The ROYAL MARSDEN
NHS Foundation Trust

Translocations and clonality detection in lymphoproliferative disorders by capture-based Next-generation sequencing

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NHS

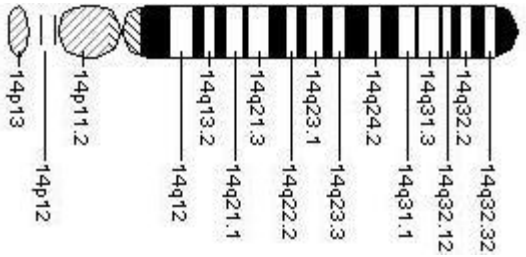
EuroClonality

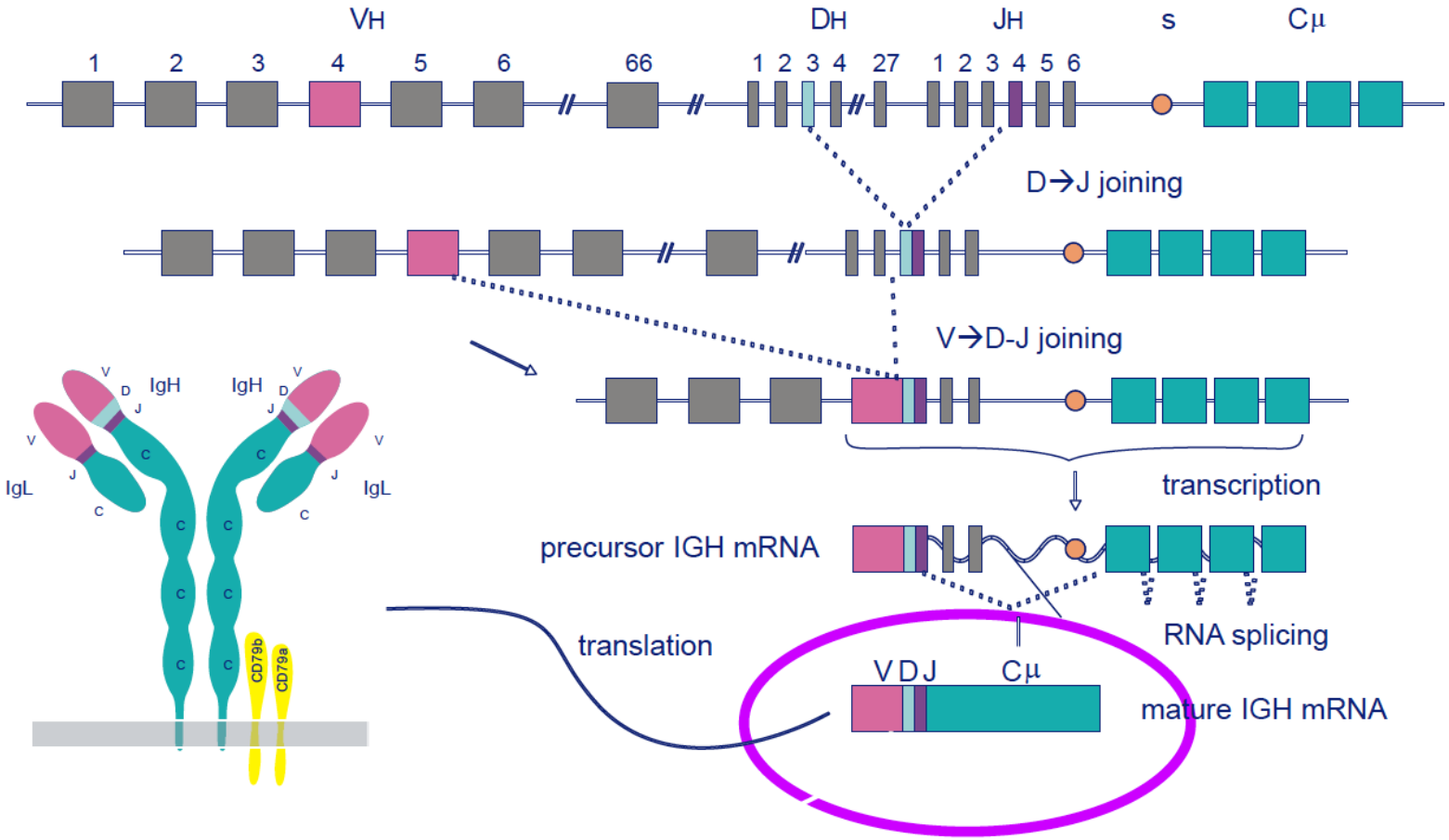
Detection and characterization of clonal IG/TR rearrangements and translocations

Provides critical information in lymphoproliferative neoplasms

- Ascertaining the clonal nature of lymphoid proliferations
- Characterization of translocations in lymphomas and leukemias
- Characterization of CDR3 regions for MRD target identification and stereotyping analysis
- Analysis of immune repertoire in cancer and non-malignant disorders

IGH rearrangements



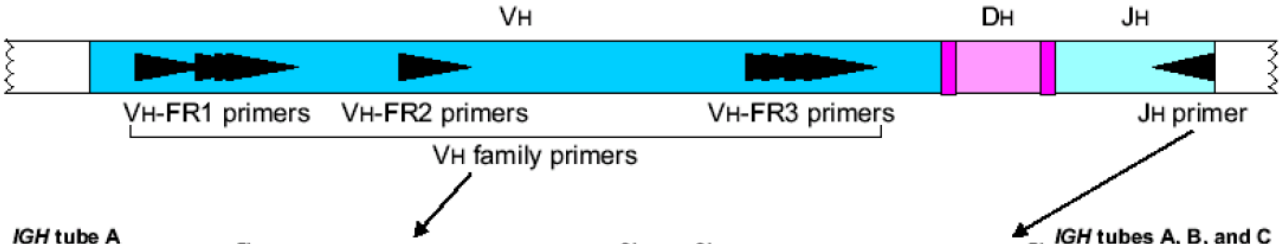


IGH rearrangements: combinatorial diversity

junctional region

IGHV3-21 (germline)	insertion	IGHD3-3 (germline)	insertion	IGHJ4-1 (germline)
TGTATTACTGTGCGAGA		GTATTACGATTTTTGGAGTGGTTATTATACC		ACTACTTTGACTACT
TGTATTACTGT	AGGC	CGATTTTTGGAGTGGTTATTATA	GTCCA	TGACTACT
TGTATTACTGTGCG	TATCCGGA	TTA CGATTTTTGGAGTGGTTATTATAC	CGATCG	CTTTGACTACT
TGTATTACTGTGC	CCGGACTG	TTTTGGAGTGGTTATTATACC	GGT	ACTACTTTGACTACT
TGTATTACTGTGCGAG	CTGAGTC	TATTACGATTTTTGGAGTGGTTAT	CGTAGCGTA	TTTGACTACT
TGTATTACTG	ACATCGA	CGATTTTTGGAGTGGTTATTATA	CGTAG	ACTTTGACTACT
TGTATTACTGTGCG	CGT	TACGATTTTTGGAGTGGTTATTAT	GGCTAAGG	TGACTACT
TGTATTACTGTGCGA	CCGG	TTACGATTTTTGGAGTGGTTATTATACC	CGGAGC	TACTTTGACTACT
TGTATTACT	GATG	GATTTTTGGAGTGGT	GGTTC	ACTACTTTGACTACT
TGTATTACTGTGCG	TTCA	ATTACGATTTTTGGAGTGGTTATTATA	CGATCGA	CTTTGACTACT
TGTATTACTGTGC		TTTGGAGTGGTTATTATA	CC	ACTTTGACTACT
TGTATTACTGTGCGAGA	GGCTAG	ATTACGATTTTTGGAGTGGTTATTATACC	GTCG	GACTACT
TGTATTACTGTG	GTCCAG	TATTACGATTTTTGGAGTG	CCGTAG	CTACTTTGACTACT
TGTATTA	CCGGA	CGATTTTTGGAGTGGTTATTATA	C	ACT
TGTATTACTGTGC	ACGC	ATTACGATTTTTGGAGTGG	GTACG	TTTGACTACT
TGTATTACTGTGCGA	CGTA	GTATTACGATTTTTGGAGTGGTTATTATACC	GGCA	ACTTTGACTACT

Detection of IG/TR rearrangements by PCR



***IGH* tube A**

VH1-FR1	(1-2)	(-252)	GGCCTCAGTGAAGGTCTCCTGCAAG
VH2-FR1	(2-5)	(-284)	GTCTGGTCCTACGCTGGTGAAACCC
VH3-FR1	(3-7)	(-256)	CTGGGGGGTCCCTGAGACTCTCCTG
VH4-FR1	(4-4)	(-256)	CTTCGGAGACCCTGTCCCTCACCTG
VH5-FR1	(5-51)	(-255)	CGGGGAGTCTCTGAAGATCTCCTGT
VH6-FR1	(6)	(-263)	TCGCAGACCCTCTCACTCACCTGTG

***IGH* tube B**

VH1-FR2	(1-2)	(-192)	CTGGGTGCGACAGGCCCCTGGACAA
VH2-FR2	(2-5)	(-190)	TGGATCCGTCAGCCCCCAGGGAAGG
VH3-FR2	(3-7)	(-189)	GGTCCGCCAGGCTCCAGGGAA
VH4-FR2	(4-4)	(-188)	TGGATCCGCCAGCCCCCAGGGAAGG
VH5-FR2	(5-51)	(-190)	GGGTGCGCCAGATGCCCGGGAAAGG
VH6-FR2	(6)	(-194)	TGGATCAGGCAGTCCCCATCGAGAG
VH7-FR2	(7)	(-192)	TTGGGTGCGACAGGCCCCTGGACAA

***IGH* tube C**

VH1-FR3	(1-2)	(-55)	TGGAGCTGAGCAGCCTGAGATCTGA
VH2-FR3	(2-5)	(-54)	CAATGACCAACATGGACCCTGTGGA
VH3-FR3	(3-7)	(-57)	TCTGCAAATGAACAGCCTGAGAGCC
VH4-FR3	(4-4)	(-48)	GAGCTCTGTGACCGCCGCGGACACG
VH5-FR3	(5-51)	(-69)	CAGCACCGCCTACCTGCAGTGGAGC
VH6-FR3	(6)	(-63)	GTTCTCCCTGCAGCTGAACTCTGTG
VH7-FR3	(7)	(-69)	CAGCACGGCATATCTGCAGATCAG

Leukemia (2003) 17, 2257–2317

www.nature.com/leu

LEADING ARTICLE

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

JJM van Dongen[1], AW Langerak[1], M Brüggemann[2], PAS Evans[3], M Hummel[4], FL Lavender[5], E Delabesse[6], F Davi[7], E Schuuring[8,9], R García-Sanz[10], JHJM van Krieken[11], J Droese[2], D González[10], C Bastard[12], HE White[5], M Spaargaren[13], M González[10], A Parreira[14], JL Smith[5], GJ Morgan[3], M Kneba[2] and EA Macintyre[6]

Open

Leukemia (2012) **26**, 2159–2171

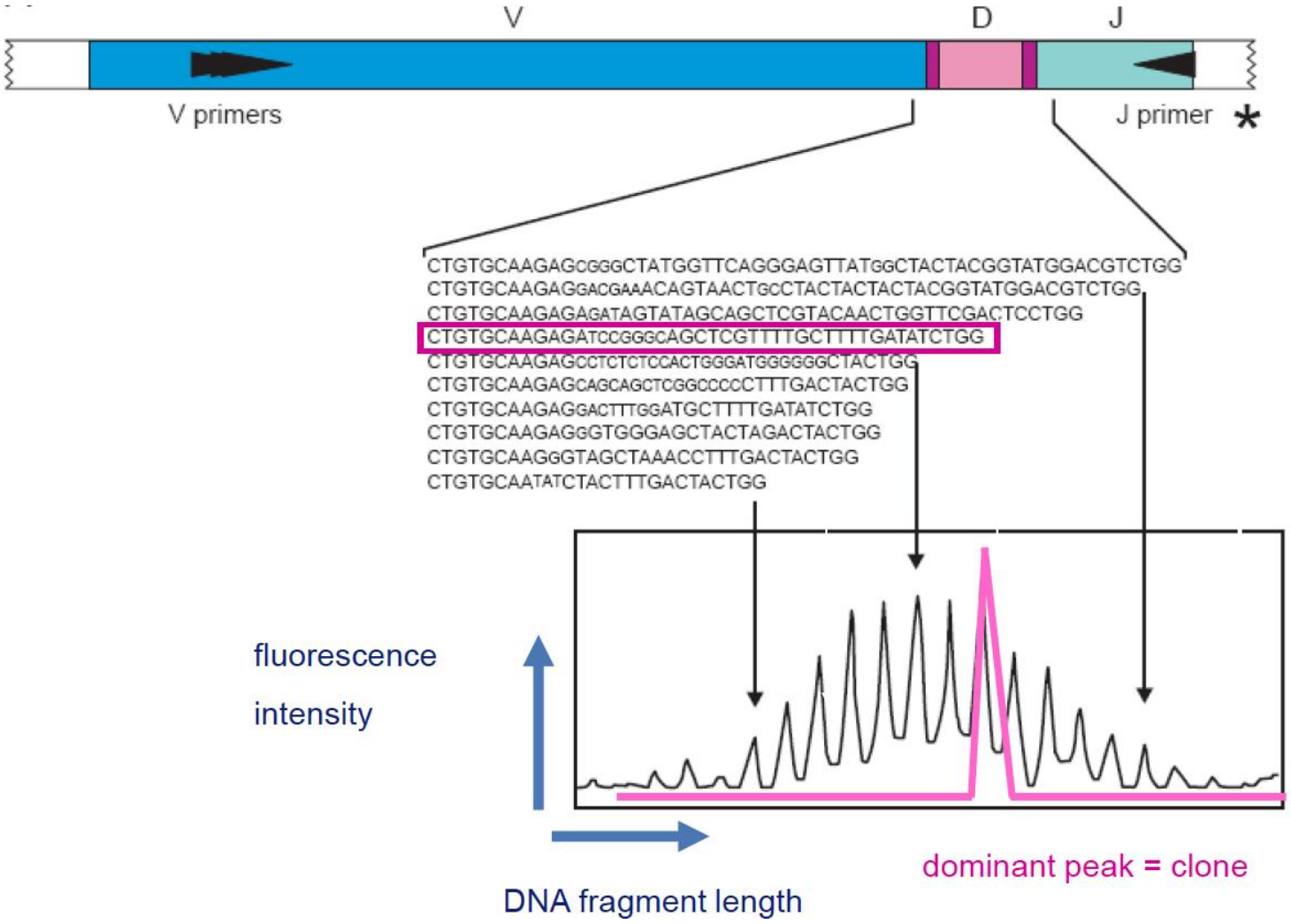
www.nature.com/leu

REVIEW

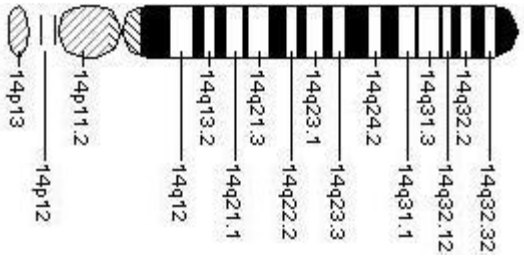
EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations

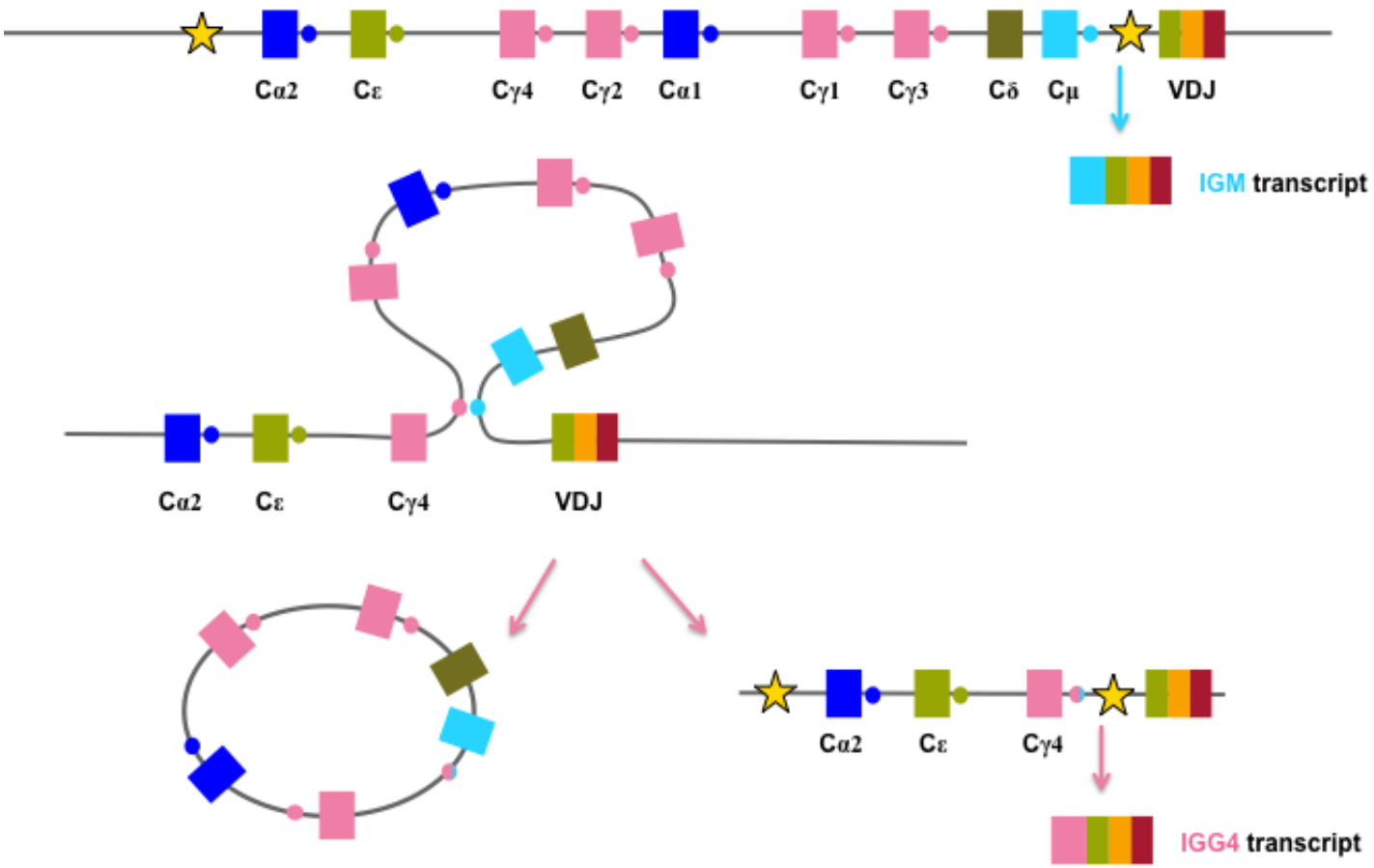
AW Langerak[1], PJTA Groenen[2], M Brüggemann[3], K Beldjord[4], C Bellan[5], L Bonello[6], E Boone[7], GI Carter[8], M Catherwood[9], F Davi[10], M-H Delfau-Larue[11], T Diss[12], PAS Evans[13], P Gameiro[14], R Garcia Sanz[15], D Gonzalez[16], D Grand[17], Å Håkansson[18], M Hummel[19], H Liu[20], L Lombardia[21], EA Macintyre[22], BJ Milner[23], S Montes-Moreno[24], E Schuuring[25], M Spaargaren[26], E Hodges[27] and JJM van Dongen[1]

Detection of IG/TR rearrangements by PCR



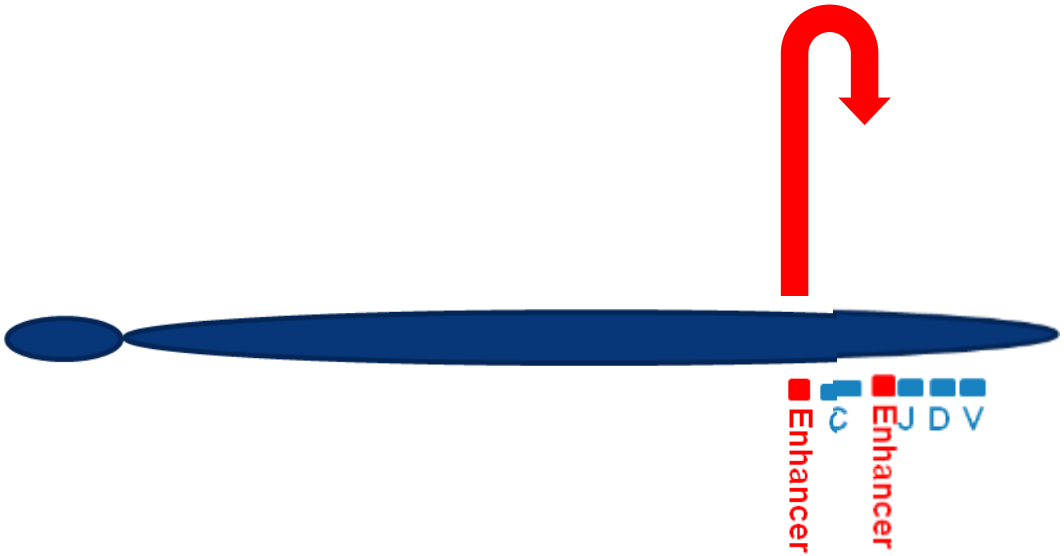
IGH Isotype Switching





IGH translocations lead to oncogene over-expression

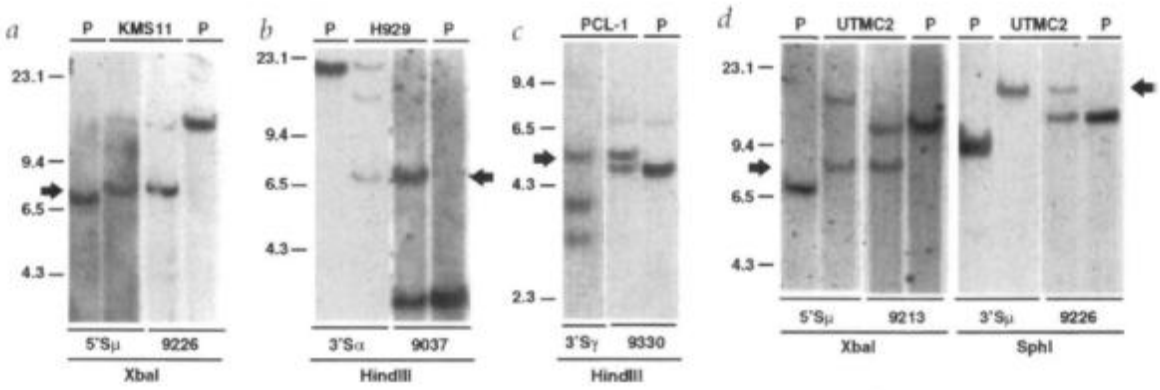
Chr.14



Chr.4

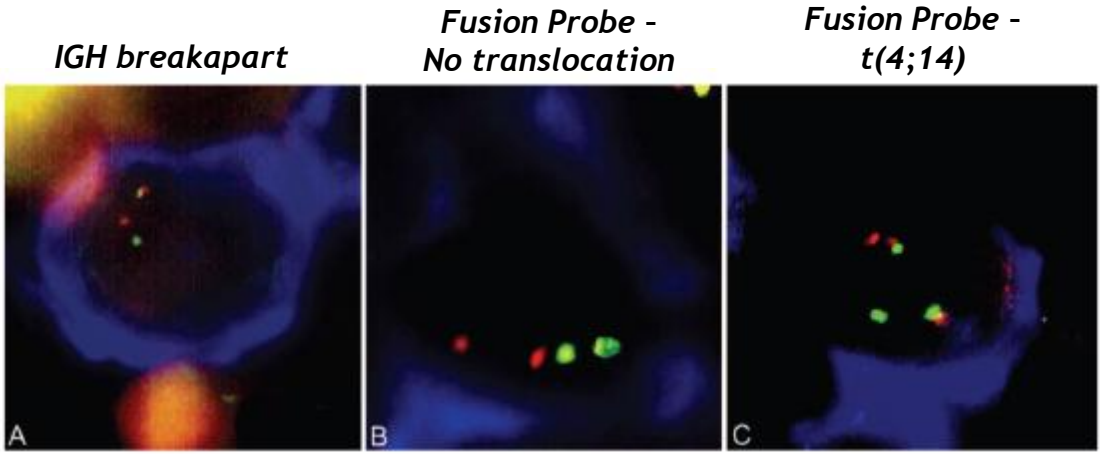
Translocation Detection

Southern blotting



FISH

Break-apart probes for IGH followed by dual fusion probes for the partner chromosomes



AIM: to develop a comprehensive NGS tool for lymphoproliferative disorders

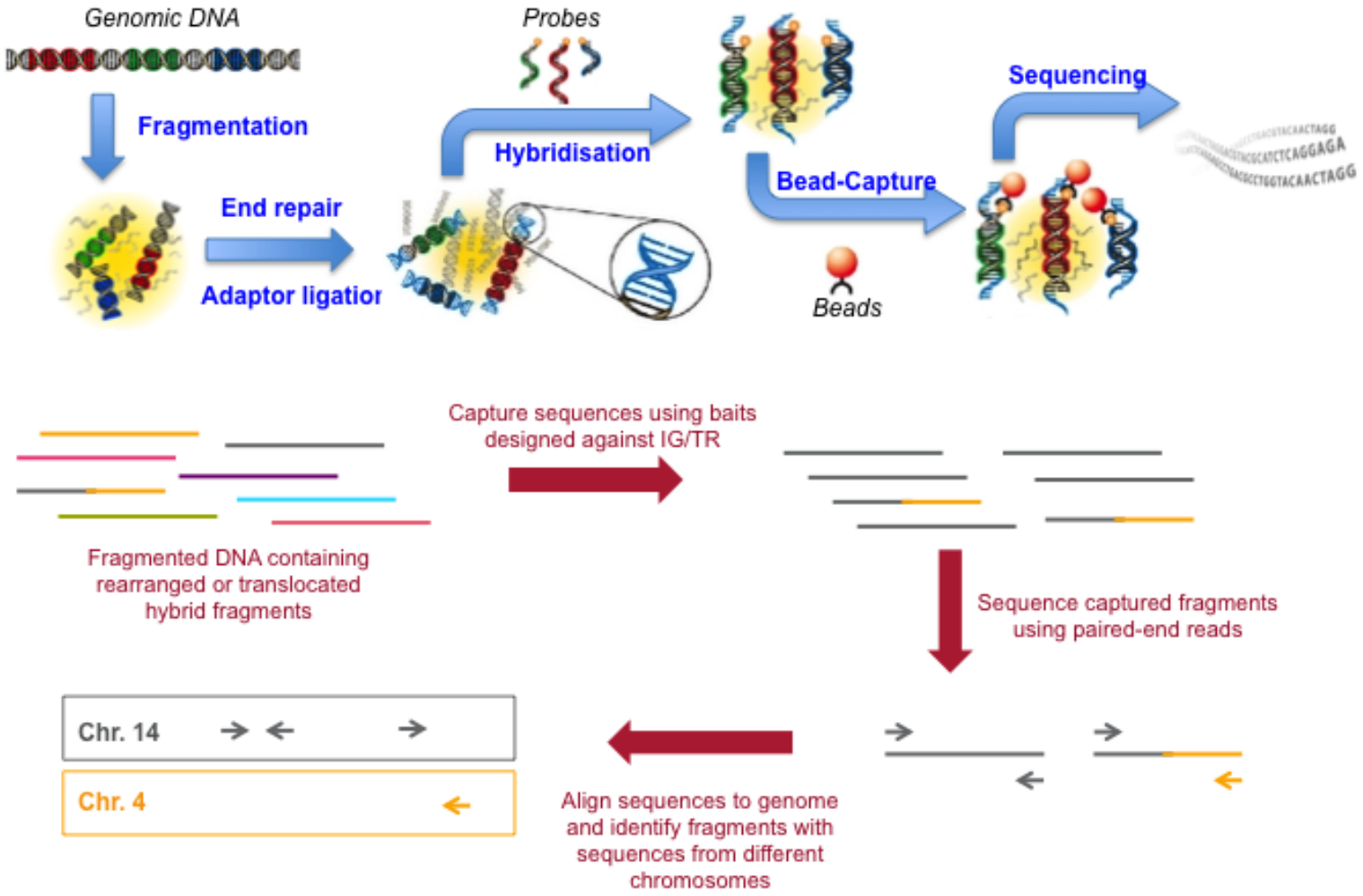
EuroClonality

- Detection of clonality by analysing V(D)J rearrangements
- Detection of IG and TR translocations
- Detection of diagnostic, prognostic and predictive genetic mutations in lymphoid disorders
- Detection of clinically-relevant amplifications and deletions in lymphoid disorders

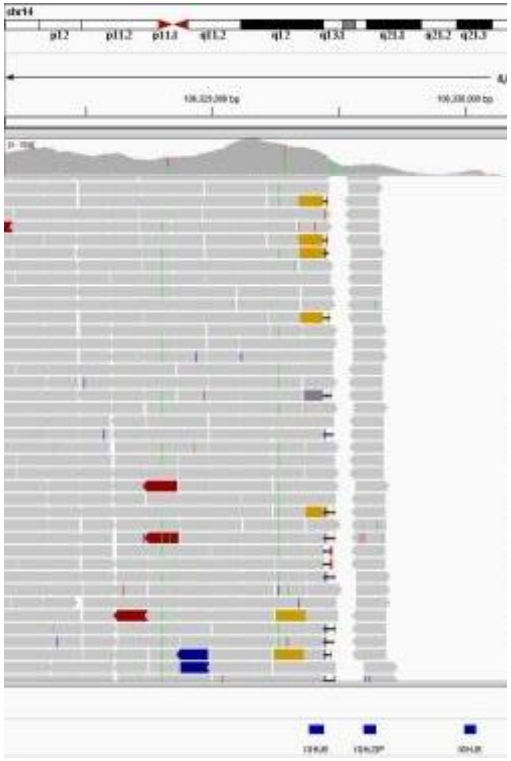
EuroClonality SeqCap EZ pilot: design

- 30 clonal samples from EC-NGS consortium laboratories
- Including different B and T cell disorders, with well characterised samples by FISH for translocations and/or V(D)J sequencing
- B-ALL, T-ALL, SMZL, CLL, BURKITT, MM, FL, DLBCL
- EC-NGS panel performed in all samples and compared with original results
- Baits covered all V, D and J genes including switch regions within the constant region (~180kb in total)

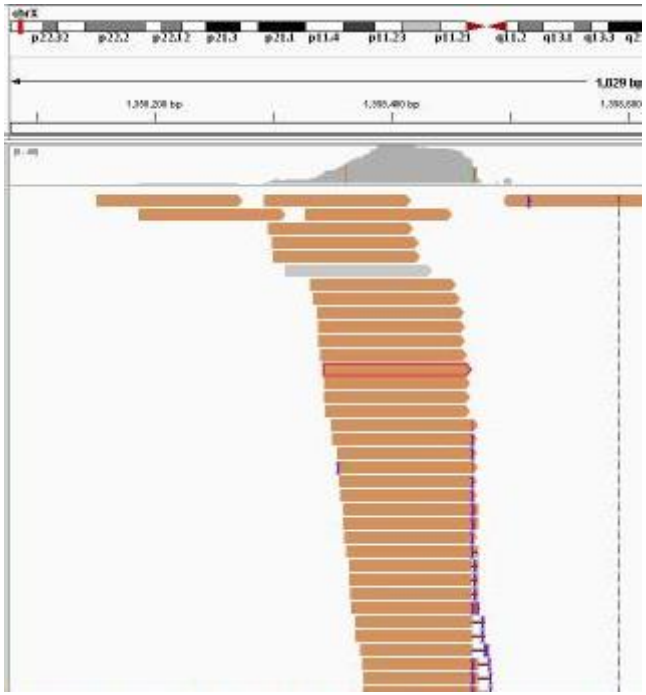
Euro-Clonality SeqCap EZ lymphoid panel



Detection of chromosomal translocations using the Euro-Clonality SeqCap EZ lymphoid panel



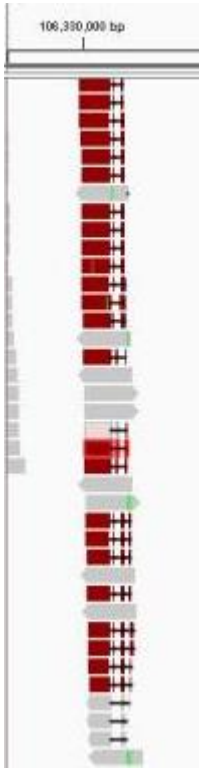
IGHJ6 (chromosome 14)



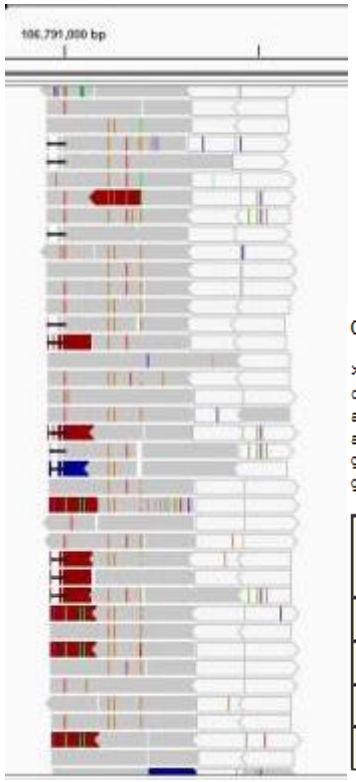
5' CRLF2 (chromosome X)

IGHD3-9 (chr 14)

Detection of V(D)J rearrangements using the Euro-Clonality SeqCap EZ lymphoid panel



IGHJ5



IGHV3-30

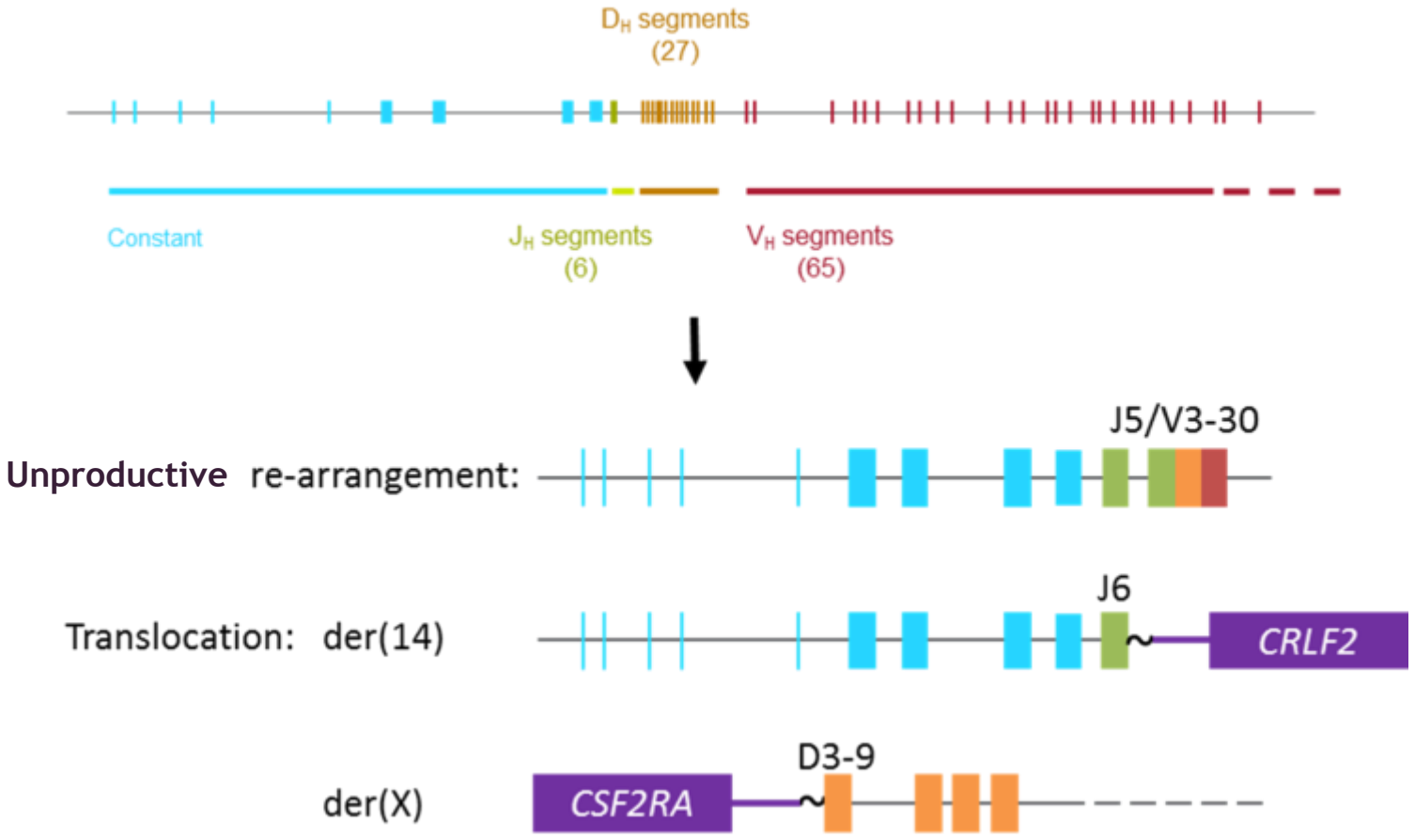
IMGT-V Quest

Complementary reverse sequence compared with the human IG set from the IMGT reference directory

```
>mdx (complementary reverse)
ctggagtgggtggcagttatatcatatgatggaagcaataaatactacgcagactccgtg
aagggccgattcaccatctccagagacaattccaagaacacgctgtatctgcaaatgaac
agcctgagagctgaggacacggctgtgtattactgtgcgagagatgcctccgagggacga
gctgctatcggaactggttcgacccctggggccagggaaccctggtcaccgtctcctcag
gtgagtcctcaccaccccctctctgagt
```

Result summary:	Unproductive IGH rearranged sequence (out-of-frame junction)		
V-GENE and allele	Homsap IGHV3-30-3*01 F, or Homsap IGHV3-30-3*02 F	score = 775	identity = 100.00% (156/156 nt)
J-GENE and allele	Homsap IGHJ5*02 F	score = 237	identity = 96.08% (49/51 nt)
D-GENE and allele by IMGT/JunctionAnalysis	Homsap IGHD2-2*01 F	D-REGION is in reading frame 2	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[X.6.38.11]	[X.8.X]	CARDASEGRAAI#NWFDPW

Schematic representation



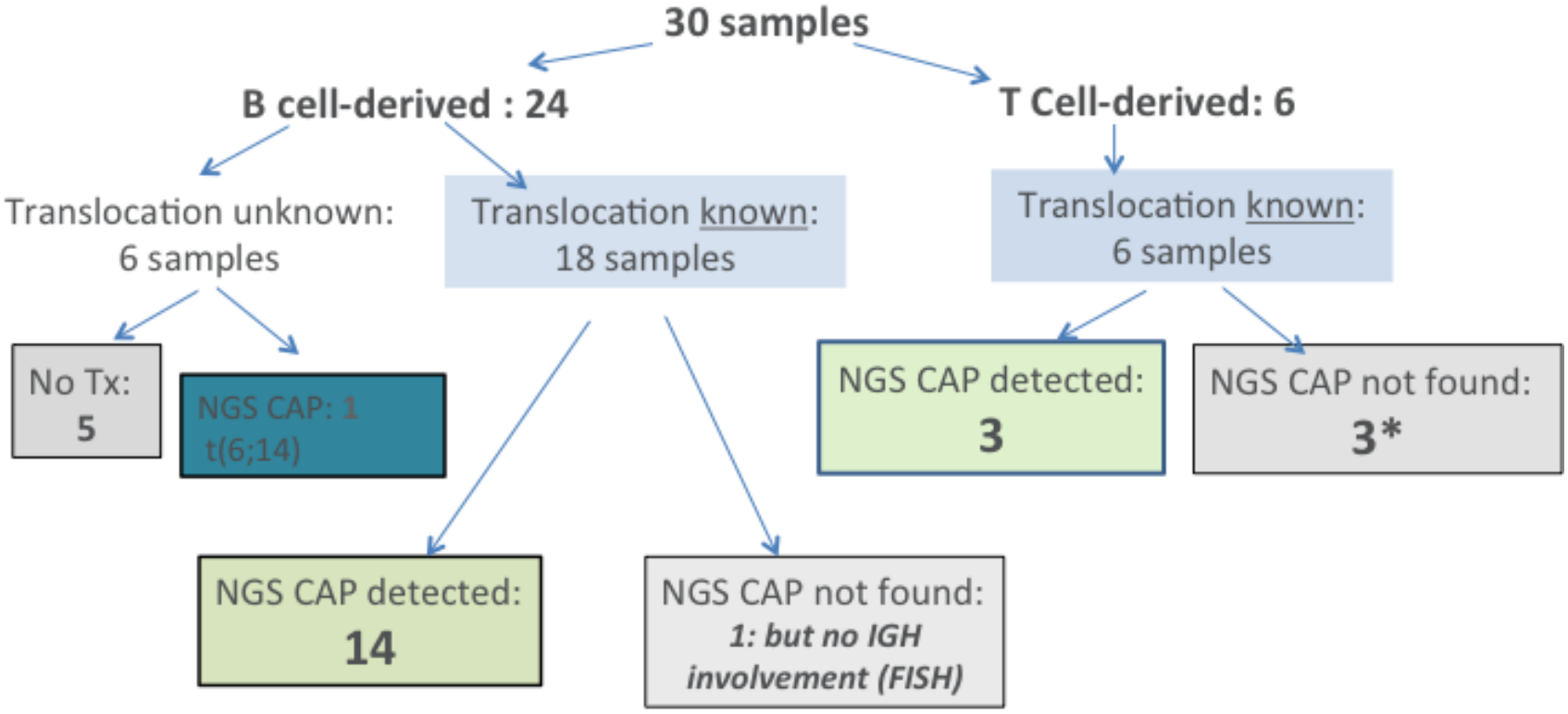
EuroClonality SeqCap EZ pilot: Translocations

		NGS Capture results			
		der(IG/TR)		der(partner chromosome)	
Diagnosis	Karyotyping/ FISH result	Break 1	Break 2	Break 1	Break 2
B-ALL	t(X;14)	*IGHJ6*	*5' CRLF2*	*IGHD3-9*	*5' CRLF2*
B-ALL	t(Y;14)	*IGHJ5*	*5' CRLF2*	*IGHD6-19*	*5' CRLF2*
BL	t(8;14)	na	na	*IGHA1sw*	*MYC* intron 1
BL	t(8;14)	*IGH sw*	*5' MYC*	*IGH sw*	*5' MYC*
BL	t(8;14)	*IGHJ4*	*5' MYC*	na	na
BL	t(8;14)	*IGHA1sw*	*MYC* intron 1	*IGHA1 sw*	*MYC* intron 1
CLL	t(2;14)	*IGHM sw*	*5' BCL11A*	*IGHM sw*	*5' BCL11A*
CLL	t(14;18)	*IGHJ6*	*BCL2 MBR*	na	na
CLL	t(14;18)	*IGHJ5*	*BCL2 MCR*	*IGHD2-15*	*BCL2 MCR*
CLL	t(14;16)	na	na	*IGHD2-2*	chr16:69479932
CLL	t(1;14)	*IGH sw*	*chr1:206286226*	*IGH sw*	*chr1:206286210*
SMZL	t(5;14)	*IGHM sw*	chr5:88608990	*IGHM*	chr5:88608986
SMZL	t(6;14)	*IGHA2 sw*	*CCND3*	na	*na*
DLBCL	IGH break	*IGHM sw*	*IRF4*	*IGH sw*	na
DLBCL	t(14;18)	*IGHJ5*	*BCL2*	*IGHV3-21*	*BCL2*
T-ALL	inv14/t(14;14)	*TRDD3*	na	*BCL11B*	na
T-ALL	t(7;10)	*TRBJb2.5*	na	*TLX1*	na
T-NHL	inv7	*TRGV8*	na	*TRBJb2.7*	na

EuroClonality SeqCap EZ pilot: V(D)J rearrangements

		IGH						IGK				IGL	
		Allele 1			Allele 2			Allele 1		Allele 2		Allele 1	
Diagnosis	Results	IGHV	IGHD	IGHJ	IGHV	IGHD	IGHJ	IGKV	IGKJ	IGKV	IGKJ	IGLV	IGLJ
BL	Sanger Seq.	3-15	3-22	4	x	3-16	4	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	3-15	3-22	4	x	3-16	4	1-9	2	D1-13	2	None detected	
BL	Sanger Seq.	3-23	na	4	x	x	x	4-1	3	x	x	n/a	n/a
	NGS	3-23	4-23	4	x	x	x	4-1	3	x	x	None detected	
BL	Sanger Seq.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	3-72	6-13	4	x	3-22	4	1-5	4	VK3-11-Kde		None detected	
CLL	Sanger Seq.	3-30	2-2	4	x	x	x	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	3-30	na	4	x	x	x	4-1	3	VK1-8-Kde		None detected	
CLL	Sanger Seq.	5-51	4-17	4	x	x	x	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	5-51	4-17	4	x	x	x	1-16	4	x	x	None detected	
CLL	Sanger Seq.	4-34	5-18	6	x	x	x	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	4-34	5-18	6	x	2-8	4	4-1	2	x	x	None detected	
CLL	Sanger Seq.	4-61	6-19	5	5-51	5-12	4	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	4-61	6-19	5	5-51	5-12	4	2-30	2	4-1	3	2-14	3
SMZL	Sanger Seq.	2-5	6-19	2	x	x	x	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	2-5	6-19	2	x	x	x	2-28	2	x	x	None detected	
CLL	Sanger Seq.	4-39	6-13	5	x	x	x	n/a	n/a	n/a	n/a	n/a	n/a
	NGS	4-39	6-13	5	x	3-16	4	1-39	4	1-17	1	None detected	

EuroClonality SeqCap EZ pilot: Summary



3 FAILS

*Design of baits did not include *TCRBJb1= accounts for 3 of the 3 samples*

EuroClonality SeqCap EZ pilot: conclusions

- **A capture based protocol is a feasible approach for the simultaneous detection of clonality, translocations, mutations and CNV in lymphoproliferative disorders**

- A pan-European validation within the EC-NGS is planned for 2015 in well-characterised samples by FISH (translocations) and PCR-Sanger sequencing (clonality + genetic mutations)

- A bioinformatics pipeline specific for IG/TR and translocations from capture data is being validated

Acknowledgments

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EuroClonality

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Monica Brüggemann

Mark Catherwood

Christiane Pott

Kostas Stamatopoulos

ERIC
european research initiative on CLL

TP53 Network
http://www.ericll.org/pages/tp53network

TP53 mutational analysis

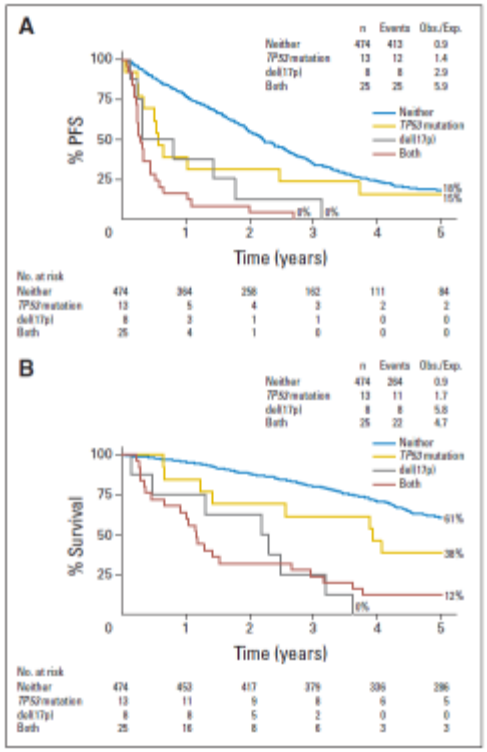
Aim: To improve the performance of TP53 mutation analysis and increase availability to allow better management of 17p-/TP53mut CLL

TP53 network:

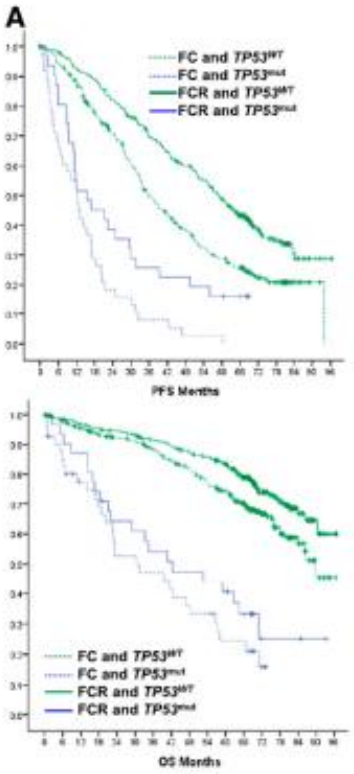
Certifying labs: Brno (CZ) and Ulm (DE)

Training centres per country: *Czech Republic, France, Germany, Greece, Italy, Spain, Nordic countries and the UK*

17p-/TP53mut Poor outcome with FC/FCR



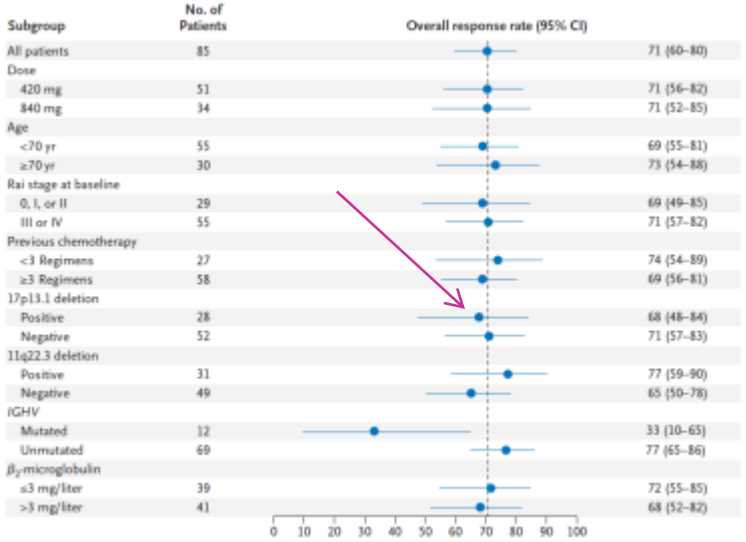
LRF CLL4 Gonzalez 2011 J Clin Onc

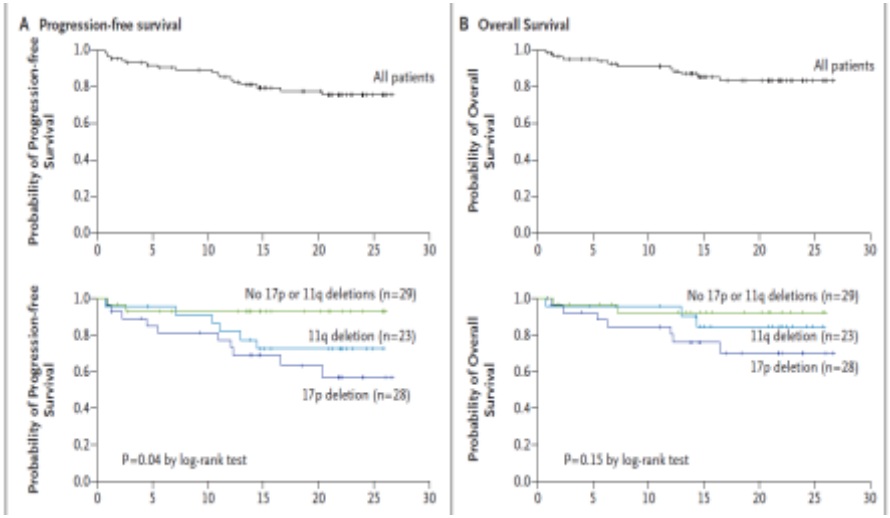


German CLL8 Stilgenbauer Blood 2014
Zenz 2010 J Clin Onc

New treatment options for 17p-/*TP53*mut CLL: Ibrutinib

- Ibrutinib (BTK inhibitor)
- Approved by FDA and EMA as front-line treatment for *17p-/TP53mut*





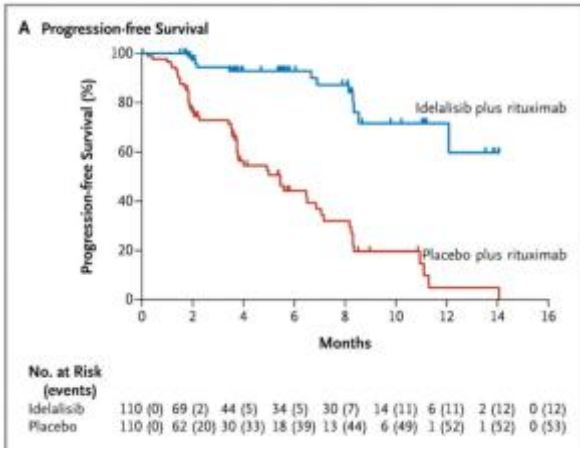
Overall response to Ibrutinib according to subgroup

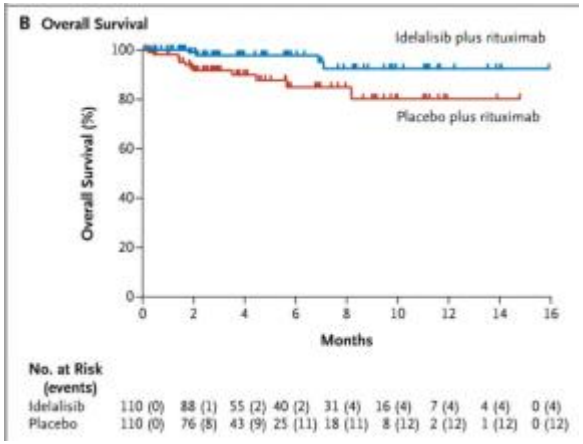
Byrd JC et al. N Engl J Med. 2013 Jul 4; 369(1): 32-42.

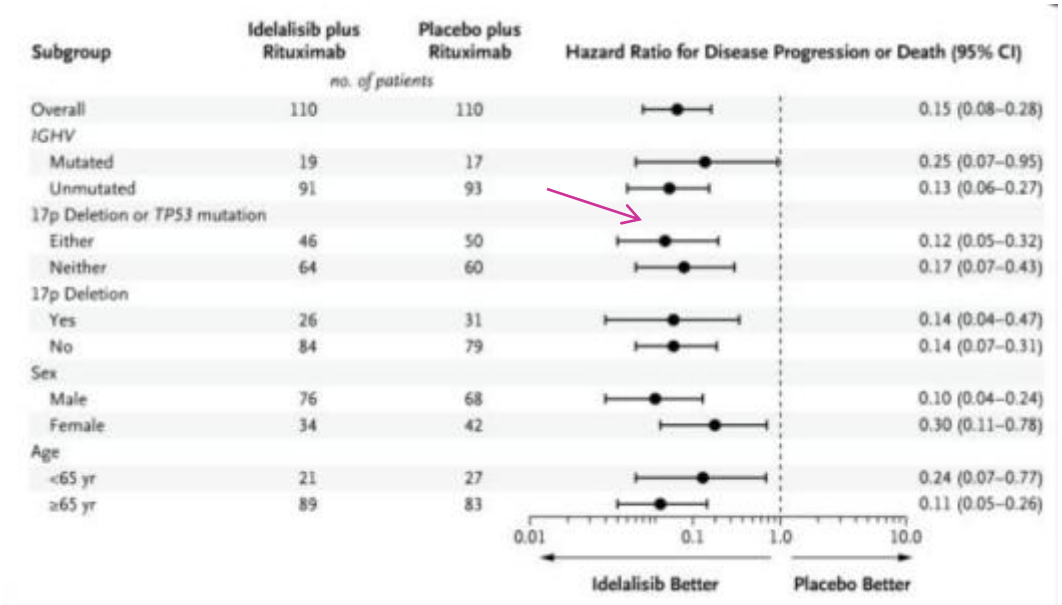
RESONATE study: Improved PFS and OS in previously treated patients

New treatment options for 17p-/*TP53*mut CLL: Idelalisib + Rituximab

- Idelalisib (PI3Kδ inhibitor)
- Approved by EMA as front-line treatment for *17p-/TP53mut*







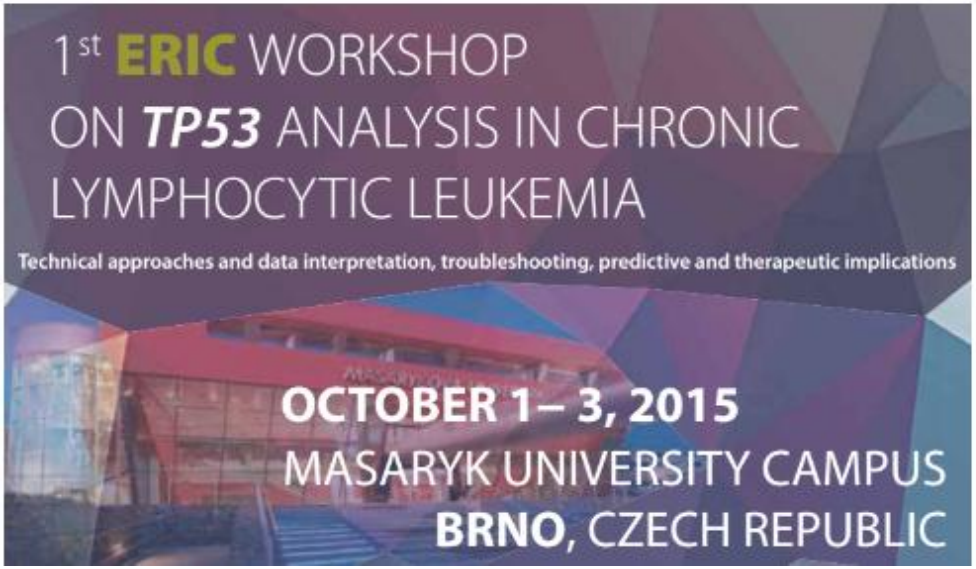
Furman RR et al N Engl J Med. 2014 March 13; 370(11): 997–1007

ERIC
european research initiative on CLL

TP53 Network
http://www.ericll.org/pages/tp53network

Aims of *TP53* network

- Education
- Selection of appropriate techniques
- Establish Quality control procedure
- Workshop Brno:



Role of national training centres

- Coordination the activities of ERIC/TP53 network
- Provide technical support: on-site training, sample exchange
- Offer to perform diagnostic testing during implementation
- Contribute to the online helpdesk

- Contact details:
- Dörte Wren/David Gonzalez-de-Castro
- Molecular Diagnostics Royal Marsden Sutton UK

- Dorte.wren@icr.ac.uk
- David.Gonzalez-de-Castro@icr.ac.uk

Venetoclax (AbbVie/Roche)

- Breakthrough therapy designation granted by FDA for CLL with 17p deletion
- Efficacy study in relapsed/refractory CLL or untreated 17p- ongoing

New treatment options for 17p-/*TP53*mut CLL: BCL2 inhibitor (ABT-199, Venetoclax)

- EHA 2015: Venetoclax + Rituximab:
- Patients previously treated (n=49)
- Overall response 84%, 41% complete response or CR with incomplete marrow recovery; MRD negativity in 65% (evaluated in 20pts)

Best Response		
	All pts n=49	**del17p n=9**
ORR, n (%)	41 (84)	7 (78)
CR/CRi	20[a] (41)	3[b] (33)
PR	20 (41)	4 (44)
nodular PR (nPR)	1 (2)	0 (0)
PR unconfirmed[c]	4 (8)	1 (11)
Stable disease (SD)	1 (2)	0 (0)
Progressive disease (PD)	2 (4)	0 (0)
Discontinued prior to assessment[d]	1 (2)	1 (11)

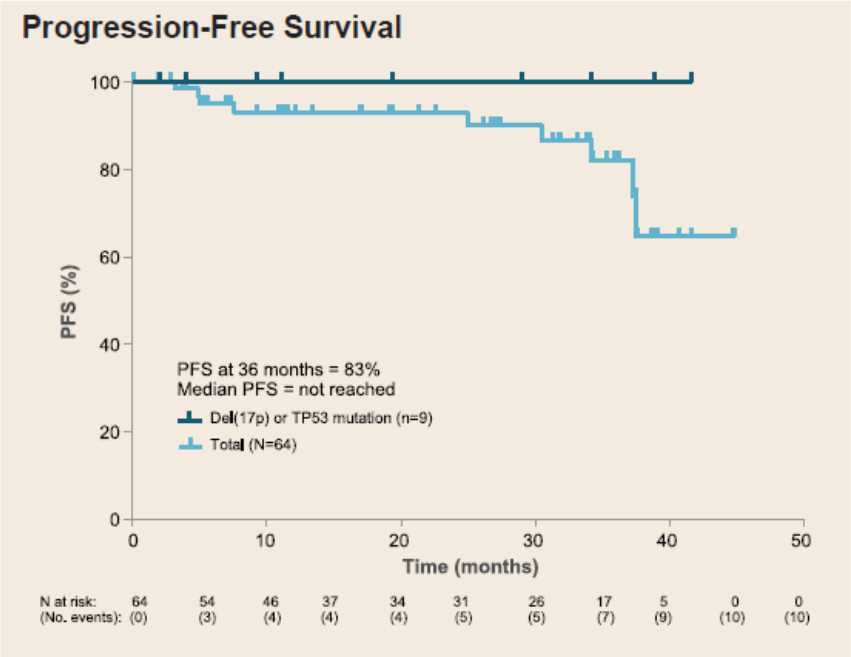
[a]6/20 CRi;
[b]2/3 CRi;

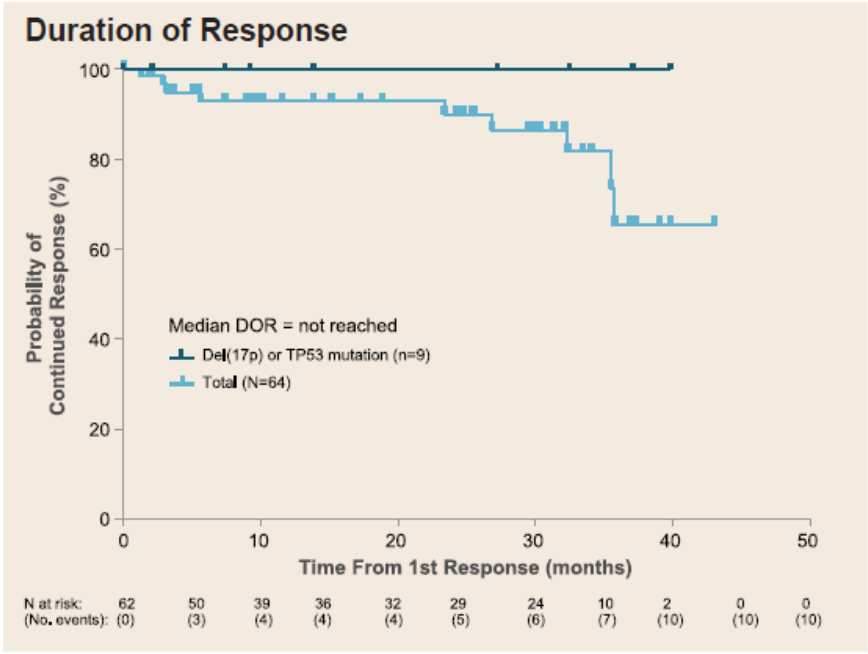
Roberts AW EHA 2015 oral presentation/abstract

comp

- Ibrutininb (Imbruvia): Pharmacyclics, Inc + Johnson & Johnson's Janssen Pharmaceutical
- Idelalisib (Zydelig), Gilead Sciences

Idelalisib


Progression-Free Survival
PFS (%)
100
80
60
40
20
0
PFS at 36 months = 83%
Median PFS = not reached
Del(17p) or TP53 mutation (n=9)
Total (N=64)
0
10
20
30
40
50
Time (months)
N at risk: 64 54 46 37 34 31 26 17 5 0 0
(No. events): (0) (3) (4) (4) (4) (5) (5) (7) (9) (10) (10)


Duration of Response
Probability of Continued Response (%)
100
80
60
40
20
0
Median DOR = not reached
Del(17p) or TP53 mutation (n=9)
Total (N=64)
0
10
20
30
40
50
Time From 1st Response (months)
N at risk: 62 50 39 36 32 29 24 10 2 0 0
(No. events): (0) (3) (4) (4) (4) (5) (6) (7) (10) (10) (10)

Euro-Clonality SeqCap EZ lymphoid panel