Molecular Diagnosis

Oncologic Diagnosis

Oncology: Molecular Targets

- Detection of Clonal Populations
  - Antigen Receptor Gene Rearrangement
  - EBV clonality
    - Southern Blotting Methods
    - PCR methods
- Identification of Tumor-specific genetic alterations
  - Translocations (Chromosomal rearrangements):
    - W/o fusion genes
    - w/ fusion genes
  - 'Oncogene mutations/amplifications
  - Tumor suppressor gene mutatons/deletions
  - Specific chromosomal losses
  - Alterations in genomic methylation
- Gene Expression Profiling

Antigen Receptor Gene Rearrangement

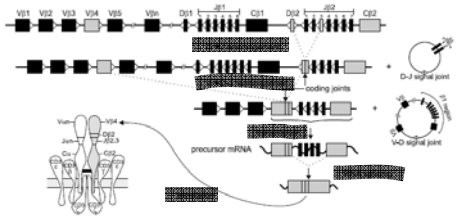
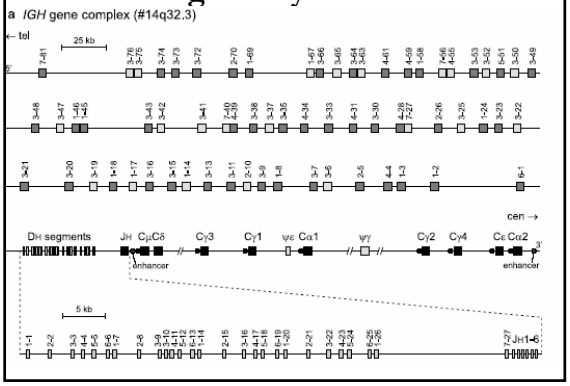
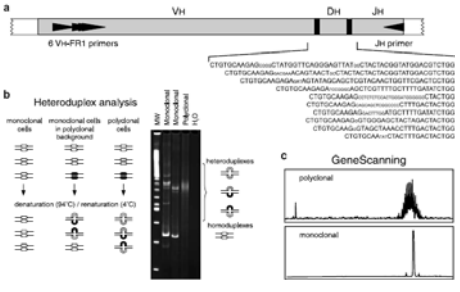


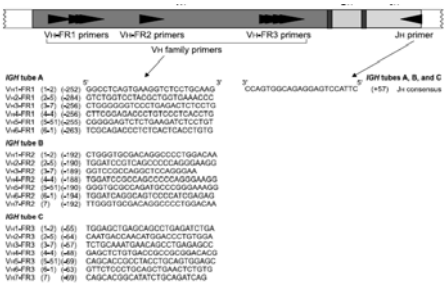
Figure 1 Schematic diagram of sequential rearrangement steps, transcription, and translation of the *TCRB* gene. In this example, first a Dβ2 to Jβ2.3 rearrangement occurs, followed by Vβ4 to Dβ2–Jβ2.3 rearrangement, resulting in the formation of a Vβ4–Dβ2–Jβ2.3 coding joint. The rearranged *TCRB* gene is transcribed into precursor mRNA, spliced into mature mRNA, and finally translated into a TCRβ protein chain. The two extrachromosomal TRECs that are formed during this recombination process are indicated as well; they contain the D-J signal joint and V-D signal joint, respectively.

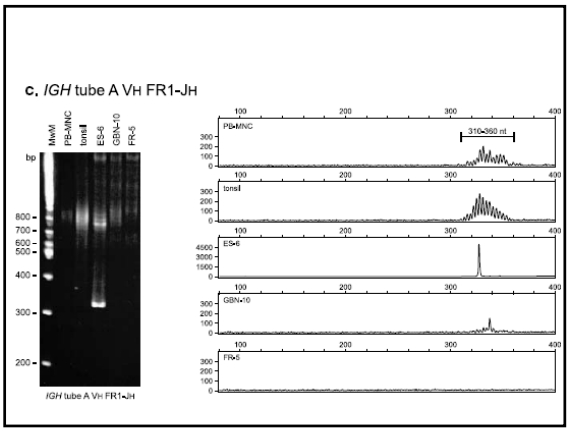
Ig heavy chain



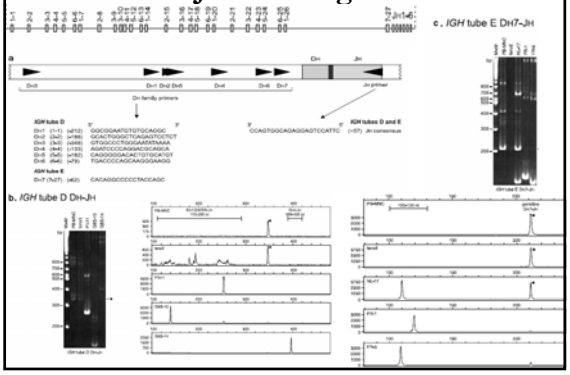
Antigen Receptor Gene Rearrangement







DH-JH rearrangement



PCR paraffin vs frozen

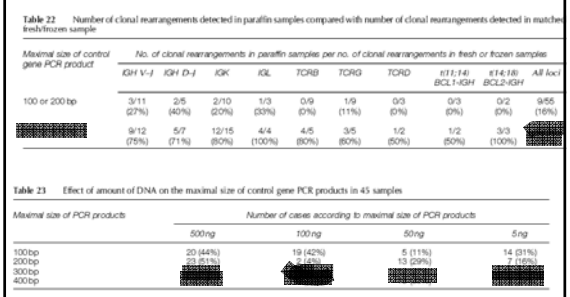


Table 22 Number of clonal rearrangements detected in paraffin samples compared with number of clonal rearrangements detected in matched fresh/frozen sample

Maximal size of control gene PCR product	No. of clonal rearrangements in paraffin samples per no. of clonal rearrangements in fresh or frozen samples									
	IGH V–J	IGH D–J	IGK	IGL	TCRB	TCRG	TCRD	t(11;14) BCL1-IGH	t(14;18) BCL2-IGH	All loci
100 or 200 bp	3/11 (27%)	2/5 (40%)	2/10 (20%)	1/3 (33%)	0/9 (0%)	1/9 (11%)	0/3 (0%)	0/3 (0%)	0/2 (0%)	9/55 (16%)
[redacted]	9/12 (75%)	5/7 (71%)	12/15 (80%)	4/4 (100%)	4/5 (80%)	3/5 (60%)	1/2 (50%)	1/2 (50%)	3/3 (100%)	[redacted]

Table 23 Effect of amount of DNA on the maximal size of control gene PCR products in 45 samples

Maximal size of PCR products	Number of cases according to maximal size of PCR products			
	500 ng	100 ng	50 ng	5 ng
100 bp	20 (44%)	19 (42%)	5 (11%)	14 (31%)
200 bp	23 (51%)	[redacted]	13 (29%)	7 (16%)
300 bp	[redacted]	[redacted]	[redacted]	[redacted]
400 bp	[redacted]	[redacted]	[redacted]	[redacted]

Clonality PCR vs Southern

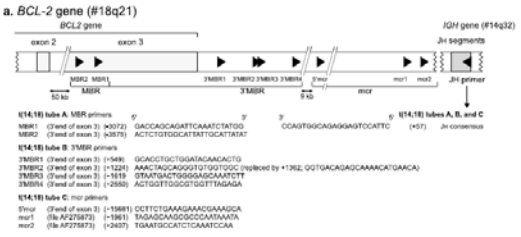
Table 26. Concordance between multiplex PCR results and Southern blot analysis results (PCR/SB) on Ig/TCR gene rearrangements per subcategory of included frozen samples

Diagnosis	IGH[a]	IGK	IGL	TCRB	TCRG	TCRD
Prefollicular ($n = 8$)	C: 8/8 P: 0/0	C: 8/8 P: 0/0	C: 4/4 P: 4/4	C: 2/4[c] P: 4/4	C: 0/0 P: 8/8	C: 0/0 P: 8/8[e]
B-CLL ($n = 16$)	C: 15/16 P: [redacted]	C: 16/16 P: 0/0	C: 5/5 P: 9/11	C: 1/1 P: 15/15	C: 0/0 P: 16/16	C: 2/2 P: 14/14
Postfollicular ($n = 25$)	C: [redacted] P: 0/0	C: [redacted] P: [redacted]	C: 3/5 P: 19/20	C: 2/4 P: 21/21[d]	C: 0/0 P: [redacted]	C: 0/0 P: 24/25[f]
All B-cell malignancies ($n = 49$)	C: 45/49 P: 0/0	C: 43/48 P: 0/1	C: 12/14 P: 32/35	C: 5/9 P: 40/40	C: 0/1 P: 46/48	C: 2/2 P: 46/47
T-cell malignancies ($n = 18$)	C: 2/2 P: 15/16[d]	C: 0/0 P: 17/18	C: 0/0 P: 17/18	C: 17/17[d] P: 1/1	C: 15/16[d] P: 1/2	C: 2/3 P: 14/15[f]
Reactive samples ($n = 15$)	C: 0/0 P: 15/15	C: 0/0 P: 15/15	C: 0/0 P: 15/15	C: 0/0 P: 14/15	C: 0/0 P: 15/15	C: 0/0 P: 15/15
Miscellaneous ($n = 8$)	C: 3/3 P: 3/5	C: 2/2 P: 4/6	C: 0/0 P: 6/8	C: 3/3 P: 5/5[e]	C: [redacted]	C: 1/1 P: 5/7
All samples ($n = 90$)	C: [redacted] P: [redacted]	C: [redacted]	C: 12/14 P: 70/76	C: [redacted]	C: [redacted]	C: 5/6 P: 80/84

Translocations w/o gene fusion

Tumor	Translocation	Activated Gene	Mechanism of Activation
B-All/Burkitt	t(8;14)(q24;q32)	MYC	Relocation to IgH locus
Large Cell Lymphoma	t(3;14)(q27;q32)	BCL6	Relocation to IgH locus
Mantle Cell Lymphoma	t(11;14)(q13;q32)	Cyclin D1	Relocation to IgH locus
Follicular B-cell lymphoma	t(14;18)(q32;q21)	BCL2	Relocation to IgH locus
T-cell ALL	t(8;14)(q24;q11)	MYC	Relocation to TCR α/δ locus
T-cell ALL	t(1;14)(p32;q11)	TAL1	Relocation to TCR α/δ locus

BCL2-IgH gene rearrangement



Translocations w/fusion product: hematologic tumors

TUMOR	Translocation	Gene fusion
Chronic myelogenous leukemia	t(9;22)	BCR-ABL(p210)

Acute promyelocytic leukemia	t(15;17);
	t(11;17)(q23;q21);
	t(5;17) (q35;q21);
	t(11;17)(q13;q21)
	der(17)
		PML-RAR
		PLZF-RAR
		NPM-RAR
		NUMA-RAR
		STAT5b-RAR

AML	t(8;21)(q22;q22)	AML1-ETO
AML and ALL (esp. infants and post-Rx)	11q23	MLL-(~30 partners)
Anaplastic large cell lymphoma (pediatric)	t(2;5)(p23;q35)	NPM-ALK
ALL	t(9;22)	BCR-ABL(p190)
MALT lymphoma	t(11;18)	API2-MLT

Translocations w/chimeric products: solid tumors

Tumor	Translocation	Product
Ewing's Sarcoma	t(11;22); t(21;22); t(7;22); t(12;22)	EWS/FLI1; EWS/ERG; EWS/ETV1; EWS/ETV4
Alveolar Rhabdomyosarc.	t(1;13); t(2;13)	PAX7/FKHR; PAX3/FKHR
Synovial sarcoma	t(X;18)	SYT/SSX1
DSRCT	t(11;22)	EWS/WT1
Myxoid/round cell liposarcoma	t(12;22)	CHOP/FUS
Clear cell sarcoma soft parts	t(12;22)	EWS.ATF-1
Extraskeletal myxoid chondrosarc	t(9;22)	EWS/TEC

Detection of Gene Fusions

- RT-PCR:
  - Detection of fusion transcripts.
    - Present only in tumor cells, not in normal cells.
    - Marker of tumor volume.
    - "Real-time" methods used to follow change in tumor burden.
      - Sensitivity approximately 1:10K
    - "Nested" RT-PCR to detect minimal residual disease
      - Sensitivity approximately 1:1M

Oncogene Mutations/amplification

- HER-2 amplification in breast CA
  - Poor prognosis
  - Response to Herceptin (Trastuzumab)
- KIT mutations in Gastrointestinal Stromal Tumor
  - Diagnostic of tumor
  - Specific mutations associated with sensitivity/resistance to Gleevec (Imatinib).

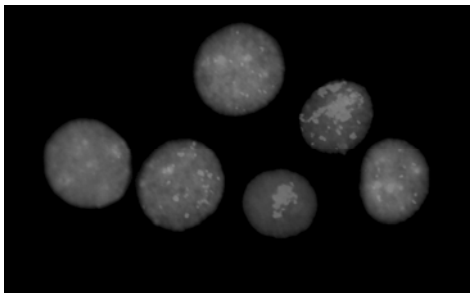
Oncogene Mutations/amplification

- nMYC amplification in Neuroblastoma
  - Poor prognosis, important criterion in classification of risk category.
- EGFR mutations in Lung Carcinoma
  - Predict response to gefitinib (Iressa), but NOT (so far) Erlotinib (Tarceva)
  - Seen more commonly in females, East Asians, and never smokers.

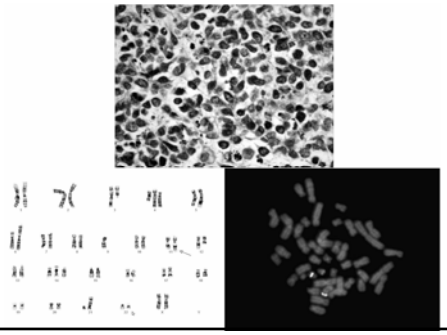
Loss of Heterozygosity in tumors

- Tumor suppressor genes:
  - Need to lose two copies. Often one copy lost by mutation, and the second by loss of the chromosomal locus, or by gene conversion ("loss of heterozygosity").
- Oligodendrogliomas:
  - Loss of Chromosome 1p and 19q
    - Diagnostic value
    - Predicts chemosensitivity.

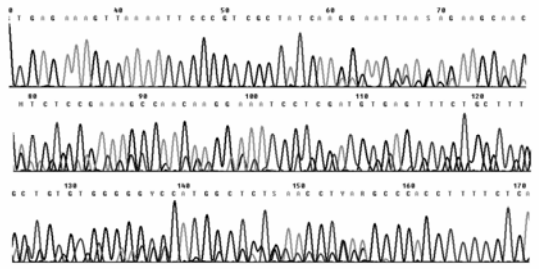
HER2 amplification: FISH



Ewing's Sarcoma: FISH



EGFR mutation: 15bp deletion



Gene Expression Profiling

- Label total RNA from a tumor
- hybridize to chip w/ ≥ 25,0000 cDNAs/oligonucleotides.
  - Expression profile unique to tumor type.
  - ? Predict behavior
  - ? Identify origin of mets
  - ? Identify targets for therapy.

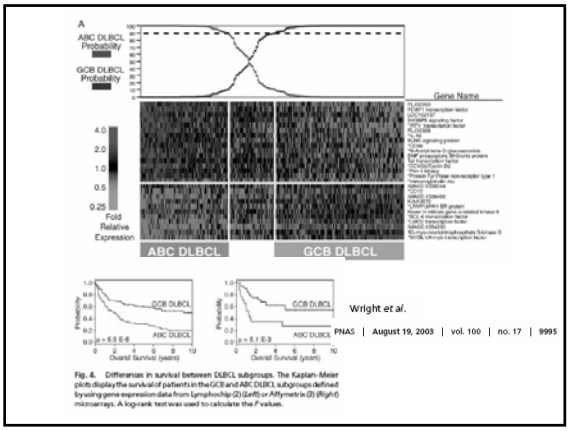


Fig. 4. Differences in survival between DLBCL subgroups. The Kaplan–Meier plots display the survival of patients in the GCB and ABC DLBCL subgroups defined by using gene expression data from Lymphochip (2) (Left) or Affymetrix (3) (Right) microarrays. A log-rank test was used to calculate the P values.