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 method superior to flow cytometry in hairy cell leukaemia monitoring. It also describes a novel inventive MasterMix that has been developed (patent pending) specifically to improve the sensitivity of allele specific PCR.

16 Hairy Cell Leukemia 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).

The sensitivity of the quasa® test was significantly higher than for flow cytometry, 62.5% vs 31%. The negative predictive value of the quasa® test was also significantly higher than for flow cytometry, 40% vs 26.6%. Both methods had 100% specificity and positive predictive value as there were no false positives.

Both technologies were able to successfully monitor residual disease decline from less than 0.1% down to zero. In addition the patient identified with the highest mutational percentage by quasa® (patient no.6) has since relapsed illustrating the technology's power as a diagnostic tool in residual disease monitoring.

Introduction

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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 0.02% by flow cytometry but at 6 weeks the mutation was undetectable by both methods. This result highlights the value of MRD monitoring by quasa® to assess response to treatment. Interestingly, the patient with the highest mutational percentage by quasa® (patient no.6) has since developed pancytopenia due to disease relapse highlighting also the value of quasa® monitoring for relapse surveillance. This patient has previously demonstrated disease refractoriness to Cladribine and Pentostatin but achieved partial response with Rituximab; he is currently being considered for treatment with the BRAF-V600E-selective inhibitor, vemurafenib [8]

Materials and Method

We investigated 16 HCL patients and 4 healthy controls. The patient characteristics and results are summarised in table 1. All participants gave written informed consent in accordance with the Declaration of Helsinki.

Multiparameter flow cytometry (MFC) was performed on peripheral blood using a four coloured BD FACS Canto II. Classification of HCL positive cells was based on monotypic surface immunoglobulin bright co-expression of CD20, CD22, CD11c and dim CD103 expression. A minimum of 10,000 events were recorded and the number of positive events were calculated as a percentage of the mononuclear cell population.

DNA was extracted from peripheral blood using Qiagen DNeasy kit following the manufacturers' instructions. Samples were analysed by quasa® according to an established standard operating procedure and in triplicate repeats (Primer Design Ltd, Southampton, UK). Briefly, the percentage level of the BRAF-V600E mutation was calculated using mutation-specific primer amplified DNA relative to wildtype-specific primer amplified DNA. The combined results of the flow cytometry and quasa® are contained in table 1 along with the patient clinical details.

	Age	Sex	Years since diagnosis	Lines of previous treatment	Years since last treatment	quasa® result	Flow result
1	67	M	0	Cladribine	0	0.09%	0.02%
2	53	F	13	Spleenectomy, pentostatin	12	<0.05%	0%
3	71	F	4	Cladribine	8	<0.05%	0%
4	75	M	7	Interferon, pentostatin	7	<0.05%	0%
5	79	M	13	Pentostatin, interferon, rituximab	1	0.12%	0%
6	71	M	10	Cladribine twice, pentostatin, rituximab	3	0.22%	0.01%
7	55	F	9	Cladribine twice	5	Negative	0%
8	65	M	5	Cladribine	5	0.19%	0.01%
9	68	F	13	Interferon, pentostatin	12	Negative	0%
10	82	M	25	Pentostatin twice	9	0.14%	0.02%
11	50	M	9	Pentostatin	9	Negative	0%
12	77	M	25	Interferon, pentostatin	17	Negative	0%
13	70	M	3	Cladribine	3	<0.05%	0%
14	63	M	7	Interferon, pentostatin	7	<0.05%	0.1%
15	65	M	2	Cladribine	2	Negative	0%
16	65	M	2	Cladribine	2	Negative	0%

Results

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 <0.05-0.22%) as shown in table 1. All four control samples from healthy individuals tested negative using both methods.

The sensitivity of the quasa® test was significantly higher than for flow cytometry, 62.5% vs 31%. The negative predictive value of the quasa® test was also significantly higher than for flow cytometry, 40% vs 26.6%. Both methods had 100% specificity and positive predictive value as there were no false positives.

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Conclusions

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References

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Allele Specific 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 homologue bases. The net effect is to drastically improve the specificity of an allele specific PCR reaction.

Example data below. quasa® BRAF V600E allele specific primers were tested on synthetic templates representing 100% mutant sequences (Dark blue) and 100% Wild type sequences (Light blue).

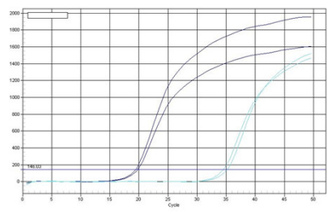


Fig A: quasa® with traditional qPCR MasterMix. Mis-priming occurs on wild type template at around cycle 32 (Light blue traces). Thus the window of specificity is around 18 Ct values.

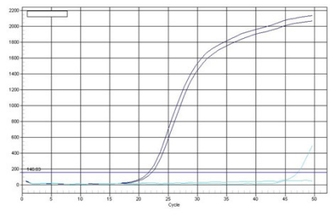
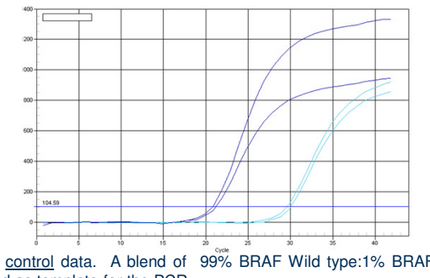


Fig B: quasa® with allele specific PCR mastermix. Mispriming occurs on wild type template at around cycle 46 (Light blue traces). Thus the window of specificity is around 25 Ct values. This represents >100 fold increase in specificity in the assay.

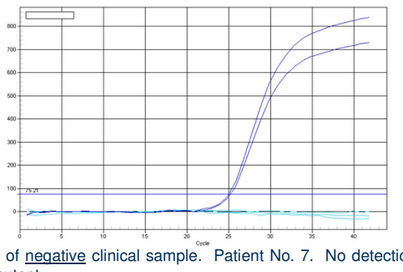
Licensing enquires for both quasa and the allele specific PCR MasterMix should be directed to Dr Jim Wicks: jim@primerdesign.co.uk

Example quasa® clinical data

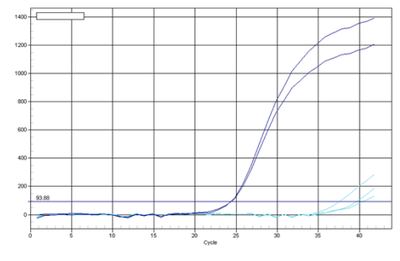
Dark blue traces: BRAF WT specific primer/probe set; Light blue traces: BRAF V600E specific primer/probe set



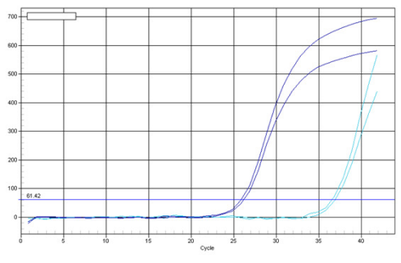
Positive control data. A blend of 99% BRAF Wild type:1% BRAF V600E was used as template for the PCR.



Example of negative clinical sample. Patient No. 7. No detection of BRAF V600E mutant.



Example positive clinical sample. Patient No. 1. Residual levels of BRAF V600E detected at levels of 0.09%.



Example positive clinical sample. Patient No. 6. Residual levels of BRAF V600E detected at levels of 0.22%.

Primerdesign