Biological standards developed in the EuroClonality-NGS Working Group

Frederic Davi & Anton W Langerak

on behalf of the EuroClonality-NGS WG

AIRRc Biological Resources meeting

December 7, 2020

Who are we ?

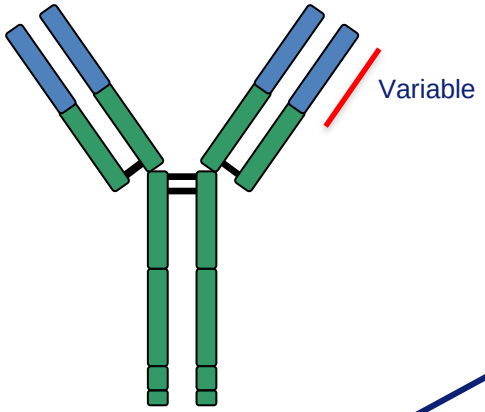
EuroClonality

- Academic consortium started in 1996 based on BIOMED-2 grant *PCR-based clonality studies for early diagnosis of lymphoproliferative disorders*

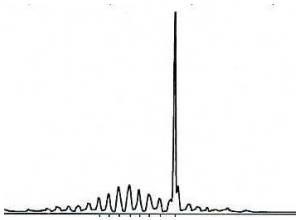
- Collaborative study of 48 European institutes (including 30 PCR labs) in 7 national networks
- Key publications in 2003 and 2007 on immunogenetic assays (IG/TR clonality/repertoire; low throughput assays)
- Renamed into EuroClonality (scientific foundation); sustainability model based on valorization of collective IP http://www.euroclonality.org
- Key tasks:

  1) educational & diagnostic service; 2 EQA service; 3) innovation

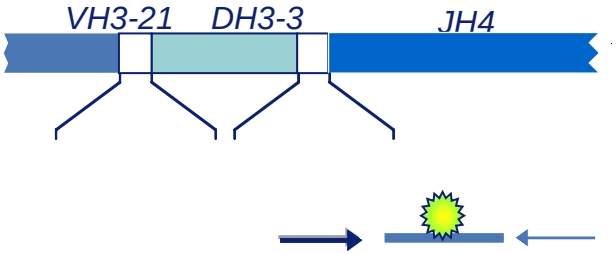
Immunogenetics applications



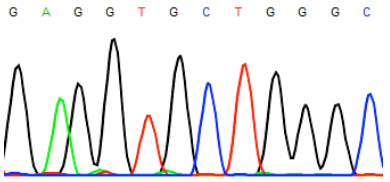
Clonality



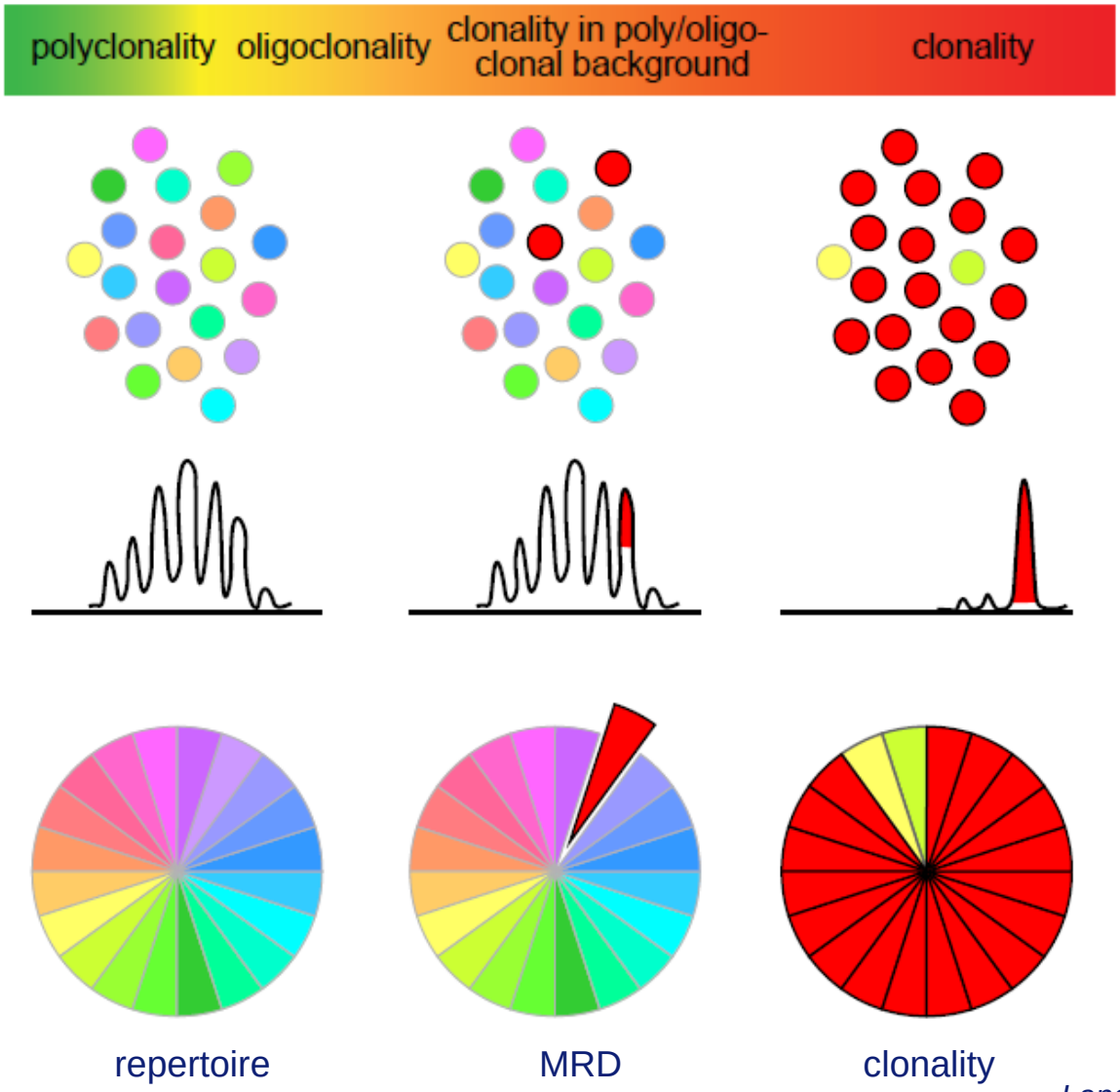
Minimal residual disease



IGHV mutational status



HiT immunogenetic applications …



Langerak, J Immunol 2017

EuroClonality-NGS Working Group

EuroClonality

ESLHO
european scientific foundation
of laboratory hemato oncology

ERIC
european research initiative on CLL

EuroClonality

EuroMRD

NGS EuroClonality

→ EuroClonality-NGS main objective :
develop, ***standardize***, and ***validate*** IG/TR NGS assays for clinical applications

EuroClonality-NGS Working Group - PI's

EuroClonality

A.W. Langerak (Rotterdam, NL), chair

J.J.M. van Dongen (Leiden, NL)

P. Groenen (Nijmegen, NL)

M. Brüggemann /C. Pott (Kiel, DE)

M. Hummel (Berlin, DE)

D. Gonzalez (Belfast / Sutton, UK)

F. Davi (Paris, FR)

E. Macintyre (Paris, FR)

R. Garcia Sanz (Salamanca, ES)

F. Fend (Tübingen, DE)

the IgCLL group

ERIC european research initiative on CLL

K. Stamatopoulos (Thessaloniki, GR)

L. Sutton (Stockholm, SE)

EuroMRD

G. Cazzaniga (Monza, IT)

J. Trka (Prague, CZ)

J.Hancock /J.Moppett (Bristol/London,UK)

M. Ladetto (Torino, IT)

Bioinformatics

ARReST: N. Darzentas (Brno,CZ/ Kiel,DE)
Vidjil: M. Giraud (Lille, FR)
IMGT: V. Giudicelli (Montpellier, FR)
INAB-CERTH: F. Psomopoulos (Thess,GR)

Partners

N. Paust (Freiburg, DE) Hahn Schickard

ESLHO (project management)

M. Bitter and B. Lubbers

EuroClonality-NGS Working Group

EuroClonality

EuroClonality NGS

Steering committee
(coordinator: Langerak)

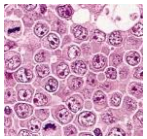
Langerak, Brüggemann, Cazzaniga, Darzentas, Davi, Gonzalez, Groenen, Hummel, Macintyre, Pott and Stamatopoulos

Workpackage	Primer design					
	IGH V-J	IGH D-J	IGK	TRB	TRG	TRD
Leaders	Pott Garcia Sanz	Davi Stamatopoulos	Groenen Langerak	Brüggemann Hummel	Cazzaniga Van Dongen	Macintyre
Workpackage	Bioinformatics					
Leader	Darzentas					
Core projects	1. MRD	2. Clonality (amplicon-based)	3. Clonality (capture-based)	4. Repertoire (amplicon-based)		
Leaders	Brüggemann Pott	Groenen Hummel	Gonzalez	Stamatopoulos Davi		

IG clonality feasibility study

EuroClonality

Workflow IG-NGS clonality assessment



Frozen-tissue or FFPE sample

↓

DNA isolation

Multiplex PCR
(IGHV-IGHD-IGHJ FR3,
IGHD-IGHJ, IGKV-IGKJ,
IGKV/Intron-KDE)

- DNA purification
- Adaptor ligation
- Library preparation and amplification

Sequence run
Ion Torrent

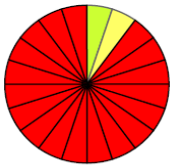
Bioinformatics analysis
with
ARResT/Interrogate

Multiplex PCR for IG-NGS clonality analysis

IGHV-IGHD-IGHJ	**IGHD-IGHJ**	**IGKV-IGKJ** **IGKV/Intron-KDE**
Forward primers: 22 x IGH-V-FR3	Forward primers: 8 x IGH-D	Forward primers: 11 x IGK-V IGK-INTR
Reverse primers: 3 x IGH-J	Reverse primers: 3 x IGH-J	Reverse primers: IGK-DE 3 x IGK-J
PCR mixture: 40 ng DNA 0.2/0.4 µM primers 1 x GeneAmp buffer 200 µM dNTP 1.5 mM $MgCl_2$ 0.5 U AmpliTaq Gold	PCR mixture: 40 ng DNA 0.2 µM primers 1 x GeneAmp buffer 200 µM dNTP 2 mM $MgCl_2$ 0.5 U AmpliTaq Gold	PCR mixture: 40 ng DNA 0.2 µM primers 1 x GeneAmp buffer 200 µM dNTP 1.5 mM $MgCl_2$ 0.5 U AmpliTaq Gold

1 x 10 min 94°C → 30 x (30 sec 92°C, 40 sec 60°C, 40 sec 72°C) → 1 x 30 min 72°C, 1 x 10 min 20°C

Pool reaction mixtures 1:1:1



Scheijen, Leukemia 2019;33:2227

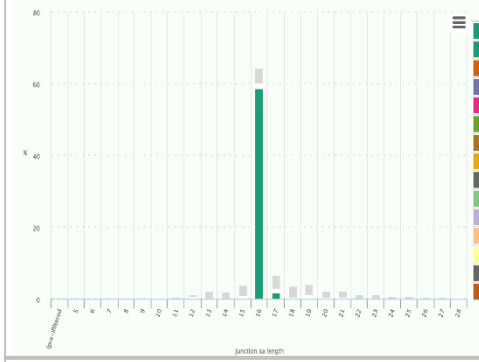
Clonality reporting usermode ARResT/Interrogate

EuroClonality

WS19_181 **IGH VJ FR3** **Reads: 113521**

VJ:Vh-(Dh)-Jh junction class set

clonotypes over junction aa lengths, top50 coloured (tip: hover / click)



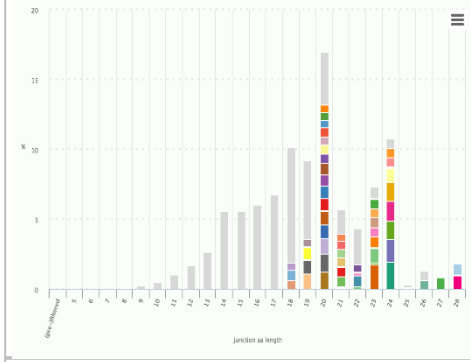
top15 clonotypes over junction aa lengths

genes and segmentation	seq	len	reads	norm.%
~V3 -1/23/-2 J4	CSKALTITVLYYFDYW	16	66635	58.70
~V3 -1/23/-2 J4	CSKALTITVLYYFDYW	17	1967	1.73
V3-23=V3-23D -1/23/-2 J4	CSKALTITVLYYFDYW	16	337	0.30
~V3 -1/23/-2 J4	CSKALTITMFYYFDYW	16	247	0.22
V4-39 -1/30/-5 J2	CARRSRPYGDYVRGYFDLW	19	210	0.18
V4-39 -0/21/-1 J4	CARHYLHSQLAYFDYW	16	193	0.17
V2-70=V2-70D -3/24/-6 J6	CARTIMSIAARDYYYGMDVW	20	185	0.16
V6-1 -0/20/-6 J6	CARDRGLKIRDYYYGMDVW	19	184	0.16
V4-39 -1/16/-11 J6	CARLVVRGVTTMDVW	15	179	0.16
V1-2 -1/36/-16 J4	CARGPDTYYYDSSGA	15	178	0.16
~V3 -0/10/-2 J4	CSKEVLYYFDYW	12	172	0.15
V2-70=V2-70D -0/27/-2 J3	CARIRRGVGSSWFHAFDIW	19	166	0.15
V2-26 -2/49/-8 J6	CARIRDMGKREQQLAPPSPLYYYGMDVW	28	162	0.14
V3-15 -1/17/-8 J4	CTTVEWMAGDYW	12	161	0.14
V2-70=V2-70D -3/24/-15 J6	CARRSIGPTHTNGMDVW	17	161	0.14

WS19_181 **IGHD** **Reads: 7374**

DJ:Dh-Jh junction class set

clonotypes over junction aa lengths, top50 coloured (tip: hover / click)



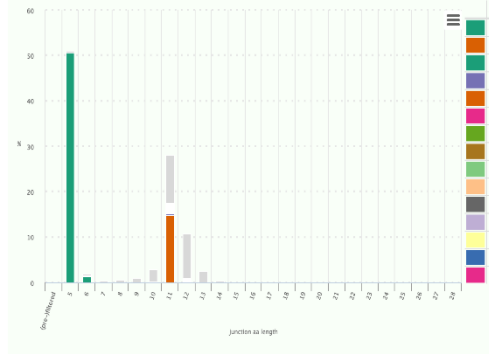
top15 clonotypes over junction aa lengths

genes and segmentation	seq	len	reads	norm.%
D3-22 -7/14/-2 J6	VVLL***WLPSQLPTTRYYGMDVW	24	146	1.98
D3-10 -7/20/-12 J6	VVLLWFGELPLYVNR##YGMDVW	23	129	1.75
D3-16 -7/9/-2 J6	VVL*LRLGELSPSLYYYYYGMDVW	24	118	1.60
D3-3 -0/4/-0 J6	VVLRFLEWLLYP##F*YYYYGMDVW	24	104	1.41
D3-10 -6/24/-0 J5	VVLLWFGELSLWLSIVRGNWFDPW	24	99	1.34
D3-3 -5/9/-0 J6	VVLRFLEWLFHI##YYYYYGMDVW	24	97	1.32
D3-22 -3/12/-4 J1	VVLL***WLLPSTR*YFQHW	20	93	1.26
D3-22 -0/5/-3 J2	VVLL***WLLLRS#WYFDLW	20	92	1.25
D3-22 -7/16/-0 J4	VVLL***WLLNPTPYYFDYW	20	82	1.11
D3-22 -8/14/-3 J3	VVLL***WFLTSG#AFDIW	19	81	1.10
D3-3 -5/13/-7 J6	VVLRFLEWLLDQRG#YYYYGMDVW	23	77	1.04
D5-12 -6/11/-4 J6	VVDIVATTRL#YYYYGMDVW	20	74	1.00
D3-9 -6/11/-0 J6	VVLRYFDWL*MRY#YYYYGMDVW	24	74	1.00
D5-12 -5/52/-8 J4	VVDIVATSAGEGPRIL*WW*LLPT#DYW	28	74	1.00
D3-9 -3/18/-6 J4	VVLRYFDWLVWGPWASVDYW	20	73	0.99

WS19_181 **IGK V-J** **Reads: 150686**

VJ:Vk-Jk junction class set

clonotypes over junction aa lengths, top50 coloured (tip: hover / click)



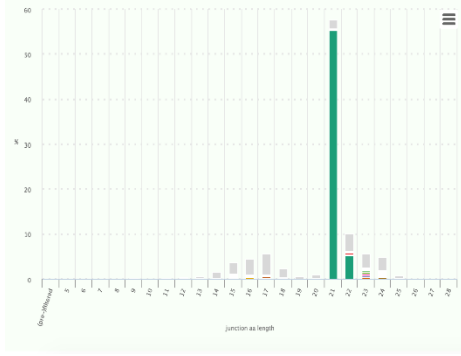
top15 clonotypes over junction aa lengths

genes and segmentation	seq	len	reads	norm.%
V2D-26 -14/3/-8 J5	CMQDR	5	76309	50.64
V3-20=V3D-20 -6/3/-0 J1	CQQYGSSVWTF	11	22433	14.89
V2D-26 -14/3/-8 J5	CMQDR	6	2158	1.43
V6D-21 -0/1/-4 J5	CHQSSSLPHTF	11	334	0.22
V3-20=V3D-20 -6/3/-0 J1	CQQYGSSVWTF	12	245	0.16
V3-20=V3D-20 -6/3/-0 J2	CQQYGSSVWTF	11	199	0.13
V4-1 -1/1/-2 J1	CQQYYSTP##TF	12	178	0.12
V4-1 -0/0/-3 J1	CQQYYSTPPTF	11	167	0.11
V2-29 -14/3/-8 J5	CMQDR	5	144	0.10
V4-1 -1/0/-1 J2	CQQYYSTP#CSF	12	141	0.09
V4-1 -1/0/-2 J1	CQQYYSTPRTF	11	140	0.09
V3-20=V3D-20 -1/0/-2 J4	CQQYGSSPLTF	11	139	0.09
V1-37=V1D-37 -1/0/-2 J1	GQRTYNAPRTF	11	138	0.09
V3-20=V3D-20 -3/0/-0 J1	CQQYGSSPWTF	11	136	0.09
V3-20=V3D-20 -6/3/-0 J2	CQQYGSSVWTF	12	136	0.09

WS19_181 **IGK V/Intron-Kde** **Reads: 16234**

Vk/intron-Kde junction class set

clonotypes over junction aa lengths, top50 coloured (tip: hover / click)

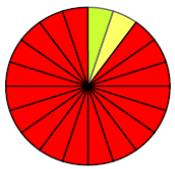


top15 clonotypes over junction aa lengths

genes and segmentation	seq	len	reads	norm.%
intron -0/1/-10 Kde	PCVCPINAAVASFPDDGSPGR	21	8969	55.25
intron -0/1/-10 Kde	PCVCPINAAVASFPDDGSPGR	22	863	5.32
intron -2/0/-3 Kde	PCVCPIDAAVASFP##PSGSPGR	23	79	0.49
intron -0/0/-4 Kde	PCVCPIDAAVASFPD#PSGSPGR	23	62	0.38
intron -3/0/-1 Kde	PCVCPINAAVASFP#SPSGSPGR	23	59	0.36
intron -2/0/-3 Kde	PCVCPINAAVASFP##PSGSPGR	23	56	0.34
intron -0/0/-2 Kde	PCVCPIDAAVASFPD#SPSGSPGR	24	50	0.31
V1-37=V1D-37 -2/0/-2 Kde	GQRTYNAPSPSGSPGR	16	49	0.30
intron -0/0/-4 Kde	PCVCPINAAVASFPD#PSGSPGR	23	46	0.28
V2D-26 -0/0/-3 Kde	CMQDAQDP##PSGSPGR	17	45	0.28
V6-21=V6D-21 -8/0/-11 Kde	CHQSRSGSPGR	11	43	0.26
V7-3 -3/6/-14 Kde	CLQSKNFPR#SPGR	14	40	0.25
V7-3 -0/0/-18 Kde	CLQSKNFP##GR	12	38	0.23
V4-1 -3/0/-5 Kde	CQQYYST#PSGSPGR	15	37	0.23
intron -0/2/-10 Kde	PCVCPINAAVASFPDDWHPGR	21	37	0.23

EuroClonality-NGS purpose developed pipeline

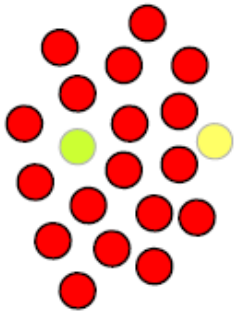
ARResT/Interrogate



IG/TR MRD marker screening study

EuroClonality

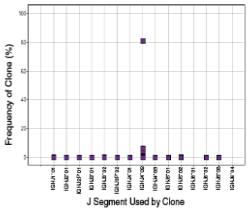
Screening of diagnostic sample



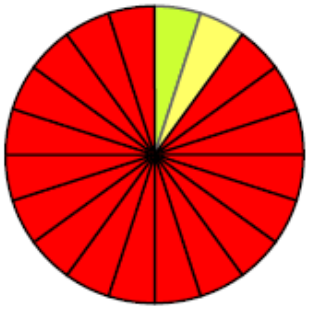
IG/TR Multiplex PCR

Preparation of amplicons for HTS

HTS



Bioinformatic identification of index-sequence



Brüggemann, Leukemia 2019;33:2241

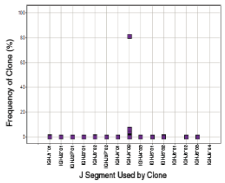
MRD analysis

Courtesy: M. Brueggemann

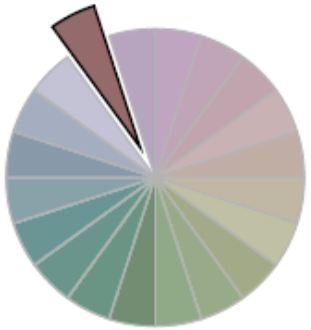
IG/TR Multiplex PCR

Preparation of amplicons for HTS

HTS

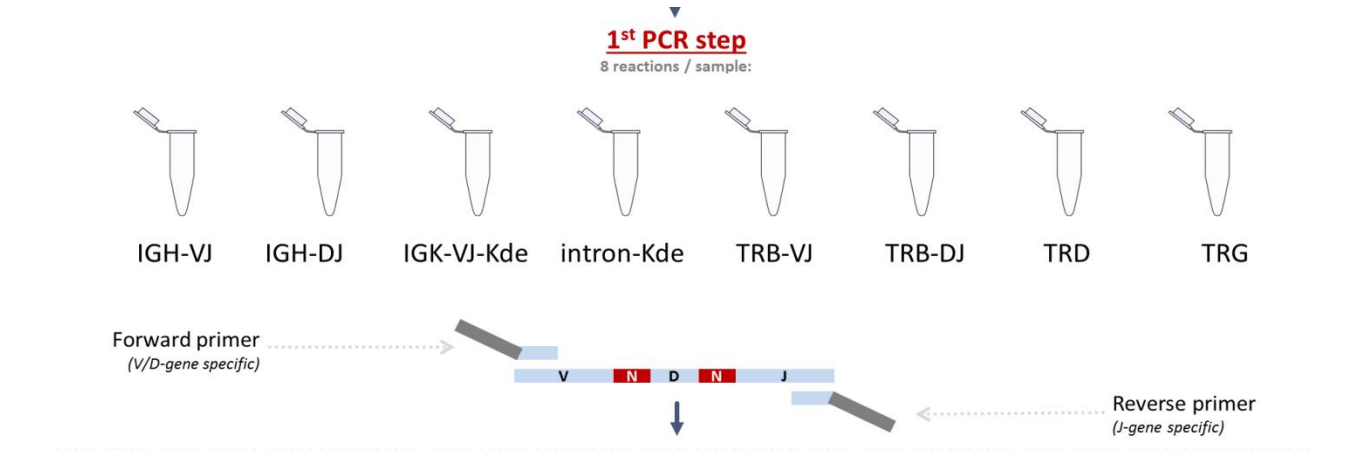


Bioinformatic search for index-sequence



Ongoing work

IG/TR protocol (2-step)

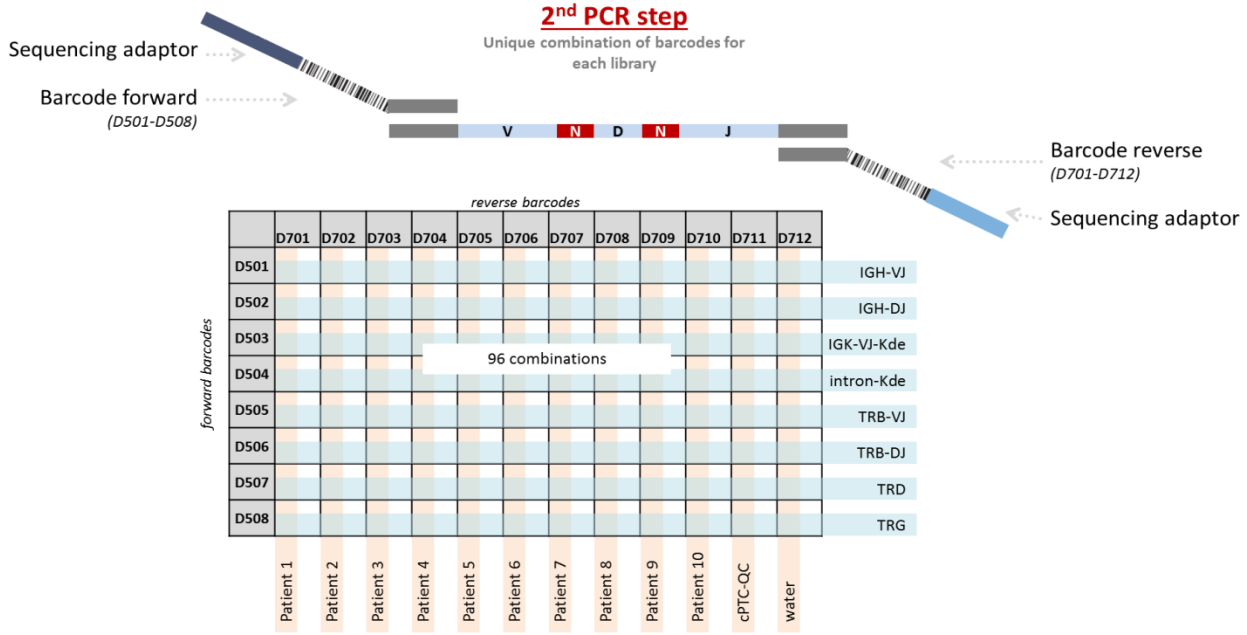


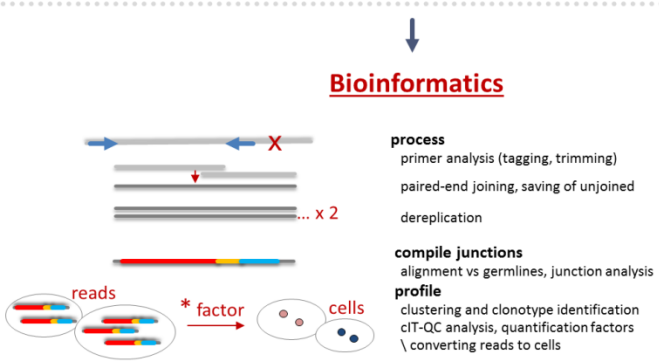
IGH-V-FR1-A-1	(IGHV1-2*01)	(-277)	CTGGGGCTGAGGTGAAGAAG
IGH-V-FR1-B-1	(IGHV5-51*01)	(-282)	GCAGTCTGGAGCAGAGGTGAAAA
IGH-V-FR1-C-1	(IGHV2-5*01)	(-297)	TCACCTTGAAGGAGTCTGGTCC
IGH-V-FR1-D-1	(IGHV3-7*01)	(-295)	AGGTGCAGCTGGTGGAGTC
IGH-V-FR1-E-1	(IGHV3-23*01)	(-296)	GAGGTGCAGCTGTTGGAGTC
IGH-V-FR1-F-1	(IGHV4-4*01)	(-272)	CCAGGACTGGTGAAGCCTTC
IGH-V-FR1-G-1	(IGHV4-34*01)	(-278)	CAGTGGGGCGCAGGACTGTT
IGH-V-FR1-H-1	(IGHV4-4*01)	(-272)	CCAGGACTGGTGAAGCCTCC
IGH-V-FR1-I-1	(IGHV6-1*01)	(-302)	GTACAGCTGCAGCAGTCAGG
IGH-V-FR1-J-1	(IGHV7-4-1*01)	(-286)	GCTGGTGCAATCTGGGTCTG
IGH-V-FR1-K-1	(IGHV1-45*01)	(-250)	CCTCAGTGAAGGTTTCCTGCAAGG
IGH-V-FR1-L-1	(IGHV2-26*01)	(-265)	AAACCCACAGAGACCCTCACGCTGAC
IGH-V-FR1-M-1	(IGHV3-16*01)	(-256)	CTGGGGGGTCCCTGAGACTCTCCTG
IGH-V-FR1-N-1	(IGHV4-31*01)	(-259)	CTTCACAGACCCTGTCCCTCACCTG
IGH-V-FR1-O-1	(IGHV6-1*01)	(-263)	TCGCAGACCCTCTCACTCACCTGTG

CTTACCTGAGGAGACGGTGACC	(+68)	(IGHJ6*01)	IGH-J-A-1
CTCACCTGAGGAGACAGTGACC	(+58)	(IGHJ2*01)	IGH-J-B-1

Brüggemann, Leukemia 2019;33:2241

IG/TR protocol (2-step) + bioinformatics





EuroClonality-NGS purpose developed pipeline

ARResT/Interrogate

Knecht, Leukemia 2019;33:2254
Brüggemann, Leukemia 2019;33:2241

ARTICLE

Minimal resdiual disease

Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study

Monika Brüggemann[1] · Michaela Kotrová[1,2] · Henrik Knecht[1] · Jack Bartram[3] · Myriam Boudjogrha[4] · Vojtech Bystry[5] · Grazia Fazio[6] · Eva Froňková[2] · Mathieu Giraud[7] · Andrea Grioni[6] · Jeremy Hancock[8] · Dietrich Herrmann[1] · Cristina Jiménez[9] · Adam Krejci[5] · John Moppett[10] · Tomas Reigl[5] · Mikael Salson[7] · Blanca Scheijen[11] · Martin Schwarz[1] · Simona Songia[6] · Michael Svaton[2] · Jacques J. M. van Dongen[12] · Patrick Villarese[13] · Stephanie Wakeman[8] · Gary Wright[3] · Giovanni Cazzaniga[6] · Frédéric Davi[4] · Ramón García-Sanz[9] · David Gonzalez[14] · Patricia J. T. A. Groenen[11] · Michael Hummel[15] · Elizabeth A. Macintyre[13] · Kostas Stamatopoulos[16] · Christiane Pott[1] · Jan Trka[2] · Nikos Darzentas[1,5] · Anton W. Langerak[17] · on behalf of the EuroClonality-NGS working group

Leukemia 2019;33:2241

ARTICLE

Minimal residual disease

Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS

Henrik Knecht[1] · Tomas Reigl[2] · Michaela Kotrová[1] · Franziska Appelt[1] · Peter Stewart[3] · Vojtech Bystry[2] · Adam Krejci[2] · Andrea Grioni[4] · Karol Pal[2] · Kamila Stranska[2,5] · Karla Plevova[2,5] · Jos Rijntjes[6] · Simona Songia[4] · Michael Svatoň[7] · Eva Froňková[7] · Jack Bartram[8] · Blanca Scheijen[6] · Dietrich Herrmann[1] · Ramón García-Sanz[9] · Jeremy Hancock[10] · John Moppett[11] · Jacques J. M. van Dongen[12] · Giovanni Cazzaniga[4] · Frédéric Davi[13] · Patricia J. T. A. Groenen[6] · Michael Hummel[14] · Elizabeth A. Macintyre[15] · Kostas Stamatopoulos[16] · Jan Trka[7] · Anton W. Langerak[17] · David Gonzalez[3] · Christiane Pott[1] · Monika Brüggemann[1] · Nikos Darzentas[1,2] · on behalf of the EuroClonality-NGS Working Group

Leukemia 2019;33:2254

ARTICLE

Lymphoma

Next-generation sequencing of immunoglobulin gene rearrangements for clonality assessment: a technical feasibility study by EuroClonality-NGS

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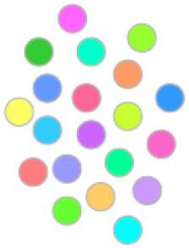
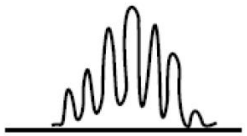
Leukemia 2019;33:2227

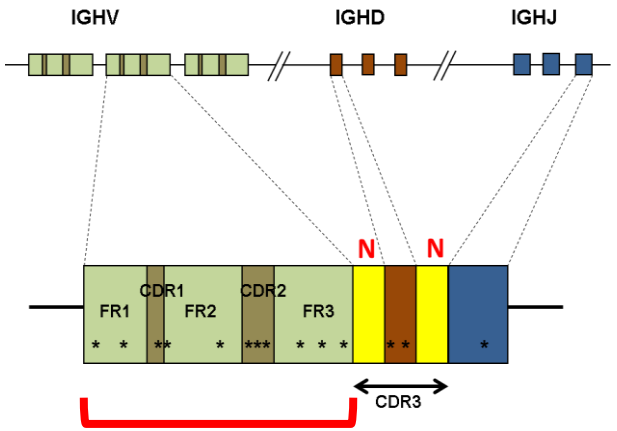
Repertoire: clinical applications for malign B cells

EuroClonality

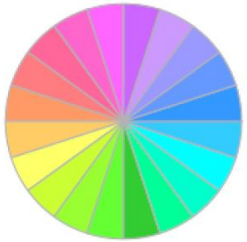




IGHV mutational status in CLL

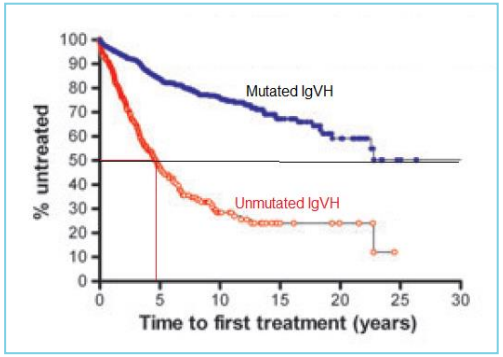


% identity to germline – 2% threshold



repertoire

TTFT



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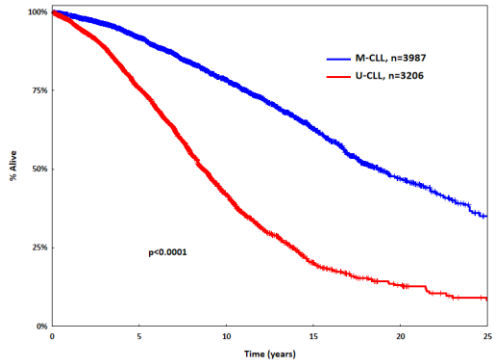
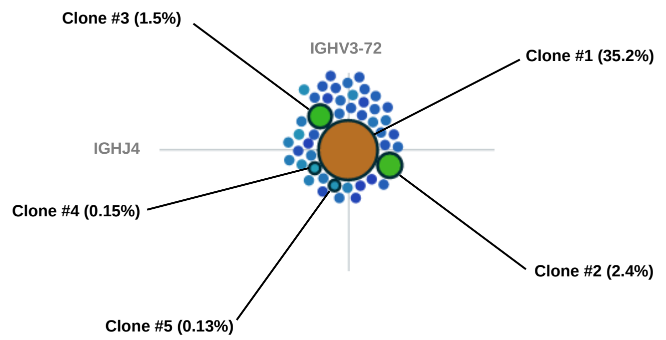


Repertoire: insights into pathogenesis of malign B cells

EuroClonality

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Clone #1  -c------------------------...------------------------------------------------------------............------------------------------------------------
Clone #2  --------a-----------------...--------------------------------------------------------a---............------------------------------------------------
Clone #3  ------------t-------------...--------------------------------------------------------a---............------------------------------------------------
Clone #4  -c------------------------...------------------------------------------------------------............------------------------------------------------
Clone #5  -c------------------------...------------------------------------------------------------............------------------------------------------------

          -------------->__________ CDR2-IMGT ________<------------------------------------------------- FR3-IMGT ---------------------------------------
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Clone #1  ------------------------------------------------------------------...-----------------g--------------a----------t-----------g-------------------------
Clone #2  -----------------------------------------------------------------g...--------------------------------a------------------------------------------------
Clone #3  -----------------------------------------t-----------------------g...------------g-------------------a----------t-------------------------------------
Clone #4  ------------------------------------------------------------------...-----------------g--------------a----------t-----------g-------------------------
Clone #5  ------------------------------------------------------------------...-----------------g--------------a----------t-----------g-------------------------

          -----------> CDR3-IMGT
          gtgtattactgtgctagaga
Clone #1  -------------------tgggatattgtactagtactagctgccgtaaccgtcttgactactggggccagggaaccctggtcaccgtctcctcag  Homsap IGHJ4*02 F
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Davi et al, Leukemia 2020

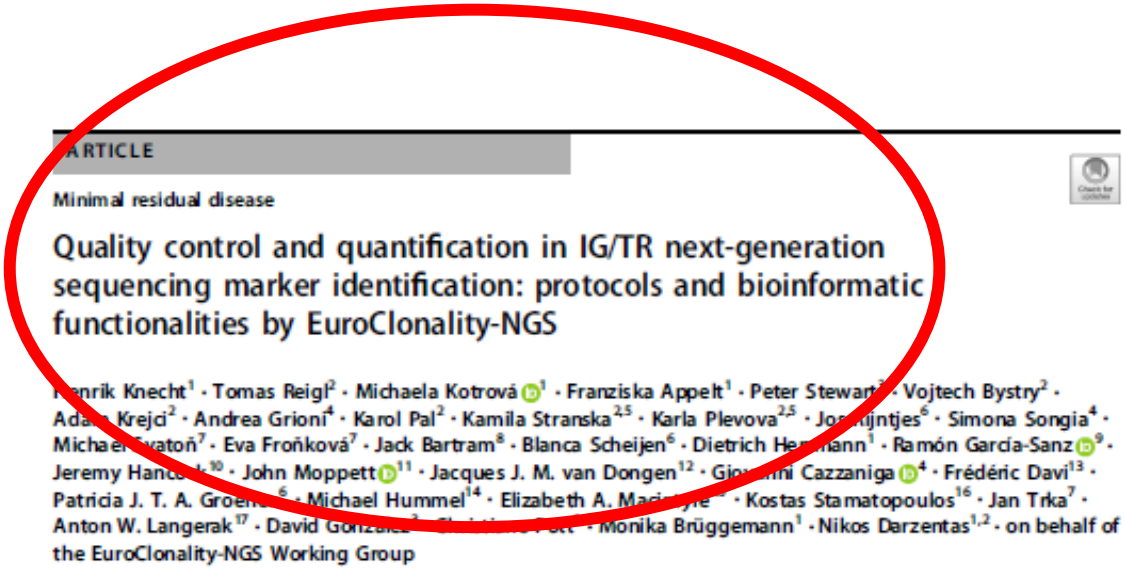
Our ongoing work …

EuroClonality

- IG clonality biological validation (~200 B cell proliferations), *submitted*
- TR clonality biological validation (~200 T cell proliferations)
- IG/TR/CNV/SNV capture validation (280 lymph.prol.), *submitted*
- 1-step IG / TR clonality in hematopathology (FFPE, FF)
- 1-step IG / TR marker screening & MRD in ALL / NHL
- microfluidics based library preparation for ALL marker screening
- leader-based IGHV analysis in CLL

- Standardization (SOPs), interpretation guidelines, training, education

Quality control samples / biol. standards



ARTICLE

Minimal residual disease

Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS

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Knecht, Leukemia 2019;33:2254; Darzentas, in preparation

What did you make?

- Strategy
- Applications

Polyclonal Target Quality Control (PT-QC)

- 1:1:1 mixture of **genomic DNA of blood MNC, thymus, tonsil,** representing a full repertoire of IG/TR rearrangements
- Application: assess performance biases or unusual amplification shifts in a sequencing run by **tracking primer distribution/usage** and comparison with **stored reference profiles**

What did you make?

- Strategy
- Applications

human B / T cell line catalogue

- ~60 **tumor cell lines** fully characterized for IG/TR rearrangements
- Application: reference catalogue

central In-tube Quality/Quantification Control (cIT-QC)

- mixture of B/T cell line DNA with **selected IG/TR rearrangements**
- Application: enables conversion from reads to cells, highlights obvious over-/under-amplification (normalizing abundance)

Pro/Con for the design

- Parameters taken into consideration
  - genomic DNA-based approach
  - gene usage (in-tube control)
  - coverage of all IG / TR (polyclonal target control)
  - concentration / copies (in-tube control)

N.B. standards developed for DNA-based approaches, but could be transformed into RNA-based immunogenetics approaches (provided that rearrangements are transcribed)

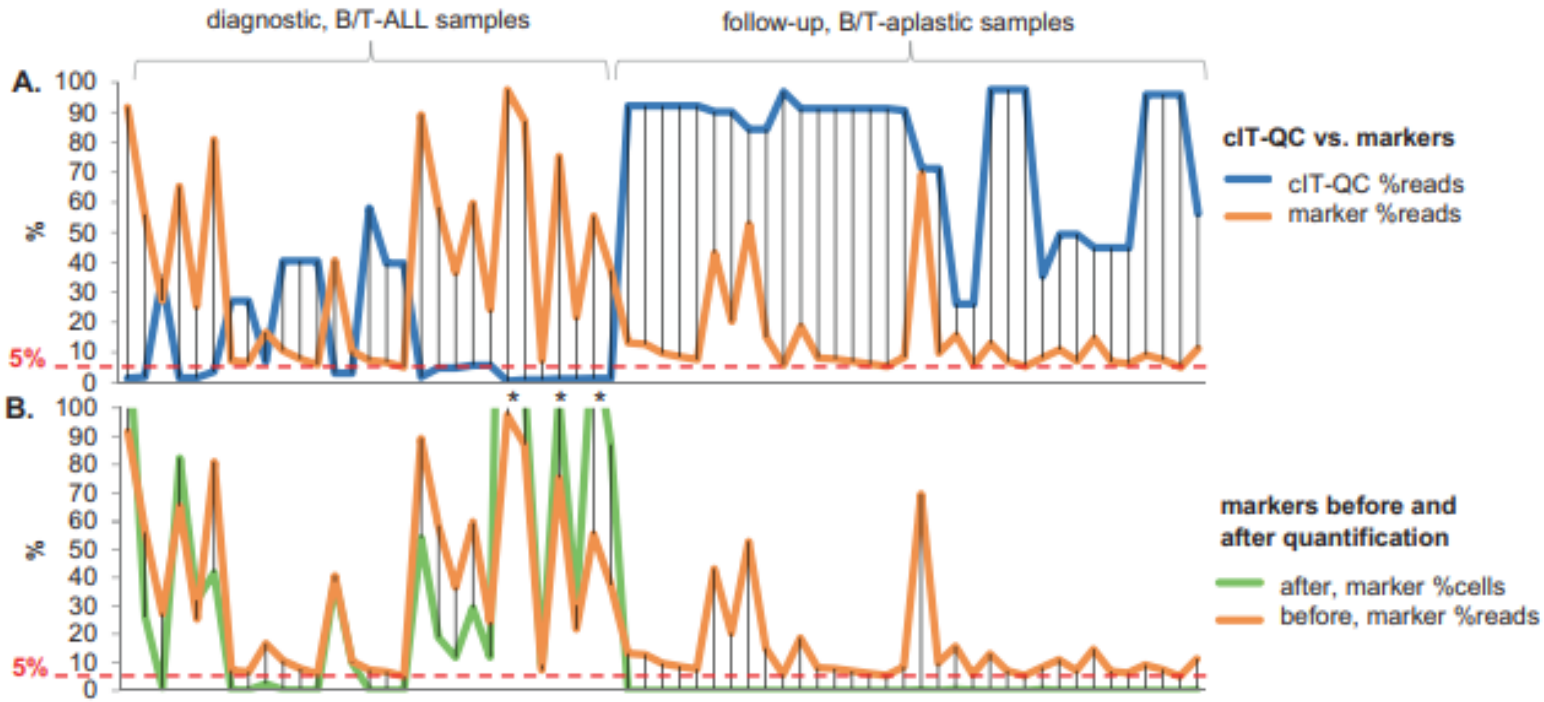
Conclusion/Results

- Quick review of key results
  - human cell line catalogue → ~60 cell lines
  - full characterization of IG/TR rearrangements by:
    - multiplex amplicon PCR-based NGS protocol
    - capture NGS protocol
    - Sanger sequencing

Darzentas, in preparation

Conclusion/Results

- Quick review of key results
  - cIT-QC: 9 cell lines (6B/3T) → 46 rearrangements
  - every target covered at least 3-4 times



Knecht, Leukemia 2019;33:2254

Lessons Learned/Recommendations

- What would you do differently, if you were to repeat this project?
  - Consider best cell lines in view of broad (commercial) availability
  - Work on standards with equimolar coverage of rearrangements