european research initiative on CLL

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

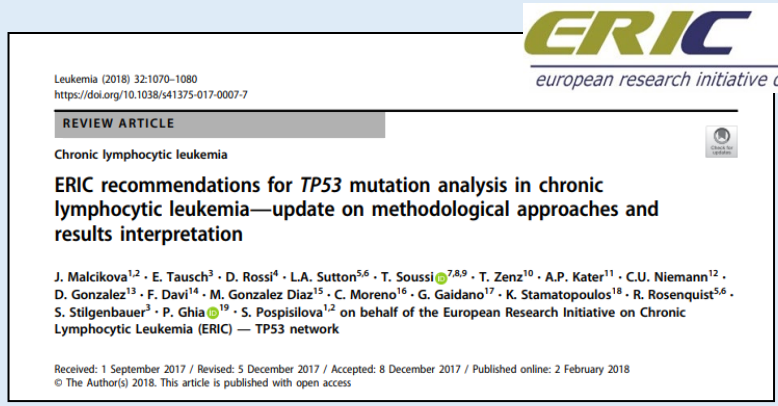
49th General Assembly of ERIC Members at EHA

25th EHA Annual Congress

June 11, 2020

BACKGROUND

Should we decrease 10% (5%) cut-off for reporting TP53 mutations?
If yes, how much?



PROJECT AIMS

TECHNICAL ASPECT – WHAT IS A SAFE CUT-OFF FOR RELIABLE DETECTION IN ROUTINE DIAGNOSTICS?

- To compare NGS results among laboratories performing NGS detection of *TP53* mutations in CLL with detection limit of 1% VAF

CLINICOBIOLOGICAL ASPECT – WHAT IS THE LOWEST SIZE OF MUTATED CLONE THAT BRINGS HARM TO THE PATIENT IF NOT PROPERLY TREATED?

- To confirm prognostic impact of low-VAF *TP53* variants in patients entering first-line treatment, both for unmut-IGHV and mut-IGHV

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TECHNICAL ASPECT – WHAT IS A SAFE CUT-OFF FOR RELIABLE DETECTION IN ROUTINE DIAGNOSTICS?

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HOW TO

PHASE 1:

Inter-laboratory comparison of NGS results obtained from the set of reference samples = METHODICAL HARMONIZATION

PHASE 2:

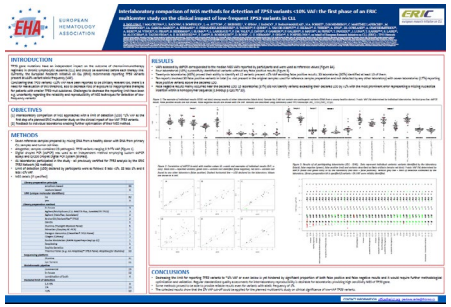
DATA COLLECTION - results of TP53 analysis using NGS with detection limit ~1% VAF

Required data: Results of TP53 analysis using NGS methodology with detection limit ~1% VAF. Corresponding clinical and laboratory data (disease course and routine prognostic markers)

RESULTS OF PHASE 1:

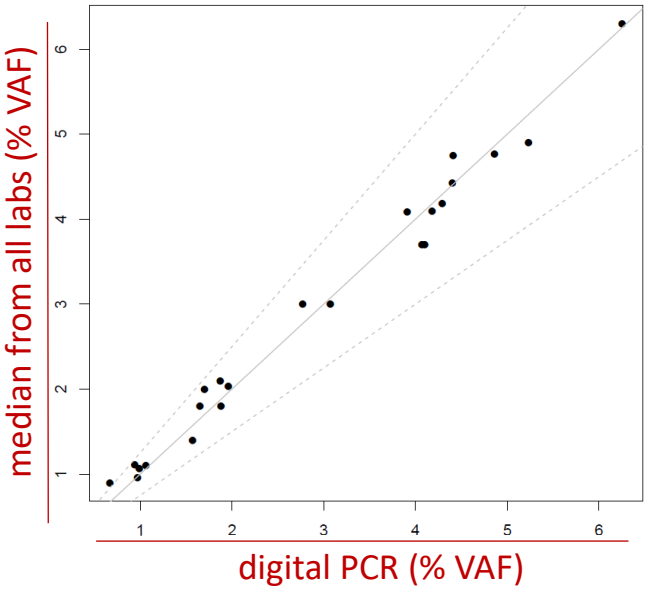
Inter-laboratory comparison of NGS results obtained from the set of reference samples

Abstract/E-poster No. EP659



sample	variant (c)	variant (p)	ddPCR [%]
mTP53_1	c.166G>T	p.(E56*)	3.9
	c.524G>A	p.(R175H)	4.3
	c.580C>T	p.(L194F)	4.1
	c.743G>A	p.(R248Q)	2.8
	c.853G>A	p.(E285K)	4.2
mTP53_2	none	none	-
mTP53_3	c.166G>T	p.(E56*)	0.9
	c.524G>A	p.(R175H)	1.1
	c.580C>T	p.(L194F)	1.0
	c.743G>A	P.(R248Q)	0.7
	c.853G>A	p.(E285K)	1.0
mTP53_4	c.173del	p.(P58Qfs*65)	6.3
	c.949dup	p.(Q317fs*20)	5.2
mTP53_5	c.559+1G>A	p.?	1.7
mTP53_6	c.173del	p.(P58fs*65)	1.9
	c.949dup	p.(Q317fs*20)	1.6
mTP53_7	c.337T>G	p.(F113V)	2.0
	c.569C>T	p.(P190L)	4.4
	c.626_627del	p.(R209fs*6)	4.1
	c.672+1G>T	p.?	4.4
	c.685_689del	p.(C229fs*9)	3.1
	c.741_742delinsTT	p.(R248W)	4.9
	c.817C>T	p.(R273C)	1.9
	c.949C>T	p.(Q317*)	1.7

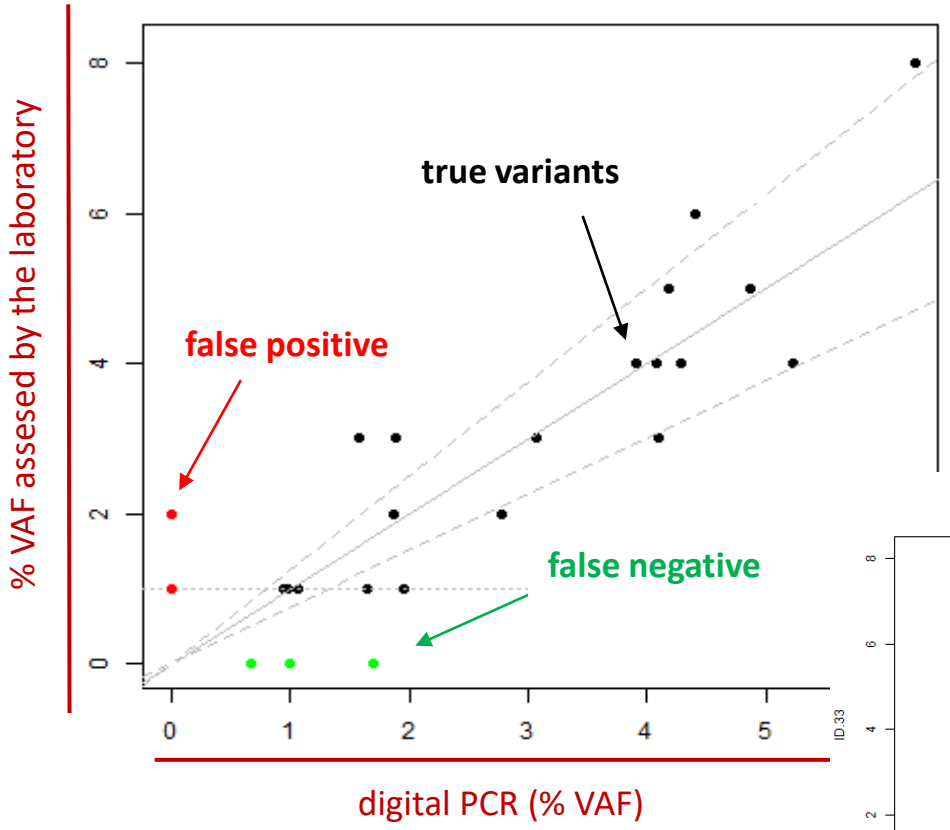
- 7 samples with 23 variants 0.7 – 6.3 % VAF
- 43 participants from 18 countries registered, 41 sent results
- Wide range of commercial and *in-house* NGS approaches
- Limit of detection 1% prevailed
- VAF assessed independently by ddPCR: good correlation with median values

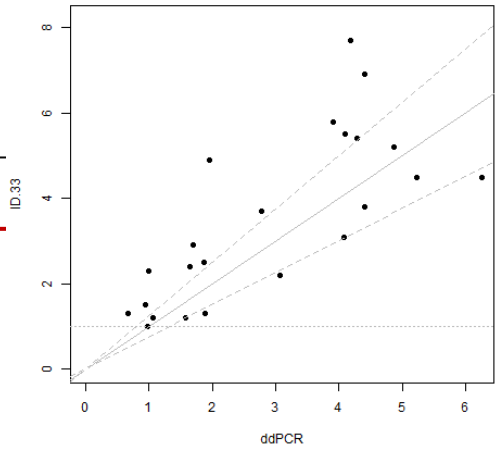


2018 November 1: Invitation
2019 May 10: Samples sent
2019 May 23: 1st results
2019 November 20: Last results
2020 March 1: EHA abstract
2020 June 10: Personalized reports

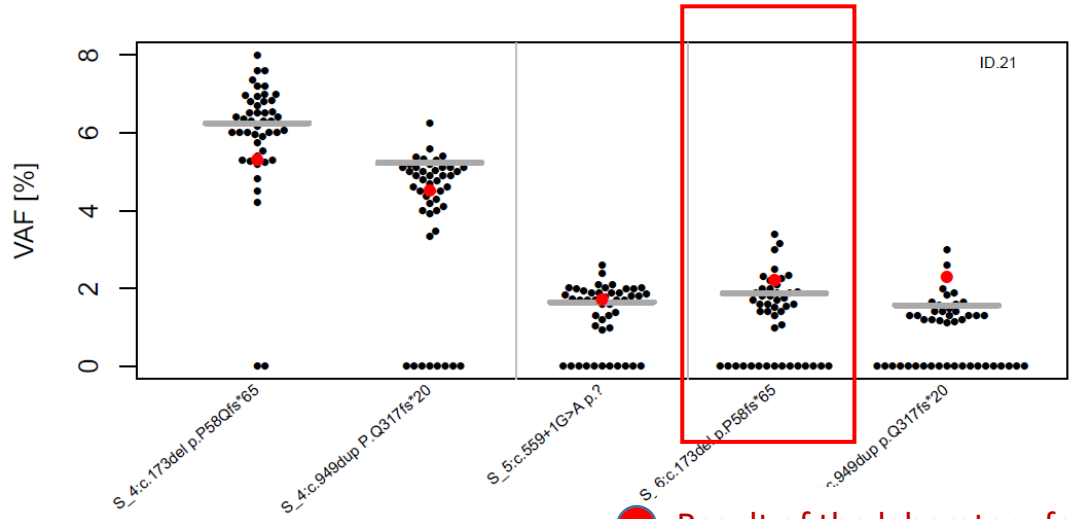
PHASE 1: PERSONALISED REPORTS

Inter-laboratory comparison of NGS results obtained from the set of reference samples





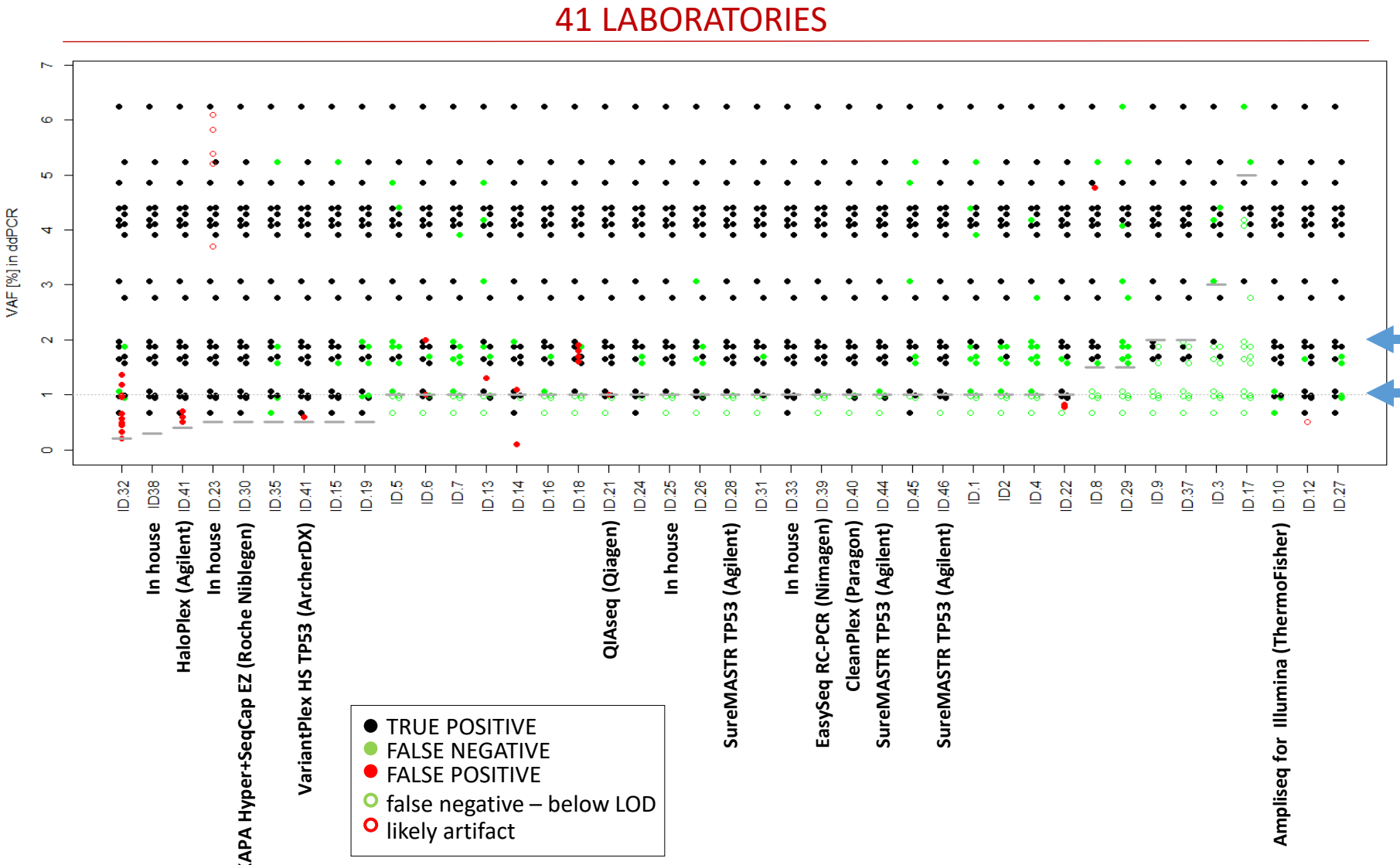
A VARIANT, e.g.c.173del



- Result of the laboratory for given variant
- Results of other laboratories
- ddPCR result

PHASE 1: OVERALL RESULTS

Inter-laboratory comparison of NGS results obtained from the set of reference samples



Cut-off	Total*	Not found*	False pos*
2% VAF	396	5.6%	1.0%
1% VAF	627	13.2%	3.3%

*only laboratories decaring LOD ≤1% VAF (N=33) were considered

PHASE 2: WHAT`S COMING NEXT?

DATA COLLECTION - results of TP53 analysis using NGS with detection limit ~1% VAF

2%

1. Do you wish to participate in the data collection part of the ERIC study? This would require to provide data from a consecutive set of patients entering first-line therapy before 2016 analyzed for TP53 mutations by a reliable NGS methodology with a threshold ≤2%VAF.

2. If yes, which deadline is feasible for you to provide the data?

3. If yes, how many patient samples do you plan to include, approximately?

4. In the case that the harmonization phase showed some shortcomings of your NGS approach, are you able to improve it?

NGS results: variants, VAF
Disease course: dates of diagnosis, therapy, follow-up
Laboratory data: IGHV, FISH

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