MYC Rearrangement in B-Cell Precursor Acute Lymphoblastic Leukaemia

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Introduction

- Acute lymphoblastic leukemia (ALL) is a type of cancer that affects white blood cells. These cancerous lymphoblasts inhibit normal bone marrow function, leading to anaemia and infection. 0.1% of ALL patients harbour a *MYC* rearrangement with B-cell precursor ALL (BCP-ALL)[2]
- Treatment of ALL considers a range of prognostic factors such as white cell count (WCC), age, immunophenotype and cytogenetics, which stratify patients into low, medium or high risk groups (Figure 1).[3] Mature B-ALL treatment involves a short and intensive regimen of chemotherapy (with high-dose methotrexate, cytarabine and cyclophosphamide)[3]. BCP-ALL treatment includes induction and maintenance therapy given over 3 years for boys and 2 years for girls.
- *MYC* is an oncogene that is overexpressed in Burkitt's lymphoma (BL), where it has been shown to be essential for BL cell survival and proliferation.[1] The aberrant expression of *MYC* arises from a translocation between chromosome 8 and the immunoglobulin heavy (*IgH*) or light chains (*IgL* or *Igk*) respectively; t(8;14), t(2;8) and t(8;22).
- As evidenced in Figure 1, the characterisation of genetic rearrangements in ALL has prognostic value. The aim of this study was to characterise BCP-ALL patients with suggested involvement of *MYC*. *MYC* involvement was to be confirmed, additional clinical data gathered and a literature review to be completed to inform future decisions on the optimum clinical approach for patient treatment.

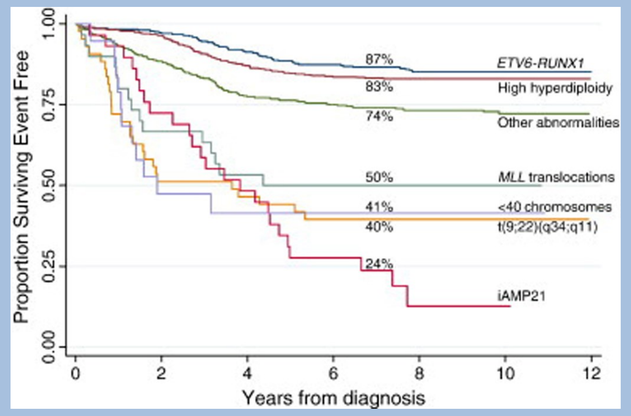

Figure 1— Clinical implications of genetic abnormalities in childhood BCP-ALL, showing high event free survival (ESF) in *ETV6-RUNX1* and high hyperdiploidy and low EFS in those with iAMP21 and t(9;22). The EFS of BCP-ALL patients with *MYC* involvement warrants further investigation. [8]

Methods

- Forty seven patients were identified from a patient database to have a cytogenetically visible abnormality in the region in which *MYC* resides on chromosome 8 (8q24).
- Fluorescence *in-situ* hybridization (FISH) was performed on patients with fixed cells to verify the presence of the *MYC* translocation.
- FISH uses oligonucleotides with fluorophores attached or "fluorescent probes" to bind to specific gene sequences on the chromosome. With the *MYC* breakapart probe (Cytocell), a red and green probe is designed to bind to opposite ends of the *MYC* gene as seen in Figure 2. A summary of the FISH process is shown in Figure 3.

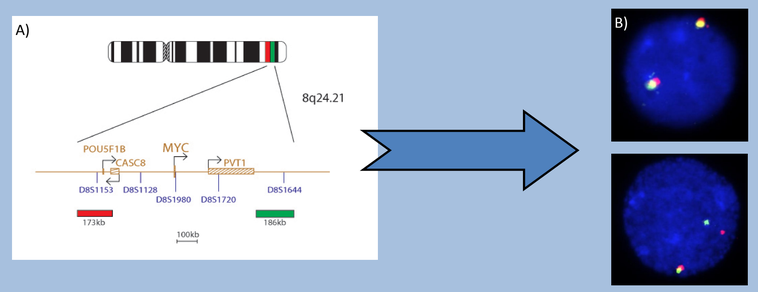

Figure 2—FISH for *MYC* rearrangements. (a) The site of binding of the commercial *MYC* breakapart probe by Cytocell. [7] **(b)** Interphase cells with 0R0G2F (Fusion—Overlapping green and red signals) *MYC* probe signals, signifying a normal cell (Top) and (Bottom) a Myc rearrangement is seen with 1R1G1F signals, the translocation has led to the separation of a green and red signal.

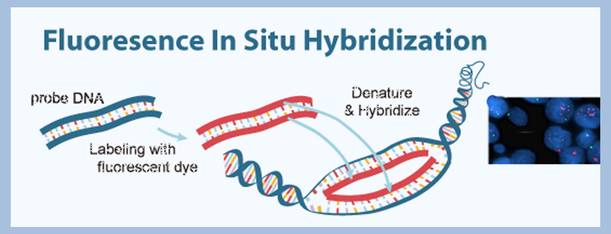

Figure 3— FISH involves hybridisation of fluorescent probes to target DNA which is analysed through fluorescent microscopy. [9]

Results

- Sixteen patients were identified to have *MYC* rearrangement as seen in Table 1. A further 8 patients were deemed positive due to t(8;14) and t(8;22) being observed in their karyotype. Sixteen were paediatric patients (range 0-17 years old) and 8 adult patients (range 28-64 years old) altogether. The most prevalent translocation observed was the t(8;22), an *IgL-MYC* translocation, seen in eleven patients.
- Of the twelve paediatric patients confirmed by FISH (Table 1), eight had survival data available. 6 patients were still alive 3 years post diagnosis. Insufficient data was available from adult patients to draw any conclusions. 1 patient died from primary organ failure and another never achieved complete remission (CR) upon treatment.
- Of the sixteen patients, an average of 57% of cells exhibited a breakapart signal pattern as seen in Table 1.
- No significant difference was seen between the *MYC*-positive and *MYC*-negative patients in terms of immunophenotype (Table 2) or length of time from diagnosis to death.

Patient ID	Age	Gender	WCC	Karyotype	% cells rearranged
22450	0	Male	N/A	47,XY,+del(1)(p1?3),**t(3;8;14;13)(q27;q24;q32;q1?4)**[6]	81
22901	0	Female	N/A	46,XX,t(7;19;11)(q11.2;p13,3;q23)[2]/46,idem,**t(8;22)(q24;q11)**[6]/46,XX[2]	39
979	1	Female	18.6	47,XX,+i(1)(q10),**t(8;14)(q24;q32)**[8]/ 47,XX,ins(1)(q21),+ins(1)(q21), der(2)t(1;2)(q21;q33),**t(8;22)(q24;q11)**,+der(8)**t(8;22)(q24;q11)**,add(14)(p11)[cp14]	55
25578	2	Male	N/A	46,XY,**t(8;22)(q24;q11)**[21]/46,XY[2]	20
26683	5	Male	N/A	47,XY,**t(8;22)(q24;q11)**,+21c[8]/47,XY,+21c[4]	34
25729	7	Female	9	49,X,add(X)(p22),der(5)t(5;21)(p15;q11),del(6)(q21q25),**t(8;14)(q24;q32)**,add(9)(p?21),+11,+13,-14,+der(14)**t(8;14)(q24;q32)**,**t(18;22)(q21;q11)**,t(19;22)(q13;q11),+der(?)t(?;1)(?;q12)[10]	85
5892	7	Male	8.2	46,XY,**t(8;14)(q24;q11)**[3]	74
6008	10	Male	**43.6**	46,XY,**t(2;8)(p12;q24)**,del(9)(p?21),?del(13)(q1?),dup(21)(q?)[9]/ 46,XY[2]	98
492	11	Male	**9.2**	46,XY,**t(8;14)(q24;q32)**[20]/ 46,XY[1]	62
22293	16	Male	N/A	46,XY,der(1)t(1;7)(p36-1;q11-2),**t(8;14)(q24;q11)**[30]/46,XY[15]	36
4469	16	Male	**14.6**	47,XY,**t(1;8)(q25;q24)**,der(6)t(X;6)(q2?;q2?),?del(9)(p22p24), der(12)inv(12)(p?q?)del(12)(p1?2p13),t(12;21)(p13;q22),+der(21)t(12;21)(p13;q22)[4]/ 47,idem,-18,+der(18)(t(4;18)(?;q?)[3]	26
27424	17	Male	N/A	46,XY,t(2;14)(p13;q12),**t(8;22)(q24;q11)**[6]/46,XY[2]	16
3545	28	Female	**21**	**60-65**,XX,+X,+X,+1,**t(2;8)(p12;q24)**,+t(2;8)(p12;q24),+3,+4,+5,+6,+7,+11,+12,+13, der(14)t**(14;18)(q32;q21)**,+der(14)t**(14;18)(q32;q21)**,+14,+15,i(17)(q10),+18,+20,+20,+21[cp4]	81
21844	39	Female	N/A	46,XX,**t(8;14)(q24;q11)**,**t(14;18)(q32;q21)**,add(14)(p11)[10]	96
4214	52	Male	**130.5**	46,XY,dup(6)(q23q27),**t(8;22)(q24;q11)**,dup(12)(q13q14),del(13)(q3),**t(14;18)(q32;q21)**, del(17)(p1?p1?2)[9]/ 46,XY[2]	42
25131	64	Female	9.1	50,XX,der(5)t(1;5)(q2;q2),+6,+7,**add(8)(q24)**,**+add(8)(q24)**,-9,add(10)(q24),-13,add(13)(p1),**t(14;18)(q32;q21)**,add(17)(p1),**+der(18)t(14;18)**,+20,+mar[cp10]	65

Table 1—FISH confirmed *MYC* positive patients with accompanying ancillary information, arranged by age. Patient ID in bold were confirmed with partner gene FISH (*IgH* and *IgL*) WCC—White cell count. Red and green indicates the rearrangement that involves *MYC* or *BCL2,* respectively.

		Pre-B Marker	B-Cell CD Markers				T-Cell CD Markers			Myeloid Markers		
RegId	**Blast count**	**CD34**	**CD10**	**CD19**	**CD20**	**cIGM**	**CD2**	**CD3**	**CD7**	**CD33**	**CD13**	**MYC Status**
3725		4	64	57	59		11		5	17		Non-confirmed +
3725 (BM Rel)		4	24	26			5	5	9	37	43	
484	95	71	97	98			99	1	1	1	4	Non-confirmed +
4469		24	40	50			37	33	36	21	46	Confirmed +
26683		74	96	90	1		5	4	2	2	3	Confirmed +
26683 (Rel?)		47	52	45								
492	96	1	17	67			29	99	8	7	7	Confirmed +
979	91	3	78	80			95	7	11	0	0	Confirmed +
1053	95	52	81	80			10		9	6	57	-
1447	100	2	87	87			13		9	3	2	-
2154	97	6	71	68			1		1	0	0	-
3525	>95	38	64	83		15	2		3	0	0	-
12801			12	6			36	13		60	54	-
7171	80	95	85	84	-		8	-	8			-
4354	16 (Day 8)	+	+/-	+	+	0						-
299	98	99	90	90			99	0	5	10	10	-
450	96	5	74	72			99	22	20	70	52	-
4408	82	0	92	92	1	-	0	0	3	69	93	-
21620		93	21	91	90	0	4	5	6	1	46	-

Table 2 — Available immunophenotype data of *MYC* positive and negative patients. The CD markers are used to identify the type of lymphoblasts present and the immunophenotype serves as the basis for diagnosis. Most patients are strongly positive for B-Cell markers. T-Cell markers were unexpectedly seen in a small group of patients.

Discussion

- This is the largest study to investigate *MYC* rearrangements in BCP-ALL to date.
- Twenty four patients were confirmed to be *MYC* positive by FISH. The most common translocation was t(8;22) whereas a previous study[2] found the t(8;14) translocation to be most prevalent.
- Outcome data show a trend towards a favourable outcome in the *MYC* positive patients reported here, however this warrants further investigation in a larger cohort in order to understand the impact the *MYC* rearrangement has on the prognosis.
- Other confounding factors may contribute to the outcome of these patients including other cytogenetic abnormalities, age, WCC, response to treatment, presence of minimal residual disease and additional gene deletions[4-6].
- Immunophenotype data collected on this cohort shows that all patients have positive staining for B-cell markers. Interestingly, both positive and negative patients also show positive staining for T-cell and myeloid markers.
- In a previous study of 5 paediatric patients[2], upon discovery of a *MYC* rearrangement, these patients were switched to a mature B-ALL treatment protocol. All patients achieved complete remission. The patient cohort we studied received BCP-ALL treatment with no protocol switch, but also showed a trend towards a favourable outcome.
- Further studies will be conducted on an expanded patient cohort to include confirmation of *BCL2 (*green in Table 1) and *BCL6* involvement, alongside single nucleotide polymorphism arrays to investigate copy number alterations present in the genomes of these patients. The intention is to expand our knowledge on this rare subgroup of BCP-ALL patients.

References and Acknowledgements

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