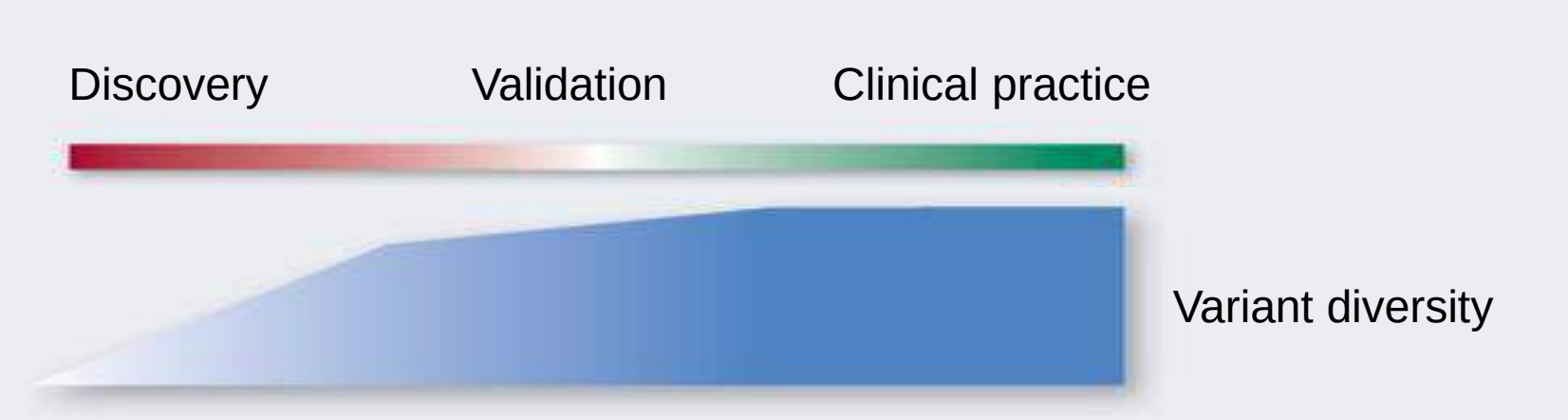
Reporting TP53 gene analysis results in CLL

ERIC
european research initiative on CLL

2nd **ERIC WORKSHOP**
ON **TP53** ANALYSIS
IN CHRONIC
LYMPHOCYTIC LEUKEMIA

Mutations in TP53 -
From discovery to clinical practice in CLL



*Leroy at al, Cancer Research Review 2017

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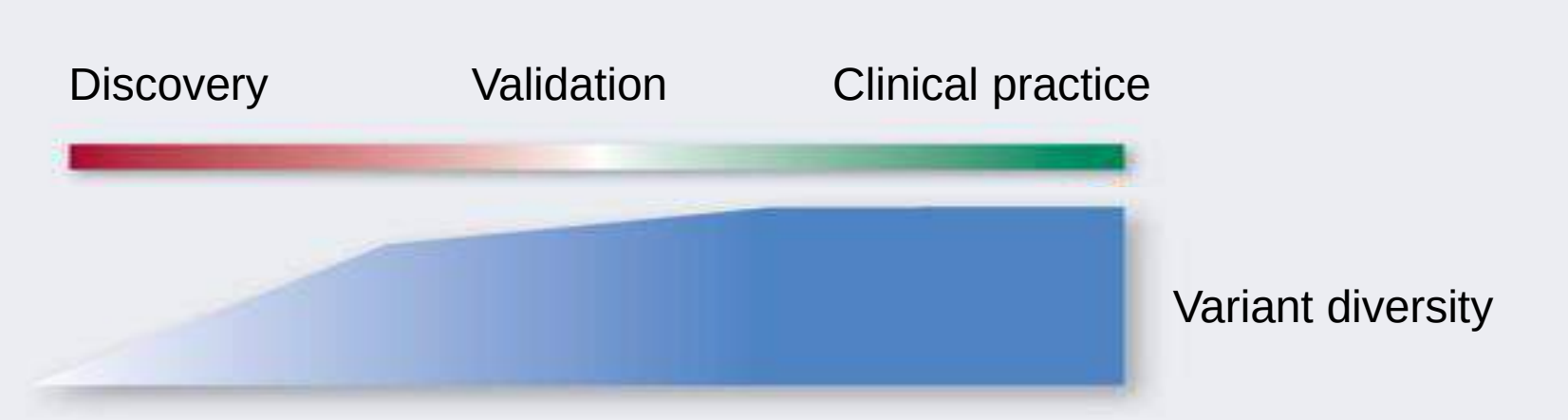

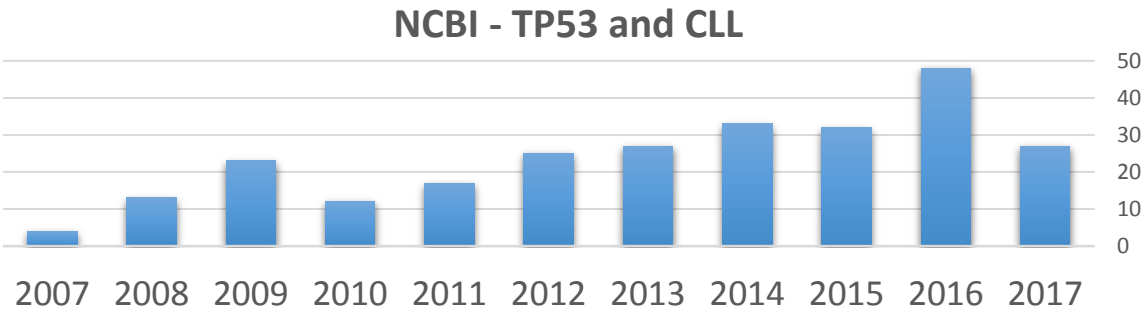

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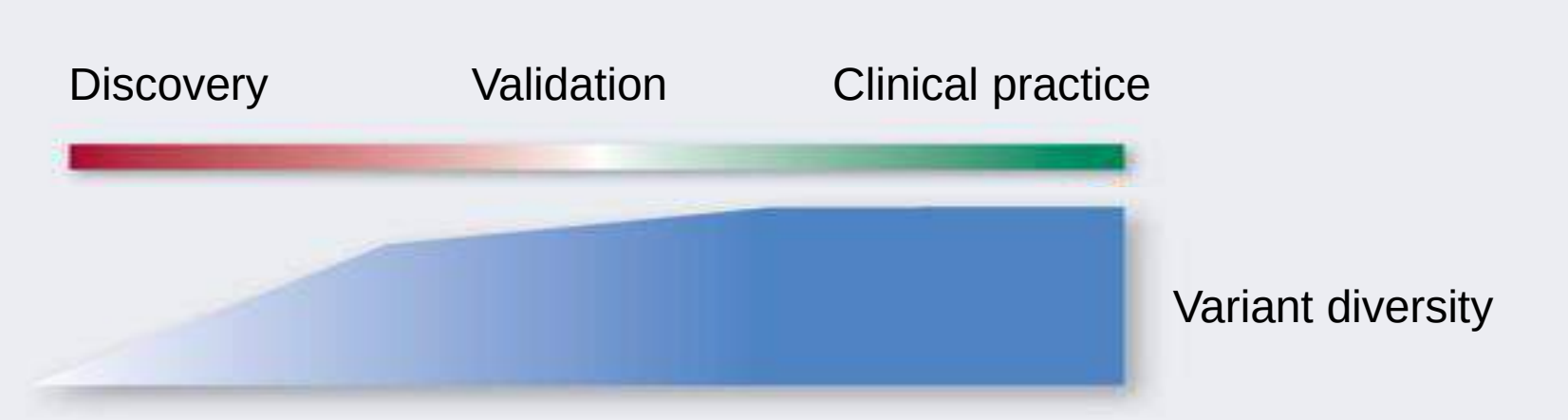

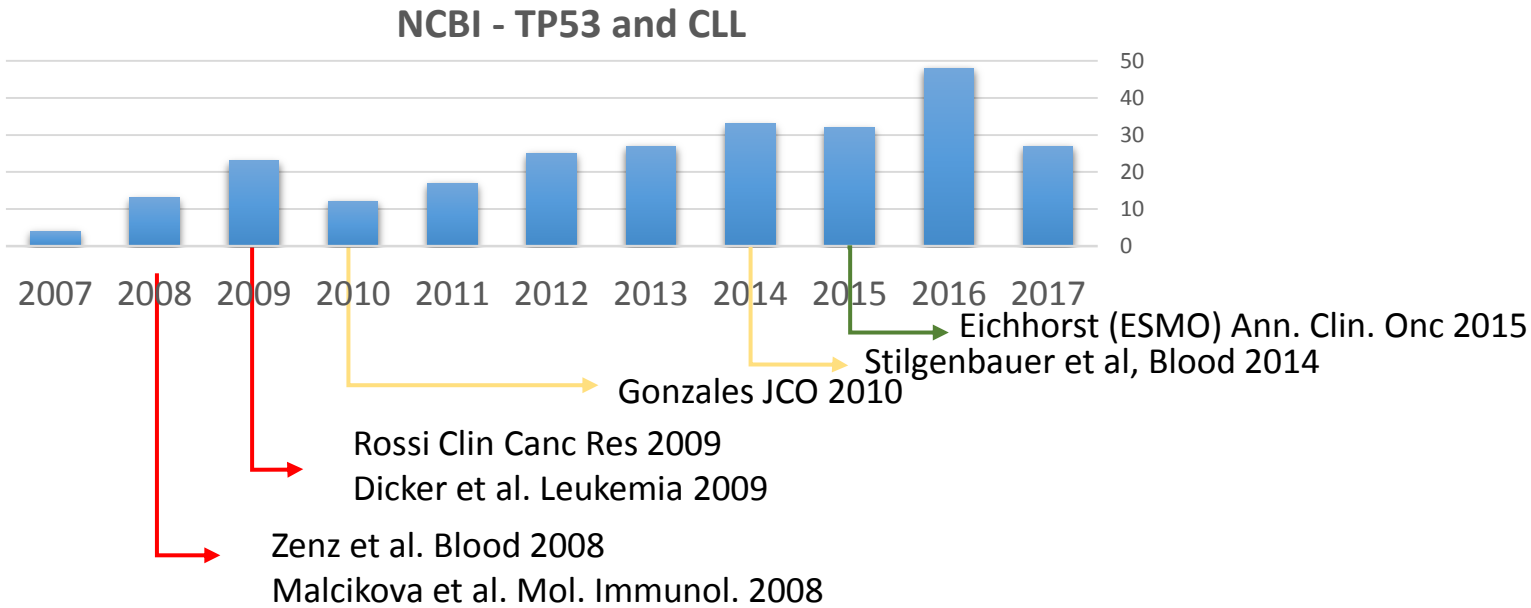

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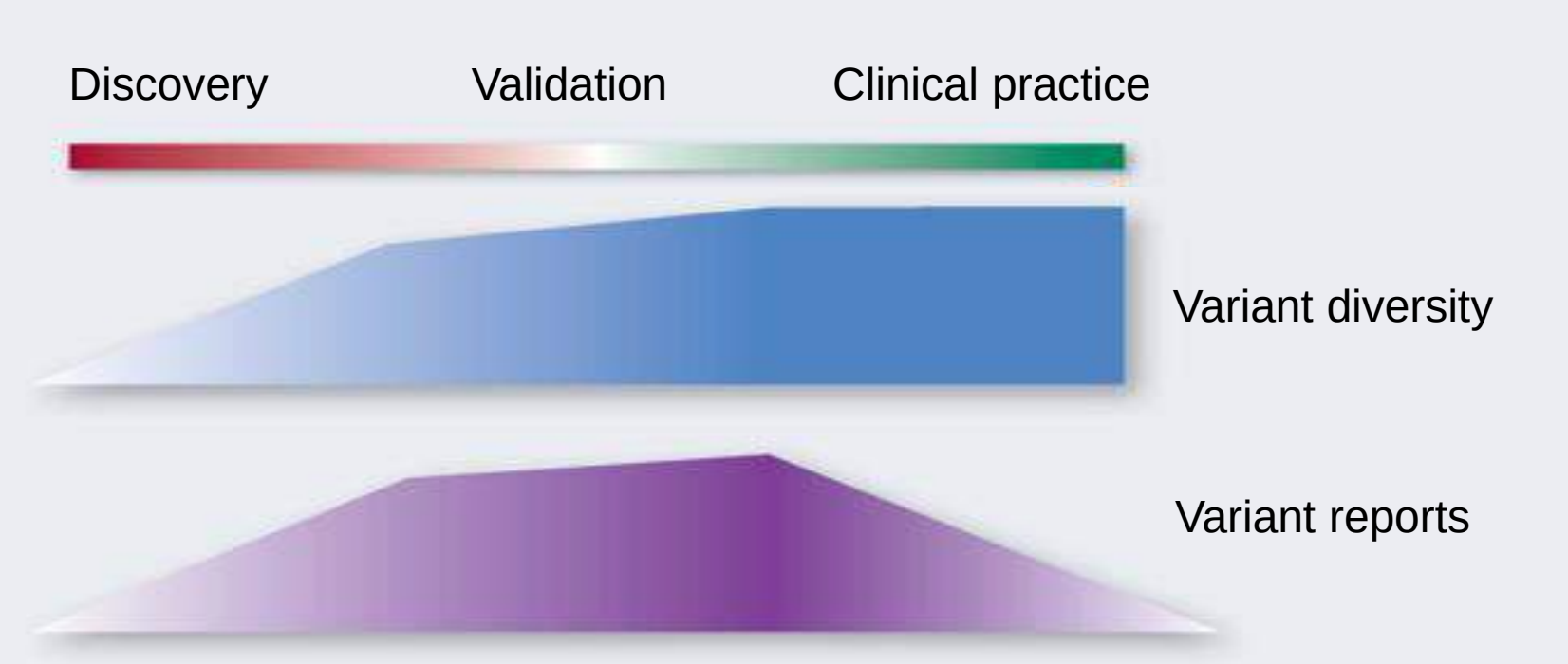
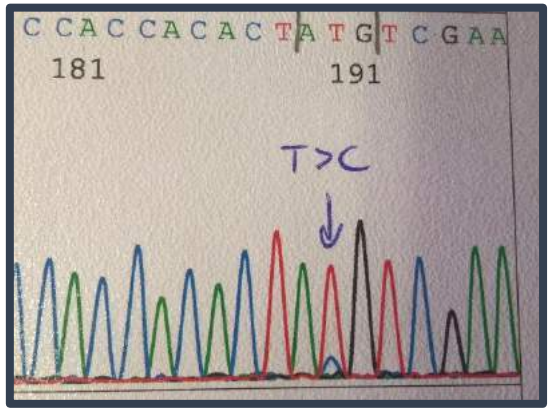
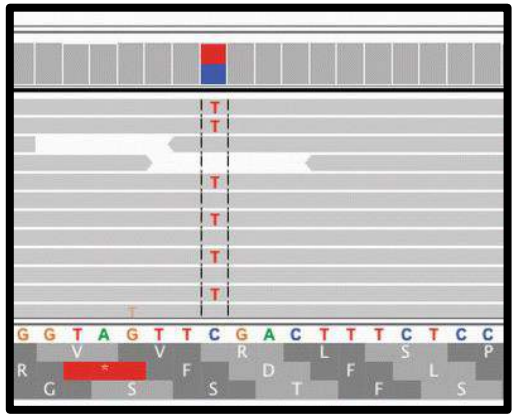


From variant detection to clinical report

Sequencing

“Detection”





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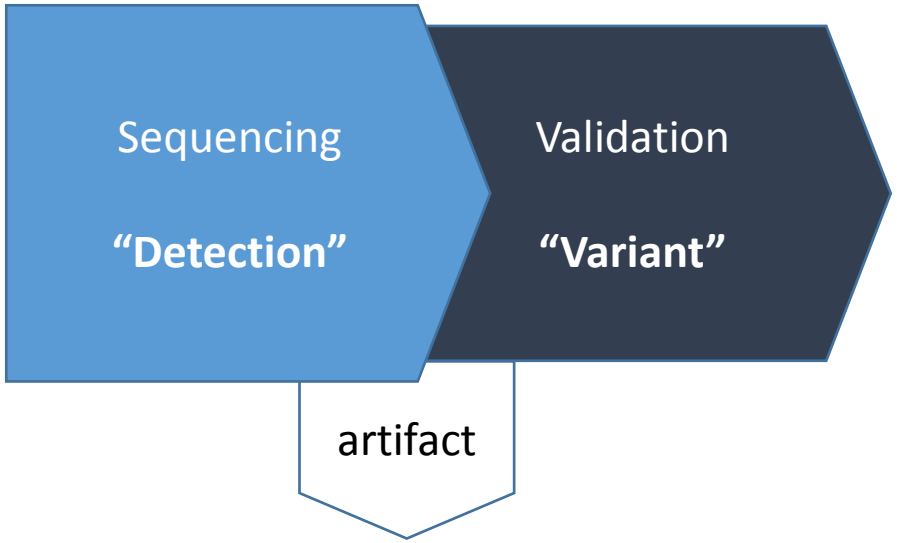


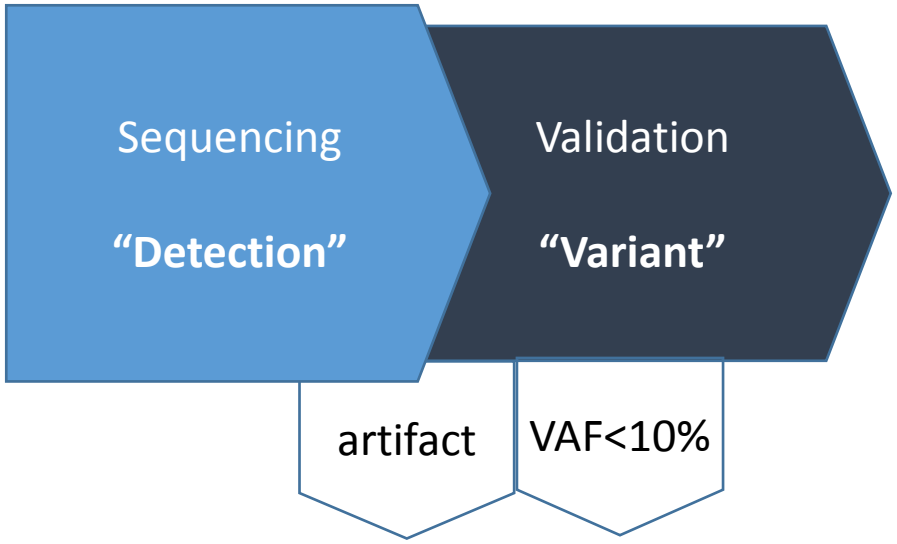
Sanger:

Enzyme Slippage
Dye Blob
DNA loop/hairpin
Contamination

NGS:

PCR dublicates
First bp read
Low Q30/ Low Coverage
Contamination

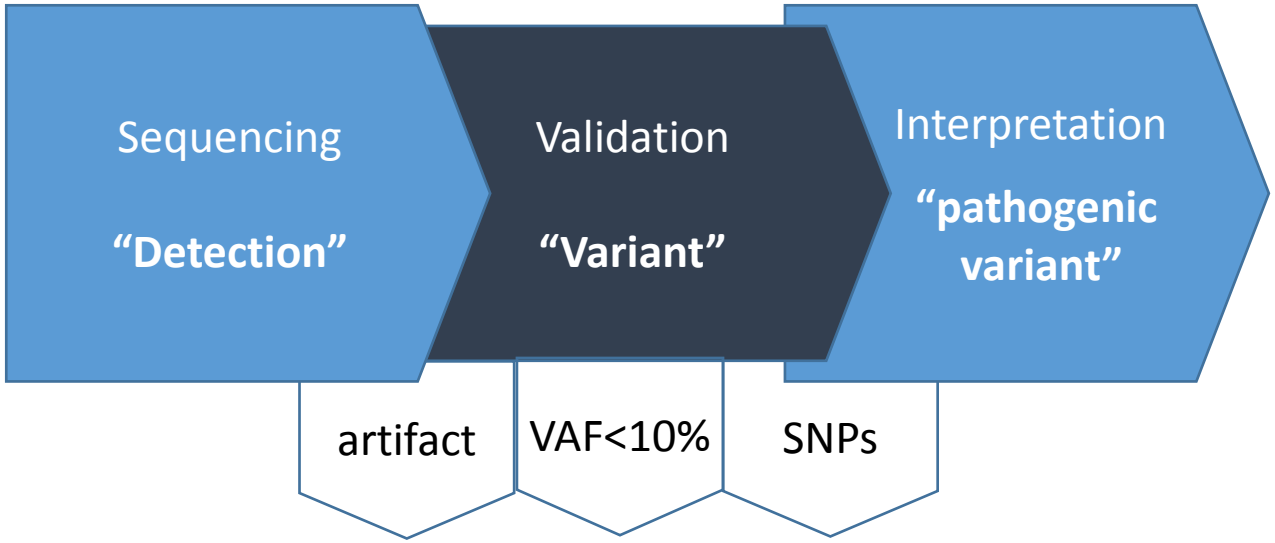
From variant detection to clinical report



omit variants with VAF <10% / not confirmable via Sanger

1) Higher risk for false positive results
2) Clinical significance remains unclear

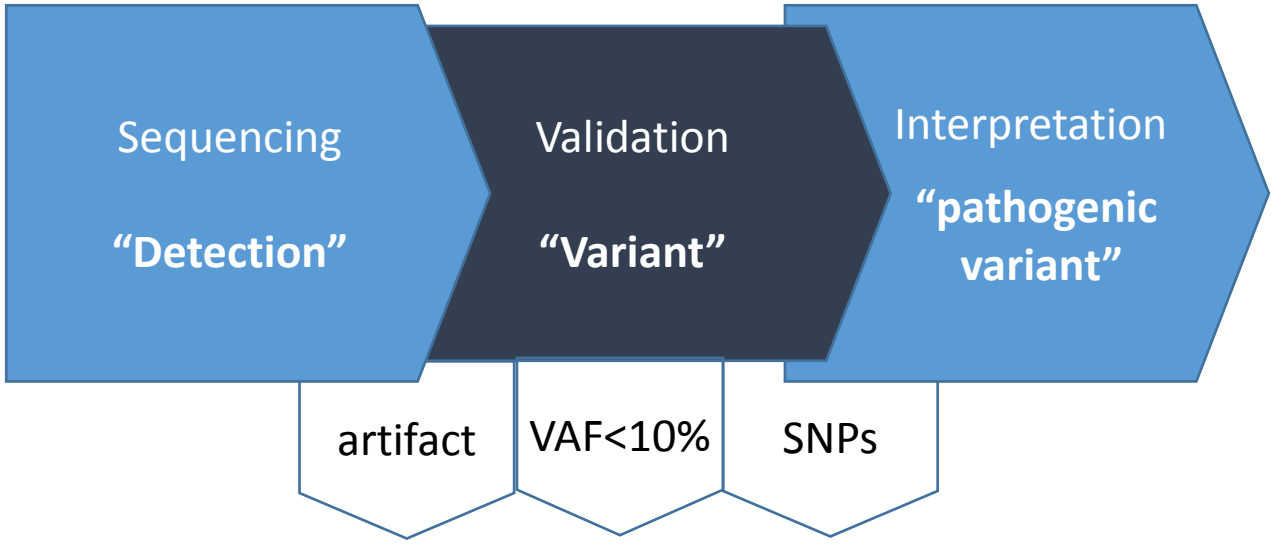
From variant detection to clinical report



"Single nucleotide polymorphisms" or just "polymorphisms" are variants with a frequency of >1% in healthy. 97 known in TP53, only 5 exonic missense TP53 SNPs.

Rest is "rare non-disease coding variants" or "rare non-pathogenic variants".

From variant detection to clinical report



pathogenic or not?

=> Samesense, missense, intronic, exonic, splice site...
=> Databases (dbSNP, 1000g, IARC, COSMIC, SESHAT)
=> Prediction tools (SIFT, POLYPHEN)
=> Non-tumor control (CD19neg, saliva, remission sample)

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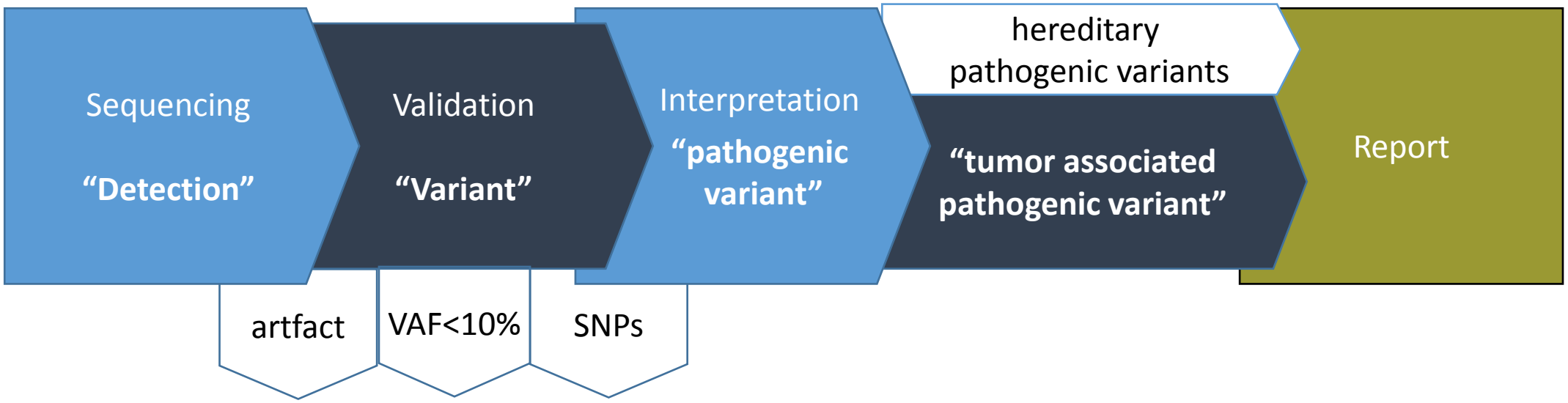

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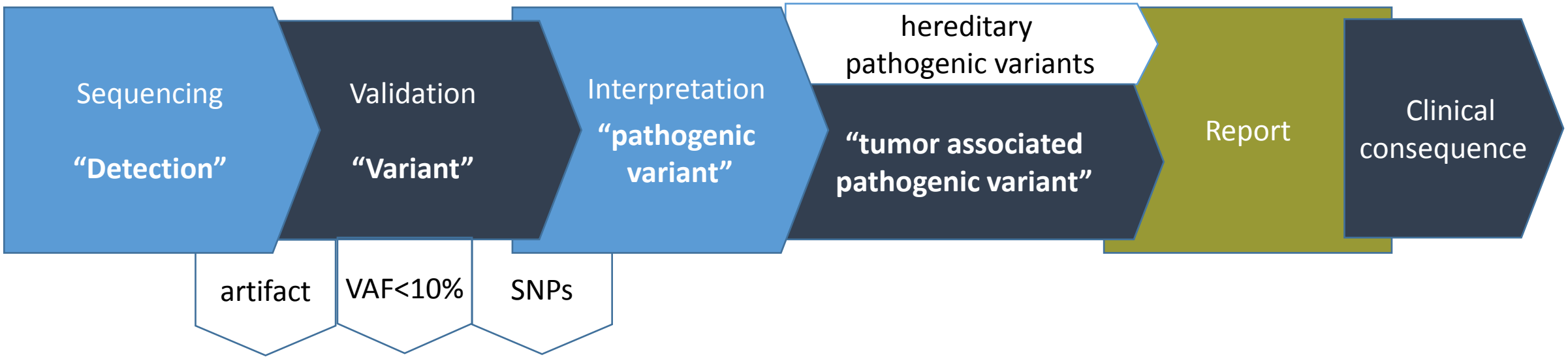

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Evidence based variant categorization

Tier I: Variants of Strong Clinical Significance

Therapeutic, prognostic & diagnostic

Level A Evidence
FDA-approved therapy
Included in professional guidelines

Level B Evidence
Well-powered studies with consensus from experts in the field

Tier II: Variants of Potential Clinical Significance

Therapeutic, prognostic & diagnostic

Level C Evidence
FDA-approved therapies for different tumor types or investigational therapies
Multiple small published studies with some consensus

Level D Evidence
Preclinical trials or a few case reports without consensus

Tier III: Variants of Unknown Clinical Significance

Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases
No convincing published evidence of cancer association

Tier IV: Benign or Likely Benign Variants

Observed at significant allele frequency in the general or specific subpopulation databases
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"Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer" a Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists"
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Mutation

rare in TP53

SNP/ rare non patho-genic variant

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Li et al., Journal of Molecular Diagnostics 2016

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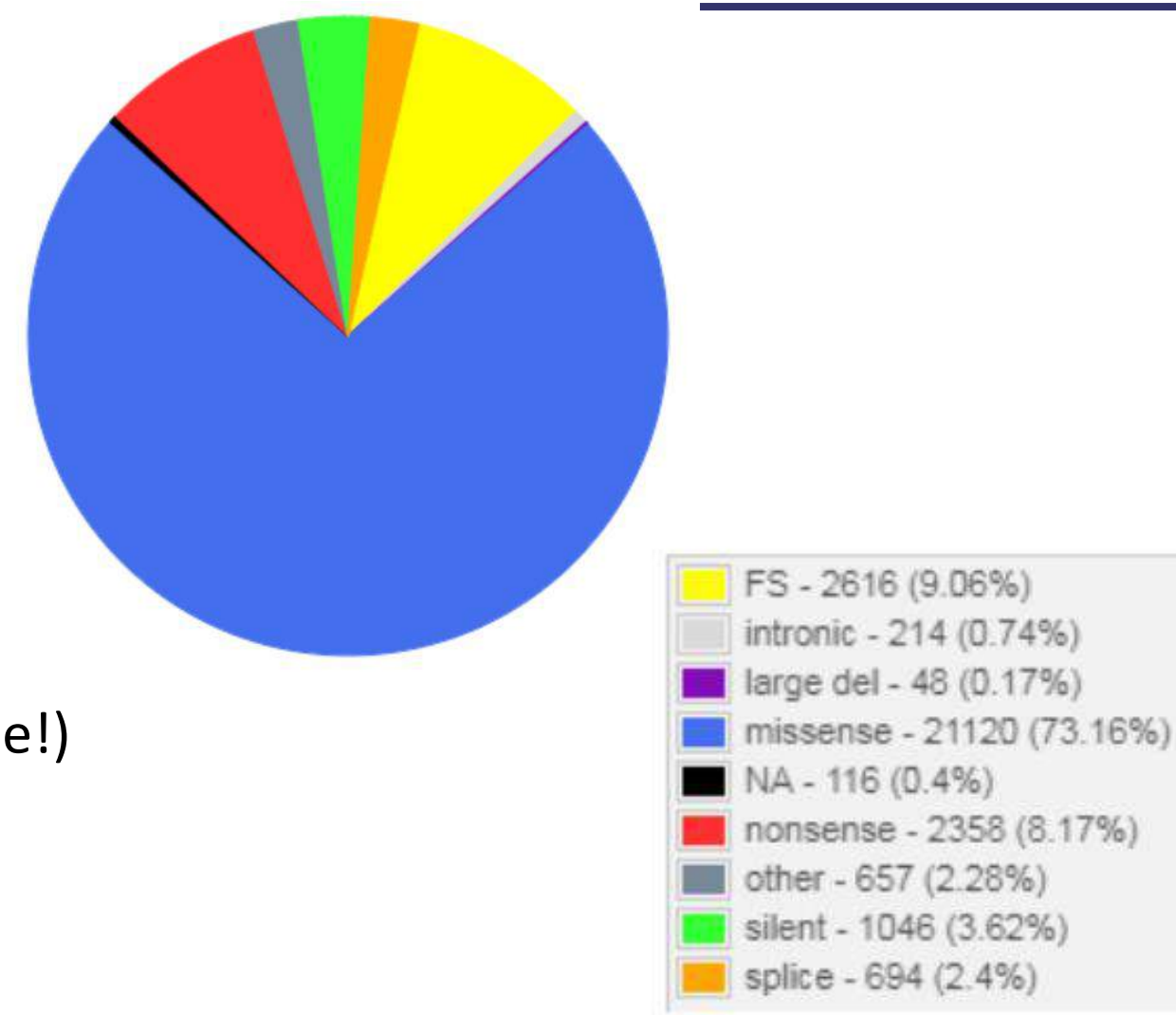
TP53 mutation type

Missense, Silent, Nonsense?

~ 73% of mutations are missense and the pathogenic value unclear (database!)
~17% of mutations are nonsense or frameshift=> generally pathogenic (database!).
~2% are splice site => pathogenic

Silent and intronic mutations are generally of no pathogenic effect.
However very few variants have proven effect on splicing and RNA processing (Database!)



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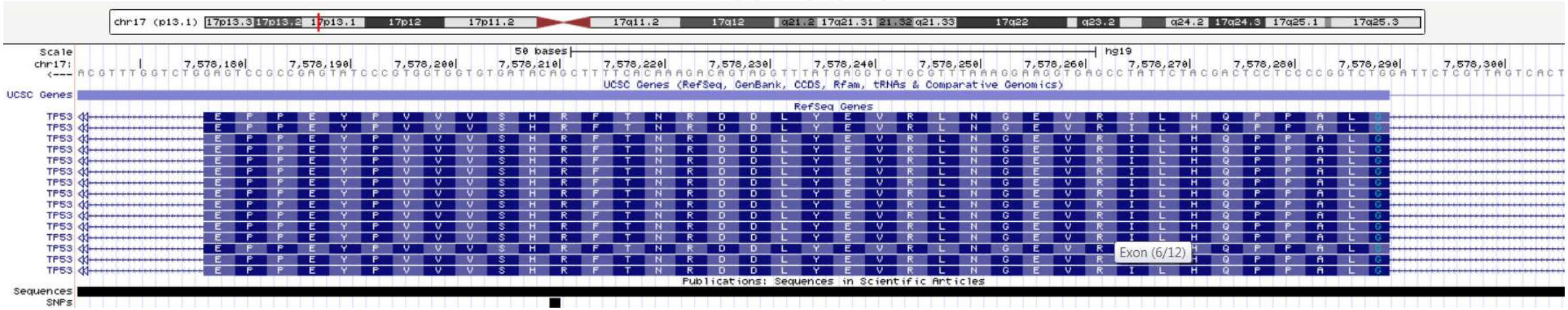
TP53 mutation type (cont'd)

Exonic, intronic, splice site?

TP53 – exon 6



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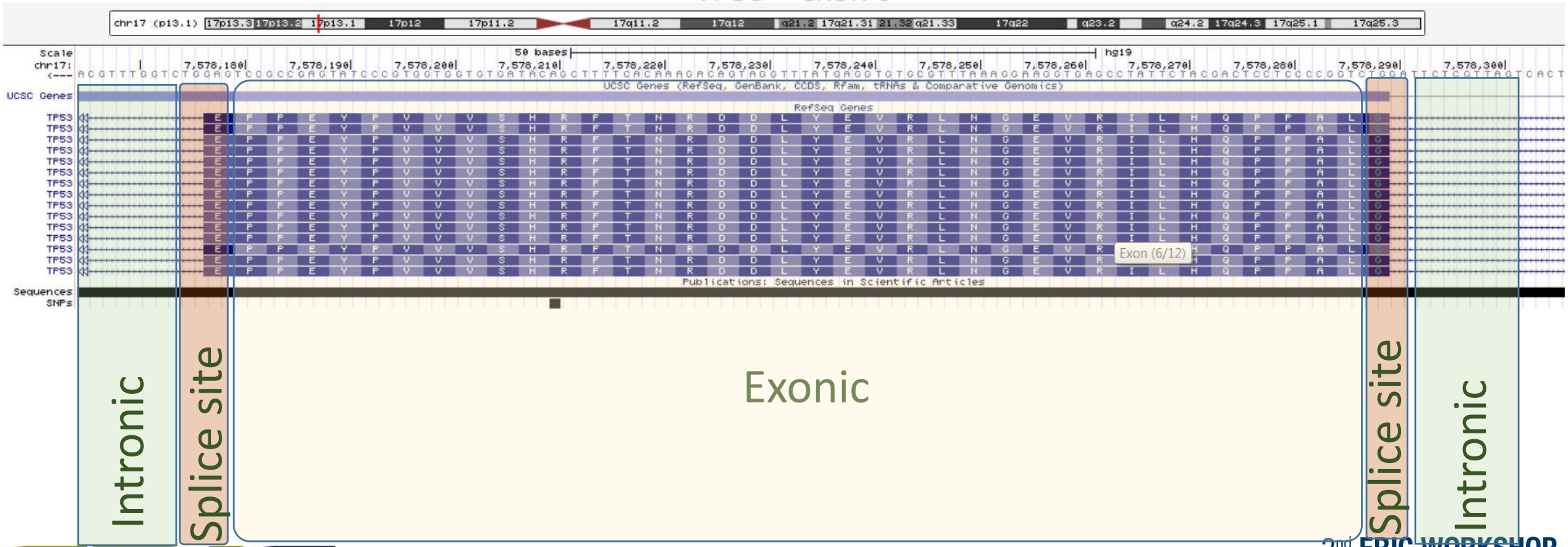
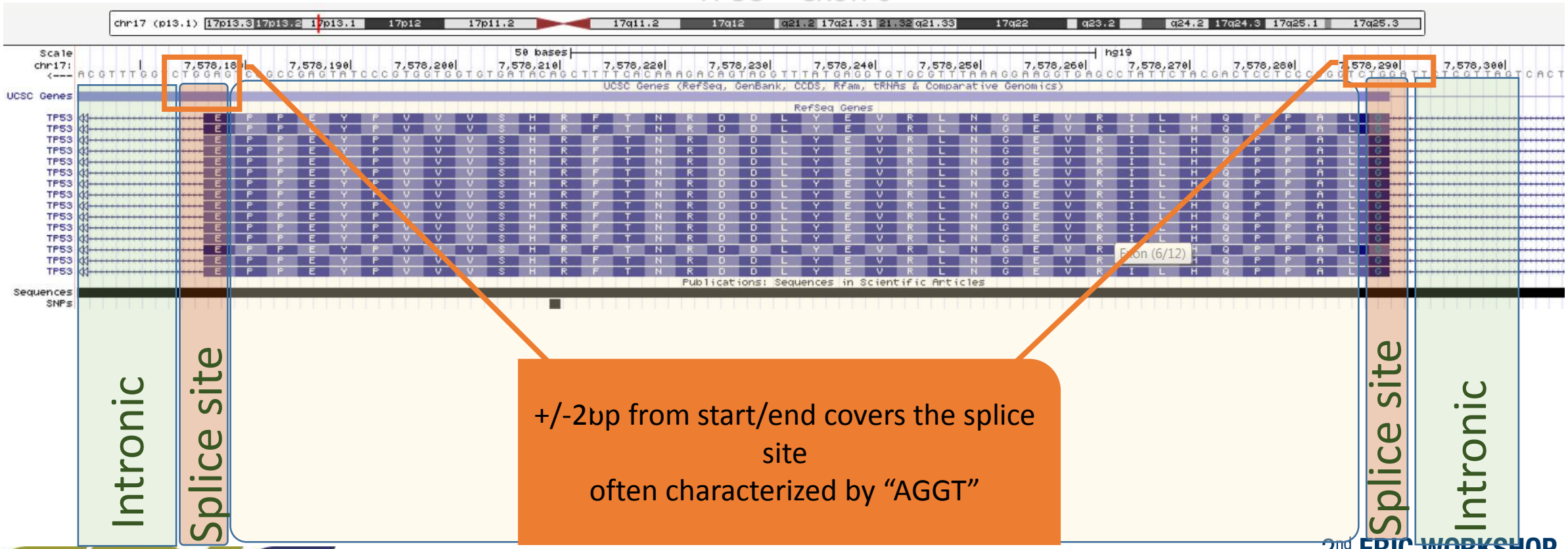


TP53 mutation type – splice site mutations

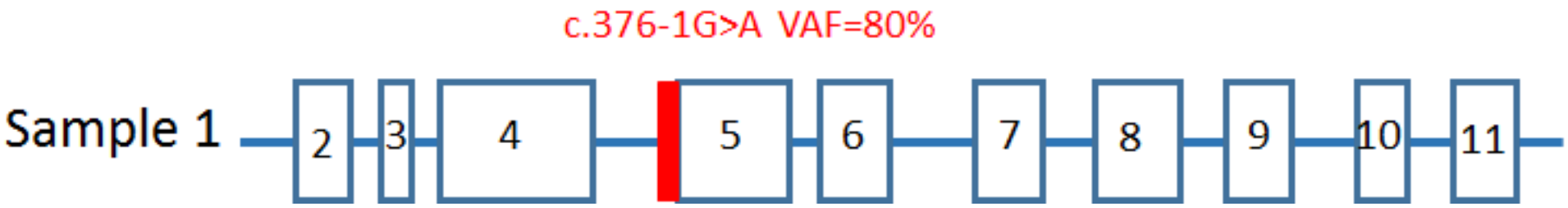
Exonic, intronic, splice site?

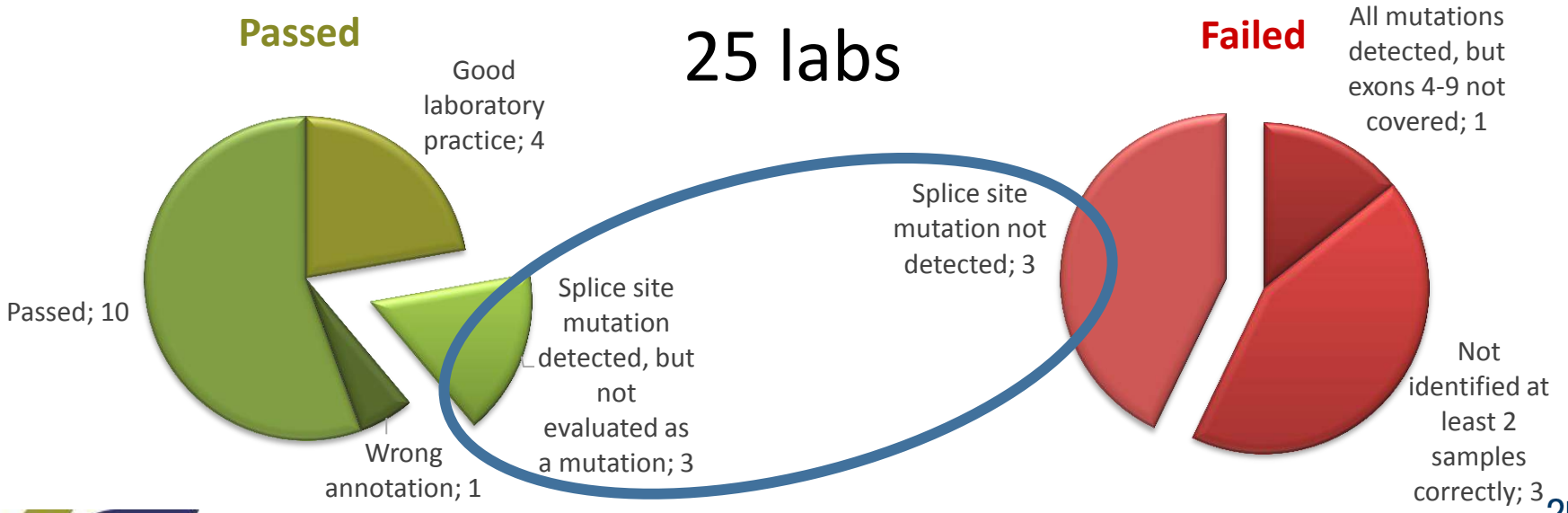
TP53 – exon 6



+/-2bp from start/end covers the splice site
often characterized by “AGGT”

ERIC TP53 certification – splice site mutations (cont'd)





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TP53 mutation analysis – pitfalls in variant identification

Missense, Silent, Nonsense?

“This nonsense mutation / stop codon leads to an amino acid substitution.”

p. R213R: “The TP53 variant c.639A>G, which lead to an amino acid change in codon 213.”

TP53 mutation analysis – databases

Population databases:

1000 Genomes Project, Exome Variant Server, dbSNP

	Allele
Variation Class:	SNV: single nucleotide variation
RefSNP Alleles:	A/C/G (REV)
Allele Origin:	A:germline C:germline G:germline
Ancestral Allele:	C
Variation Viewer:	VarView
Clinical Significance:	With Benign ,Uncertain significance,drug-response allele [ClinVar]
MAF/MinorAlleleCount:	G=0.3400/41119 (ExAC) G=0.4571/2289 (1000 Genomes) G=0.3700/4811 (GO-ESP) G=0.4264/12416 (TOPMED)

P72R

SNP / Polymorphism

TP53 mutation analysis – databases (cont'd)

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Variation Viewer:	VarView
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MAF/MinorAlleleCount:	T=0.000008/1 (ExAC)

TP53 mutation analysis – databases (cont'd)

<u>Cancer-specific variant databases</u>

Catalog of Somatic Mutations in Cancer (COSMIC)

IARC TP53 mutation database

Show 50 entries Search:

cDNA description	Protein description	Exon number	Effect	TA Class	SIFTclass	Somatic count	Germline count	CellLine count	Validated SNP	SNP link
c.524G>A	p.R175H	5-exon	missense	non-functional	deleterious	1216	75	79	no	28934578

Showing 1 to 1 of 1 entries First Previous 1 Next Last

Previous More features Cell-lines Mutation distribution Tumor distribution Download selected Dataset

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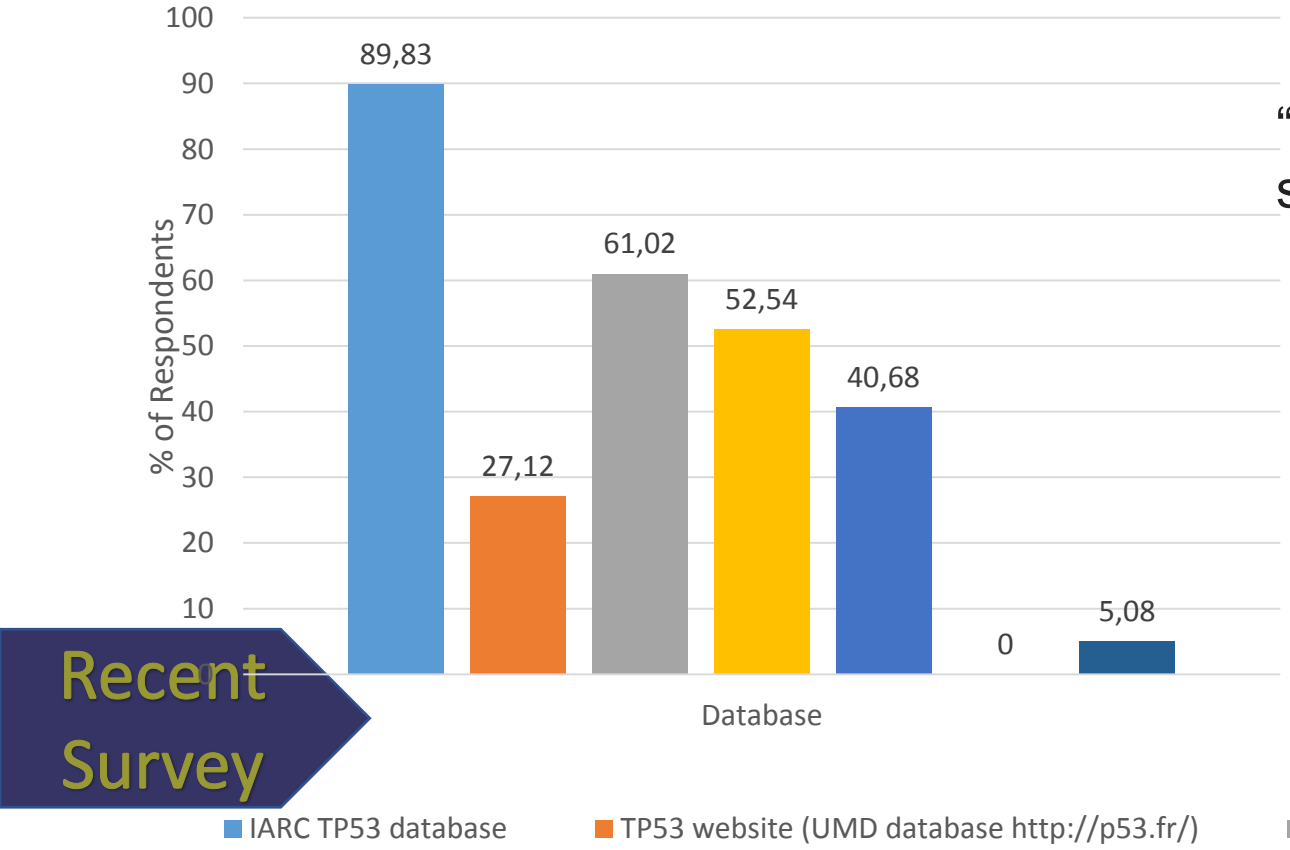
TP53 mutation analysis – pitfalls in variant identification

What database do you use for mutation validation?

Polymorphisms and Mutations



Recent Survey

". P72R: "This SNP is likely benign, possible clinical significance affecting drug response."

"p.Pro72Arg (rs1042522) is related with a higher risk of breast cancer, and is not associated with Systemic Lupus Erythematosus in Caucasians, African-Americans, and Asian children and adult."

TP53 mutation analysis – pitfalls in variant identification

Prediction tools

SIFT prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences

Polyphen-2 possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations

Specificity of only 60-80% i.e. SIFT result for BRAF V600E => Benign

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TA class

Overall transcriptional activity (TA) on 8 different promoters

Missense mutations are classified as "non-functional" if the median of promotor activity is <=20, "partially functional" if the median is >20 and <=75, "functional" if the median is >75 and <=140, and "supertrans" if the median is >140.

TP53 mutation analysis – databases (cont'd)

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TP53 mutation analysis – non-tumor control

Non-tumor control

derived from tumor free material (saliva, buccal swap, bone marrow) or enriched / purified from same sample (purity control recommended) or sample from a time point of no measurable disease.

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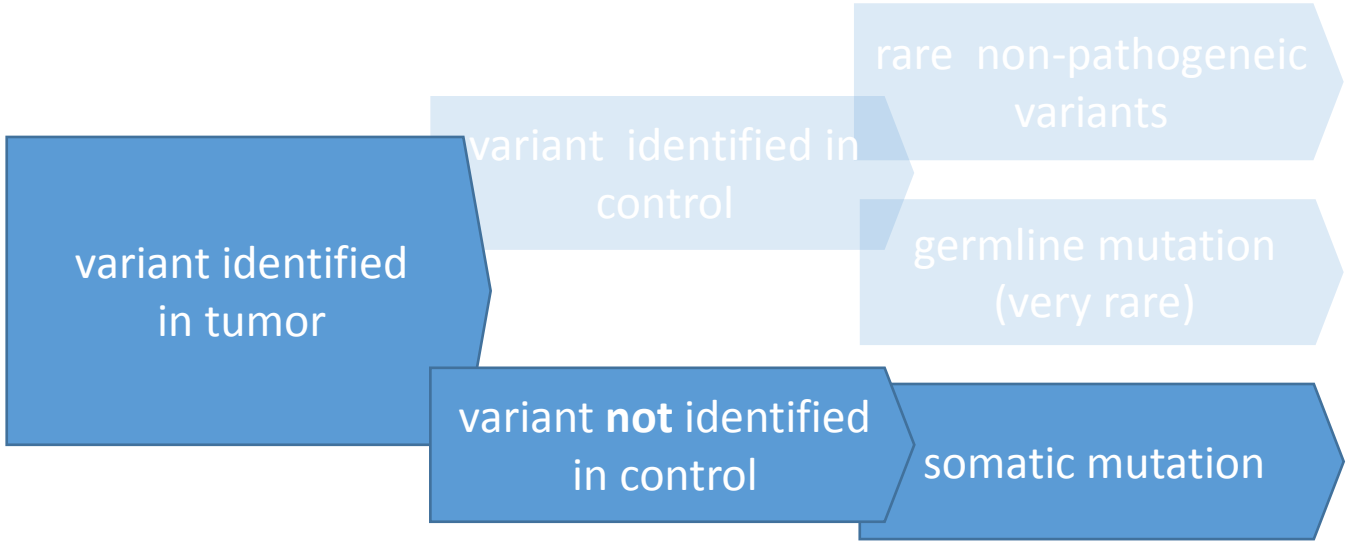

Pitfalls:

tumor contamination of non-tumor material

haematopoetic progenitor cells not considered

Should I look for germline variants?

<u>Non-tumor control</u>

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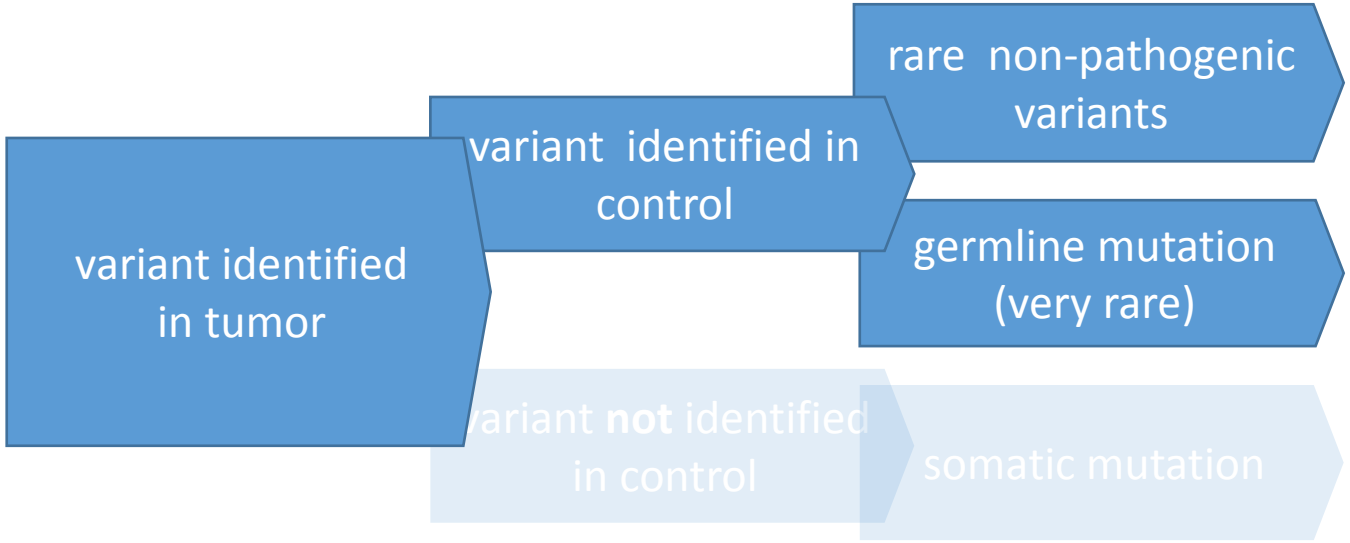

Should I look for germline origin of a variant?

Clinical suspicious?
Variant fraction of 50% (or 100%)?
Variant with high prevalence in patients with Li Fraumeni (like) syndrome.

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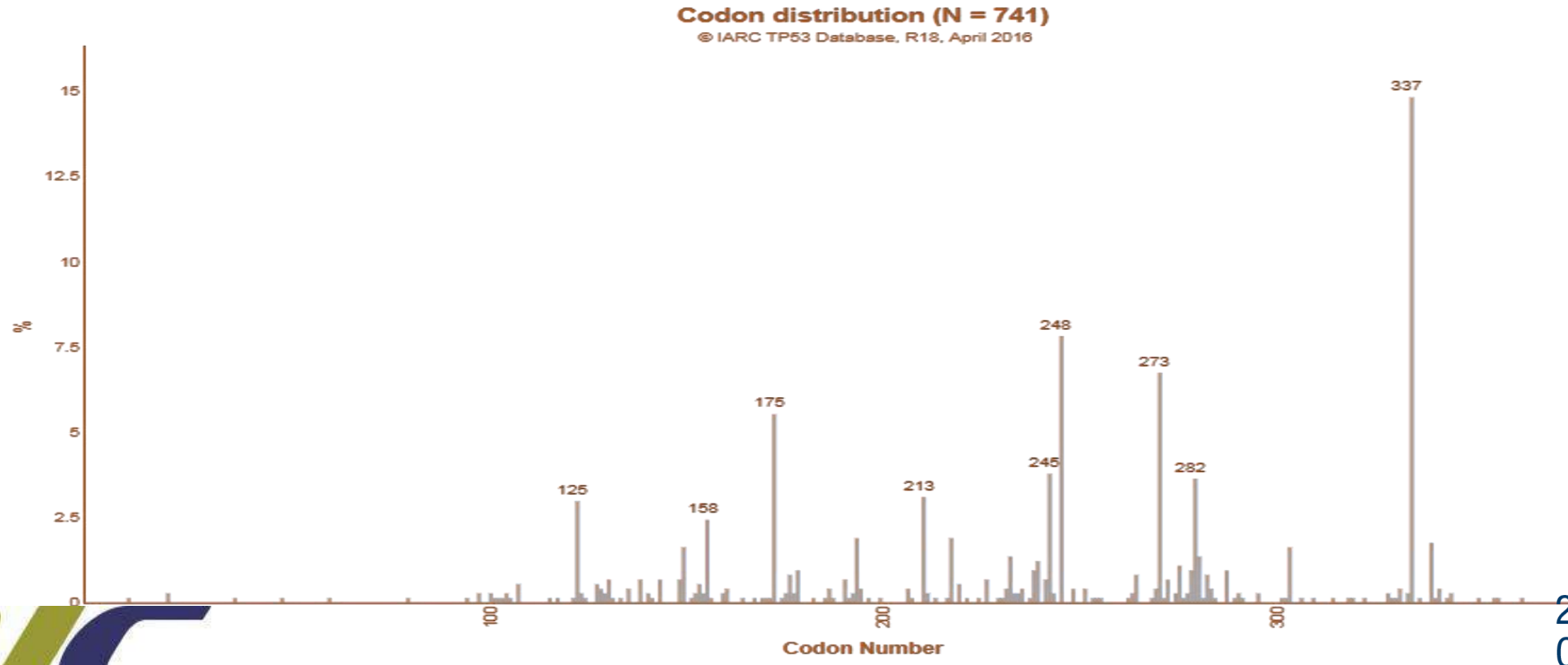

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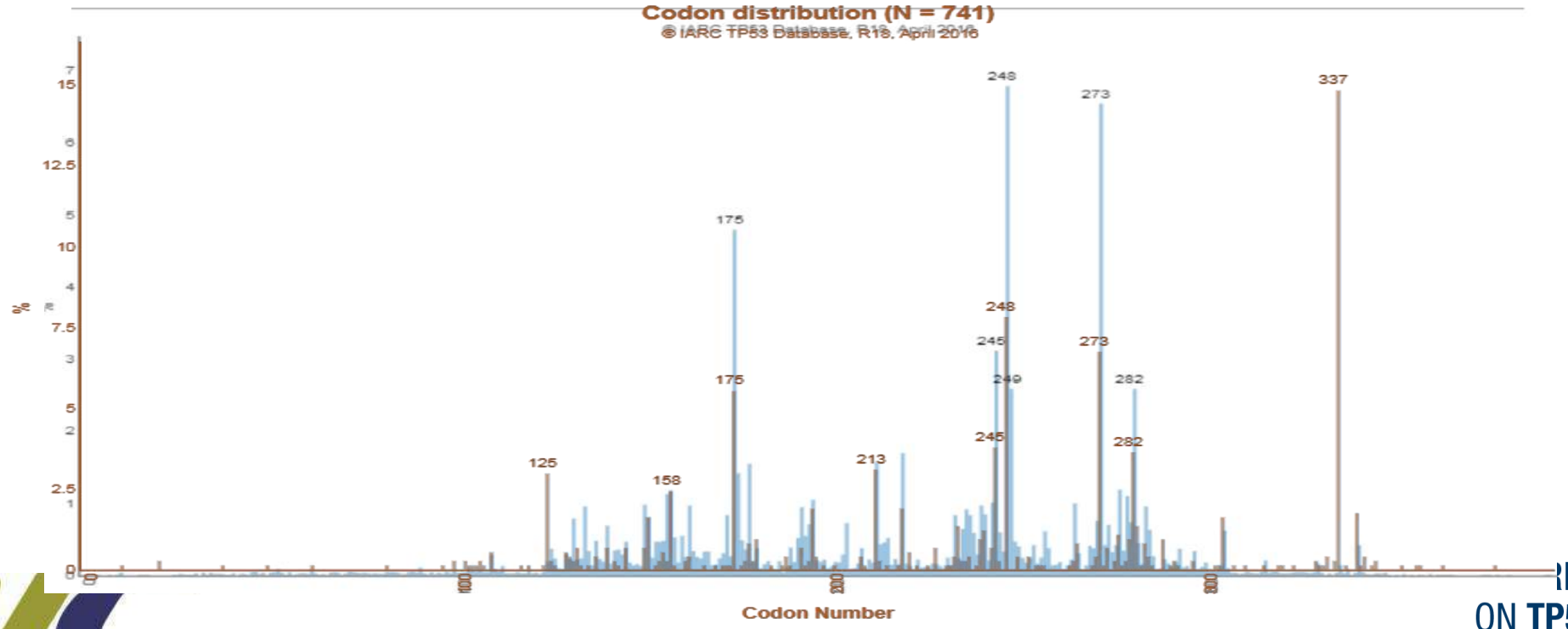

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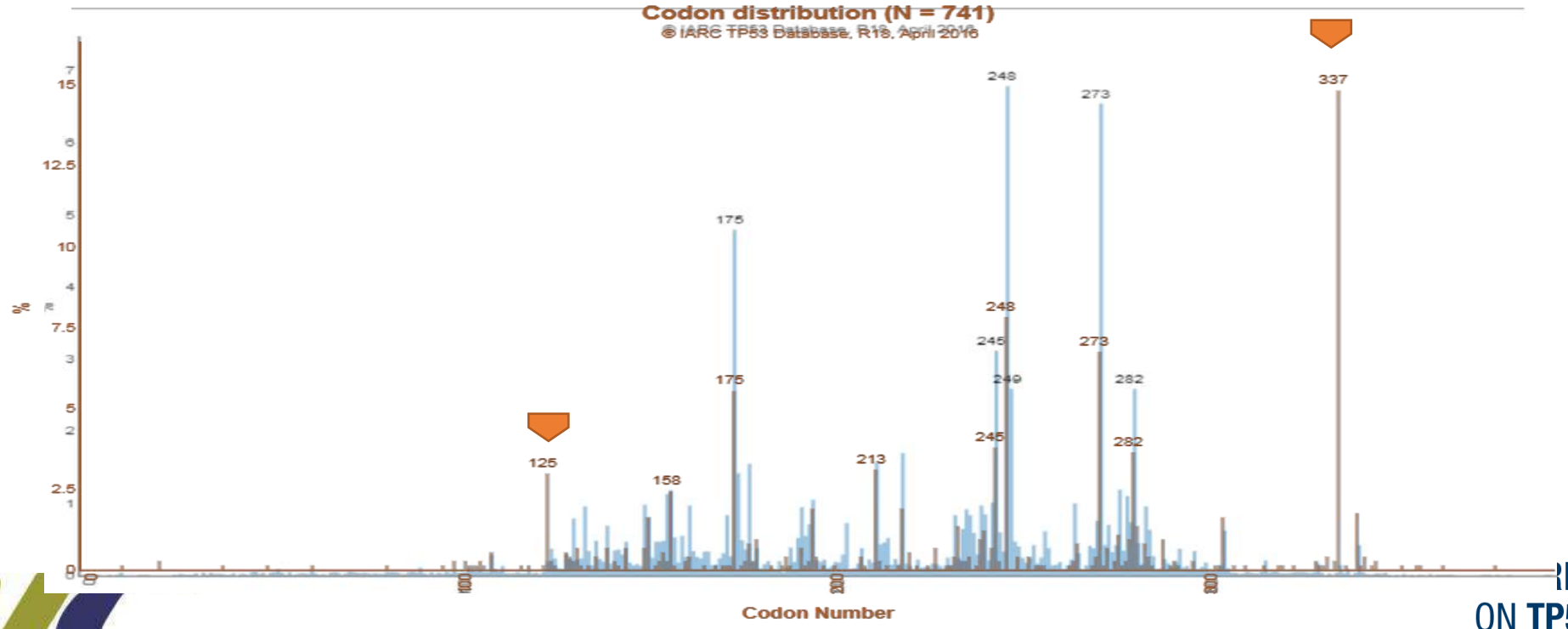

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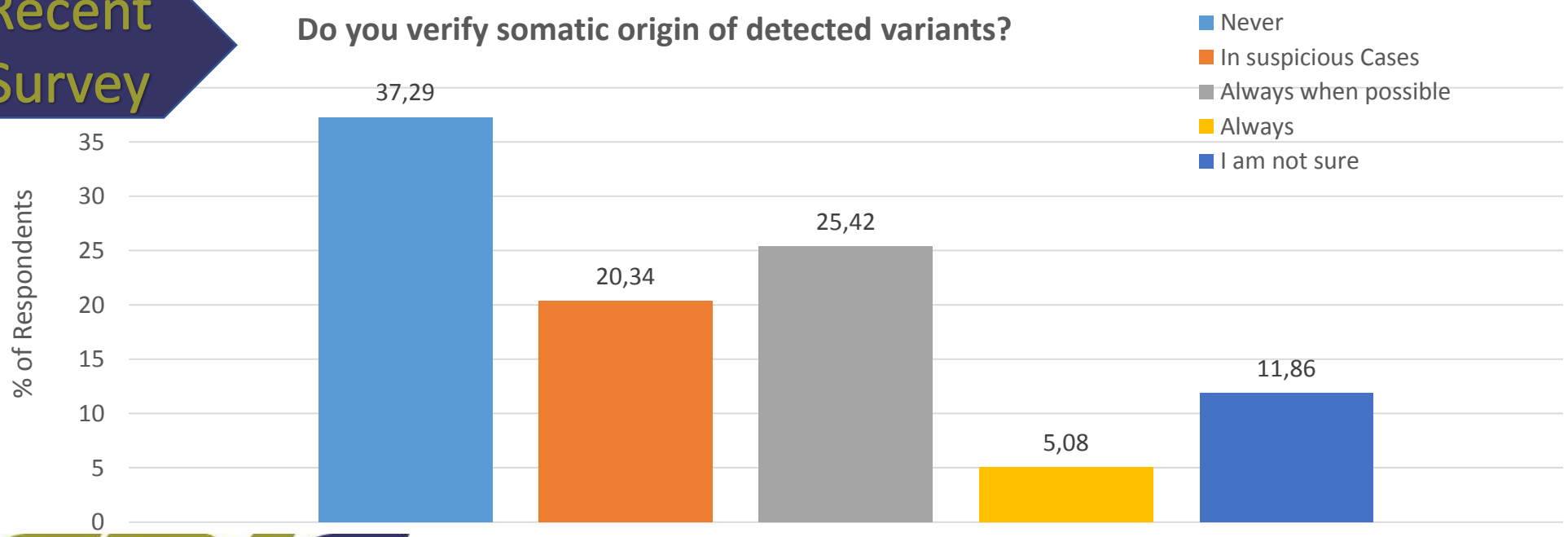

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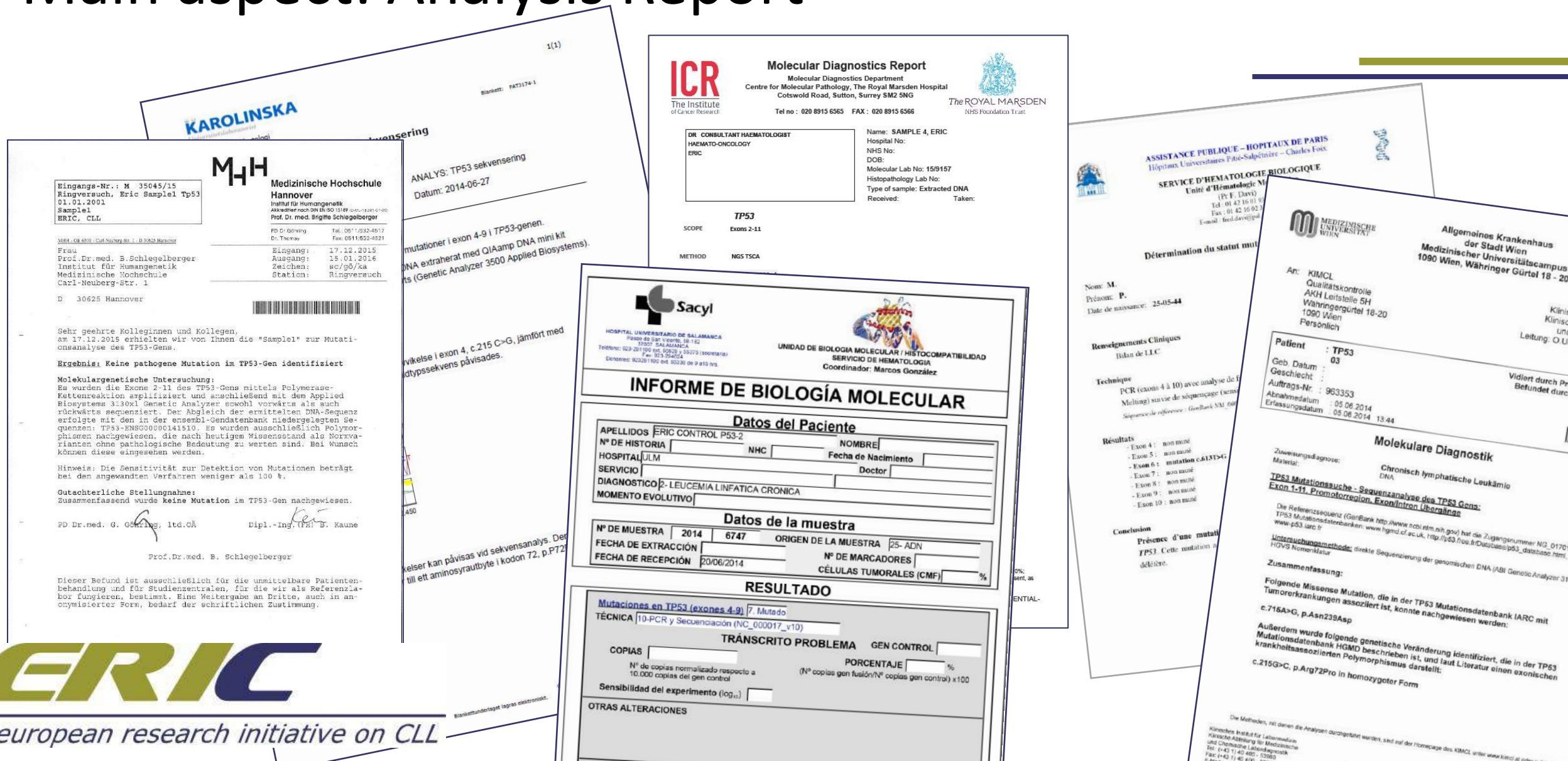
Main aspect: Analysis Report



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Reporting of TP53 variants - Requirements

Lab name / Contact details

Lab ID

Responsible Person

Университет "Св. Кирил и Методиј" - Скопје ФАРМАЦЕВСТКИ ФАКУЛТЕТ
University "Ss.Cyril and Methodius" - Skopje FACULTY OF PHARMACY

RESULT

of sequence analysis of exons 5-9 of the TP 53 gene

Date:	Sample received:	11 July 2014
	Analysis:	12 – 15 July 2014
	Results issued:	23 July 2014
Lab number:	ERIC 1	
Patient:	ERIC-1	
Physician:	ERIC Accreditation in TP53 Mutation Analysis July 2014	
Diagnosis:	CLL	
Analysis:	TP53 mutation analysis	
Sample:	DNA, 6 µl (0.2µg/µl)	
Method:	DNA sequencing of PCR amplified fragments containing coding and 5' and 3' flanking sequences of exons 5 to 9 of the TP 53 gene	
Detection Limit:	15% of mutant allele in a background of normal DNA	
Sensitivity:	>90%	
Specificity:	100%	
Positive predictive value:	100%	
Negative predictive value:	>90%	
Reference sequences:	NM_000546; SwissProt #P04637	
Result:	No functional sequence variants present in exons 5, 6, 7, 8 and 9 of the TP 53 gene c.672+62A>G homozygote (rs1625895) *no functional effect*	
Mutant allele content:	N/A	
Comment:	The analysis does not exclude the presence of functional sequence variants in exons 1,2,3,4, 10 and 11 (present in <10% of CLL patients with mutated TP53 gene). The analysis does not exclude the presence of functional sequence variants with allelic content of <15% in the analyzed exons.	
References:	www-p53.iarc.fr ; Pospisilova S. et al. Leukemia (2012) 26, 1458-1461	

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Head of the Laboratory: Prof. Aleksandar Dimovski MD, PhD

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european research initiative on CLL

2nd ERIC WORKSHOP ON TP53 ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Reporting of TP53 variants - Requirements

Lab name / Contact details

Date of sample acquisition

Patient Identifier

Sample ID

Responsible Person

Универзитет "Св. Кирил и Методиј" - Скопје ФАРМАЦЕВСТКИ ФАКУЛТЕТ
University "Ss.Cyril and Methodius" - Skopje FACULTY OF PHARMACY

RESULT

of sequence analysis of exons 5-9 of the TP 53 gene

Date:	Sample received:	11 July 2014
	Analysis:	12 – 15 July 2014
	Results issued:	23 July 2014
Lab number:	ERIC 1	
Patient:	ERIC-1	
Physician:	ERIC Accreditation in TP53 Mutation Analysis July 2014	
Diagnosis:	CLL	
Analysis:	TP53 mutation analysis	
Sample:	DNA, 6 µl (0.2µg/µl)	
Method:	DNA sequencing of PCR amplified fragments containing coding and 5' and 3' flanking sequences of exons 5 to 9 of the TP 53 gene	
Detection Limit:	15% of mutant allele in a background of normal DNA	
Sensitivity:	>90%	
Specificity:	100%	
Positive predictive value:	100%	
Negative predictive value:	>90%	
Reference sequences:	NM_000546; SwissProt #P04637	

Result: No functional sequence variants present in exons 5, 6, 7, 8 and 9 of the TP 53 gene

c.672+62A>G homozygote (rs1625895)
no functional effect

Mutant allele content: N/A

Comment: The analysis does not exclude the presence of functional sequence variants in exons 1,2,3,4, 10 and 11 (present in <10% of CLL patients with mutated TP53 gene).

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References: www-p53.iarc.fr ; Pospisilova S. et al. Leukemia (2012) 26, 1458-1461

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Reporting of TP53 variants - Requirements

Lab name / Contact details

Date of sample acquisition

Patient Identifier

Type of analysis

Type of material and technique

(Sensitivity / Specificity)

Exons covered & Reference Seq

Analysis details

Responsible Person

Универзитет "Св. Кирил и Методиј" - Скопје ФАРМАЦЕВСТКИ ФАКУЛТЕТ
University "Ss.Cyril and Methodius" - Skopje FACULTY OF PHARMACY

RESULT

of sequence analysis of exons 5-9 of the TP 53 gene

Date:	Sample received: 11 July 2014 Analysis: 12 – 15 July 2014 Results issued: 23 July 2014
Lab number:	ERIC 1
Patient:	ERIC-1
Physician:	ERIC Accreditation in TP53 Mutation Analysis July 2014
Diagnosis:	CLL
Analysis:	TP53 mutation analysis
Sample	DNA, 6 µl (0.2µg/µl)
Method	DNA sequencing of PCR amplified fragments containing coding and 5' and 3' flanking sequences of exons 5 to 9 of the TP 53 gene
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Reporting of TP53 variants - Requirements

Result

- Lab name / Contact details
- Date of sample acquisition
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- Interpretation
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- Responsible Person

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RESULT

of sequence analysis of exons 5-9 of the TP 53 gene

Date:	Sample received: 11 July 2014 Analysis: 12 – 15 July 2014 Results issued: 23 July 2014
Lab number:	ERIC 1
Patient:	ERIC-1
Physician:	ERIC Accreditation in TP53 Mutation Analysis July 2014
Diagnosis:	CLL
Analysis:	TP53 mutation analysis
Sample	DNA, 6 µl (0.2µg/µl)
Method	DNA sequencing of PCR amplified fragments containing coding and 5' and 3' flanking sequences of exons 5 to 9 of the TP 53 gene
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Reporting of TP53 variants - Requirements

Result

1) Mutated or Unmutated TP53 status
2) Type of mutation (missense, nonsense)
3) Use HGVS to describe mutations

chr17.hg19:g.7578406C>T or alternatively
NG_017013.2:g.17463G>A
c.524G>A (NM_000546.5)
p.R175H

Reporting of TP53 variants - Requirements

Result

1) Mutated or Unmutated TP53 status
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chr17.hg19:g.7578406C>T or alternatively
NG_017013.2:g.17463G>A
c.524G>A (NM_000546.5)
p.R175H

Interpretation

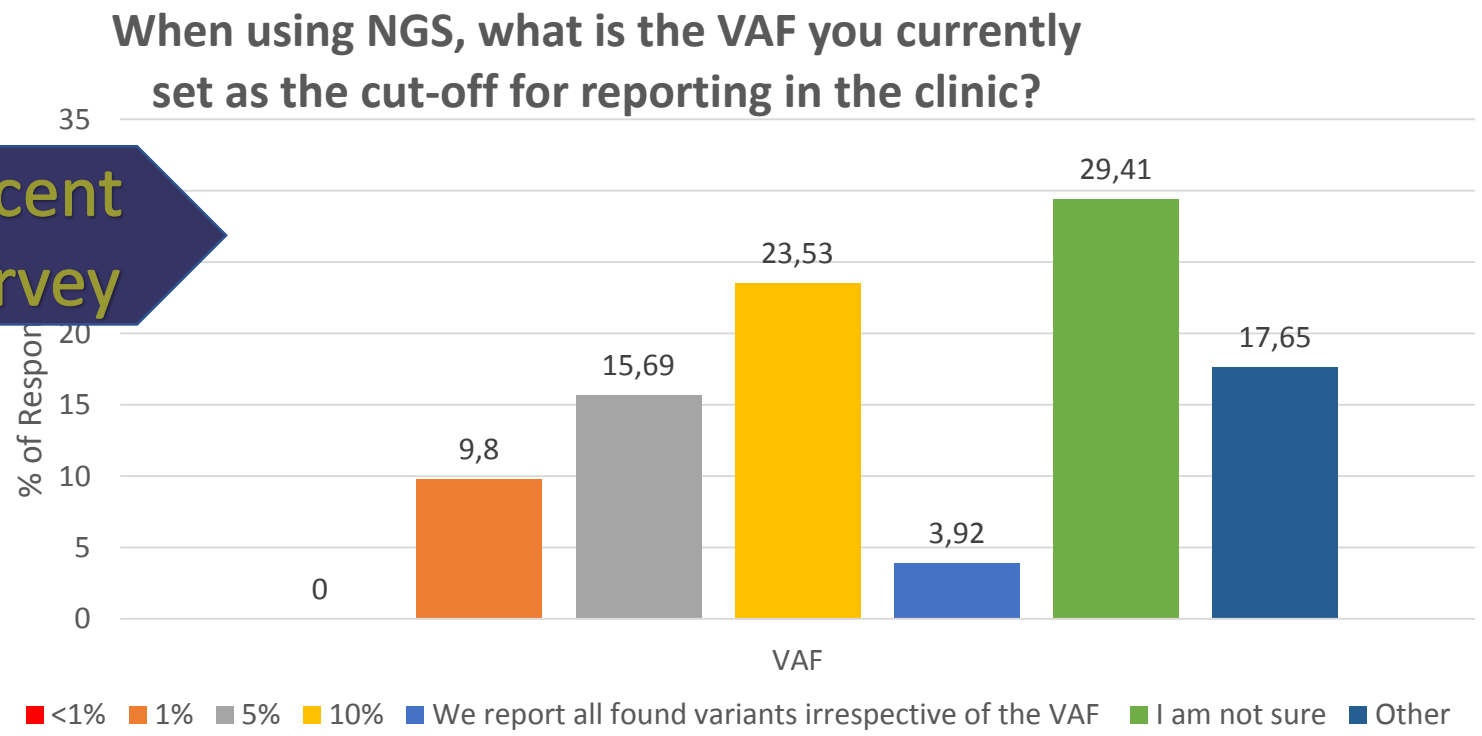
summary of the finding in the context of the current state of knowledge (reference)

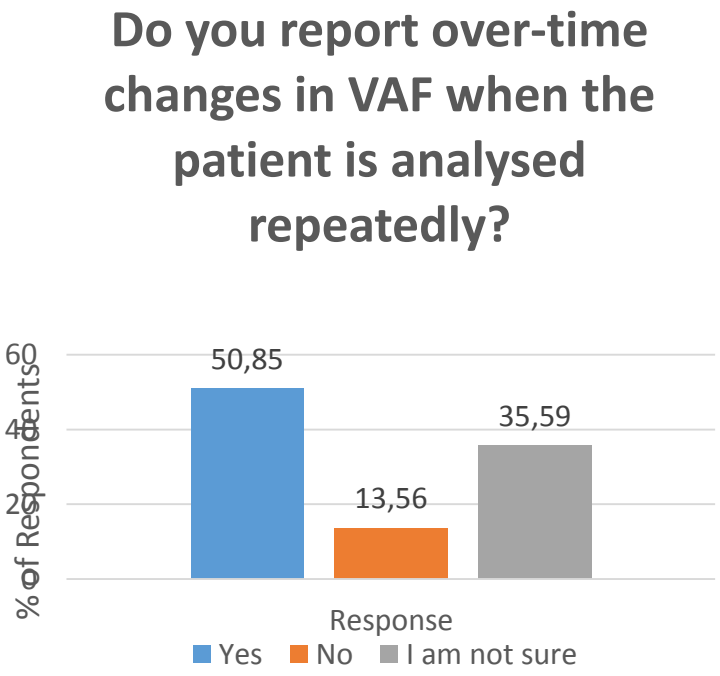
Reporting of TP53 variants – Optional results

Variant fraction	**Allelic fraction or cancer cell fraction?** Consider tumor content For NGS directly from variant calling > PCR duplicates can give wrong results (>UMIs) > chromosomal Aberrations give wrong results For Sanger only estimation possible Both: Understimation of subclonal TP53 mutations

Reporting of TP53 variants – Optional results

Recent Survey





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Reporting of TP53 variants – Optional results

SNP	**No clinical impact – no clinical need for reporting** Causing confusion: (*"This SNP is likely benign, possible clinical significance affecting drug response."*) Actually providing germline data (Patient identification) **=> Not recommended**

Reporting of TP53 variants – Optional results

Recent Survey

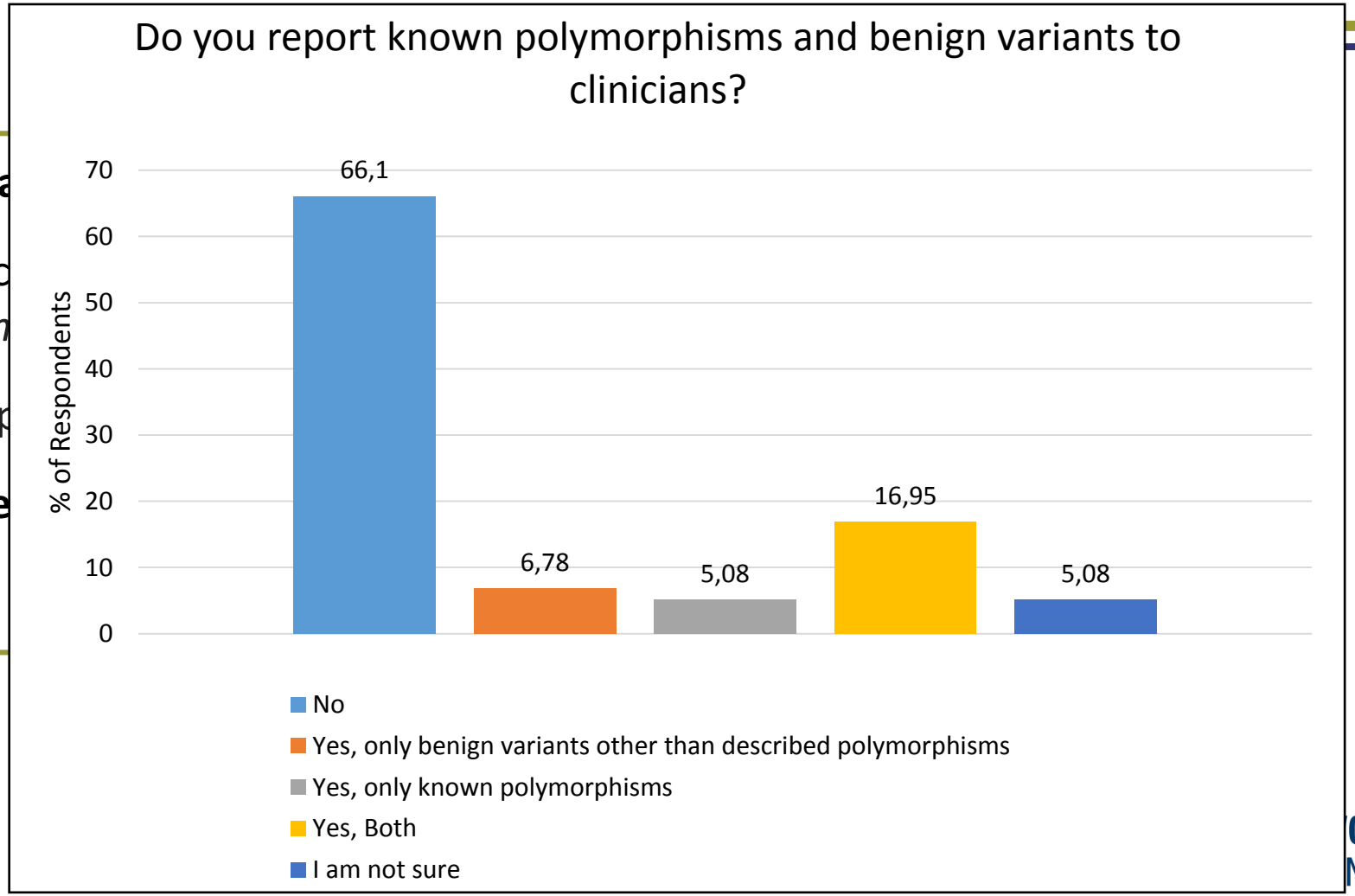
SNP

No clinica

Causing c
significan

Actually p

=> Not re



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Reporting of TP53 variants - Requirements

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- Type of analysis
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RESULT

of sequence analysis of exons 5-9 of the TP 53 gene

Date:	Sample received: 11 July 2014 Analysis: 12 – 15 July 2014 Results issued: 23 July 2014
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Acknowledgements

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