Setting standards: ERIC harmonization activities

Sarka Pospisilova

ERIC International Meeting
New frontiers in CLL Research
25-27 October 2018
Barcelona

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european research initiative on CLL

Aims of ERIC harmonization activities

- To **promote and/or standardize the assessment of IGHV gene mutational status and the *TP53* gene aberrations** for diagnostic, prognostic and predictive purposes, ultimately improving CLL patient care

- To **educate the hematological community** about the need to apply standardized and consistent methods based on the state-of-the-art, including the most innovative bioinformatics tools

- To **certify the quality of the appropriate techniques** utilized by diagnostic laboratories to ensure reliable and comparable results across different institutions in Europe and elsewhere and **provide relevant guidelines / recommendations** to the international scientific community

- To increase physicians' awareness of the need to test all CLL patients requiring therapy in order to select the **most appropriate treatment** for each case

ERIC harmonization activities

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The ERIC harmonization activities are addressed to all scientific personnel working in laboratories performing diagnostics of CLL patients

- **who have never performed the analyses in the past,**
- **who have recently introduced novel diagnostics and need reassurance on the correctness of the procedure,**
- **who are already experienced in the use of appropriate diagnostics and need official certification of quality control for applied methodologies**

ERIC harmonization activities

ERIC Networks:

- IG Network
- *TP53* Network

Currently running ERIC Certification activities:

- IG analysis (3 rounds completed, round 4 in progress)
- *TP53* mutation analysis (6 rounds completed, round 7 in progress)
- MRD: ERIC has obtained funding support to provide reagent kits for a further harmonisation project that will aim to expand access to MRD testing. This will run in parallel with a program to provide education and certification in MRD analysis.

On-line support:

- On-line Help desk for immunoglobulin gene sequence analysis
- On-line *TP53* Help desk: support for *TP53* mutation analysis

Publications of methodical recommendations: http://www.ericll.org/eric-recommendations/

- Recommendations on IGHV gene mutation analysis:

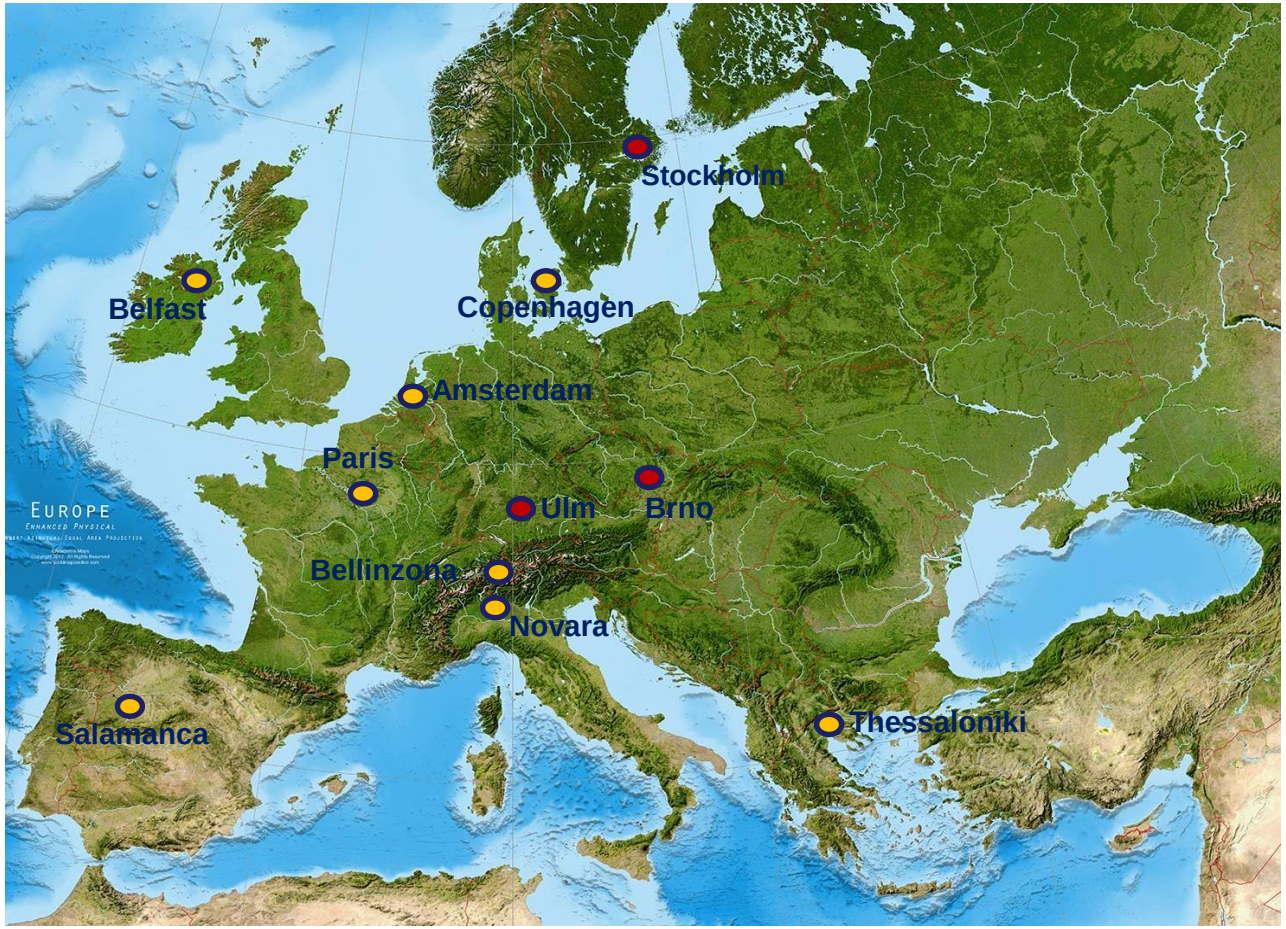
  Ghia et al., Leukemia 2007, Langerak et al., Leukemia 2011, Rosenquist et al., Leukemia 2017
- Recommendations on *TP53* gene mutation analysis:

  Pospisilova et al., Leukemia 2012, Malcikova et al., Leukemia 2018
- Recommendations on MRD analysis: *Rawstron et al., Leukemia 2007, Leukemia 2013, Leukemia 2016*

TP53 Network & *TP53* Certification

ERIC *TP53* NETWORK

The TP53 Network consists of **11 Reference Centres** and **3 Certifying Centres**



CERTIFYING CENTRES
Czech Republic, Brno
Germany, Ulm
Sweden, Stockholm

REFERENCE CENTRES
Czech Republic, Brno
Denmark, Copenhagen
France, Paris
Germany, Ulm
Greece, Thessaloniki
Italy, Novara
Spain, Salamanca
Sweden and Nordic Countries
Switzerland, Bellinzona
The Netherlands, Amsterdam
United Kingdom, Belfast

ERIC *TP53* NETWORK

european research initiative on CLL

STRUCTURE:

Training Centres

- Stephan Stilgenbauer, Eugen Tausch, Ulm, Germany
- Sarka Pospisilova, Jitka Malcikova, Sarka Pavlova, Brno, Czech Republic
- Richard Rosenquist, Lesley Ann Sutton, Stockholm, Sweden and Nordic countries
- Kostas Stamatopoulos, Thessaloniki, Greece
- Gianluca Gaidano, Novara, Italy
- Fred Davi', Paris, France
- Ramon Garcia Sanz, Salamanca, Spain
- Carsten Niemann, Copenhagen, Denmark
- Arnon Kater, Amsterdam, Netherlands
- Davide Rossi, Bellinzona, Switzerland
- David Gonzalez de Castro, Belfast, U.K.

Certifying Centres

Stephan Stilgenbauer, Eugen Tausch, Ulm, Germany
Sarka Pospisilova, Jitka Malcikova, Sarka Pavlova, Brno, Czech Republic
Richard Rosenquist, Lesley Ann Sutton, Stockholm, Sweden

For more information on the *TP53* Network:

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www.ericll.org/tp53network

TP53 Network

BACKGROUND

ERIC has a longstanding interest in the standardization and harmonization of diagnostic techniques.

READ MORE

AIMS OF THE NETWORK

ERIC aims to promote and/or advance the assessment of TP53 gene aberrations for diagnostic purposes.

READ MORE

STRUCTURE OF THE NETWORK

The TP53 Network consists of 11 Reference Centres and 3 Certifying Centres.

READ MORE

CERTIFIED CENTRES

ERIC is proud to announce that it currently has 125 certified centres in 25 different countries!

READ MORE

CERTIFICATION ROUNDS

5 certification rounds have been completed. Certification rounds are held twice a year.

READ MORE

PARTICIPATION FORM

Please complete the Participation Form which remains active for the whole year by clicking on read more.

READ MORE

ONLINE HELP DESK

If you would like to request assistance from the TP53 Help Desk, please click on read more and fill out the following form.

READ MORE

GUIDANCE TOOLS

Useful documents: Manual, TP53 Analysis Report, Certification Requirements & Free Software for Sanger Analysis Data.

READ MORE

FAQ

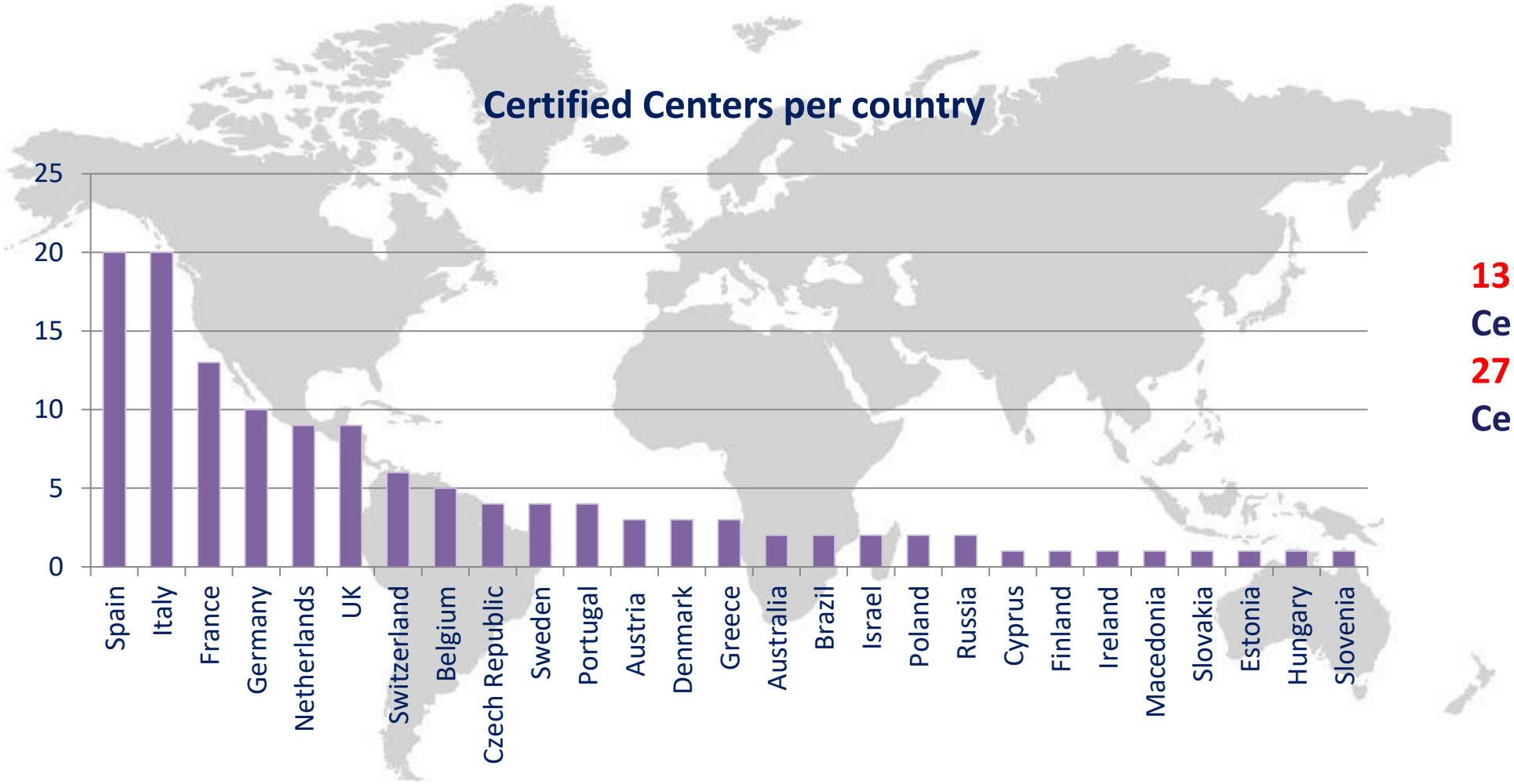
Click on read more to see Frequently asked questions about the TP53 certification process

READ MORE

TP53 Certification Rounds

- As a general rule, ERIC holds **2** certification rounds per year.
- Laboratory can certify either for Sanger sequencing or for NGS in one round.
- To participate, please complete the **Online Participation Form.**
  This remains active throughout the entire year (TP53 Network Section).
- Up to 40 labs are accepted per round. Others are prioritized in the next round (as well as those who failed).
- After confirming their participation and shipping address, laboratories obtain samples and instructions for analysis. Results should be submitted online.

TP53 Certification: 6 completed rounds



131
Certified Laboratories
27
Certified Countries

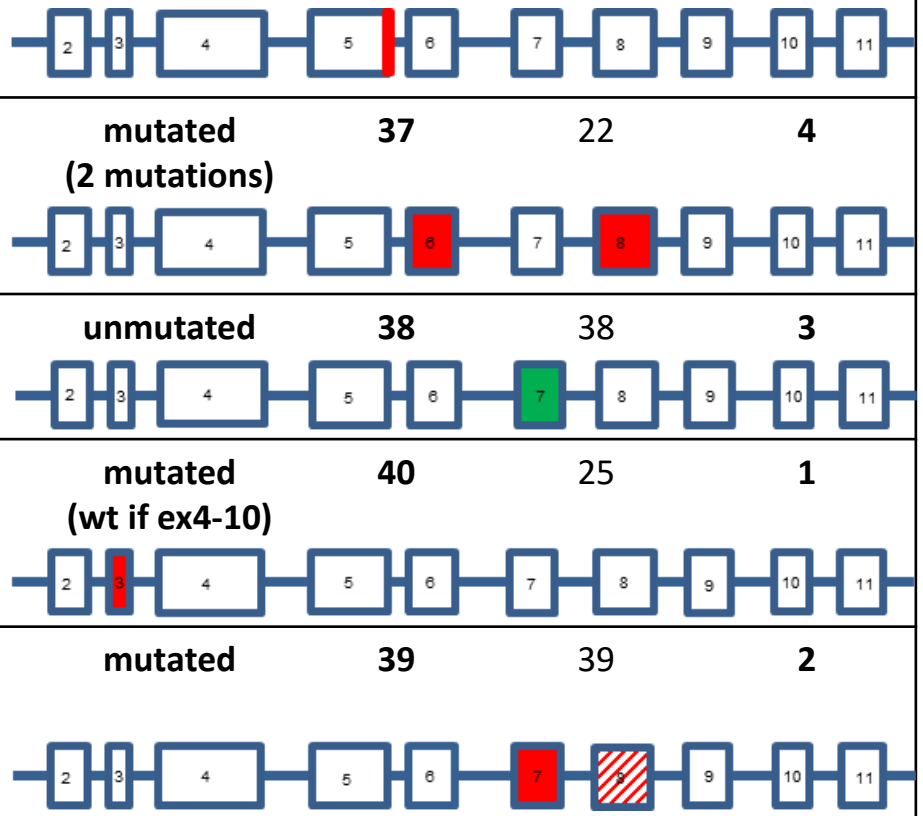
TP53 Certification Requirements

Requirement	Your result	Minimal requirements	GLP requirements
Participating in ERIC TP53 survey	✓	✓	✓
Correct identification of all TP53 variants within declared detection limit	✓	✓	✓
Correct result interpretation	✓	✓	✓
Providing lab specific report	✓	✓	✓
Covering at least exon 4-10	✓	✓	✓
Correct variant description	✓		✓
Covering at least exon 2-11	✓		✓
Analysis finished and submitted within 28 days	✓		✓

GLP = Good Laboratory Practice

TP53 Certification: Examples from Round 6

Sample	Variant	Variant type	Allelic frequency	Conclusion*	Passed	Passed GLP	Failed
1	c.559+1G>A p.?	**splice**	100%	**mutated**	**30**	28	**11**
2	c.582_583insG p.(I195fs) c.902dupC p.(G302fs)	**insertion** **duplication**	65% 29%	**mutated** **(2 mutations)**	**37**	22	**4**
3	c.704A>G p.(N235S)	**missense, functional**	50%	**unmutated**	**38**	38	**3**
4	c.78delT p.(P27fs)	**deletion**	52%	**mutated** **(wt if ex4-10)**	**40**	25	**1**
5	c.747G>C p.(R249S), c.841G>A p.(D281N) c.817C>T p.(R273C),	**missense** **missense, minor** **missense, minor**	25% 2,8% 1,3%	**mutated**	**39**	39	**2**



- *mutated ~ pathogenic/likely pathogenic variant(s)*
- *unmutated ~ benign variant or variant of unknown significance*

GOOD LABORATORY PRACTISE

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Diploma

Internal ERIC certification on the assessment of *TP53* mutations

Institution

Technique: Sanger Sequencing

PASS: The laboratory meets highest ERIC standards for TP53 mutation detection. In 5 CLL samples TP53 mutation status was assessed correctly.

1st January 2015

Date of Validation – Valid for 2 years

Prof. Emilio Montserrat
Spain

Prof. Sarka Pospisilova
Czech Republic

Dr. Eugen Tausch
Germany

Prof. Stephan Stilgenbauer
Germany

European Reference Laboratories:

University Hospital Brno,
Center of Molecular Biology and Gene Therapy,
Dept. of Internal Medicine - Hematology and Oncology,
Cernopolni 9, 625 00 Brno, CZECH REPUBLIC

Universitätsklinik Ulm
Klinik für Innere Medizin III
Albert-Einstein-Allee 23
Ulm - GERMANY

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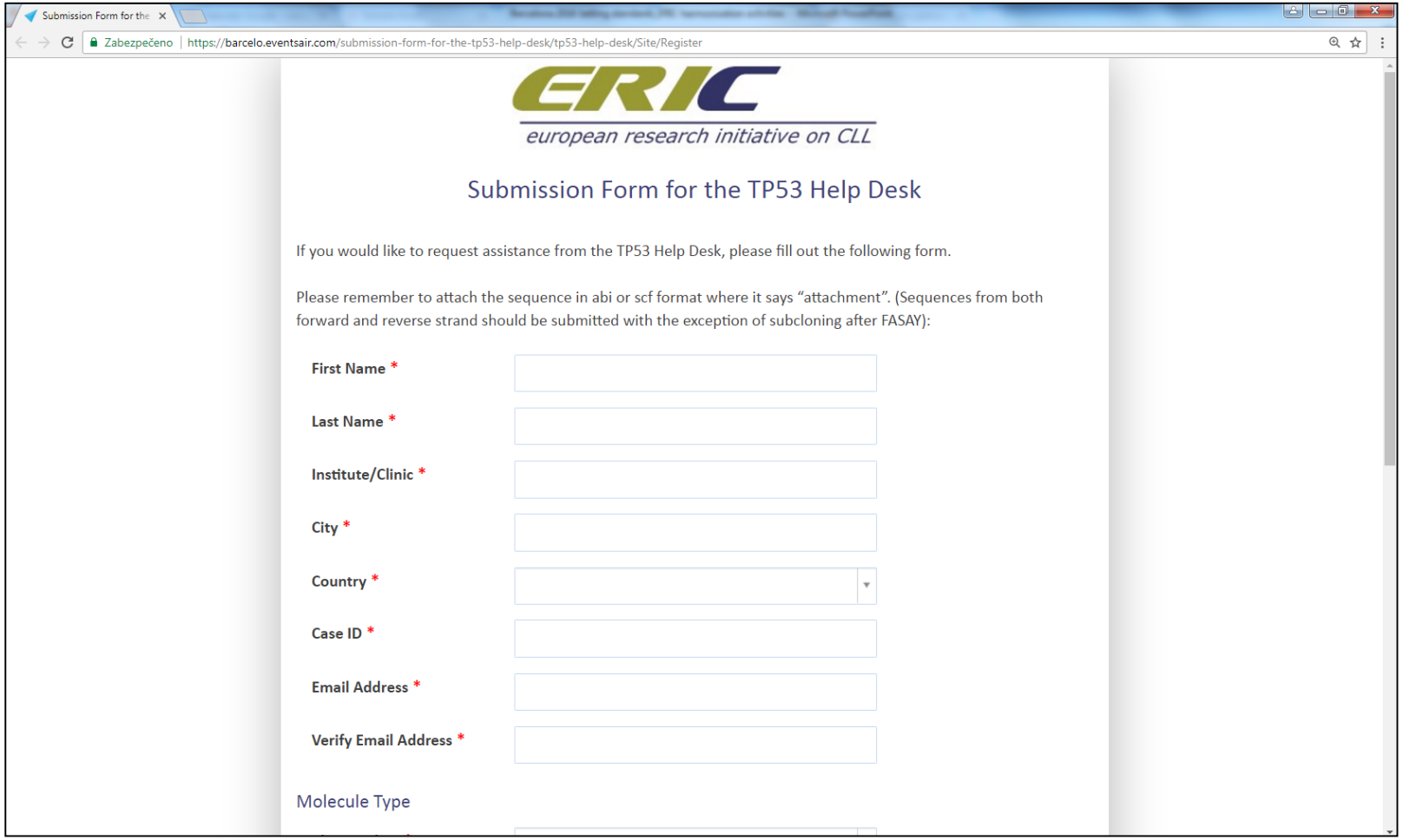
TP53 Certified Center

TP53 Certification: Round 7 and 8

- **Round 7** has already started. Participants are currently confirming their shipping details and samples will be sent soon.
- 40 participants has been accepted. Applicants that do not participate in this Round (over 40) will be given priority in the next round.
- Interested laboratories for Round 8 should complete the online participation form as soon as possible.
- **Round 8** is due to open in spring 2019. Specific information regarding this Round will be added to the ERIC website and a newsletter will be circulated to ERIC Members nearer to the date.

TP53 Help desk:
online support for TP53 mutational analysis

http://www.ericll.org/tools/



ERIC TP53 Network Project Announcement:

MULTICENTER STUDY ON PROGNOSTIC AND PREDICTIVE IMPACT OF *TP53* VARIANTS BELOW 10% VAF

Main activities:

- 1. METHODICAL HARMONIZATION
  Inter-laboratory comparison of NGS results obtained from the set of reference samples
- 2. DATA COLLECTION
  - NGS for *TP53* with detection limit at least 1%
  - Consecutive samples of CLL patients entering first-line therapy with follow-up ≥4 years

Aims:

- To compare NGS results among laboratories performing NGS detection of *TP53* mutations in CLL with detection limit of 1% VAF
- To confirm prognostic and predictive impact of low-VAF *TP53* variants in patients entering first-line treatment
- Depending on the results, possibly update recommendations on minor *TP53* variant detection, validation and reporting
- Recognize factors affecting expansion of *TP53* mutations (IGHV status, therapy, VAF, cytogenetics)

For further information → ERIC office or pospisilova.sarka@fnbrno.cz

IG Network & IG Certification

ERIC IG Network

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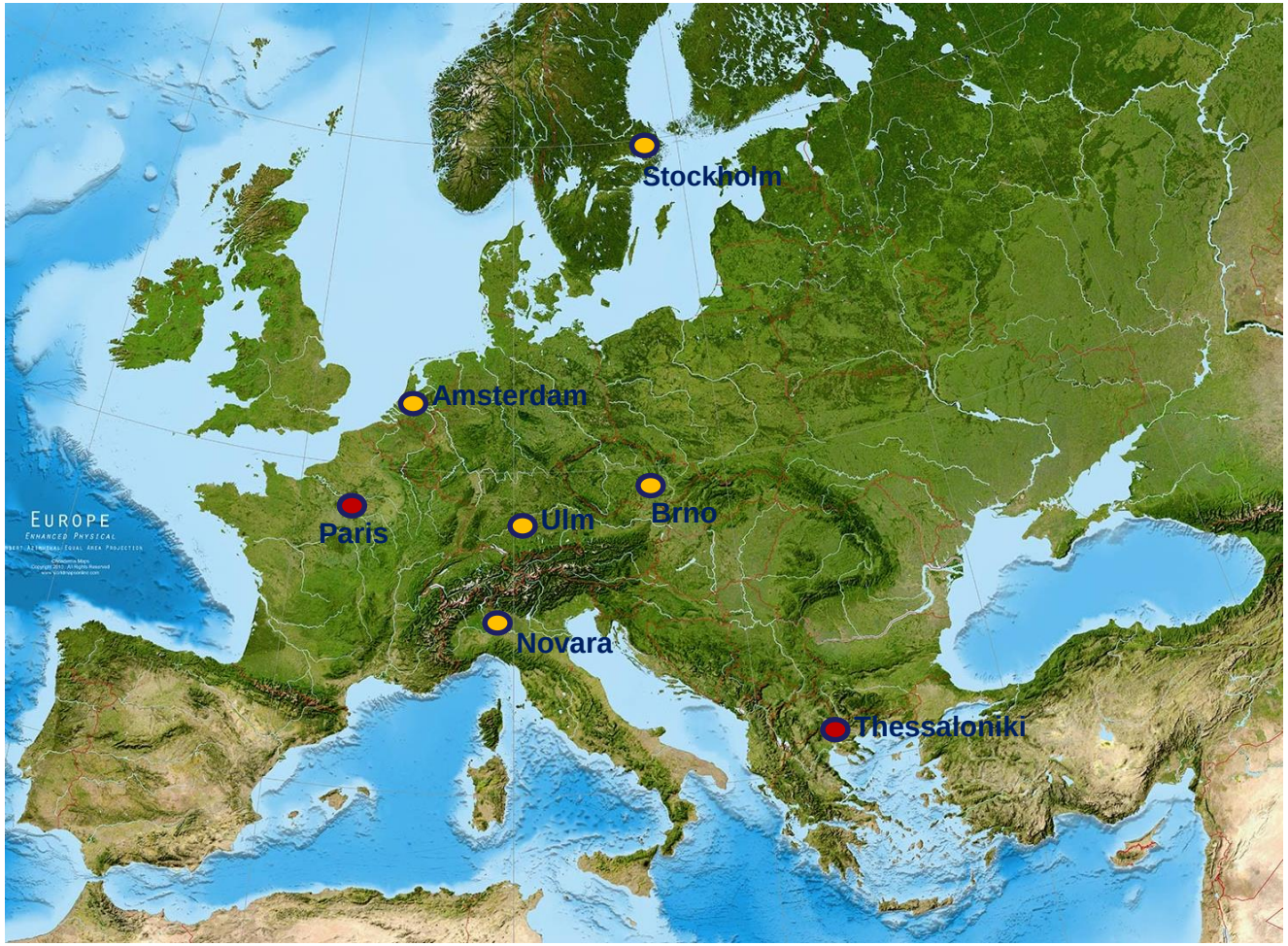
IG Network consists of **7 Reference Centres** and **2 Certifying Centres**



- **CERTIFYING CENTRES**
  Greece, Thessaloniki
  France, Paris

- **REFERENCE CENTRES**
  Greece, Thessaloniki
  France, Paris
  Czech Republic, Brno
  Germany, Ulm
  Italy, Novara
  The Netherlands, Amsterdam
  Sweden and Nordic Countries, Stockholm

Summary of IG Certification Rounds 1-4

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IG Certified Center

Round 1
38 Labs Passed
12 Labs Failed

Round 2
29 Labs Passed
3 Labs Failed

Round 3
15 Labs Passed
8 Labs Failed

Round 4
Evaluation in progress

80 Certified Centres

23 Unsuccesful centres

29 Certified Countries

Unsuccessful centres *- some centres failed in more than one round*
Certified centres *- some centres certified in more than one round*

Certified Centres in 29 Countries

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IG Certified Center

We have certified laboratories in five different continents!
Asia, Australia, Europe, North America and South America.

ARGENTINA	AUSTRALIA	AUSTRIA	BELGIUM
CYPRUS	CZECH REPUBLIC	DENMARK	FINLAND
FRANCE	GERMANY	GREECE	HUNGARY
IRELAND	ISRAEL	ITALY	MACEDONIA
NORWAY	POLAND	PORTUGAL	RUSSIA
SLOVAKIA	SLOVENIA	SPAIN	SWEDEN
SWITZERLAND	TAIWAN	THE NETHERLANDS	THE UNITED KINGDOM
THE UNITED STATES			

Update on Round 3 of IGHV Certification

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IG Certified Center

Project details: Project lead by: CERTH/INAB (Greece)

Original Applicants: 26

Participating centers: 23

Number of participating countries: 10

TP53 Certification: Example from Round 3

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IG Certified Center

Sample ID	IGHV gene and allele	IGHD gene and allele	IGHJ gene and allele	IGHV gene % identity to germline	SHM status	Comments
ERIC 3-1	IGHV3-21*01	IGHD3-16*02	IGHJ6*02	97.2	borderline	stereotyped subset #2
ERIC 3-2	IGHV1-69*06	IGHD3-3*01	IGHJ6*06	100	unmutated	
ERIC 3-3	IGHV4-34*02	IGHD5-24*01	IGHJ6*02	92.6	mutated	stereotyped subset #4
ERIC 3-4	IGHV4-39*01	IGHD6-13*01	IGHJ5*02	100	unmutated	stereotyped subset #8
ERIC 3-5	IGHV3-15*01	IGHD6-13*01	IGHJ4*02	98.3	unmutated	

IG Certification Requirements: Example from Round 3

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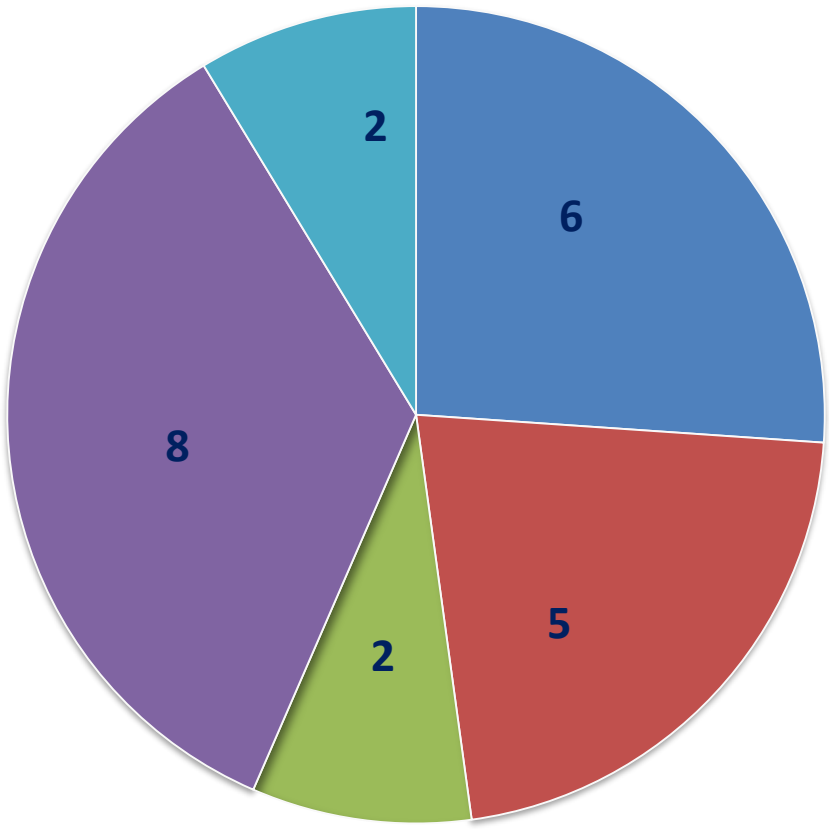
IG Certified Center

Accreditation requirements and your result	Minimal requirements	Good laboratory practice requirements
Participating in ERIC IG survey	✓	✓
Covering VH FR1 – VH CDR3 of all samples	✓	✓
Raise caution about the borderline somatic hypermutation status of ERIC3-1	✓	✓
Identification of sample ERIC3-1 as belonging to stereotyped subset #2	✓	✓
Identification of sample ERIC3-2 as IGHV-unmutated	✓	✓
Identification of sample ERIC3-3 as IGHV-mutated	✓	✓
Identification of sample ERIC3-3 as belonging to stereotyped subset #4	✓	✓
Identification of sample ERIC3-4 as IGHV-unmutated	✓	✓
Identification of sample ERIC3-4 as belonging to stereotyped subset #8	✓	✓
Identification of sample ERIC3-5 as IGHV-unmutated	✓	✓
Correct annotation	✓	✓
Provided Lab specific report	✓	✓
Correct interpretation	✓	✓
Finished and submitted analysis before the deadline		✓

IG Certification Results: Example from Round 3



Number of applicants

- Passed
- Failed
- Provisionally passed
- Repeat Analysis
- Repeat FASTA sequence

PROVISIONALLY PASSED

2 labs were asked to send us their revised lab reports within 1 month
All passed

REPEAT ANALYSIS

8 labs were asked to repeat analysis
6 passed
2 failed

REPEAT FASTA SEQUENCES

2 labs were asked to send us the FASTA sequences
1 passed
1 sent sequences but did not complete analysis

FINAL RESULTS of ROUND 3

Passed: 15

Failed: 8

Total: 23

IG Certification - Application to Round 4

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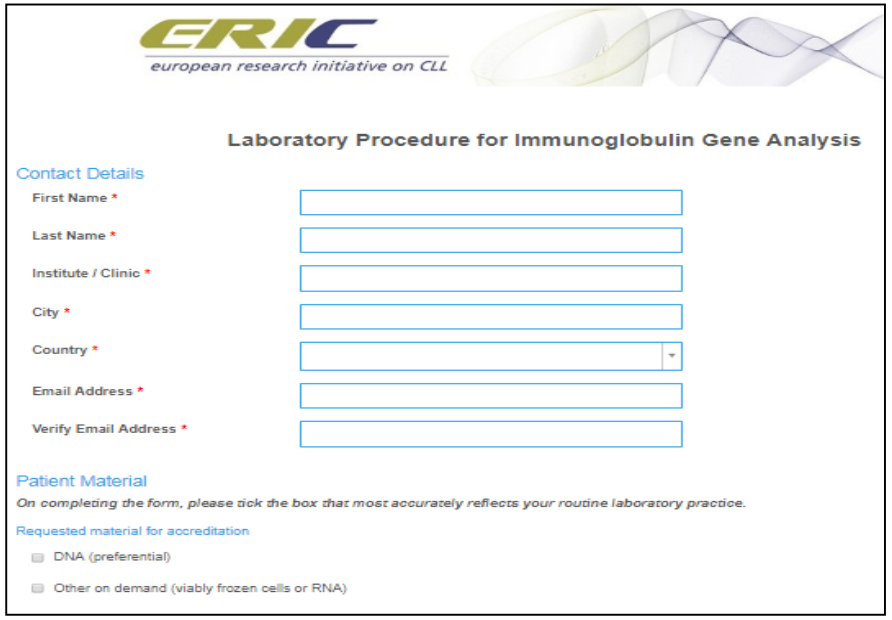
IG Certified Center

Project to be lead by: CERTH/INAB

Deadline to apply: Please complete the Online Participation Form on the IG Network Section which remains active for the whole year

For further information: http://www.ericll.org/ignetwork/



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Laboratory Procedure for Immunoglobulin Gene Analysis

Contact Details

First Name *

Last Name *

Institute / Clinic *

City *

Country *

Email Address *

Verify Email Address *

Patient Material

On completing the form, please tick the box that most accurately reflects your routine laboratory practice.

Requested material for accreditation

- DNA (preferential)
- Other on demand (viably frozen cells or RNA)

Online support
for immunoglobulin gene sequence interpretation

http://www.ericll.org/tools/

ERIC workshops and meetings on harmonization activities

Recent Regional Educational Workshops Organized by ERIC

- Precision medicine in chronic lymphocytic leukemia: What is the role of biomarkers?
  Tel Aviv | July 04, 2018

- Biomarker-guided management of chronic lymphocytic leukemia
  Moscow | April 13, 2018

- Biomarkers in chronic lymphocytic leukemia: the art of synthesis
  Belgrade | March 16-17, 2018

Belgrade

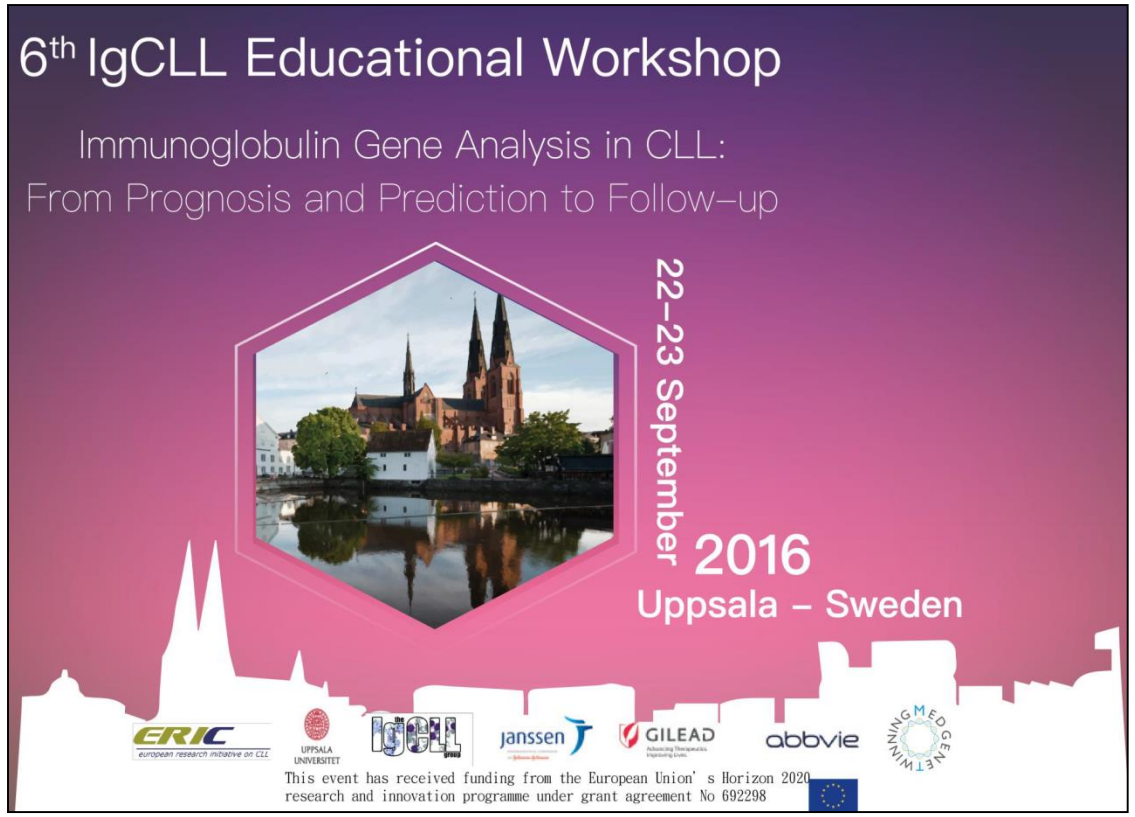
Moscow

ERIC Educational Workshops on *IGHV* analysis in CLL

- Immunogenetics in chronic lymphocytic leukemia in the NGS era
  Single-day Workshop organized by ERIC and Euroclonality-NGS
  Rotterdam | 24 November 2017

- ERIC Workshop on IG Sequence Analysis
  Tel Aviv | 23 May 2017

- 6th Educational IGCLL workshop, Uppsala,
  Uppsala | 22-23 September 2016



ERIC Educational Workshops on *TP53* analysis in CLL

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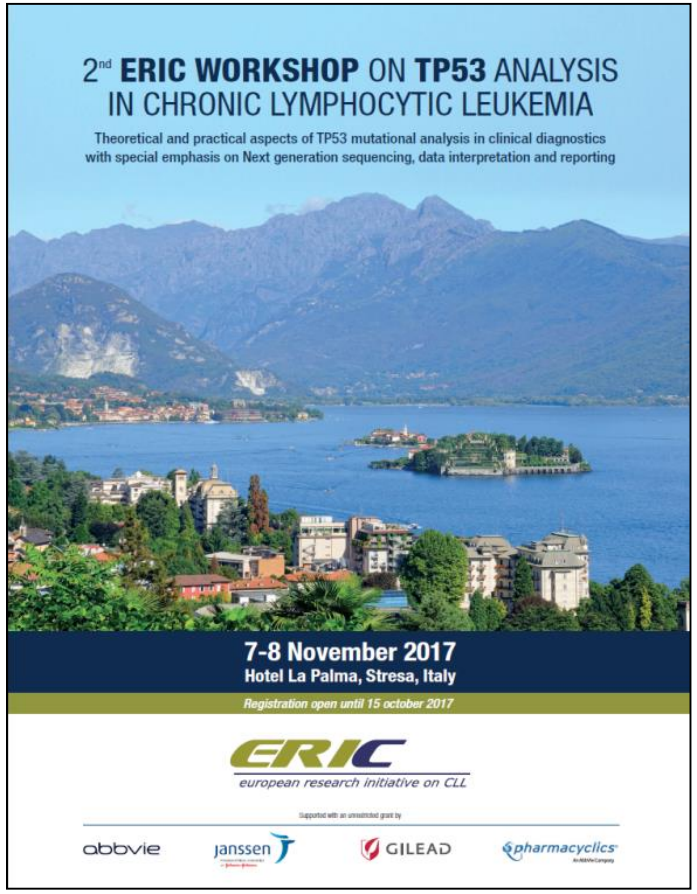
1st ERIC Workshop on TP53 analysis in CLL (Brno, CZ, 2015)

2nd ERIC Workshop on TP53 analysis in CLL (Stresa, IT, 2017)



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TP53 Certified Center

Paolo Ghia

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Arnon Kater

Carol Moreno

Emili Montserrat

ERIC office

THANK YOU VERY MUCH FOR YOUR ATTENTION !