clinical outcome. Illmer *et al.*[8] also showed that 4 of 5 t(8;21) or inv(16) AML patients with a *JAK2* mutation had early relapses within 20 months after diagnosis. Taken together, these results suggest that mutations in the *JAK2*, *KIT* and *FLT3* genes are associated with unfavorable clinical outcome in patients with t(8;21) AML.

Our study also implies that patients with *RTK* and *JAK2* mutations may benefit from allogeneic HSCT. Three patients with mutations received allogeneic HSCT after relapse and have achieved continuous second CR. Three patients in each group also received allogeneic HSCT at the first CR. As a consequence, 6 out of 9 patients with AML harboring *KIT*, *FLT3* and *JAK2* mutations who continued CR received allogeneic HSCT. When patients who underwent HSCT were censored at the date of the HSCT, the 6-year overall survival in patients with mutations was 25% compared to 62% in those without mutations (p=0.1368) (Figure 1B). These findings are of significant clinical import as activating mutations in *KIT*, *FLT3* and *JAK2* could be potential therapeutic targets for specific tyrosine kinase inhibitors and JAK2 pathway inhibitors in patients with t(8;21) AML harboring the mutations.

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JAK1 mutation analysis in T-cell acute lymphoblastic leukemia cell lines

T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of T-cell precursors that mainly occurs in children and adolescents. A variety of oncogenic events that are involved in the pathogenesis of T-ALL have been identified, including *NOTCH1* and *PTEN* mutations, overexpression of *TAL1*, *LYL1* and *TLX1*, and deletion of *CDKN2A* (p16).[1] Apart from mutations in *FLT3* and *NRAS*, and chromosomal aberrations generating the NUP214-ABL1 fusion, mutations that drive proliferation and survival of T-ALL cells are still unknown in the majority of patients. Recently, activating point mutations in the *JAK1* gene were identified in patients with ALL, and rarely also in acute myeloid leukemia (AML) patients.[2-4] In T-ALL, *JAK1* mutations were identified in approximately 20% of adult T-ALL cases, with a much lower frequency in childhood T-ALL.[2] These mutations are very heterogeneous in the sense that they are dispersed over several *JAK1* domains, and differ in their ability to transform hematopoietic cells and to activate downstream signaling pathways such as the STAT, PI3K and MAPK cascades.[2-4]

Leukemia cell lines with mutations in *FLT3, JAK2* and *NOTCH1* have been described as useful models for preclinical testing of small molecule inhibitors.[5-8] Given the recent identification of *JAK1* mutations in T-ALL, we investigated if *JAK1* mutations could be detected in a panel of 18 common T-ALL cell lines. By sequencing of the JAK1 open reading frame at cDNA level in these cell lines, we identified 2 transcript variants, one non-synonymous substitution, as well as several synonymous substitutions (Table 1).

A first transcript variant was identified in the HPB-ALL cell line (Figure 1A, 1B). This transcript lacks nucleotides 2896-2967, encoding amino acids 966-989 that are located between the P-loop and the activation loop in the kinase domain. When sequencing HPB-ALL genomic DNA, we could not detect the presence of a deletion, but we detected a single nucleotide change (2897 A>T) generating a novel GT splice donor site in

exon 21 (Figure 1C). As a consequence, the second half of exon 21 is spliced out, resulting in the absence of amino acids 966-989 in the protein. Due to the fact that this splice site is not 100% effective, a normally spliced JAK1 transcript is also present harboring a 2897 A>T nucleotide change with corresponding E966V amino acid change (Figure 1A, 1D).

To examine the functional consequences of the identified variants, the JAK1(Del966-989) and JAK1(E966V) variants, as well as wild type *JAK1* and the known activating mutant JAK1(R724H), were expressed in 293T and in interleukin 3 (IL3) dependent Ba/F3 cells. While we could confirm transformation of Ba/F3 cells to IL3 independent proliferation by JAK1(R724H), as was reported by Flex *et al.*, the E966V and Del(966-989) variants were unable to confer IL3 independence to Ba/F3 cells (Figure 1E). Western blot analysis of JAK1 in 293T cells showed that the E966V and Del(966-989) variants displayed weak JAK1 auto-phosphorylation, comparable to wild type *JAK1*, and in contrast to the strongly phosphorylated R724H mutant (Figure 1F). Furthermore, Western blot analysis of STAT5 phosphorylation in Ba/F3 cells showed increased phospho-STAT5 signal only in the R724H mutant (*data not shown*). In agreement with these findings, JAK1 phosphorylation in HPB-ALL, the cell line expressing JAK1 E966V and Del(966-989), was not increased as compared to other T-ALL cell lines. *JAK1* was found to be

Table 1. Overview of synonymous and non-synonymous variations detected in the T-cell acute lymphoblastic leukemia cell lines analyzed in this study (nucleotides are numbered relative to the start codon).

Cell lines	Nucleotide(s) *	Exon	Protein level
DND-41, JURKAT	546 A>G	6	No change
MOLT-14, MOLT-16	579 T>C	6	No change
DND-41, JURKAT	1590 C>T	11	No change
P12-ICHIKAWA, SUP-T1	1977 C>G	14	No change
PEER, BE-13, JURKAT, LOUCY, HSB-2	2049 C>T	15	No change
DND-41	2097 C>G	15	No change
ALL-SIL, MOLT-14, MOLT-16, P12-ICHIKAWA, SUP-T1	2199 A>G	16	No change
LOUCY	3093 T>C	22	No change
MOLT-14, MOLT-16	3096 G>A	22	No change
HPB-ALL	2897 A>T	21	E966V
MOLT-4, RPMI-8402	648-1178	7,8	Lacking kinase, pseudokinase, SH2 and half of FERM domain
HPB-ALL	2896-2967	21	Lacking amino acids 966-989 in kinase domain

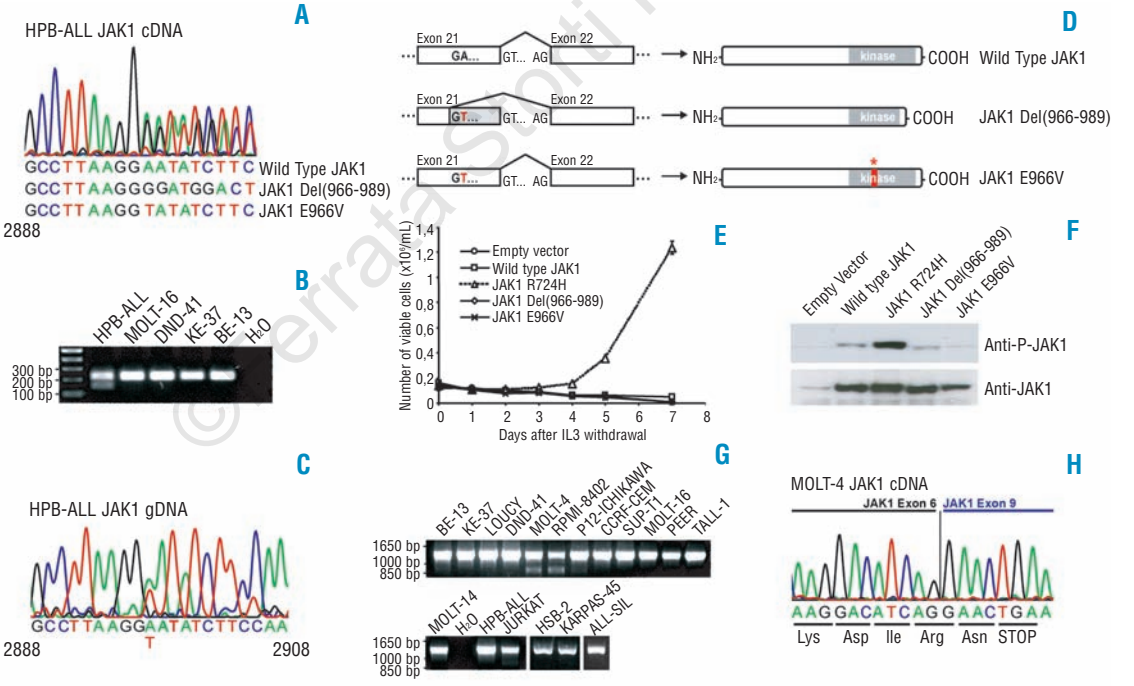

Figure 1. Analysis of JAK1 sequence variants detected in T-ALL cell lines. (A) Chromatogram corresponding to the JAK1 transcript in the HPB-ALL cell line indicating the presence of wild type JAK1 and two variant transcripts. (B) RT-PCR performed with primers in exon 21 and 22 confirms the presence of an alternative transcript in HPB-ALL. (C) Genomic DNA sequence of JAK1 in the HPB-ALL cell line reveals a 2897A>T substitution. (D) Scheme illustrating the mechanism for generating the observed transcript variant in the HPB-ALL cell line. (E) Proliferation of Ba/F3 cells expressing the indicated constructs in absence of IL3 (IL3 was removed at day 0). JAK1 Wild Type, R724H, Del(966-989) and E966V cDNA sequences were amplified by PCR from T-ALL cell lines and cloned into the pMSCV-GFP vector. All constructs were verified by sequencing. Data are presented as mean ± st dev. (F) Western blots illustrating that only the JAK1(R724H) mutant displays increased JAK1 phosphorylation as compared to wild type JAK1 when overexpressing the indicated constructs in HEK293T cells. (G) RT-PCR in T-ALL cell lines with primers amplifying the 5' region of JAK1 reveals the presence of shorter JAK1 transcripts in the MOLT-4 and RPMI-8402 cell lines. (H) Chromatogram representing the sequence of the 850 bp band amplified from cDNA of the MOLT-4 cell line that is shown in part G. of this figure. The chromatogram shows fusion of exon 6 and 9, resulting in frame-shift and premature stop-codon formation.

expressed in all examined T-ALL cell lines (*data not shown*). These data show that it remains critically important to test potential mutant forms of kinases to discriminate between driver and passenger mutations, as was also shown recently in the context of *FLT3*.[9]

In the MOLT-4 and RPMI-8402 cell lines, we identified another transcript variant, which lacks exons 7 and 8 (nucleotides 648-1178). This results in a shift in the open reading frame with generation of a premature stop codon in exon 9. Consequently, a truncated form of *JAK1* is expressed in these cell lines, lacking the entire kinase, pseudokinase and SH2 domain as well as part of the FERM domain (Figure 1G, 1H). Due to the absence of a kinase domain, no further functional studies were performed on this variant.

To investigate if the 18 T-ALL cell lines were dependent on JAK1 signaling for their proliferation and survival, we examined the effect of treatment with a small molecule JAK inhibitor (JAK inhibitor I, Calbiochem, San Diego, CA, USA). This compound inhibited JAK1 autophosphorylation in control cells with an IC_{50} value of 100 nM (*data not shown*). The majority of T-ALL cell lines were completely insensitive to treatment with this inhibitor (IC_{50} values ≥10 μM), while other T-ALL cell lines (ALL-SIL, SUP-T1, DND-41, TALL-1) displayed an increased sensitivity with IC_{50} values below or around 1 μM. In none of the T-ALL cell lines was proliferation completely inhibited at a concentration of 10 μM of the JAK inhibitor (*data not shown*). Despite the presence of *JAK1* variants in the cell lines HPB-ALL, MOLT-4 and RPMI-8402, these cell lines were not more sensitive to JAK1 inhibition, confirming that the observed variants were unlikely to contribute to *JAK1* activation. As a final experiment, we knocked down JAK1 expression in the HPB-ALL and RPMI-8402 cell lines using a JAK1 siRNA (Validated Stealth siRNA, Invitrogen, Carlsbad, CA, USA), which again confirmed that these T-ALL cell lines were not dependent on *JAK1* expression for their survival and proliferation (*data not shown*).

Flex *et al*. reported association of hyperactive *JAK1* mutants with advanced age at diagnosis in ALL patients. Unfortunately, we were not able to identify a T-ALL cell line with an activating mutation in JAK1 that could be used as a T-cell model to study JAK1 signaling and study the effect of JAK1 inhibition. As most of the cell lines we tested correspond to samples from childhood/adolescent T-ALL, the results obtained in this study are in line with the data presented by Flex *et al*. Furthermore, our observation that concentrations in the range of 1 μM or more of a JAK kinase inhibitor are required to inhibit T-ALL cell lines indicate that T-ALL cell lines in general are not critically dependent on *JAK1* activity for their proliferation and survival.

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A father and his son with systemic AL amyloidosis

In systemic AL amyloidosis, fibrils are derived from a monoclonal immunoglobulin light chain produced by a plasma cell clone in the bone marrow.[1] AL amyloidosis may be associated with multiple myeloma but more commonly the plasma cell clone is not malignant. A monoclonal gammopathy of undetermined significance (MGUS) may have preceded the development of amyloidosis. AL amyloidosis is often recognized and diagnosed at a late stage thereby giving the patients an average 10-14 months survival if they do not respond to treatment.[2] Even though it was one of the first types of amyloidosis biochemically characterized, still little is known about how the light chains cause disease, why they are deposited in certain organs and why there is an enormous variation in patients' clinical symptoms and outcome.

There are several types of familial amyloidoses usually inherited dominantly. The most recognized one is familial amyloidotic polyneuropathy where a mutation in the transthyretin (TTR) gene leads to an amyloidogenic protein variant.[3] AL amyloidosis has not been considered to be hereditary[4-6] and to date no two identical amyloidogenic light chains have been identified.