Quantitative Allele Specific Amplification (quasa®) in residual disease monitoring of Hairy Cell Leukaemia

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Abstract

This study describes the successful molecular surveillance of the BRAF V600E mutation in peripheral blood by quantitative allele specific amplification (quasa®) and proves the residual tumour frequency in hairy cell leukaemia. The study also describes a novel Allele Specific MasterMix that has been developed providing primers specifically to improve the sensitivity of allele specific PCR.

Hairy Cell Leukaemia patients and four healthy control subjects were studied. The power of a novel allele specific PCR approach as a molecular surveillance tool was compared to the current benchmark technology: Multiparameter flow cytometry (MFC).

Results

The sensitivity of the quasa® test was significantly higher than for flow cytometry: 83% vs 71%. The negative predictive value of the quasa® test was also significantly higher than for flow cytometry, 80% vs 64%. Both methods had 100% specificity and positive predictive value as there were no false positives.

Conclusions

Five patients tested positive by flow cytometry defined as ≥0.01% events meeting the co-expression criteria (range 0.01-0.1%). Six patients tested negative by quasa®, with 10 testing positive (range 0.02-0.22%) as shown in table 1. All four control samples from healthy individuals tested negative using both methods.

Materials and Method

This study investigated 14 HCL patients and 6 healthy controls. The patient characteristics and results are summarised in table 1. All patients gave written informed consent in accordance with the Declaration of Helsinki.

Multiparameter flow cytometry (MFC) was performed on peripheral blood using a four colour BD FACS Canto II. Classification of HCL positive cells was based on a panel of surface markers: light chain restriction, CD19, CD20, CD23, CD45, CD138, CD26, and CD56. A minimum of 10000 events were recorded for each patient.

One patient in our study (patient no.1) was tested at 3 weeks and 6 weeks post initial Cladribine treatment. At 3 weeks the mutation was 0.09% by quasa® and 0.10% by flow cytometry but at 6 weeks the mutation was undetectable by both methods. This result highlights the value of MRD monitoring by quasa® as an early response to treatment. Interestingly, the patient with the highest mutational percentage by quasa® (patient no.7) has since relapsed illustrating the technology’s power as a diagnostic tool in residual disease monitoring.

Example quasa® clinical data

Dark blue traces: BRAF WT specific primer/probe set; Light blue traces: BRAF V600E specific primer/probe set

Example quasa® PCR MasterMix

A patent application has been filed on the Allele Specific PCR MasterMix. The technology centres around replacing one or more of the standard dNTPs within the MasterMix with homology bases. The effect of this is to dramatically improve the specificity of allele-specific PCR reactions.

Example data below. quasa® BRAF V600E allele specific primers were tested in synthetic DNA templates containing 100% mutant sequences (Dark blue), and 100% wild type sequences (Light blue).

Example qPCR clinical sample. Patient No. 7. No detection of BRAF V600E.

Example qPCR clinical sample. Patient No. 6. Residual levels of BRAF V600E at ≤0.01%.

Example qPCR clinical sample. Patient No. 1. Residual levels of BRAF V600E at ≤0.01%.

Fig A: quasa® with traditional PCR MasterMix. Measuring across a wild type sequence at around cycle 30 there is significant overlap. Touch the window of specificity at around 15 Ct values.

Fig B: quasa® with allele specific PCR MasterMix. Measuring across a wild type sequence at around cycle 30 there is significant overlap. Touch the window of specificity at around 15 Ct values.

References