Validation of genetic abnormalities in the *MYB* gene in T-ALL detected by MLPA

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Introduction

- Copy number abnormalities (CNAs) that affect a series of genes have been implicated in T-cell Acute Lymphoblastic Leukaemia (T-ALL) development and may have prognostic significance
- Multiplex Ligation-Dependent Probe Amplification (MLPA) can be used to detect CNAs in several genes associated with a disease
- Duplication of the *MYB* oncogene has been found in 8.4% of T-ALL patient samples and now requires further biological assessment [1]
- **Project aim:** Evaluate the reliability and accuracy of MLPA for the detection of copy number alterations in the *MYB* gene

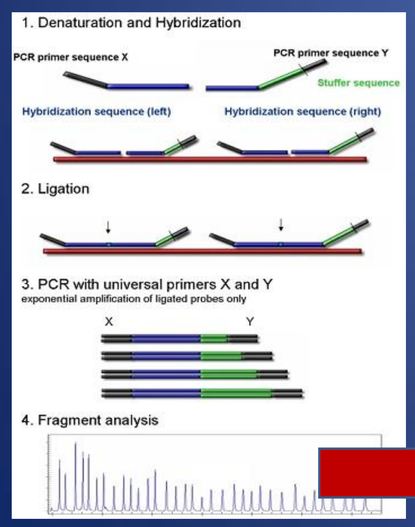
Methods

- MLPA was performed on 7 cell lines using the P383 T-ALL kit® (MRC-Holland©), which contains probes for exons 2, 6 and 16 of *MYB*
- Real-time quantitative PCR (qPCR) was performed using primers for the same regions of *MYB* covered by the MLPA probes

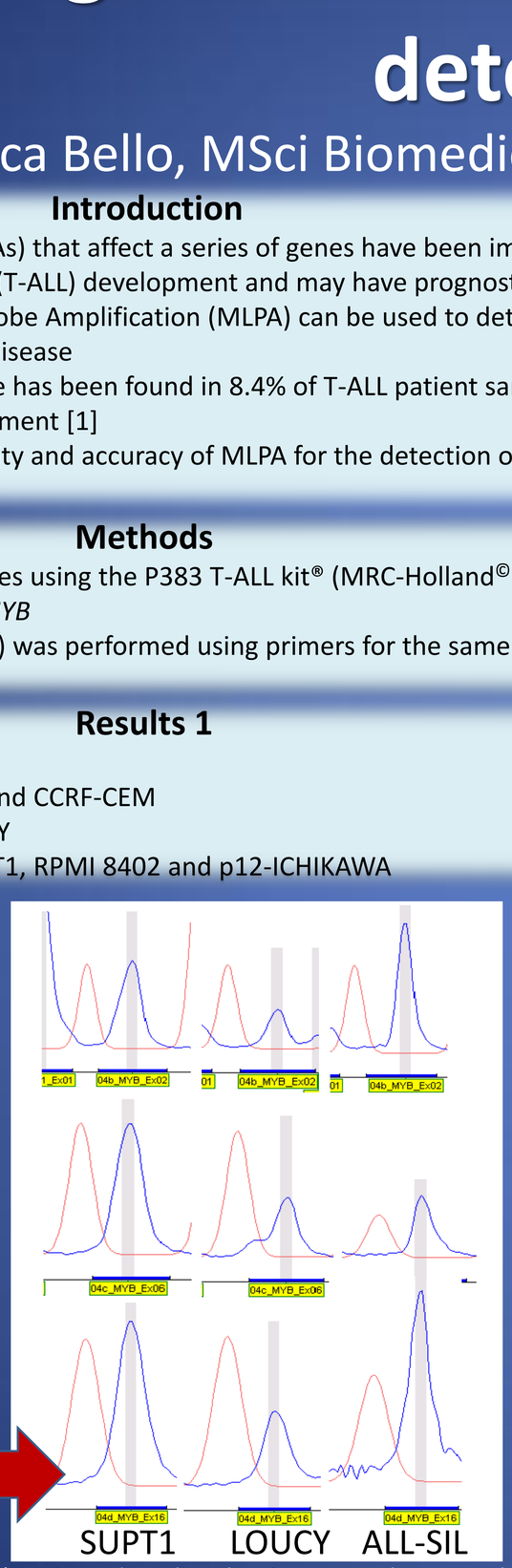
Results 1

- MLPA detected:
  - i) gain of *MYB* in ALL-SIL and CCRF-CEM
  - ii) a *MYB* deletion in LOUCY
  - iii) no CNAs of *MYB* in SUPT1, RPMI 8402 and p12-ICHIKAWA

1A)



1B)



1A) The various steps of MLPA technique . 1B) *MYB* raw data plots showing a normal copy number, gain and deletion of *MYB* in SUPT1, LOUCY and ALL-SIL, respectively

Results 2

- qPCR confirmed:
  - *i)* *MYB* gain in ALL-SIL, CCRF-CEM and in exons 6 and 16 of RPMI 8402
  - ii) a normal *MYB* copy number in SUPT1 , p12-ICHIKAWA and exon 2 of RPMI 8402
  - *iii)* *MYB* deletion in LOUCY

	MYB (exon 16)/*ALAS1*	*MYB* (exon 16)/*RPLP0*	*MYB* (exon 16)/*TRFC*	Interpretation
ALL-SIL	1,3112	1,3514	2,0696	Gained
LOUCY	0,4446	0,3617	0,6188	Deleted
SUPT1	0,7021	0,6055	1,4972	Normal

Table 1: qPCR results showing the copy number of *MYB* exon 16 when measured relative to three endogenous controls (*ALAS1*, *RPLP0* and *TRFC*)

Results 3

- MLPA vs. qPCR: Discrepant results were reported for RPMI 8402, in which a *MYB* gain was identified for exons 6 and 16 by qPCR only

	qPCR			MLPA		
	ex2	ex6	ex16	ex2	ex6	ex16
SUPT1						
LOUCY						
ALL-SIL						
CCRF-CEM						
RPMI 8402						
p12-ICHIKAWA						

Table 2: summary of results obtained by qPCR and MLPA on various cell lines showing a normal (black), deleted (red) and gained (green) *MYB* copy number

Conclusion and discussion

- This study demonstrated that MLPA can be considered a reliable technique for the detection of copy number abnormalities in T-ALL.
- The discrepant results reported in this study and compared to previous reports, need investigating further.
- The use of this technique in the analysis of MYB CNAs in recently diagnosed T-ALL patients can provide further information about copy number alterations, relevant in determining the therapeutic approach.

References:

[1] Lahortiga I, Keersmaecker K, Van Vlierberghe P *et al.* Duplication of the *MYB* oncogene in T cell acute lymphoblastic leukaemia. Nature Genetics. 2007; 35(5): 593-595.