CHAPTER 15

Normal and Pathological V(D)J Recombination:
Contribution to the Understanding of Human Lymphoid Malignancies

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Abstract

The majority of haematological cancers involve the lymphoid system. They include acute lymphoblastic leukemias (ALL), which are arrested at variable stages of development and present with blood and bone marrow involvement and chronic leukemias, lymphomas and myelomas, which present with infiltration of a large variety of hematopoietic and non hematopoietic tissues by mature lymphoid cells which express a surface antigen receptor. The majority involve the B-cell lineage and the vast majority have undergone clonal rearrangement of their Ig and/or TCR rearrangements. Analysis of Ig/TCR genomic V(D)J repertoires by PCR based lymphoid clonality analysis within a diagnostic setting allows distinction of clonal from reactive lymphoproliferative disorders, clonal tracking for evidence of tumor dissemination and follow-up, identification of a lymphoid origin in undiagnosed tumors and evaluation of clonal evolution. Ig/TCR VDJ errors are also at the origin of recombinase mediated deregulated expression of a variety of proto-oncogenes in ALL, whereas in lymphoma it is increasingly clear that IgH containing translocations result from abnormalities other than VDJ errors (somatic hypermutation and/or isotype switching). In addition to this mechanistic contribution to lymphoid oncogenesis, it is possible that failure to successfully complete expression of an appropriate Ig or TCR may lead to maturation arrest in a lymphoid precursor, which may in itself contribute to altered tissue homeostasis, particularly if the arrest occurs at a stage of cellular expansion.

Introduction

Approximately 5% of human cancers overall and over 70% of haematological cancers involve the lymphoid system, with the majority involving the B-cell lineage. Lymphoid cancers include immature, "blastic" lymphoid proliferations which involve essentially the blood and/or bone marrow (Acute Lymphoblastic Leukemia or ALL), mature lympho-proliferations involving predominantly secondary lymphoid organs (non-Hodgkin's Lymphomas or NHL) or blood and bone marrow (chronic lymphocytic leukemias or CLL) and expansions of plasmocytes, with predominant bone marrow and tissue involvement (multiple myeloma or MM). Dysimmune states such as Hodgkin's disease or Angioimmunoblastic lymphadenopathy (AILD), at the interface

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between reactive immune disorders and lymphoid malignancies, also exist. Whether these are classified as lymphoid malignancies or not often depend on the techniques available for their characterization, notably analysis of the V(D)J status of their immunoglobulin (Ig) and T-cell Receptor (TCR) loci by techniques which will be collectively referred to as lymphoid clonality analysis here. Lymphoid malignancies are also frequently characterized by V(D)J recombinase errors which lead to transcriptional deregulation of lymphoid "oncogenes" by juxtapositioning to, most commonly, Ig or TCR regulatory sequences. This represents a lymphoid specific form of "physiological genetic instability" which includes V(D)J recombinase errors and abnormalities of isotype switching and/or somatic mutation. Only the former will be considered here; they are collectively, if imprecisely, referred to as V(D)J translocations. Such errors can be considered to be, at a minimum, mechanistic elements involved in lymphoid oncogenesis. It is, however, possible that failure to successfully complete fabrication and expression of an appropriate Ig or TCR may in itself represent an oncogenic event within the multistage process that is now recognized to preceed clinical presentation of the majority of human cancers.

Since lymphoid cancers represent homogeneous populations arrested at different stages of development, they provide invaluable models for the study of molecular and cellular events leading to interruption of lymphoid development. Within this context, "reading the language" of Ig/TCR rearrangements can provide useful information regarding the type of lymphoid (sub)population involved, the stage of maturation arrest and the chromatin accessibility of the different Ig/TCR loci. It should however be emphasised that the pheno/genotype of the bulk lymphoid cancer is not necessarily synonymous, but most probably downstream, to the lymphoid cancer stem cell. Since any detectable clonal V(D)J rearrangement or translocation suggests at a minimum that the Ig/TCR loci were accessible during preceeding stages of lymphoid oncogenesis, such rearrangements represent useful fingerprints of upstream oncogenic events. We have undertaken to review these different, but interlinked, applications of the analysis of normal and abnormal V(D)J coding joint repertoires applied to understanding of lymphoid malignancies and their dysimmune close relatives. Such an approach is by definition nonexhaustive and we apologies to all individual contributors which we have only referenced indirectly, in the interests of brevity. We will not discuss therapeutic aspects of V(D)J manipulation, nor analysis of transcribed, functional V(D)J repertoires and will only briefly touch on detection of signal junction rearrangements.

Diagnostic Clonality Analysis

Molecular analysis of Ig/TCR genomic repertoires in diagnostic evaluation of (suspected) human lymphoid malignancies was initially performed by Southern blot analysis,[1-3] but was progressively replaced from the 1980s onwards by PCR analysis from DNA.[4-9] Both are based on the principal that reactive lymphoproliferations are associated with polyclonal Ig/TCR repertoires whereas the majority of lymphoid cancers demonstrate clonal, homogeneous rearrangements of Ig and/or TCR loci,[10] with the pattern of clonal rearrangements reflecting the lymphoid lineage involved and its stage of maturation arrest.[10-13]

Technical and Practical Aspects

Southern blotting predominantly reflected homogeneous V and J segment usage whereas PCR V(D)J amplification also exploits heterogeneity of VDJ junctional sequences at the third complementarity determining region (CDR3). The longer the CDR3, the easier the distinction of clonal and polyclonal rearrangements.[14] Detection of VDJ, DJ, VD DD and DJ rearrangements are possible if appropriate primers are used.[15] The majority of diagnostic systems use consensus primers directed to relatively conserved framework regions, often in a multiplex format.[15] Predictably, the risk of false negative results is dependent on the complexity of the repertoire (Table 1) and the degree of homology between the V, D and J primers and their target sequences. The other main factor contributing to false negativity is somatic mutation involving PCR primer target sequences but others include: presence of inhibitors; analysis of uninvolved tissue and DNA degradation of fixed tissues.

Table 1. Human Ig/TCR repertoires, combinatorial complexity and chromosomal localisation

	Number of Germline Encoded Segments					
	V	D	J	Approximate CDR3 Length(bp)	Number of N Regions	Chromosomal Localization
IgH	46-52	27	6	50	1-2	14q32.3
IgK	31-36	0	5	10	1	2p11.2
Igλ	30-33	0	4	10	1	22q11.2
TCRδ	7	3	4	5-50	1-4	14q11.2
TCRα	45-47	0	50	10	1	14q11.2
TCRγ	9	0	5	10	1	7q14
TCRβ	39-47	2	13	10	1-2	7q34

The number of V segments varies. Certain Vα/δ segments can rearrange to both TCRδ and TCRα loci. Number of N region varies with incomplete VD, DD or DJ rearrangements.

Distinction of clonal, oligoclonal and polyclonal PCR products is based on either nondenaturing polyacrylamide gel electrophoresis (PAGE), usually under conditions encouraging heteroduplex formation, or "genescan" sizing of fluorescent PCR products. The former has the advantage of optimising distinction of clonal homoduplexes from polyclonal heteroduplexes but requires optimal PAGE conditions. Genescan sizing allows precise information regarding clonal product size, useful for molecular follow-up and comparison of different samples from a given tumor and can allow identification of V and J segment usage if differently labelled primers are used (Fig. 1). Under qualitative conditions, both have an approximate sensitivity of 1-5%, although this depends on the position of clonal and polyclonal populations, since a clonal population which is situated at the peak of the Gaussian distribution of polyclonal PCR products will be detected with lower sensitivity than one which is either larger or smaller than these fragments (Fig. 1). Quantitation of clonal rearrangements by real-time PCR is possible using CRD3 specific probes, or more usually primers (Fig. 2) (ref. 16 and references there in) This requires sequencing of diagnostic material and has been developed essentially for follow-up of patients with ALL. In general, diagnostic strategies aim only to distinguish clonal from polyclonal populations and do not attempt to identify segment usage. Judicious use of appropriately situated, variably labelled fluorescent primers allows identification of V, D and J segments from a limited number of multiplex PCR, based on PCR product size and fluorescence.[17] "Reading the language" of Ig/TCR rearrangements in this way can contribute to identification of the stage of maturation arrest and lineage affiliation. Such analyses do not allow determination of functional, in-frame rearrangement, unless combined with sequence analysis.

Diagnostic PCR have been developed for all loci other than TCRα. The most widely used loci for diagnostic clonality analysis are IgH VDJ and TCRγ VJ, since both rearrange relatively early during normal B and T-lymphoid development respectively, including in all subsets of each lineage. Backup loci for the B-cell lineage include Igκ and IgH DJ rearrangements, whereas Igλ clonality analysis within a diagnostic setting is complex and rarely adds additional information. For suspected T-cell malignancies, TCRγ can be complemented by TCRβ VDJ analysis, which is a more appropriate target than TCRδ; due to the deletion of this locus during TCRα rearrangement and the consequent risk of pseudo-clonality from rare residual TCRδ rearrangements. Use of TCRδ is essentially restricted to clonality analysis in ALL and rare suspected TCRγδ lymphoproliferative disorders. Details regarding the incidence and patterns of Ig/TCR rearrangements in the main categories of lymphoproliferative disorders (LPD) can be found in Table 2.[15,18-24] Succinctly, mature B lineage LPD rearrange IgH and

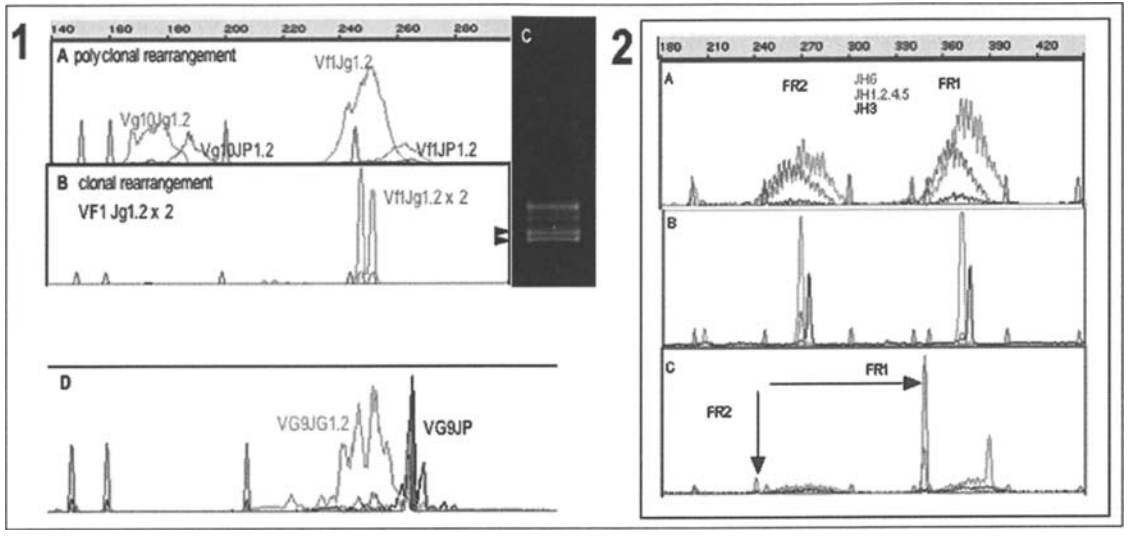

Figure 1. Lymphoid clonality analysis. Genescan and heteroduplex images of TCR and IgH genomic repertoires. 1)- Analysis of multiplex TCRγ rearrangement by PCR from DNA using fluorescent primers and genescan (GS) evaluation (left) compared to nondenaturing PAGE analysis of heteroduplex (HD) PCR products (right): A = polyclonal rearrangements. B and C = Clonal bi-allelic Vfl-Jγ1/2 rearrangements in a B-cell precursor ALL, analysed by GS (B) or HD (C). Slow migrating clonal heteroduplexes are seen when both alleles undergo rearrangement with the same Vγ and Jγ segments, but different CDR3. Homoduplexes are indicated by arrows. The use of differently labelled primers allows identification of the V and J segments used on the basis of PCR product size and colour, as shown. Size markers are in red. D = polyclonal TCR Vγ9-Jg rearrangement (green) and canonical Vγ9JP (black). The latter represents a selected, functional repertoire, as evidenced by the 3bp spacing between peaks, as seen with IgH, but not with other TCRγ rearrangements. 2)- Analysis of multiplex multifluorescent IgH rearrangement by PCR from DNA using fluorescent primers and genescan evaluation. Polyclonal (A) and clonal (B and C) rearrangements are shown. The use of 3 differently labelled JH primers (JH1,2,4,5 in blue, JH6 in green and JH3 in black) gives improved resolution in polyclonal rearrangements, the majority of which use JH4-6 segments. IgH rearrangements are amplified with both FR1 and FR2 consensus primers in separate reactions but analysed together, in order to increase clonal informativity in cases having undegone somatic mutation. For example, of the 2 B-cell precursor ALLs shown , sample B demonstrates a major JH6 and a minor JH3 rearrangement which are informative with both FR1 (right) and FR2 (left) VH primers. For sample C, in contrast, the major JH1,2,4,5 rearrangement is amplified efficiently from the FR1 but poorly from the FR2 primer and the minor JH6 rearrangement is only seen with FR1. A color version of this image is available at www.landesbioscience.com/curie. The uniform intensity, red peaks coorespond to size markers.

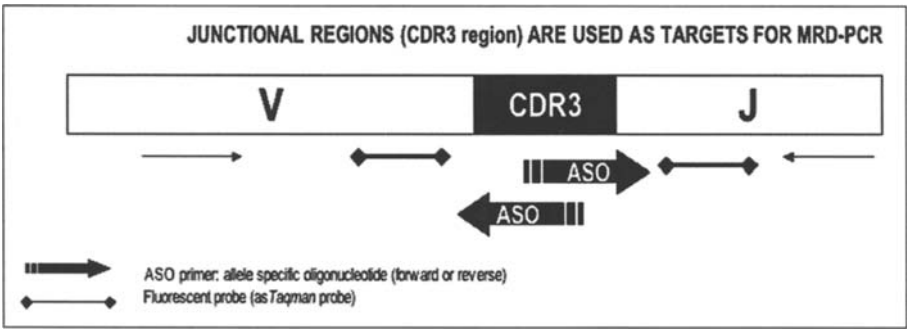

Figure 2. Real time quantitative Ig/TCR CDR3 specific strategies. Quantification by RQ-PCR of the tumor load or minimal residual disease (MRD). For each Ig/TCR rearrangement, the junctional region is amplified, sequenced and several "clone-specific" primers or allele specific oligonucleotides (ASO) are designed. Specific CDR3 specific primers are then used for clone specific amplification of follow-up material using CDR3 and V, D or J primers and V or J Taqman probes. Quantification is performed using a standard curve constructed from the RQ-PCR assay by serial dilutions of patient's blasts in a peripheral blood mononuclear cell pool (10^{-1} to 10^{-5}).

Igκ (VJ or Kappa deleting element—KDE) in the vast majority of cases, with extensive repertoires which lead to little risk of false positive results and a risk of false negative results which is proportional to the degree of somatic mutation. Mature T lineage LPD rearrange TCRγ and TCRβ and occasionally TCRδ. The restricted repertoire of TCRγ VJ rearrangements leads to a risk of false positive detection of pseudoclonality, particularly if PAGE conditions are suboptimal.[15] PAGE heteroduplex analysis is preferable to fluorescent genescan analysis in a diagnostic setting, since there is a lower risk of false positives. The presence of canonical "invariant" rearrangements, such as Vγ9-JP rearrangements in circulating TCRγδ lymphocytes, can also be erroneously interpreted as indicating clonal expansion by inexperienced operators and for this reason not all diagnostic multiplex strategies include a JP (also referred to as Jγ1.2) specific primer.[15] Two classifications for human TCRγ V and J segments exist.[15] The presence of minor normal clonal/invariant populations is well recognised in circulating CD8+ T-lymphocytes from older individuals and in reactive disorders such as lymphomatoid papulosis. The risk of false positive results can be minimised by simultaneous use of TCRβ analysis[19] and restriction of these analyses to high throughput laboratories, in order to maximise experience. Interpretation of lymphoid clonality profiles should be undertaken in close interaction with the prescribing physician or pathologist and with knowledge of the clinical context.

Cross lineage rearrangements, also referred to as "illegitimate rearrangements" (Ig rearrangements in a T LPD or vice versa) are rare in mature LPD. They are common in acute lymphoblastic leukemias, with the majority of B lineage ALL demonstrating TCRγ rearrangement and/or TCRδ or, more rarely TCRβ rearrangements. Ig rearrangements in T-ALL are less common and are preferentially found in the TCRγδ lineage.[25-27] Illegitimate rearrangements reflect the fact that ALLs remain recombinase competent and consequently rearrange all loci in an accessible chromatin configuration. Rearrangement patterns differ with oncogenic subtype, with stage of maturation arrest and with patient age. Amongst B-cell precursor ALLs, for example, relatively mature cases which express Ig cyt μ rarely demonstrate TCRγ rearrangements, whereas the majority of CD10+ cyt μ- ETV6-RUNX1 or BCR-ABL cases do so.[28,29] Details of Ig/TCR rearrangement profiles in ALL can be found in Table 2. Detection of lymphoid clonality is rarely required to make a diagnosis of ALL and is essentially used for molecular follow-up (see below).[16] Extensive sequence analysis of these clonal rearrangements has, however, allowed accumulation of a large databank allowing analysis of V, D and J segment usage and CDR3 diversity, which may eventually lead to improved understanding of the pathogenic stages leading to ALL development.

Table 2. Approximate incidence of clonal Ig/TCR rearrangement in lymphoid malignancies, as detected by PCR from DNA. Only diagnostic PCR targets are cited

	IgH	IgK	Igλ	TCRδ	TCRγ	TCRβ
B-Cell Proliferation						
BCP-ALL	90	30	20	30	60	30
CLL	100	100	30	10	20	25
Non-Hodgkin's Lymphoma						
FL	90	85	20	5	5	5
MCL	100	100	45	5	10	10
BLBCL	85	80	30	15	15	20
MZL	95	80	30	10	15	20
T-Cell Proliferation						
T-ALL	5	0	0	50	90	90
T-LGL	0	5	5	30	95	95
AILT	30	30	5	35	90	90

Abbreviations: BCP-ALL: B-cell precursor Acute Lymphoblastic Leukemia; CLL: Chronic Lymphocytic leukemia; FL: Follicular Lymphoma; MCL: Mantle Cell Lymphoma; BLBCL: Diffuse Large B-Cell Lymphoma; MZL: Marginal Zone Lymphoma; T-ALL: T-cell acute lymphoblastic leukaemia; T-LGL: T-Large Granular lymphocytic leukaemia; AILT: Angioimmunoblastic T-Cell Lymphoma.

Clinical Applications

Diagnostic clonality analysis is mainly used to distinguish reactive, polyclonal LPD from clonal, probably but not necessarily, malignant LPD. Once a clonal population has been identified, it is possible to track this clone in different tissue samples, in order to assess dissemination at diagnosis, or to determine clonal identity at relapse. Clonal tracking has also been used within a minimal residual disease setting in ALL and certain NHL, once apparent complete remission has been obtained, to stratify individual patient management, based on the cinetics of response to remission induction at diagnosis. Succinctly, clonal tracking with CDR3 specific probes, used within a strictly standardised, quantitative setting, allow the detection of minor clonal poulations with a reproducible sensitivity of at least 10^{-4} (1 malignant cell amongst 10000 normal cells). It has also been used to "back-track" preclinical development of ALL, in conjuction with molecular oncogenic markers, allowing the identification of leukemic clones many years before clinical presentation, including in postnatal samples prior to development of pediatric ALL.[30,31]

Recombinase Mediated Oncogenesis

Analysis of structural chromosomal abnormalities by classical morphological karyotyping in lymphoid malignancies allowed the identification of recurrent translocations involving the Ig loci in B lymphoid malignancies and TCR loci in T-cell malignancies. The advent of molecular techniques led to identification of the Ig/TCR partner genes and the demonstration that karyotypic analysis largely underestimated the incidence and complexity of these rearrangements. The large number of partner genes identified has allowed numerous insights into normal and pathological lymphoid development and function, but their very number precludes their description here and readers are invited to consult the following reviews on the subject.[32-36] Only general aspects relevant to V(D)J rearrangement will be detailed here. Within the context of lymphoid

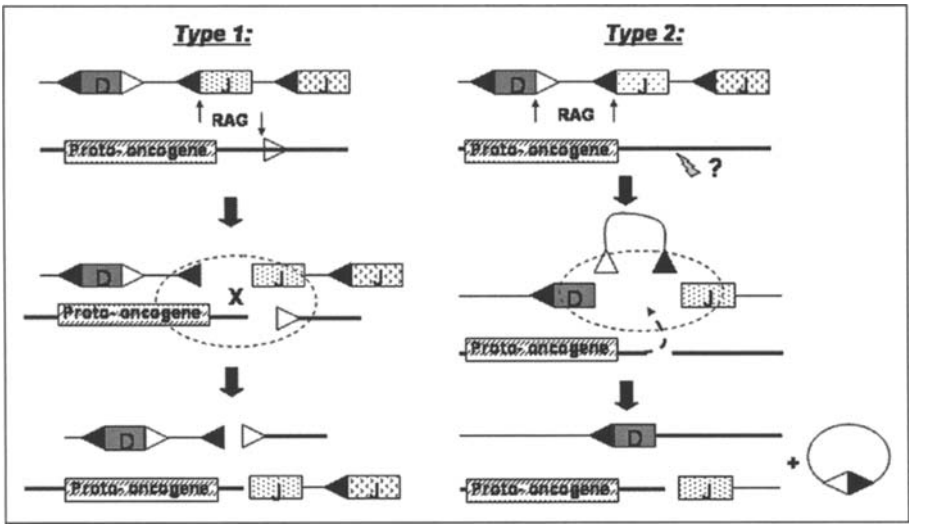

Figure 3. Type 1 and 2 Ig/TCR rearrangements. Recognition signal sequences (RSS) are represented by triangles at V, D and J segment extremities. Gray triangles represent cryptic RSS. In Type I rearrangements, RAG targets both the bona-fide and the cryptic RSS. In Type 2 junctions, the break in the proto-oncogene is targeted by unknown mechanisms. In both cases, there is excision of intervening DNA, in the signal joint shown at the bottom right hand corner.

malignancies, the term "illegitimate" rearrangement is usually reserved for cross-lineage intralocus rearrangements, such as the TCR rearrangements identified in B lineage ALL described above. "Trans-rearrangement" refers to rearrangement between distinct Ig and TCR loci, abnormalities which have been principally described in patients with Ataxia Telangiectasia.[37] V(D)J translocations usually implies structural karyotypic abnormalities involving a proto-oncogene and an Ig or TCR locus (Fig. 3 and Table 3). The increasing recognition of recombinase mediated deregulation of genes with no involvement of an Ig/TCR locus, including those resulting from microscopic, intragenic rearrangements not associated with evident karytoypic abnormalities, justifies use of the more general term "recombinase mediated oncogenesis". Comparative genomic hybridization has demonstrated that in pediatric B lineage ALL, many of these deletions involve genes which regulate B-cell development, including TCF3 (also known as E2A), EBF1, LEF1, IKZF1 (IKAROS) and IKZF3 (AIOLOS).[38] At least a proportion of these are mediated by the recombinase.

Recombinase mediated events can occur at the site of any RSS-like sequence which is in an accessible chromatin configuration during recombinase activity. One of the best studied examples outside the lymphoid oncogenesis context is deletions of the HPRT locus.[39-42] These have been used as a measure of genomic instability, some of which are mediated by the recombinase complex. Within the present context, only V(D)J recombinase mediated events with oncogenic potential will be detailed. The role of recombinase abnormalities in Ig/TCR rearrangements is illustrated by their high incidence in patients with Ataxia Telangiectasia and similar disorders.[43]

VDJ Errors in Lymphoid Malignancies

During lymphoid development, recombinase activity targeted to recombination signal sequences (RSS) would ideally be restricted to legitimate targets within Ig/TCR loci and all genes controlling tissue homeostasis would be protected from this lymphoid specific form of "physiological genomic instability". The existence, however, of a large number of RSS-like sequences throughout the genome (10 million or 1 cryptic RSS every 1-2 kb on average) means that non-specific targeting of RAG1 can induce double stranded breaks outside Ig/TCR loci, leading to

Table 3. Deregulation of lymphoid oncogenes by Ig/TCR juxtapositioning in ALL

Oncogene (Ig/tcr Partner Genes)	Protein Family Group	Translocations Involved	References
B-ALL	**Translocation Involving Ig Genes**		
ID4	Inhibitor of DNA binding(ID)HLH	t(6; 14)(p21; q32)	61
LHX4	LIM-homeodomain	t(1; 14)(q25; q32)	60
BCL9	Not identified	t(1; 14)(q21; q32)	59
IL3	4H Cytokine	t(5; 14)(q32; q32)	62
c-Myc	bHLH-Zip	t(8; 14)(q24; q32)	58,55
		t(2; 8)(p12; q24)	56
		t(8; 22)(q24; q11)	57
CEBP	bZIP	t(14; 19)(q32; q13);	63
		t(8; 14)(q11; q32);	
		Inv(14)(q11; q32)/	
		t(14; 14)(q11; q32)	
		t(14; 20)(q32; q13)	
T-ALL	**Translocation Involving TCR Genes**		
HOXA cluster	Class I homeodomaincontaining	Inv(7)(p15q34)t(7; 7)	90,91
TLXI(HOXII)	Class II homeodomaincontaining	t(7; 10)(q34; q24)	65,66
		t(10; 14)(q24; q11)	
TLX3 (HOXIIL2)*	Class II homeodomaincontaining	t(5; 14)(q35; q32)	86,87
LMO1	LIM-only domain	t(11; 14)(p15; q11)	68
LMO2	LIM-only domain	t(11; 14)p13; q11),	69
		t(7; 11)(q35; q13)	71
TAL1	b HLH Type II	t(1; 14)(p32; q11),	100
		t(1; 7)(p32; q34)	91
TAL2	b HLH Type II	t(7; 9)(q34; q32)	75
LCK	SRC family of tyrosine kinase	t(1; 7)(p34; q34)	95,101
BHLHB1	b HLH Type II	t(14; 21)(q11.2; q22)	76
LYL1	b HLH Type II	t(7; 19)(q34; p13)	74
CCND2	D-type cyclin	t(7; 12)(q34; p13)	102
		t(12; 14)(p13; q11)	103
NOTCH1	Notch receptor family	t(7; 9)(q34; q34.3)	94

**TLX3* is included despite the fact that the predominant t(5; 14) involves BCL11B, not IgH, since these *BCL11B-TLX3* translocations are mediated by the recombinase and since rare translocations involving *TLX3* and *TCRα/δ* are described.

intergenic rearrangements and deregulation of genes by juxtapositioning to Ig/TCR regulatory sequences (promoters or enhancers).[44] This can lead to increased expression or nonextinction of the juxtaposed "proto-oncogene" by promoter/enhancer substitution or by separation of coding sequences from negative regulatory elements. Only those rearrangements which lead to deregulated tissue homeostasis will be associated with lymphoid malignancies. If the deregulated genes induce a survival or proliferative advantage or a block to maturation, the clone bearing the translocation will be transformed, or at least immortalised. Based on these considerations, V(D)J errors will only occur in cells which are recombinase competent and will target proto-oncogenes which are accessible during this phase of recombinase activity.

It is increasingly recognised that the transcriptional and phenotypic profile observed in a cancer at diagnosis is not necessarily identical, but is probably more mature, when compared to the cancer initiating or stem cell. Genetic modifications which occur in this cancer stem cell are, however, transmitted to all clonal descendants. Within this context, both bona-fide Ig/TCR rearrangements and recombinase mediated oncogenic rearrangements detected in diagnostic material can represent genetic fingerprints of earlier events which have occurred in lymphoid cancer stem cells, or in intermediate malignant precursor populations. If such markers are present in the majority of the tumor at diagnosis, it is likely that they reflect an upstream event during oncogenic development, wheras those present in minor subclones are more likely to represent downstream events occurring in tumor subclones. The capacity to accurately evaluate the proportion of cells demonstrating a given marker depends on the techniques used. Briefly, molecular PCR and CGH based techniques using extracted DNA are poorly adapted to precise quantification and cytogenetic analysis of mitotic material is biased by potential nonrepresentativity of the cells undergoing mitosis under the culture conditions used. FISH analysis of interface nuclei has the advantage of being cell based, but is only applicable to certain oncogenic markers, not to V(D)J rearrangements and is heavily dependent on the quality of material analysed (bare nuclei vs. tissue sections, for example). Given these reserves, detection of an Ig or TCR rearrangement in an apparently nonlymphoid cancer, implies prior exposure of malignant precursors to recombinase activity. Identification of Ig/TCR rearrangements in Acute Myeloid leukaemia, for example, is preferentially found in cases with MLL gene rearrangement, with the MLL fusion transcript partners being associated with different Ig/TCR profiles.[45] Similarly, detection of a recombinase mediated oncogenic marker implies chromatin accessibility of the partner gene during a phase of recombinase competence prior to tumor development. What level of qualitative and/or quantitative recombinase competence and/or RAG1/2 activity is required for these recombinase errors is not clear. Rearrangement of TCRδ and TCRγ can occur in the presence of much lower levels of RAG1 activity than that required for TCRβ rearrangement [46] and it is possible to induce TCRδ rearrangement in kidney cells in the presence of E2A and HEB.[47,48]

Categories of Recombinase Errors

Two categories of recombinase errors are recognised:[44,49,50] Type I rearrangements demonstrate breaks at RSS at both loci, one of which is usually an Ig or TCR; in Type II rearrangements, only the Ig/TCR break is mediated by RAG and the mechanisms targeting the double stranded break on the partner gene are incompletely understood (Fig. 3). Once generated, this DNA fragment becomes included in the recombinase complex, with the translocation resulting from a DNA repair error, rather than mistargetting of the recombinase. A recombinase mediated error is characterized by i) involvement of an Ig/TCR locus; ii) recurrent genomic breakpoints; iii) identification of a bona-fide RSS-like sequence at the breakpoint on the partner chromosome iv) addition of nongermline encoded nucleotides at the translocation breakpoint and v) generation of a signal joint. Recombinase mediated translocations were first identified in B lymphoid non-Hodgkin's lymphoma (NHL) with the t(14; 18) translocation involving IgH and BCL2.[51-53] Translocations involving Ig loci preferentially involve the IgH locus and are found in relatively mature, sIg+ lymphomas. These translocations are essentially Type II and primarily involve abnormalities of class switch and somatic hypermutation; [54] as such, they are beyond the scope of this article, which is restricted to V(D)J recombinase errors

in immature lymphoproliferative disorders, essentially ALL. A proportion of these abnormalities are also found in certain lymphomas, notably those involving *MYC* in Burkitt's lymphoma and those involving *HOX11/TLX1* in T-lymphoblastic lymphoma.

VDJ Deregulation with Oncogenic Potential

Ig translocations are found in approximately 1% of B lineage ALL, when they are virtually restricted to mature, sIg + cases. Partner genes include MYC,[55-58] BCL-9,[59] LHX4,[60] ID4,[61] IL3[62] or the different members of the CEBP family[63] (Table 3). In contrast, chromosomal abnormalities involving the TCR loci are among those most frequently encountered in T-ALL. Most involve the TCRα/δ locus on chromosome 14q11 or more rarely, TCRβ on chromosome 7q34; [35,64] rearrangements involving TCRγ are exceptional. The first TCR translocations to be described in T-ALL were those involving HOX11/TLX1 at chromosome 10q24[65-67] and LMO1/2 on chromosome 11p.[68-71] The incidence of TCR translocations by classical, morphological karyotyping was underestimated and it was only with the advent of screening by FISH that the true incidence was appreciated. Screening for TCR translocations demonstrated that approximately 45% of T-ALLs demonstrate translocations, including a minority with as yet unidentified partners. Predominant known TCR partner genes can be divided into those of the bHLH, LMO and HOX/TLX families.

The most commonly encountered bHLH partner is TAL1/SCL, which was initially described in the rare t(1; 14)(p32; q11).[72] Much more frequent is the SIL-TAL1 recombinase mediated intrachromosomal deletion, which places the entire TAL1 coding sequence under control of the SIL promoter.[73] SIL-TAL1 deletions are found in 20% of pediatric and 5-10% of adult T-ALLs. Other bHLH translocations include the rare t(7; 19)(q34; p13),[74] t(7; 9)(q34; q32)[75] and t(14; 21)(q11; q22)[76] involving LYL-1, TAL2 and bHLHB1 respectively. The frequent involvement of members of the bHLH family of transcriptional regulators is coherent with the fundamental role of bHLH proteins in regulation of T and B lymphoid lineage development . This is further emphasised by the fact that the LMO proteins deregulated by TCR juxtapositioning in translocations involving LMO1 (11p15)[68] or LMO2 (11p13)[69,71] form part of a complex which also includes TAL1 and its bHLH partner, E2A.[77-81]

Deregulation of homeobox gene expression is increasingly recognised in T-ALL. The orphan homeobox gene, HOX11/TLX1, is predominantly involved in the t(10; 14)(q24; q11) and more rarely the t(7; 10)(q34; q24).[65-67] Forced expression of TLX1 in murine bone marrow gives rise to T-ALL-like malignancies with long latency, suggesting that other events are necessary to induce leukemia; but with TLX1 expression representing an early event.[82,83] TLX1 regulates the G1/S checkpoint of T-ALL via its biding capability to the protein serine/threonine phosphatases PP2A and PP1.[84,85] Chromosomal translocations t(10; 14)(q24; q11) involving TLX1 are amongst the clearest example of recombinase involvement in T-ALL. Deregulated expression of HOX11L2/TLX3 is frequently found in pediatric T-ALL, due in most cases to a t(5; 14) involving the TLX3 locus at 5q35 and CTIP2/ BCL11B at 14q32, 7000 kb proximal to the IgH locus.[86,87] Despite the absence of Ig/TCR involvement, this translocation is mediated by the recombinase and rare translocations involving TLX3 and TCRα/δ have been described.[88] TLX3 has very close homology to TLX1, as evidenced by microarray studies showing that TLX1 and TLX3 T-ALLs cluster together.[89,90] TCR translocations involving the HOXA cluster on chromosome 7 predominantly involve the TCRβ locus, leading to a cryptic intrachromosomal inversion.[90,91] Another common abnormality in T-ALL is deletion of the p16/INK4/Cdk2 gene; [92,93] this is recombinase mediated in at least a proportion of cases. Other rare recombinase mediated abnormalities include translocations involving TCRβ and Notch1 in the t(7; 9)(q34; q34)[94] and t(1; 7)(q34; q34) involving LCK and TCRβ.[95,96]

In general, these recombinase mediated errors are restricted to T-ALLs of the TCRαβ lineage, which express RAG1 and have undergone extensive TCR rearrangement. The HOX/TLX cases are arrested prior to TCRα rearrangement, in contrast to SIL-TAL1 cases, which have undergone TCRα rearrangement on at least one allele. They are rarely found in TCRγδ expressing T-ALLs, with the exception of TLX3 expressing cases, which frequently express both

TCRγδ and cytoplasmic TCRβ.[97] Recombinase V(D)J errors are clearly an important mechanism in the development of immature T-cell malignancies. Attempts to recreate these malignancies in murine models have frequently demonstrated long latency and/or a low proportion of mice developing leukaemia/lymphoma, in keeping with multistage oncogenesis.[98,99] In keeping with this, low levels of translocations involving LMO2 have been identified in normal thymus.[49]

Conclusion

The aforementioned abnormalities represent a mechanistic role for the recombinase in development of lymphoid malignancies. It is however also possible that failure to complete production of a mature, functional Ig or TCR may favour malignant expansion, particularly if the cells are arrested at a stage when the pre B or TCR is expressed and capable of mediating ligand driven cellular expansion. The majority of acute leukaemias do not express a surface Ig/TCR, despite having undergone extensive Ig/TCR rearrangement. This failure to complete successful Ig/TCR rearrangement is likely to be at least partially at the origin of the recombinase competence and the maintenance of RAG1 expression. It is therefore at least theoretically possible that abrogation of the factors blocking completion of Ig or TCR assembly could lead to expression of the appropriate Ig/TCR at the surface, downregulation of RAG expression and possibly even leukemic cell death by differentiation. As mentioned above, a significant proportion of HOX11L2/TLX3+ T-ALLs express unusual TCRγδ receptors and cytoplasmic TCRβ. These T-ALLs maintain high levels of RAG1 transcripts, despite the expression of a surface TCR, suggesting that expression of an "inappropriate, default" TCRγδ in cells having undergone beta selection is insufficient to allow extinction of the recombinase. Exploration of the mechanisms underlying the failure to rearrange TCRα may further our understanding of T-ALL oncogenesis.

In conclusion, understanding and exploiting normal and abnormal recombinase activity can be used both in individual patient management and in understanding lymphoid oncogenesis.

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Saïda Dadi and Sandrine Le Noir have contributed equally to this work.

References

1. Beishuizen A, Verhoeven MA, Mol EJ et al. Detection of immunoglobulin heavy-chain gene rearrangements by Southern blot analysis: recommendations for optimal results. Leukemia 1993; 7(12):2045-2053.
2. Langerak AW, Wolvers-Tettero IL, van Dongen JJ. Detection of T-cell receptor beta (TCRB) gene rearrangement patterns in T-cell malignancies by Southern blot analysis. Leukemia 1999; 13(6):965-974.
3. van Krieken JH, Elwood L, Andrade RE et al. Rearrangement of the T-cell receptor delta chain gene in T-cell lymphomas with a mature phenotype. Am J Pathol 1991; 139(1):161-168.
4. Langerak AW, Szczepanski T, van der Burg M et al. Heteroduplex PCR analysis of rearranged T-cell receptor genes for clonality assessment in suspect T-cell proliferations. Leukemia 1997; 11(12):2192-2199.
5. Gonzalez M, Gonzalez D, Lopez-Perez R et al. Heteroduplex analysis of VDJ amplified segments from rearranged IgH genes for clonality assessments in B-cell non-Hodgkin's lymphoma. A comparison between different strategies. Haematologica 1999; 84(9):779-784.
6. Derksen PW, Langerak AW, Kerkhof E et al. Comparison of different polymerase chain reaction-based approaches for clonality assessment of immunoglobulin heavy-chain gene rearrangements in B-cell neoplasia. Mod Pathol 1999; 12(8):794-805.

7. Krafft AE, Taubenberger JK, Sheng ZM et al. Enhanced sensitivity with a novel TCRgamma PCR assay for clonality studies in 569 formalin-fixed, paraffin-embedded (FFPE) cases. Mol Diagn 1999; 4(2):119-133.
8. Assaf C, Hummel M, Dippel E et al. High detection rate of T-cell receptor beta chain rearrangements in T-cell lymphoproliferations by family specific polymerase chain reaction in combination with the GeneScan technique and DNA sequencing. Blood 2000; 96(2):640-646.
9. Theriault C, Galoin S, Valmary S et al. PCR analysis of immunoglobulin heavy chain (IgH) and TcR-gamma chain gene rearrangements in the diagnosis of lymphoproliferative disorders: results of a study of 525 cases. Mod Pathol 2000; 13(12):1269-1279.
10. van Dongen JJ. Analysis of immunoglobulin genes and T-cell receptor genes as a diagnostic tool for the detection of lymphoid malignancies. Neth J Med 1987; 31(5-6):201-209.
11. Gleissner B, Maurer J, Thiel E. Detection of immunoglobulin heavy chain genes rearrangements in B-cell leukemias, lymphomas, multiple myelomas, monoclonal and polyclonal gammopathies. Leuk Lymphoma 2000; 39(1-2):151-155.
12. Sen F, Vega F, Medeiros LJ. Molecular genetic methods in the diagnosis of hematologic neoplasms. Semin Diagn Pathol 2002; 19(2):72-93.
13. Langerak AW, van Krieken JH, Wolvers-Tettero IL et al. The role of molecular analysis of immunoglobulin and T-cell receptor gene rearrangements in the diagnosis of lymphoproliferative disorders. J Clin Pathol 2001; 54(7):565-567.
14. Miqueu P, Guillet M, Degauque N et al. Statistical analysis of CDR3 length distributions for the assessment of T and B-cell repertoire biases. Mol Immunol 2007; 44(6):1057-1064.
15. van Dongen JJ, Langerak AW, Bruggemann M et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003; 17(12):2257-2317.
16. van der Velden VH, Cazzaniga G, Schrauder A et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia 2007; 21(4):604-611.
17. Delabesse E, Burtin ML, Millien C et al. Rapid, multifluorescent TCRG Vgamma and Jgamma typing: application to T-cell acute lymphoblastic leukemia and to the detection of minor clonal populations. Leukemia 2000; 14(6):1143-1152.
18. van Krieken JH, Langerak AW, San Miguel JF et al. Clonality analysis for antigen receptor genes: preliminary results from the Biomed-2 concerted action PL 96-3936. Hum Pathol 2003; 34(4):359-361.
19. Droese J, Langerak AW, Groenen PJ et al. Validation of BIOMED-2 multiplex PCR tubes for detection of TCRB gene rearrangements in T-cell malignancies. Leukemia 2004; 18(9):1531-1538.
20. Evans PA, Pott C, Groenen PJ et al. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia 2007; 21(2):207-214.
21. van Krieken JH, Langerak AW, Macintyre EA et al. Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia 2007; 21(2):201-206.
22. Bruggemann M, White H, Gaulard P et al. Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies Report of the BIOMED-2 Concerted Action BHM4 CT98-3936. Leukemia 2007; 21(2):215-221.
23. Langerak AW, Molina TJ, Lavender FL et al. Polymerase chain reaction-based clonality testing in tissue samples with reactive lymphoproliferations: usefulness and pitfalls. A report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2007; 21(2):222-229.
24. Liu H, Bench AJ, Bacon CM et al. A practical strategy for the routine use of BIOMED-2 PCR assays for detection of B- and T-cell clonality in diagnostic haematopathology. Br J Haematol 2007; 138(1):31-43.
25. Asnafi V, Beldjord K, Garand R et al. IgH DJ rearrangements within T-ALL correlate with cCD79a expression, an immature/TCRgammadelta phenotype and absence of IL7Ralpha/CD127 expression. Leukemia 2004; 18(12):1997-2001.
26. Kitchingman GR, Rovigatti U, Mauer AM et al. Rearrangement of immunoglobulin heavy chain genes in T-cell acute lymphoblastic leukemia. Blood 1985; 65(3):725-729.
27. Szczepanski T, Pongers-Willemse MJ, Langerak AW et al. Ig heavy chain gene rearrangements in T-cell acute lymphoblastic leukemia exhibit predominant DH6-19 and DH7-27 gene usage, can result in complete V-D-J rearrangements and are rare in T-cell receptor alpha beta lineage. Blood 1999; 93(12):4079-4085.
28. Chen Z, Le Paslier D, Dausset J et al. Human T-cell gamma genes are frequently rearranged in B-lineage acute lymphoblastic leukemias but not in chronic B-cell proliferations. J Exp Med 1987; 165(4):1000-1015.
29. Brumpt C, Delabesse E, Beldjord K et al. The incidence of clonal T-cell receptor rearrangements in B-cell precursor acute lymphoblastic leukemia varies with age and genotype. Blood 2000; 96(6):2254-2261.

30. Greaves M. Molecular genetics, natural history and the demise of childhood leukaemia. Eur J Cancer 1999; 35(14):1941-1953.
31. Greaves M. Childhood leukaemia. BMJ 2002; 324(7332):283-287.
32. Graux C, Cools J, Michaux L et al. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia 2006; 20(9):1496-1510.
33. O'Neil J, Look AT. Mechanisms of transcription factor deregulation in lymphoid cell transformation. Oncogene 2007; 26(47):6838-6849.
34. Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. Oncogene 2007; 26(47):6766-6776.
35. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol 2005; 23(26):6306-6315.
36. Willis TG, Dyer MJ. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood 2000; 96(3):808-822.
37. Stern MH, Lipkowitz S, Aurias A et al. Inversion of chromosome 7 in ataxia telangiectasia is generated by a rearrangement between T-cell receptor beta and T-cell receptor gamma genes. Blood 1989; 74(6):2076-2080.
38. Mullighan CG, Goorha S, Radtke I et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007; 446(7137):758-764.
39. Fuscoe JC, Zimmerman LJ, Lippert MJ et al. V(D)J recombinase-like activity mediates hprt gene deletion in human fetal T-lymphocytes. Cancer Res 1991; 51(21):6001-6005.
40. Fuscoe JC, Zimmerman LJ, Harrington-Brock K et al. Deletion mutations in the hprt gene of T-lymphocytes as a biomarker for genomic rearrangements important in human cancers. Carcinogenesis 1994; 15(7):1463-1466.
41. Fuscoe JC, Zimmerman LJ, Harrington-Brock K et al. Large deletions are tolerated at the hprt locus of in vivo derived human T-lymphocytes. Mutat Res 1992; 283(4):255-262.
42. Finette BA, Kendall H, Vacek PM. Mutational spectral analysis at the HPRT locus in healthy children. Mutat Res 2002; 505(1-2):27-41.
43. Digweed M. Human genetic instability syndromes: single gene defects with increased risk of cancer. Toxicol Lett 1993; 67(1-3):259-281.
44. Marculescu R, Vanura K, Montpellier B et al. Recombinase, chromosomal translocations and lymphoid neoplasia: targeting mistakes and repair failures. DNA Repair (Amst) 2006; 5(9-10):1246-1258.
45. Dupret C, Asnafi V, Lebocuf D et al. IgH/TCR rearrangements are common in MLL translocated adult AML and suggest an early T/myeloid or B/myeloid maturation arrest, which correlates with the MLL partner. Leukemia 2005; 19(12):2337-2338.
46. Asnafi V, Beldjord K, Boulanger E et al. Analysis of TCR, pT alpha and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. Blood 2003; 101(7):2693-2703.
47. Langerak AW, Wolvers-Tettero IL, van Gastel-Mol EJ et al. Basic helix-loop-helix proteins E2A and HEB induce immature T-cell receptor rearrangements in nonlymphoid cells. Blood 2001; 98(8):2456-2465.
48. Ghosh JK, Romanow WJ, Murre C. Induction of a diverse T-cell receptor gamma/delta repertoire by the helix-loop-helix proteins E2A and HEB in nonlymphoid cells. J Exp Med 2001; 193(6):769-776.
49. Marculescu R, Le T, Simon P et al. V(D)J-mediated translocations in lymphoid neoplasms: a functional assessment of genomic instability by cryptic sites. J Exp Med 2002; 195(1):85-98.
50. Lieber MR, Yu K, Raghavan SC. Roles of nonhomologous DNA end joining, V(D)J recombination and class switch recombination in chromosomal translocations. DNA Repair (Amst) 2006; 5(9-10):1234-1245.
51. Tsujimoto Y, Gorham J, Cossman J et al. The t(14; 18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 1985; 229(4720):1390-1393.
52. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14; 18) translocation. Cell 1986; 47(1):19-28.
53. Tsujimoto Y, Croce CM. Analysis of the structure, transcripts and protein products of bcl-2, the gene involved in human follicular lymphoma. Proc Natl Acad Sci USA 1986; 83(14):5214-5218.
54. Schlissel MS, Kaffer CR, Curry JD. Leukemia and lymphoma: a cost of doing business for adaptive immunity. Genes Dev 2006; 20(12):1539-1544.
55. Dalla-Favera R, Martinotti S, Gallo RC et al. Translocation and rearrangements of the c-myc oncogene locus in human undifferentiated B-cell lymphomas. Science 1983; 219(4587):963-967.
56. Davis M, Malcolm S, Rabbitts TH. Chromosome translocation can occur on either side of the c-myc oncogene in Burkitt lymphoma cells. Nature 1984; 308(5956):286-288.
57. Hollis GF, Mitchell KF, Battey J et al. A variant translocation places the lambda immunoglobulin genes 3' to the c-myc oncogene in Burkitt's lymphoma. Nature 1984; 307(5953):752-755.

58. Manolov G, Manolova Y. Marker band in one chromosome 14 from Burkitt lymphomas. Nature 1972; 237(5349):33-34.
59. Willis TG, Zalcberg IR, Coignet LJ et al. Molecular cloning of translocation t(1; 14)(q21; q32) defines a novel gene (BCL9) at chromosome 1q21. Blood 1998; 91(6):1873-1881.
60. Kawamata N, Sakajiri S, Sugimoto KJ et al. A novel chromosomal translocation t(1; 14)(q25; q32) in preB acute lymphoblastic leukemia involves the LIM homeodomain protein gene, Lhx4. Oncogene 2002; 21(32):4983-4991.
61. Bellido M, Aventin A, Lasa A et al. Id4 is deregulated by a t(6; 14)(p22; q32) chromosomal translocation in a B-cell lineage acute lymphoblastic leukemia. Haematologica 2003; 88(9):994-1001.
62. Meeker TC, Hardy D, Willman C et al. Activation of the interleukin-3 gene by chromosome translocation in acute lymphocytic leukemia with eosinophilia. Blood 1990; 76(2):285-289.
63. Akasaka T, Balasas T, Russell LJ et al. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood 2007; 109(8):3451-3461.
64. Raimondi SC, Behm FG, Roberson PK et al. Cytogenetics of childhood T-cell leukemia. Blood 1988; 72(5):1560-1566.
65. Hatano M, Roberts CW, Minden M et al. Deregulation of a homeobox gene, HOX11, by the t(10; 14) in T-cell leukemia. Science 1991; 253(5015):79-82.
66. Dube ID, Kamel-Reid S, Yuan CC et al. A novel human homeobox gene lies at the chromosome 10 breakpoint in lymphoid neoplasias with chromosomal translocation t(10; 14). Blood 1991; 78(11):2996-3003.
67. Kennedy MA, Gonzalez-Sarmiento R, Kees UR et al. HOX11, a homeobox-containing T-cell oncogene on human chromosome 10q24. Proc Natl Acad Sci USA 1991; 88(20):8900-8904.
68. McGuire EA, Hockett RD, Pollock KM et al. The t(11; 14)(p15; q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including Ttg-1, a gene encoding a potential zinc finger protein. Mol Cell Biol 1989; 9(5):2124-2132.
69. Royer-Pokora B, Loos U, Ludwig WD. TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11; 14)(p13; q11). Oncogene 1991; 6(10):1887-1893.
70. Van Vlierberghe P, van Grotel M, Beverloo HB et al. The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. Blood 2006; 108(10):3520-3529.
71. Boehm T, Foroni L, Kaneko Y et al. The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci USA 1991; 88(10):4367-4371.
72. Bernard O, Barin C, Charrin C et al. Characterization of translocation t(1; 14)(p32; q11) in a T and in a B acute leukemia. Leukemia 1993; 7(10):1509-1513.
73. Aplan PD, Lombardi DP, Ginsberg AM et al. Disruption of the human SCL locus by "illegitimate" V-(D)-J recombinase activity. Science 1990; 250(4986):1426-1429.
74. Mellentin JD, Smith SD, Cleary ML. lyl-1, a novel gene altered by chromosomal translocation in T-cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. Cell 1989; 58(1):77-83.
75. Xia Y, Brown L, Yang CY et al. TAL2, a helix-loop-helix gene activated by the (7; 9)(q34; q32) translocation in human T-cell leukemia. Proc Natl Acad Sci USA 1991; 88(24):11416-11420.
76. Wang J, Jani-Sait SN, Escalon EA et al. The t(14; 21)(q11.2; q22) chromosomal translocation associated with T-cell acute lymphoblastic leukemia activates the BHLHB1 gene. Proc Natl Acad Sci USA 2000; 97(7):3497-3502.
77. Baer R. TAL1, TAL2 and LYL1: a family of basic helix-loop-helix proteins implicated in T-cell acute leukaemia. Semin Cancer Biol 1993; 4(6):341-347.
78. O'Neil J, Billa M, Oikemus S et al. The DNA binding activity of TAL-1 is not required to induce leukemia/lymphoma in mice. Oncogene 2001; 20(29):3897-3905.
79. Bain G, Engel I, Robanus Maandag EC et al. E2A deficiency leads to abnormalities in alphabeta T-cell development and to rapid development of T-cell lymphomas. Mol Cell Biol 1997; 17(8):4782-4791.
80. Yan W, Young AZ, Soares VC et al. High incidence of T-cell tumors in E2A-null mice and E2A/Id1 double-knockout mice. Mol Cell Biol 1997; 17(12):7317-7327.
81. O'Neil J, Shank J, Cusson N et al. TAL1/SCL induces leukemia by inhibiting the transcriptional activity of E47/HEB. Cancer Cell 2004; 5(6):587-596.
82. Hawley RG, Fong AZ, Lu M et al. The HOX11 homeobox-containing gene of human leukemia immortalizes murine hematopoietic precursors. Oncogene 1994; 9(1):1-12.
83. Hawley RG, Fong AZ, Reis MD et al. Transforming function of the HOX11/TCL3 homeobox gene. Cancer Res 1997; 57(2):337-345.

84. Kawabe T, Muslin AJ, Korsmeyer SJ. HOX11 interacts with protein phosphatases PP2A and PP1 and disrupts a G2/M cell-cycle checkpoint. Nature 1997; 385(6615):454-458.
85. Riz I, Hawley RG. G1/S transcriptional networks modulated by the HOX11/TLX1 oncogene of T-cell acute lymphoblastic leukemia. Oncogene 2005; 24(36):5561-5575.
86. Bernard OA, Busson-LeConiat M, Ballerini P et al. A new recurrent and specific cryptic translocation, t(5; 14)(q35; q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia 2001; 15(10):1495-1504.
87. MacLeod RA, Nagel S, Kaufmann M et al. Activation of HOX11L2 by juxtaposition with 3'-BCL11B in an acute lymphoblastic leukemia cell line (HPB-ALL) with t(5; 14)(q35; q32.2). Genes Chromosomes Cancer 2003; 37(1):84-91.
88. Hansen-Hagge TE, Schafer M, Kiyoi H et al. Disruption of the RanBP17/Hox11L2 region by recombination with the TCRdelta locus in acute lymphoblastic leukemias with t(5; 14)(q34; q11). Leukemia 2002; 16(11):2205-2212.
89. Ferrando AA, Neuberg DS, Staunton J et al. Gene expression signatures define novel oncogenic pathways in T-cell acute lymphoblastic leukemia. Cancer Cell 2002; 1(1):75-87.
90. Soulier J, Clappier E, Cayuela JM et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 2005; 106(1):274-286.
91. Speleman F, Cauwelier B, Dastugue N et al. A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias. Leukemia 2005; 19(3):358-366.
92. Ohnishi H, Kawamura M, Ida K et al. Homozygous deletions of p16/MTS1 gene are frequent but mutations are infrequent in childhood T-cell acute lymphoblastic leukemia. Blood 1995; 86(4):1269-1275.
93. Cayuela JM, Madani A, Sanhes L et al. Multiple tumor-suppressor gene 1 inactivation is the most frequent genetic alteration in T-cell acute lymphoblastic leukemia. Blood 1996; 87(6):2180-2186.
94. Ellisen LW, Bird J, West DC et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell 1991; 66(4):649-661.
95. Burnett RC, Thirman MJ, Rowley JD et al. Molecular analysis of the T-cell acute lymphoblastic leukemia-associated t(1; 7)(p34; q34) that fuses LCK and TCRB. Blood 1994; 84(4):1232-1236.
96. Burnett RC, David JC, Harden AM et al. The LCK gene is involved in the t(1; 7)(p34; q34) in the T-cell acute lymphoblastic leukemia derived cell line, HSB-2. Genes Chromosomes Cancer 1991; 3(6):461-467.
97. Asnafi V, Beldjord K, Libura M et al. Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy. Blood 2004; 104(13):4173-4180.
98. Larson RC, Osada H, Larson TA et al. The oncogenic LIM protein Rbtn2 causes thymic developmental aberrations that precede malignancy in transgenic mice. Oncogene 1995; 11(5):853-862.
99. Aplan PD, Jones CA, Chervinsky DS et al. An scl gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice. EMBO J 1997; 16(9):2408-2419.
100. Carroll AJ, Crist WM, Link MP et al. The t(1; 14)(p34; q11) is nonrandom and restricted to T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood 1990; 76(6):1220-1224.
101. Tycko B, Smith SD, Sklar J. Chromosomal translocations joining LCK and TCRB loci in human T-cell leukemia. J Exp Med 1991; 174(4):867-873.
102. Le Coniat M, Della Valle V, Marynen P et al. A new breakpoint, telomeric to TEL/ETV6, on the short arm of chromosome 12 in T-cell acute lymphoblastic leukemia. Leukemia 1997; 11(8):1360-1363.
103. Karrman K, Andersson A, Bjorgvinsdottir H et al. Deregulation of cyclin D2 by juxtaposition with T-cell receptor alpha/delta locus in t(12; 14)(p13; q11)-positive childhood T-cell acute lymphoblastic leukemia. Eur J Haematol 2006; 77(1):27-34.