TP53 Certification – overview and results of round 3

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ERIC ACTIVITIES ON TP53

Project on TP53 mutation analysis certification

ERIC TP53 network
ERIC Research Projects

Comprehensive profiling and analysis of TP53 mutation in CLL


Educational activities to standardize and improve the detection of TP53 gene aberrations in CLL patients in European hematological centers

AIMS:
The ERIC aims at promoting the assessment of TP53 gene aberrations for diagnostic purposes by educating the hematological community about
1) the need of performing TP53 analysis in all cases needing therapy
2) the quality of the appropriate techniques to be utilized in Diagnostic Laboratories to ensure reliable and comparable results among different institutions – TP53 CERTIFICATION

WORKING ACTIVITIES:
1) Face-to-face educational activities
A. Hands-on TP53 workshops – introduction of scientific rationale, basic principles and practical advices on TP53 analysis
B. Network of Reference laboratories - supervision and advice to single laboratories needing to set-up, improve or consolidate the diagnostic techniques for TP53 gene aberrations assessment

2) Online educational activities (www.ericll.org)
A. On-line Help desk about all aspects of the analytical procedure, from sample collection to lab report - permanent platform on the ERIC website
B. Manual on TP53 mutational analysis
C. TP53 Certification – application form and Frequently Asked Questions
ERIC TP53 NETWORK

- U.K.
- Paris
- Salamanca
- Novara
- Uppsala
- Copenhagen
- Amsterdam
- Brno
- Bellinzona
- Thessaloniki
ERIC TP53 NETWORK

STRUCTURE:

Training Centres
- Sarka Pospisilova, Jitka Malcikova, Brno, Czech Republic
- Stephan Stilgenbauer, Eugen Tausch, Ulm, Germany
- Fred Davi’, Paris, France
- Kostas Stamatopoulos, Thessaloniki, Greece
- Gianluca Gaidano, Novara, Italy
- Ramon Garcia Sanz, Salamanca, Spain
- Richard Rosenquist, Uppsala, Sweden and Nordic countries
- Carsten Niemann, Copenhagen, Denmark
- Arnon Kater, Amsterdam, Netherlands
- Davide Rossi, Bellinzona, Switzerland
- United Kingdom - pending

Certifying Centres
Sarka Pospisilova, Jitka Malcikova, Brno, Czech Republic
Stephan Stilgenbauer, Eugen Tausch, Ulm, Germany
1) Organization of future **TP53 certification** rounds

2) Organization of future **TP53 workshops** (2017)

3) **ERIC TP53 web tools**: www.ericll.org – Networks – TP53 Network
   a) FAQ on **TP53 certification**
   b) Helpdesk (ERIC office + expert in charge)
   c) Manual for **TP53 mutational analysis**

4) **CLL - TP53 mutation database** (in collaboration with T. Soussi)
   in connection with CLL Minimal Dataset Project
ERIC Project on TP53 analysis certification

- For all laboratories performing the TP53 analysis in CLL - validate different techniques on TP53 mutation analysis
- Support introduction of TP53 analysis in labs with less experience (to provide assurance of the results quality)
- Discuss the problematic issues and give individual recommendations
- Improve analysis harmonization and interaction between centers
- Provide an official document on the quality control assessment
ERIC certified Centers from all 3 rounds

Round 1: **30**  
Round 2: **16**  
Round 3: **29**  

(3 centers participated in 2 rounds with different techniques)  

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**Total Number of Certified Centers:** 72
ERIC certified Centers – 3 Rounds

72 Centers from 20 Countries
Diploma

Internal ERIC certification on the assessment of TP53 mutations

University Hospital Brno, Czech Republic

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 3 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,
Cernigolova 5, 625 00 Brno, CZECH REPUBLIC

Universitätsklinik Ulm
Klinik für Innere Medizin II
Albert-Einstein-Allee 23
Ulm - GERMANY
TP53 Certification on TP53 mutation analysis

Round 1

Samples shipped from by the University of Ulm, Germany, 2014

Total Number of Participating Centres: 37 centers delivered results in 3 months
Total Number of Participating Countries: 15
Total Number of Centers which passed: 30
Total Number of Centers which failed: 7
TP53 certification Round 1 - Sample Selection

Sample 1
- 2 3 4 5 6 7 8 9 10 11
- c.517G>T VF=47%
- WT

Sample 2
- 2 3 4 5 6 7 8 9 10 11
- c.715A>G VF=44%
- MUT

Sample 3
- 2 3 4 5 6 7 8 9 10 11
- c.370T>C VF=38%
- c.811G>T VF=41%
- MUT

Sample 4
- 2 3 4 5 6 7 8 9 10 11
- c.1028delA VF=93%
- MUT

Sample 5
- 2 3 4 5 6 7 8 9 10 11
- MUT
Round 1 - Centers with successful TP53 mutation analysis

**Australia**
East Melbourne, Peter MacCallum Cancer Centre

**Austria**
Vienna, Clinical Institute for Laboratory Medicine

**Czech Republic**
Brno, University Hospital Brno

**Denmark**
Copenhagen, Rigshospitalet

**France**
Bobigny, Hopital Avicenne
Clermont Ferrand, Chu Estaing
Créteil, Hopital Henri Mondor
Toulouse, CHU

**Germany**
Cologne, University at Cologne
Göttingen, Klinik für Hämatologie und Onkologie
Hannover, Hannover Medical School

**Greece**
Thessaloniki, Papanikolaou Hospital
Thessaloniki, INAB-CERTH

**Italy**
Bologna, University of Bologna
Brescia, lab. Biotecnotecnie
Milano, Molecular Pathology Unit
Milano, Ospedale San Raffaele
Novara, University of Eastern Piedmont
Pavia, Fondazione IRCCS Policlinico San Matteo
Perugia, University of Perugia
Vinzenca, S. Bortolo Hospital

**Spain**
Salamanca, University Hospital of Salamanca

**Sweden**
Solna, Karolinska University Hospital
Stockholm, Karolinska University Hospital
Uppsala, Clinical Genetics

**United Kingdom**
Edgbaston, Birmingham Women’s Hospital
Leicester, University of Leicester
Liverpool, University of Liverpool, Sanger
Oxford, Oxford University Hospitals NHS Trust
Sutton, The Royal Marsden NHS Foundation Trust

*Some centers sent more than one set of results as they performed two techniques (University of Liverpool and Papanikolaou Hospital Thessaloniki)*.
TP53 Certification - Round 2

Samples shipped from the University of Ulm, Germany, 2015

Total Number of Participating Centres: 27
Total Number of Participating Countries: 16
Total Number of Centers which passed: 16
Total Number of Centers which failed: 9
Total Number of Centres which didn’t send results: 2

Samples Sent to Participants: November 11, 2015
Deadline to Receive Reports: January 15, 2016
**TP53 certification Round 2 - sample selection**

- **Sample 1**
  - c.376G>A (p.Gly266Arg) VAF=80%
  - MUT

- **Sample 2**
  - c.796G>A (p.Gly266Arg) VF=60%
  - WT

- **Sample 3**
  - c.701A>G (p.Y234C) VAF=100%
  - MUT

- **Sample 4**
  - c.659A>G (p.Y220C) VAF=20%
  - MUT

- **Sample 5**
  - c.701A>G (p.Y234C) VAF=100%
  - MUT
Centers with successful TP53 mutation analysis

**Belgium**
Lieve, CHU Liege

**Denmark**
Aalborg, Aalborg University Hospital, Institute of Pathology

**France**
Paris, Hôpitaux Universitaires Pitié Salpêtrière

**Germany**
Munich, MLL Munich Leukemia Laboratory, Molecular Genetics

**Greece**
Athens, University of Athens, Hematology Lab, APPK Clinic, Laikon Hospital

**Italy**
Rome, Sapienza University, Dept. of Cellular Biotechnologies and Hematology

**Macedonia**
Skopje, UKIM - Faculty of Pharmacy

**Netherlands**
Amsterdam, Academic Medical Center, University of Amsterdam, Cytogenetics Department

**Russia**
Moscow, National Hematology Research Center, Molecular Hematology

**Slovakia**
Bratislava, National Cancer Institute, Department of Genetics

**Switzerland**
IOSI - Oncology Institute of Southern Switzerland

**Spain**
Barcelona, Vall d'Hebron University Hospital, Hematology
Madrid, Hospital Universitario 12 de Octubre, Hematology
Valencia, Hospital Clínico - Incliva, Lab Hematología - UGDG

**United Kingdom**
Belfast, Belfast City Hospital
Sutton, The Royal Marsden NHS FT, Molecular Diagnostics
TP53 Certification - Round 3

Conducted by the University Hospital Brno, Czech Republic, 2015

35 centres participated (from 17 countries)
37 results sent
2 centers participated with 2 methods

Samples Sent to Participants: March 15, 2016
Deadline to Receive Reports: May 15, 2016
Round 3 - Breakdown of Participants by Country

Number of Participants

- France: 6
- Italy: 4
- Spain: 4
- Germany: 3
- Poland: 2
- Switzerland: 2
- Sweden: 2
- Netherlands: 2
- Czech Republic: 2
- Slovenia: 2
- Russia: 1
- Portugal: 1
- Ireland: 1
- Greece: 1
- Belgium: 1
- Austria: 1
- Australia: 1
Round 3 - sample selection

Sample 1

Sample 2

Sample 3

Sample 4

Sample 5

c.560-2A>G  p.?; VAF=53%
c.949C>T  p.Q317*; VAF=98%
Polymorphism c.639A>G p.R213R; VAF=50%
c.337T>G  p.F113V; VAF=80%
c.586C>T  p.R196*; VAF=25%
c.830G>T  p.C277F; VAF=35%
Certification requirements

Sample 1
- c.560-2A>G p.?; VAF=53%

Sample 2
- c.949C>T p.Q317*; VAF=98%

Sample 3
- Polymorphism c.639A>G p.R213R; VAF=50%
- c.337T>G p.F113V; VAF=80%

Sample 4
- c.586C>T p.R196*; VAF=25%
- c.830G>T p.C277F; VAF=35%

Sample 5
- c.639A>G p.R213R; VAF=50%
- c.586C>T p.R196*; VAF=25%
Round 3 - Methods used

**Round 1**
- Sanger; 23
- NGS; 1
- SCA+ Sanger; 1
- [CELLREF]; [VALUE] FASAY + Sanger; 3
- dHPLC + Sanger; 1
- HRM + Sanger; 1

**Round 2**
- NGS; 6
- Sanger + MLPA; 1
- HRM + Sanger; 3

**Round 1**
- NGS; 14
- Sanger; 18
- dHPLC + Sanger; 1
- + Sanger; 2
- HRM

**Round 2**
- NGS; 1
- Sanger; 16
- Sanger + MLPA; 1
- HRM + Sanger; 3
Variant assment was conducted for 16 samples.

- **Passed (12 samples):**
  - Good laboratory practice
  - Only one mutation in sample 5 detected (2 samples)

- **Failed (4 samples):**
  - Splice site mutation not detected
  - Other errors (3 samples)

**Sample 1**
- Variant: c.560-2A>G p.?; VAF=53%

**Guidelines:**
- Variants in introns at positions +1/+2 or -1/-2 impair splicing and are considered pathogenic.
- Check the character of variants via IARC TP53 (or COSMIC) database.
Centers with successful TP53 mutation analysis

**Australia**
South Australia, SA Pathology

**Austria**
Linz, Elisabethinen Hospital Linz

**Czech Republic**
Olomouc, University Hospital Olomouc
Novy Jicin, Laboratore AGEL a.s.

**France**
Bobigny, Hopital Avicenne
Basse Normandie, CHU Caen
Nantes, CHU de Nantes
Montpellier, CHU Montpellier - Hopital St Eloi
Strasbourg, Strasbourg Hospital
Reims, CHU Hopital Robert Debré

**Germany**
Hanover, Hannover Medical School
Ulm, Ulm University
Cologne, Labor Dr. Wisplinghoff

**Italy**
Ferrara, University of Ferrara
Nuoro, San Francisco Hospital, ASL Nuoro
Aviano, IRCCS CRO
Torino, SC Anatomia e Istologia Patologica 1U

**Netherlands**
Maastricht, Maastricht University Hospital
Nijmegen, Radbound Uni Nijmegen Medical Centre

**Poland**
Warsaw, Medical University of Warsaw

**Portugal**
Lisbon, Portuguese Institute of Oncology of Lisbon

**Russia**
Saint Petersburg, Almazov North-West Federal Research Center

**Slovenia**
Llubljana, University Medical Centre Llubljana

**Spain**
Valencia, Hospital Clínico Universitario
Madrid, Hospital Universitario Puerta de Hierro-Majadahonda
Barcelona, Hospital de Sant Pau

**Sweden**
Goteborg, Sahlgrenska University Hospital

**Switzerland**
Lausanne, CHUV
Bern, University Hospital Inselspital Bern
### Individual feedback

<table>
<thead>
<tr>
<th></th>
<th>Your result</th>
<th>Minimal requirements</th>
<th>Good laboratory practice requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participating in ERIC TP53 survey</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Identification of sample 2 as unmutated / TP53 wt</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Identification of sample 1, 3, 4 and 5 as mutated</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Provided lab specific report</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Covering at least exon 4-9</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Correct variant annotation</td>
<td>✗</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Covering at least exon 4-10</td>
<td>✗</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Finished and submitted analysis within 28 days</td>
<td>✗</td>
<td></td>
<td>✔</td>
</tr>
</tbody>
</table>

- Individual feedback will be sent after EHA
- Presentation with results’ overview will be available on the ERIC web
- Centers that failed will have an opportunity to re-apply in the round 4
TP53 Certification – Round 4
Samples will be provided from University Hospital Brno, CZ

Deadline for applications: September 15, 2016
Samples Sent to Participants: October 15, 2016
Deadline to Receive Reports: December 15, 2016
Results Available: January 2016 (report at ELN meeting)

• Material to be sent: DNA
• Certified methods: either Sanger sequencing or NGS, not two methods in one round
• Number of participants in each round will be restricted to 40
• The supposed frequency of rounds is two per year
TP53 Certification – Round 4 - APPLICATION

- Questionnaire at [www.ericll.org](http://www.ericll.org) – Services – TP53 analysis Certification
- Applications are collected continuously

TP53 Analysis Certification

- Participation Form
- TP53 Certification Requirements
- Frequently asked questions about the TP53 certification process
ACCREDITATION OF TP53 MUTATION ANALYSIS - PARTICIPATION FORM

PERSONAL INFORMATION

Complete the requested details below.

* Applicant details
  Full Name:
  Institution:
  Department:
  Address Line 1:
  Address Line 2:
  City/Town:
  State/Province:
  Country:
  Email Address:
  Phone Number:

Next
Paolo Ghia
Emili Montserrat
Carol Moreno
Eva Kimby
Arnon Kater
Kostas Stamatopoulos
ERIC office: Martin Fox, Natalie Sorolla

Eugen Tausch
Stephan Stilgenbauer

http://www.ericll.org/
Thank you for your attention!
Ongoing ERIC meetings at EHA 2016

**ERIC TP53 Network Meeting:** Thursday June 9, 17:15 - 18:15 Room H5 at Bella Center

**EHA - ERIC Scientific Working Group Session:** CLL Genetics and beyond
Sunday June 12, 11:45 - 12:45, Hall C15 at Bella Center