and on influenza epidemics from the National Public Health Institute were linked to the cases on a regional basis (North / Central / South). The counts of AL were regressed on solar radiation and influenza using Poisson regression, controlling for overdispersion and autoregression. The analyses were conducted piecewise, with separate regressions in the dark and light months. The results were expressed as a risk ratio (RR) and its 95% confidence interval (CI).

Results. Total AL showed a bimodal monthly variation with high numbers of cases in the dark season (October-March, with the exception of December) and low numbers in the light season. The monthly pattern was significant for ALM (p~0.021) but not for ALL, except in children aged 2-4 years who showed low numbers in the dark season. People aged 65 years or more showed excessive numbers of AL during the dark season compared with the light season (April-September) (RR 1.08; CI 1.00-1.17). During the months with mean daily solar radiation of less than 19,000 kJ/m^2/d radiation was not associated with leukemia, but during the months with radiation 19,000 kJ/m^2/d or more, the risk of AML decreased significantly with increasing radiation with a lag of one month (RR 0.41 per 1000 kJ/m^2/d; CI 0.20-0.82). Independently of solar radiation, there was an increase in AML during influenza epidemics (RR 1.10; CI 1.00-1.20) compared with non-epidemic periods.

Conclusions. Our observations are compatible with the assumption that sunlight deprivation and influenza are risk factors for AL, or adequate sunlight may be a protective factor. Darkness-related deficiency of vitamin D and influenza reoccur at the same time every year and could cause successive and cooperative mutations leading to leukemia with a short latency. The finding supports Knudson's minimal two-step model and Gilliland's hypothesis of two steps in leukemogenesis.

LATE RELAPSES IN CHILDHOOD T-ALL PATIENTS – TRUE DISEASE RECURRENCE, SECONDARY T-ALL OR SECOND T-ALL?

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Background. The vast majority of relapses in childhood T-cell acute lymphoblastic leukemia (T-ALL) patients occur relatively early, usually within 2 years from diagnosis, frequently during maintenance treatment. Our previous comparative molecular analyses between diagnosis and relapse of such *classical* T-ALL (26 patients) showed totally (62%) or at least partly (38%) identical T-cell receptor (TCR) gene rearrangement patterns at both disease phases. These results confirm that the relapse clone in these patients originated from the original diagnosis clone, which became resistant to the applied treatment. In contrast to these *classical* T-ALL, two patients experienced very late T-ALL recurrences (6 and 10 years from diagnosis, respectively) and both patients displayed completely different TCR gene rearrangement sequences between diagnosis and relapse. We hypothesized that such late *relapses* of T-ALL in fact might represent second malignancies and that patients developing such second leukemias might be genetically predisposed for T-ALL development.

Material and Methods. We succeeded to investigate 15 T-ALL patients with late relapses, i.e. at least 2.5 years from initial diagnosis. The studies at the DNA level involved detailed comparison of TCR gene rearrangements between diagnosis and relapse (PCR-heteroduplex, sequencing and/or Southern blot analyses) and the detection of gene fusions involving the TAL1 gene and/or TCR genes.

Results. We found the evidence of a common clonal origin between diagnosis and relapse in nine of the 15 patients. In one case, the T-ALL had no clonal TCR rearrangements neither at diagnosis nor at relapse. Finally, in five patients TCR gene rearrangement sequences had completely changed between diagnosis and relapse, suggesting a second T-ALL rather than a relapse. We also did not observe at relapse genetic aberrations typical for secondary ALL. Moreover, most patients remained in complete remission after second-line treatment, which is unusual both for relapsed and secondary ALL.

Conclusion. Approximately one-third of late T-ALL *relapses* in fact represent second malignancies. We are currently performing further genomic analyses to identify common genetic events or common genomic features which might be related to predisposition for development of T-ALL.

GENTUZUMAB-OZOGAMICIN, CITOSINE ARABINOSIDE, G-CSF COMBINATION IN THE TREATMENT OF ELDERLY POOR PROGNOSIS ACUTE MYELOID LEUKEMIA. A MULTICENTRIC STUDY

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Backround. Gentuzumab Ozogamicin (GO) is effective as single agent in the treatment of poor risk acute myeloid leukemia (AML) patients (pts). The aim of this study was to evaluate the efficacy and safety of a chemotherapy including growth factors, cytabine and GO in the treatment of very *poor prognosis AML elderly patients.*

Patients and Treatments. In 3 Italian Hematology Departments from September 2003 to September 2006, a total of 23 pts [F/M 12/11;median age 69 years (range 58-77)] with secondary AML (sAML) were enrolled in G-AraMy protocol which was divided in two phases. In the first phase from September 2003 to December 2004 11/23 pts receiving G-AraMy-1 treatment: rhG-CSF (5 µg/kg, on days 1-8), Aracytin as continuous perfusion (100 mg/m^2 on days 4-8), GO (6 mg/m2 iv over 2 hours on day 9). In the second phase from January 2005 to September 2006 12 pts was treated according G-Ara-My-2 protocol: rhG-CSF (5 µg/kg, on days 1-8), Ara-C as continuous perfusion (100 mg/m^2 on days 2-8), GO (6 mg/m^2 iv over 2 hours on day 9). In pts who reached complete (CR) or partial remission (PR), consolidation therapy was performed. In G-Ara-My-1 this consisted of: rhG-CSF(5 µg/kg, on days 1-6), Ara-C as continuous perfusion (100 mg/m^2 on days 2-6), GO (6 mg/m^2 iv over 2 hours on day 7). G-Ara-My-2 group was consolidated with: rhG-CSF(5 µg/kg, on days 1-5), Ara-C (1000 mg/m^2 every 12 hours on days 2-5), GO (6 mg/m^2 iv over 2 hours on day 6).

Results. Among the 23 treated pts 11 (48%) presented a post-MDS AML while 12 pts (52%) had received chemotherapy for a prior malignancy (3 Hodgkin's lymphoma, 5 breast, 2 thyroid, 1 gut, 1 bladder). According to the FAB classification these 12 pts were divided into 1 M1, 8 M2, 3 M4. Ten out 23 pts (43.5%) had previously received chemotherapy for AML being relapsed (4) or primary resistant pts (6) while 13 (56.5%) were untreated pts. Cytogenetic study was performed in all pts; karyotype was at *intermediate prognosis* in 11 cases, at *worse prognosis* in 7 cases, at *good prognosis* in 2 cases. In 3 pts no metaphases were observed. All pts performed CD33$^+$ evaluation on BM, the median percentage of CD33 positive blasts was 90% (range 25%-95%). After induction and consolidation therapy 14 pts (6 group 1; 8 group 2) (61%) achieved a CR and 2 pts obtained PR. Five pts (22%) resulted refractory to treatment and 2 died during the aplasia period post induction treatment (1 due to sepsis, 1 due to cerebral haemorrhage). The most common adverse event was myelosuppression, as expected. No VOD was recorded. Seven pts (30%) developed documented infection (including pulmonary aspergillosis in 2 cases). Two pts died while in CR, 1 due to bladder cancer relapse and 1 to ischemic stroke. Nine of CR pts (39%) relapsed; at October 2006 5 pts (22%) are alive, of whom 1 are still in CR (4%). Median time to treatment failure (TTF) and median overall survival (OS) of whole population were 6.3 months (range 1-20.6+) and 7.6 months (range 1.7-20.6+) respectively. Stratifying pts according the two treatment groups median TTF was 4.4 months (range 3-10.5) in the first group and 7.2 months (range 1-20.6+) in the second (*p*-value=0.2); median OS was 6 months (range1-13.6) in the first group and 9.1 months (range 1.7-20.6+) in the second

(*p*-value= 0.05).

Conclusions. G-Ara-My protocol could be considered an useful approach for poor risk elderly AML pts considering the low reported side effects with a CR rate similar to that reported in literature. Unfortunately CR duration is brief. The modification of protocol schedule in the G-Ara-My 2 group with the addition of more aggressive consolidation therapy seems to improve the duration of CR and OS.

EFFICACY AND TOXICITY OF INTENSIVE TREATMENT FOR HIGH RISK MYELODYSPLASTIC SYNDROMES (MDS) AND SECONDARY ACUTE MYELOID LEUKEMIA (SAML): PROMISING RESULTS FROM A SINGLE CENTER EXPERIENCE

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Background. Primary causes of treatment failure in high risk MDS and sAML are resistant disease and early relapse. Response to conventional chemotherapy (standard-dose cytarabine + anthracycline ± etoposide) is usually poor; recently, fludarabine, cytarabine and G-CSF containing regimens (FLA) have shown promising results in complete remission (CR) induction, but probably do not prolong disease free survival (DFS). The potentially curative strategy is allogeneic (allo) stem cell transplantation (SCT), given after disease debulking. Consolidation with intensified-dose chemotherapy and autologous (auto) SCT can prolong DFS in pts that cannot afford allo SCT.

Aim. retrospective evaluation of efficacy and toxicy of intensive treatment comprising SCT in high-risk MDS and sAML, at our Center.

Methods. period 06/1999-11/2005; 63 patients (pts); median age 58 (22-73), 27 (43%) >60 years. Diagnosis (WHO): AML MD 28, RAEB2 11, RAEB1 5, RCMD 3, MDS/MPD 2, T-AML/MDS 14. Cytogenetics (61 evaluable): favourable 29 (48%), intermediate 11 (18%), unfavourable 21 (34%).

Results. 61 pts received induction chemotherapy (CHT): FLAG-Ida 52, FLAG 1, AraC+Daunorubicin 2, AraC+Mitoxantrone 4, other 2. Two pts were given alloSCT upfront. Overall response rate (RR) was 86% (3 pts not evaluable): CR 72%, PR 14%. RR for *de novo* and t-AML/MDS was comparable, with CR 58% and 76%, respectively (p=ns). There was no difference in RR comparing different subgroups according to diagnosis, age and cytogenetics. Thirty-seven pts received a second course of CT, 27 consolidation, 10 reinduction. Five toxic deaths were observed (8%), 4 after first induction.Thirty-three patients (52%) underwent sequential SCT: 7 autologous, 26 allogeneic (7 sib, 5 MUD, 14 haplo). At transplant 16 pts were in CR, 15 with overt disease, 2 untreated. Median age at transplant was 57 yrs. The main reason for not undergoing SCT were uncontrolled fungal infection and disease progression. TRM was 30.3% (10 pts): 6 sepsis in aplasia, 3 late TRM, 1 GvHD. Fourteen pts out of 15 transplated with overt disease obtained CR after SCT. Relapse was observed in 33 out of 48 pts who obtained CR (68%), 9 after SCT (4 auto and 5 allo). Fifteen pts who had relapsed obtained a second CR: 6 with CHT, 1 with autoSCT, 8 with alloSCT. After a median follow-up of 398 days 16 pts are alive (25%): 12 in CR, 2 after autoSCT, 9 after alloSCT, 1 after CHT; 7 of them are older than 60 years. Overall median DFS was 159 days (range 5-1498), OS 392 days (range 3-2536).

Conclusions. prolonged survival is achievable with intensive treatment in poor prognosis MDS and sAML pts, also in the elderly. Prevention of pts contamination before SCT, mainly from aspergillus, and reduction of TRM, mainly in the haplo subset, could improve pts outcome. SCT should be given shortly after reduction of the disease burden, in order to optimize the GvL effect and avoid early relapse or progression. Auto in CR is an alternative if SCT from a donor is not feasibile; to reduce the relapse rate after Auto a maintenance treatment could be proposed.

T-CELL-DEPLETED HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR SECONDARY LEUKEMIA AND MYELODYSPLASIA FROM EITHER MATCHED OR MISMATCHED RELATED DONORS

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Background. With the aim of offer an allogeneic HSCT to all patients with secondary leukemia or myelodysplasia, in 1993, we started including patients with secondary leukemia or myelodysplasia not only in our T-cell-depleted matched transplant programme but also in our T-cell-depleted mismatched transplant programme.

Patients and Methods. Here we report the results in 42 of the patients in our large series of haploidentical transplants who had secondary leukaemia or myelodysplasia and in a matched group of 21 patients who were candidates in our HLA-matched sibling transplant programme, which was started in July 1985. Median ages (range) were 44 (17-65) and 37 (7-62) years for matched and mismatched recipients, respectively. Leukemia was secondary to MDS in 46, radio-chemotherapy-related in 8. Median time from original disease to transplant and from leukemia diagnosis to transplant were 9 (range 2-168) and 10 months (range 2-60), respectively. At transplant, 30 patients were in CR, 23 in relapse and 10 had never been treated before transplantation. Cytogenetics were unavailable in 14 patients, normal in 20, abnormal in 29. TBI-based conditioning (14,4 Gy fractionated in matched or 8 Gy single fraction in mismatched) was used in 56 patients and chemotherapy-alone based protocol in 7 patients who had been previous irradiated because of prior cancer. TBI was followed by thiotepa, rabbit ATG and cyclophosphamide in the first 12 patients or fludarabine in the others. Melphalan was given instead of TBI in 7. GvHD prophylaxis consisted only of ex vivo T-cell depletion.

Results. Two patients died too early. Primary engraftment was achieved in 40/41 mismatched and 20/20 matched recipients. Grade II-IV acute GvHD occurred only in 3 mismatched patients and chronic GvHD in 2. Seventeen of the 42 mismatched transplants and 5 of the 21 matched transplant recipients died of non-leukaemic causes. The cumulative incidence of non-leukaemic mortality ranged from 30% (95% CI 15%-59%) to 52% (95% CI 33%-80%) in the mismatched group and from 0 to 40% (95% CI 20%-80%) in the matched group depending on disease status at transplant. Infections were the most common causes of the non leukemic deaths. The cumulative incidence of relapse in patients transplanted in CR from a mismatched or matched donor was 16% (95% CI %%-46%) and 0% respectively. For those in relapse at transplant, leukemia relapse was 26% (95% CI 12%-56%) and 40% (95% CI 16%-97%) in the mismatched and matched groups, respectively. Median follow-ups (ranges) for the 13 survivors of 21 matched and the 17 of 42 mismatched were respectively 77 (8-233) and 48 (2-145) months. Probability of EFS was 0.60±0.18 and 0.50±0.11 for the patients transplanted in CR from a

matched or mismatched donor, respectively. For those already in relapse at transplant, EFS was 0.59±0.14 in the matched and 0.20±0.09 in the mismatched group.

Conclusions. Patients with secondary leukaemia or myelodysplasia can benefit from a HSCT independently of whether a matched sibling is available. Extensive T cell depletion prevents GvHD without the need for a post-transplant prophylaxis. Most important, considering the median age in cases of myelodisplasia, patients between 40 and 65 years of age are not excluded from the transplant programme.

AML1 AMPLIFICATION IN SECONDARY AML

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Background. The human AML1 gene is located in the 21q22 chromosomal band. It is normally expressed in all hematopoietic lineages and acts to regulate the expression of various genes specific to hematopoiesis playing a pivotal role in myeloid differentiation. AML1 is one of the genes most frequently deregulated in leukemia through different mechanisms including translocation, mutation and amplification.[3] In the genesis of hematologic neoplasms gene amplification is a mechanism for illegitimate activation of proto-oncogenes.[4] The most common origin of extra copies of the AML1 gene is polysomy of chromosome 21 or a partial duplication of the long arm of chromosome 21, less frequently ring, isochromosome or the tandem repetition of chromosome 21.[2] Amplification of AML1 has been recently defined as a new recurrent abnormality in ALL, associated with a poor prognosis.[6] FISH with probes directed to AML1 is the only reliable method of detection. Virtually all cases reported to date have been identified using the LSI TEL-AML1 translocation probe.[5] We report a case of 12 years old girl diagnosed 5 years ago with common-B ALL. 5 years after initial diagnosis she returned with apparent relapse. Diagnosis workup excluded relapse of primary desease and revealed a completely different type of secondary leukemia.

Material and Methods. Immunophenotyping: Bone marrow samples were analyzed by flow cytometry (Coulter).

Cytogenetics. Unstimulated cultures were harvested after 24 h of cultivation in Marrowmax (Gibco) medium. Standard cytogenetics was performed using G-banding. For FISH analyses locus-specific DNA probes (Abbot) were used.

Results. The first diagnosis in 2001 based on immunophenotype ($CD10^+$,$CD19^+$,$CD45^+/CD34^+$) was common-B ALL. In second leukemia flow cytometry showed a considerably changed immunophenotype ($CD11c^+$, $CD14^+$, $CD33^+$, $CD45^+/CD34^+$, $CD117^+$, MPO^+, $CD19^-$, $CD10^-$) corresponding to AML (M4 or M5). FISH analysis by DNA probe TEL/AML1 showed a clustering of signals for AML1 gene being typical for AML1 amplification. G-banded cytogenetics revealed a karyotype with aberrations of chromosome 21.

Conclusions. AML1 gene amplification has been found essentially in childhood ALL forming a cytogenetic subgroup of ALL.[1] This chromosomal aberration is on the other hand very rare in myeloid malignancies.[1] A few cases described in the literature were older patient and had previously received therapy with alkylating agents.1,7 According to our knowledge this is the first case of secondary AML after the treatment of childhood pr B cell leukemia with AML1 gene amplification. This is additional confirmation that the role of AML1 amplification in leukogenesis is heterogeneous.

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