Table 1. Hematologic and molecular remission on treatment with As_2O_3.

Hematologic remission	90%
bcr-1 isoform (n=29)	89.6%
bcr-3 isoform (n=9)	88.8%
bcr-2 isoform (n=2)	100%
Time to hematologic remission (days)	42.6 (25-60)
bcr-1 isoform	46.5 (30-60)
bcr-3 isoform	31 (25-37)*
bcr-2 isoform	38.5 (33-44)
Time to molecular remission (days)	83.9 (51-136)
bcr-1 isoform	84.5 (51-119)
bcr-3 isoform	67.5 (60-136)°
bcr-2 isoform	84 (74-94)

*$p < 0.001$; °$p=0.25$.

of HCR was similar in patients with all isoforms though the median time to HCR was significantly shorter in those with bcr-3 than in those with the bcr-1 isoform [31 vs 46.5 days] ($p<0.001$) with no significant difference was seen in the median time to molecular remission [67.5 days bcr-3, 84.8 days bcr-1] ($p = 0.2$). Two patients relapsed 6 and 7 months after treatment: one patient achieved a second complete remission on repeat treatment with a combination of As_2O_3 and ATRA while the second died of intracranial hemorrhage. At a median follow-up of 20.3 months (range: 4-53), thirty-four patients (85%) remain in remission with a leukemia-free survival of 94.5%. As_2O_3 achieves induction remission rates similar to those produced by treatment with ATRA or a combination of ATRA and As_2O_3 with similar numbers of patients achieving molecular remission by the end of consolidation therapy.[4,5,6] Trials in patients with relapsed APL have also shown 80-90% PCR negativity by the end of consolidation.[7,8] Interestingly As_2O_3 may show anti-leukemic efficacy for many days after the drug has been stopped, as suggested by the 70% of patients who became RT-PCR negative prior to starting consolidation despite being positive at the time of hematologic remission. There does not seem to be a major difference between patients with the bcr-1 or bcr-3 isoform but larger numbers need to be studied. There are, however, no data available on the significance of isoforms in APL patients primarily treated with As_2O_3. The median follow-up in our patients in our study is too short (20 months) to evaluate late relapses and the long-term significance of the various isoforms. These preliminary data show that all patients achieving hematologic remission on primary treatment with As_2O_3 also achieve molecular remission. Ninety-five percent of patients are in molecular remission by the end of consolidation with 85% achieving long-term remission. However, follow-up studies are needed to assess the durability of remissions in these patients.

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Acute Myeloid Leukemia

Quantification of *DEK-CAN* fusion transcript by real-time reverse transcription polymerase reaction in patients with t(6;9) acute myeloid leukemia

Real-time reverse transcription polymerase reaction (RT-PCR) was used to examine *DEK-CAN* transcript levels in serial samples from three patients with t(6;9) acute myeloid leukemia treated with intensive chemotherapy. All three patients achieved short first clinical remission, but without achieving RT-PCR negativity. DEK-CAN level significantly increased in two patients before relapse, while in the third a level of 2×10^{-3} in remission bone marrow preceded relapse by 2 months.

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The t(6;9)(p23;q34) translocation which produces the *DEK-CAN* fusion gene is detected predominantly (90%) in acute myeloid leukemia (AML) with FAB type M2 or M4 and associated with basophilia.[1-3] t(6;9) is associated with a poor prognosis.[2] There have been few studies to date using this aberration as a marker for monitoring minimal residual disease (MRD).[4-6] We have developed a highly sen-

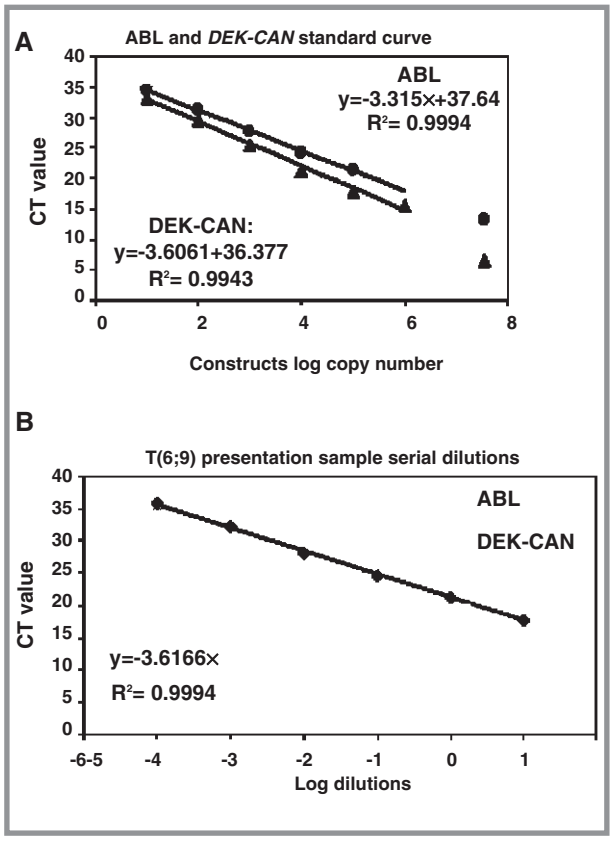

Figure 1. A: Standard curves generated from DEK-CAN and ABL constructs dilutions. B: Quantification of DEK-CAN transcript in a serial dilutions of t(6;9) presentation sample.

Table 1. Primer and probe sequences.

Oligonucleotide	*Oligonucleotide sequence*	*Product size (bp)*
D/C ConF	5'-ttaaaaagttgaagaaaccccctacag-3'	182
D/C ConR	5'-atgctgatcccactccaagtctag-3'	
Dek F	5'-ttggaagaagtcacaatgaaacaga-3'	76
Can R	5'-ggacagcaaatttcacatactgatg-3'	
Dek/CanP	5'-ttgcaaaaaggaaattcggcgcctt-3'	
AblF2	5'-tgagcctcagggtctgagtga-3	165
AblR2	5'-cctaagacccggagcttttcac-3'	
AblP2	5'-ggaactccaaggaaaaccttctcgctggacc-3'	

sitive RQ-PCR method to quantify the level of the t(6;9) transcript. Constructs for the ABL and *DEK-CAN* transcripts were prepared using the primers detailed in Table 1. The detection limit was 5 copies for the *DEK-CAN* transcript, (equivalent to 10^{-5}) (Figure 1).

Inter-run variability in RQ-PCR for *DEK-CAN* and ABL was insignificant with a standard deviation (SD) of 0.28. Serial dilutions of a presentation sample from a t(6;9) patient were also examined and showed that the protocol was linear over a wide range (y=-3.6166; R^2=0.9994). ABL and DEK-CAN were quantified using 10rmole of primers Abl2F and Abl2R and DekF and CanR and FAM-labeled

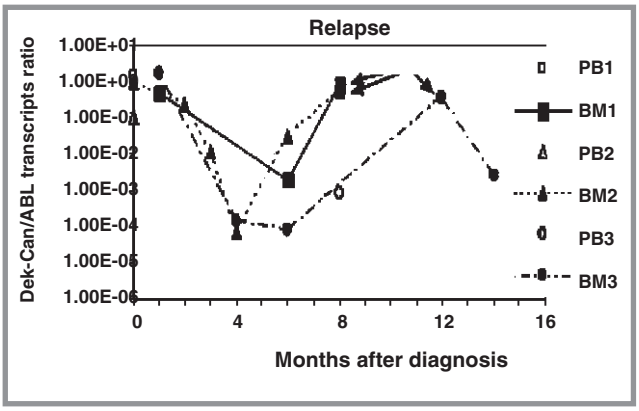

Figure 2. Sequential quantification of the Dek-Can fusion transcripts in 3 patients with t(6;9) AML at different phases of their disease.

probes AblP2 and Dek/CanP respectively. PCR amplification was performed in 251 reactions containing 1.5 mM $MgCl_2$, and 21 of cDNA. PCR cycling consisted of 3 minutes at 95°C followed by 40 cycles of 93C for 55 seconds, 56°C for 55 seconds and 72°C for 1 minute and a final hold at 72°C for 5 minutes. Samples from three t(6;9)AML patients undergoing induction and consolidation chemotherapy were investigated in this study. Follow-up by RQ-PCR of the three patients ranged from 8 to 13 months post diagnosis (Figure 2). The *DEK-CAN* levels were expressed as a ratio of DEK-CAN/ ABL.

Patient #1 (62-year old, female, AML M1). The presentation PB sample showed a 1.4 *DEK-CAN*/ABL transcript ratio. Following induction chemotherapy a BM sample showed a significant decrease in the level of fusion transcript levels (to 4.617×10^{-1}), but PCR negativity was never achieved. The level of *DEK-CAN* was 2×10^{-3} in a BM sample taken 6 months post-presentation, although cytogenetic examination showed that the sample was normal. The patient relapsed two months later.

Patient #2 (60-year old, female, AML M2). Presentation BM and PB samples showed a ratio of 1x100 and $1x10^{-1}$, respectively. After induction treatment, the DEK-CAN/ABL ratio in the BM decreased to 2.45×10^{-1}. At 3 months, the patient was in morphologic and cytogenetic remission. However RT-PCR detected 1.24×10^{-2} of the DEK-CAN/ABL transcripts in the patient's BM. Following consolidation, the *DEK-CAN* level in a BM sample taken 4 months post-presentation showed a dramatic decrease in the transcript level to a ratio of 6.8×10^{-5}, but 2 months later rose markedly to 3.2×10^{-2}, while cytogenetic and morphological examination of this sample remained normal. Eight months post-presentation the patient relapsed with 10% blasts in the BM. RT-PCR of a BM sample at the time of relapse showed a *DEK-CAN* level of 5.68×10^{-1}.

Patient #3 (30-year old, female, AML M2). A BM sample taken after induction showed a 1.7655×100 *DEK-CAN*/ABL ratio. Following further chemotherapy, the patient achieved first CR at 3 months post-presentation, and the next 2 BM samples showed ratios of *DEK-CAN*/ABL transcripts of 1.5×10^{-4} and 8.2×10^{-5}, respectively. The BM was normal both morphologically and cytogenetically at these time points. A PB sample 7.5 months post-diagnosis showed a level of 9.15×10^{-4}. Relapse occurred 4 months later with a ratio of *DEK-CAN*/ABL of 3.56×10^{-1} in BM. This patient subsequently received an autograft and the level of MRD fell to 2.5×10^{-3}.

In conclusion, this report presents the first use of RQ-PCR to accurately quantify the level of the *DEK-CAN* transcript in t(6;9)AML patients in sequential BM and PB samples from diagnosis to relapse. The protocol produces linear quantification of the *DEK-CAN* transcript over a wide range of levels. The level of the *DEK-CAN* transcript was more than 1×10^{-2} *DEK-CAN*/ABL in all patients after induction chemotherapy. After two further courses of chemotherapy, the levels fell to less than 1×10^{-4}, which indicates that chemotherapy can reduce the leukemic load, but it remains persistently detectable, albeit at a low level. All three patients had a relatively high level of MRD prior to relapse, even though the samples were morphologically and cytogenetically normal. These data support the observations made by Soekerman *et al*. and Lillington *et al*.,[2,7] that t(6;9) patients respond poorly to chemotherapy alone with a median survival of less than one year.

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Acute Lymphoblastic Leukemia

The achievement of molecular complete remission during treatment with imatinib mesylate correlates with relapse-free survival in bcr/abl-positive acute lymphoid leukemia patients

Using quantitative reverse-transcription polymerase chain reaction we investigated the significance of achieving molecular complete remission (CR) in 12 patients with bcr/abl-positive acute lymphocytic leukemia treated with imatinib. The 6 patients who achieved molecular CR had significantly better relapse-free survival (RFS) than the others (9 vs 4 months) (p=0.000). Moreover, the 6 patients with a bcr-abl/GAPDH×100,000 ratio <2 after 4 weeks of treatment had significantly better RFS (10.5 vs 4 months) (p=0.004).

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Recently, the abl protein tyrosine-kinase inhibitor imatinib mesylate has been shown to induce complete responses in 60% to 70% of patients with relapsed/refractory Philadelphia-positive acute lymphoid leukemia (ALL).[1,2] Unfortunately, the median time to progression of all patients is only 2 to 2.5 months and that of patients who respond still ony 3 months because of the development of resistance to imatinib.[2-4] Only a minority of patients remain in remission for longer periods. Interestingly, it has been shown that the amount of minimal residual disease (MRD) after 4 weeks of treatment correlates with relapse-free survival (RFS) in these patients.

Moreover, the velocity and magnitude of the increase of bcr/abl levels correlates with RFS and overall survival (OS).[5,6] Nevertheless, only few data are available concerning the ability of imatinib to induce molecular complete remission (mCR) in Philadelphia-positive ALL patients, and the significance of achieving a mCR is not completely clear. We studied 12 bcr/abl-positive ALL patients, treated with imatinib at the daily dose of 400 mg (3 cases), 600 mg (7 cases), and 800 mg (2 cases), strictly monitoring the MRD by quantitative TaqMan reverse transcription polymerase chain reaction (RT-PCR).[7] Two patients were treated while in first CR, 8 for relapsed/refractory disease and 2 were previously untreated. Ten out of the 12 patients were in CR after 4 weeks of imatinib therapy. Six achieved a mCR after 4-12 weeks; 5/10 then relapsed, 6 died of leukemia, 1 died while in CR, 3 are alive in CR, 1 is alive with disease (Table 1).

First, we investigated whether the achievement of a mCR correlated with RFS and OS. Six patients achieved a mCR, six did not. The median RFS and OS were 8 and 10.5 months in the former 6 patients and 4 and 5.5 months in the latter group, with a significant difference in terms of RFS (p=0.0006), but not of OS (p=0.29) (Figure 1). Notably, all the patients who achieved a mCR had a RFS of at least 8 months, whereas the others had a RFS not longer than 5 months. Taken together, these data show that the achievement of a mCR correlates with RFS, and the evaluation of MRD by quantitative RT-PCR allows discrimination between patients with a relatively better or worse prognosis.

Secondly, we investigated whether the MDR levels after 4 and 8 weeks of imatinib therapy correlated with RFS and OS. In particular, we considered patients with a bcr-abl/GAPDH×100,000 ratio 2 or >2. After 4 weeks of therapy, 4 patients had a small amount of MRD with a ratio 2 whereas 8 had a ratio >2. The median RFS and OS were, respectively, 10.5 and 11.5 months in the former group, and 4 and 9 months in the latter group. The difference was significant for RFS (p=0.0067). Analogous results were obtained considering the amount of MRD after 8 weeks of therapy. All the 3 patients with a bcr-abl/GAPDH×100,000 ratio <2 after 4 weeks of treatment achieved a mCR. Nevertheless, 2 patients with higher ratios at this time also later obtained a mCR. Taken together, these data confirm that the amount of MRD after 4 and 8 weeks of therapy is significant correlated with RFS.

Analogous results were obtained even if the patient who started the imatinib therapy while in molecular CR was