Variant interpretatie en rapportage

Van variantenlijst tot klinisch rapport

Astrid Eijkelenboom, PhD
Klinisch Moleculair Bioloog in de Pathologie (KMBP)

Radboudumc

Disclosure belangen spreker

(potentiële) belangenverstrengeling	Geen
Voor bijeenkomst mogelijk relevante relaties met bedrijven	nvt
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	nvt

Radboudumc

Acknowledgements

PATH project, WP2 en organisatie, in het bijzonder:
Marjolijn Ligtenberg
Ed Schuuring
Leon van Kempen

Alle deelnemers KMBP-dag 4 oktober 2019

Danielle Heideman (VUmc), Mirjam Boelens (NKI), Linda Bosch (NKI), Wendy de Leng (UMCU), Anne Jansen (UMCU), Ruud Meijers (UMCU), Clemens Prinsen (CWZ), Judith Jeuken (PAMM), Anke van den Berg (UMCG), Joost Kluiver (UMCG), Nienke Sonneveld (LUMC), Ronald van Eijk (LUMC), Paul Seegers (PALGA), Arja ter Elst (UMCG), Ina Geurts (Erasmus MC), Carel van Noesel (AMC), Erik-Jan Dubbink (Erasmus MC), Ed Schuuring (UMCG), Ingrid Vogelaar (Radboudumc, Pathologie DNA), Sjoerd van Helvert (Radboudumc), Leon van Kempen (UMCG), Marjolijn Ligtenberg (Radboudumc), Astrid Eijkelenboom (Radboudumc), Lieneke Steeghs (Radboudumc, PATH projectmedewerker), Saskia Cillessen (AMC/VUmc)

Radboudumc

KMBP meeting (4 oktober 2019)

- KMBP van 12 centra aanwezig
- Discussie variant classificatie (n.a.v. 10 casus met lastige varianten)
- Discussie: Wat te doen met 'Variants of Unknown Significance'?
- Vereisten bij rapportage fusiegen detectie

→ Werkgroep: Update richtlijn Moleculaire Verslaglegging (dd 2012)

Radboudumc

From variant list to clinical report

- Sequencing tumors, some numbers
- Variant classification in 5 classes
- 'Classification by similarity'
- From variant list to clinical report

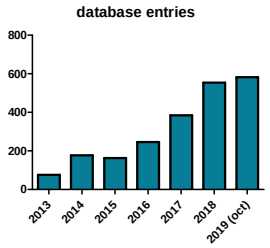
Radboudumc

From variant list to clinical report

- **Sequencing tumors, some numbers**
- Variant classification in 5 classes
- 'Classification by similarity'
- From variant list to clinical report

Radboudumc

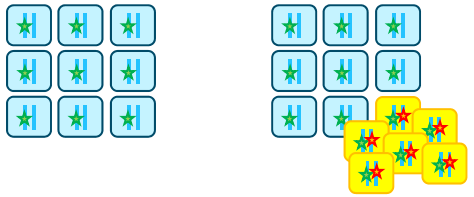
Sequencing tumors: variant classification database



Radboudumc, routine molecular diagnostics

Radboudumc

Sequencing tumors: germline + somatic variants

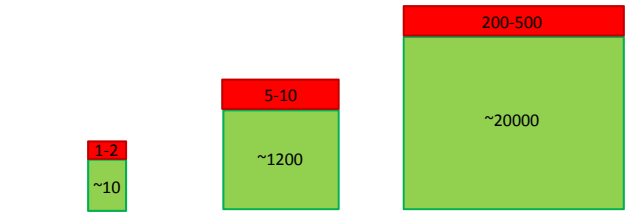


germline, (majority) benign

somatic, benign or pathogenic?
(passenger or driver?)

Radboudumc

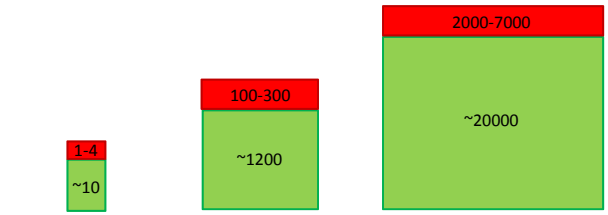
Sequencing tumors: germline + somatic variants



Estimated numbers of germline and somatic variants,
tumor with low tumor mutation burden

Radboudumc

Sequencing tumors: germline + somatic variants



Estimated numbers of germline and somatic variants,
tumor with high tumor mutation burden

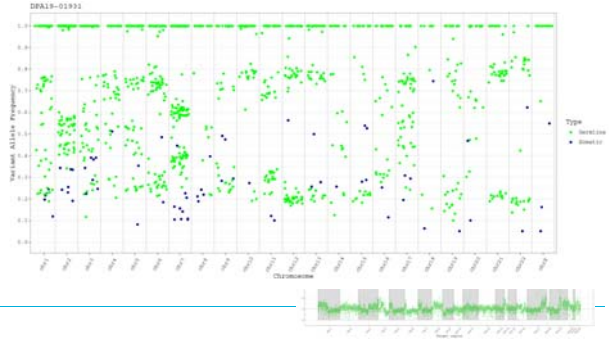
Radboudumc

Sequencing tumors: germline + somatic variants

Example TSO500: tumor with low tumor mutation burden, mostly diploid

Radboudumc

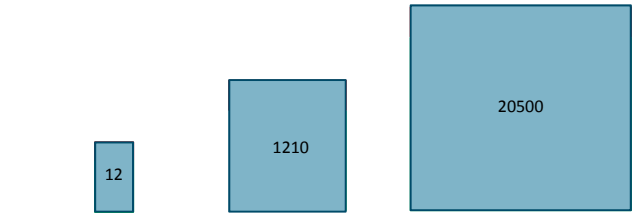
Sequencing tumors: germline + somatic variants



Example TSO500: tumor with intermediate tumor mutation burden (~30/Mb), aneuploidy

Radboudumc

Sequencing tumors: germline + somatic variants



PATH panel
(regions in) 32 genes

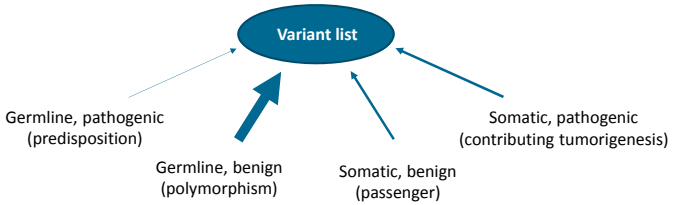
TSO500 panel
~500 genes

exome
~22000 genes

Estimated numbers of germline and somatic variants,
tumor with low tumor mutation burden

Radboudumc

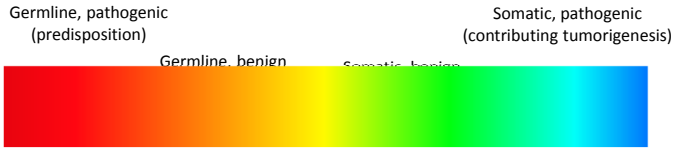
Sequencing tumors: germline + somatic variants



Lists should be prefiltered to remove artifacts

Radboudumc

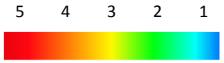
Sequencing tumors: variant classification



Radboudumc

From variant list to clinical report

- Sequencing tumors, some numbers
- **Variant classification in 5 classes**
- 'Classification by similarity'
- From variant list to clinical report



Radboudumc

Variant classification

1: Benign

2: Likely Benign

3: Variant of Unknown Significance (VUS)

4: Likely pathogenic

5: Defenitely pathogenic

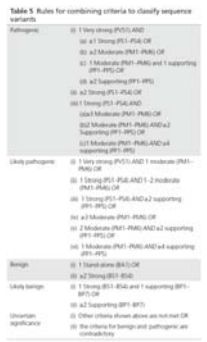
5 class system, originates from Human Genetics

Radboudumc

Variant classification

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Richards et al., 2015



Oncogenes → pathogenic = gain of function
Tumor suppressor genes → pathogenic = loss of function

Radboudumc

"De uitzondering bevestigt de regel"

Radboudumc

BRCA2 (NM_000059.3):c.9976A>T (p.(Lys3326*))

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Maar: populatie frequentie volgens gnomAD:
ALL:0.65% - AFR:0.13% - AMR:0.27% - ASJ:0.41% - SAS:0.69% - NFE:0.87% - FIN:1.09% - OTH:0.64%

Strong evidence of benign impact

BS1 Allele frequency is greater than expected for disorder

Te frequent om disease causing te zijn

Premature stop in tumor suppressor → benign

Radboudumc

CXCR4 (NM-001008540.2): c.1024dup (p.(Ser342fs))

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Geen referentie in literatuur voor deze specifieke mutatie

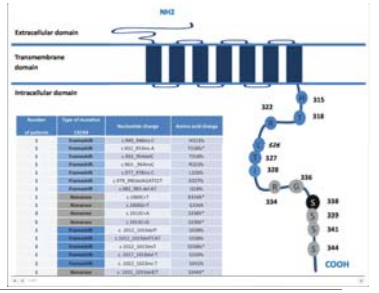
Oncogen, maar premature stops/frameshifts beschreven → verhoogde expressie

Bekend in Waldenström macroglobulinemia (WM)

Premature stop in oncogen

Radboudumc

CXCR4 (NM-001008540.2): c.1024dup (p.(Ser342fs))



Radboudumc

CXCR4 (NM-001008540.2): c.1024dup (p.(Ser342fs))

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Geen referentie in literatuur voor deze specifieke mutatie

Oncogen, maar premature stops/frameshifts beschreven → verhoogde expressie

Bekend in Waldenström macroglobulinemia (WM)

Premature stop in oncogen

Radboudumc

JAK2 (NM_004972.3): c.1849G>T (p.(Val617Phe))

Hotspot, activating mutation, commonly in myeloproliferative neoplasms, targeted with JAK inh.

Strong evidence of benign impact

BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age

But also included in multiple population databases:

gnomAD: ALL:0.034% - AFR:0.032% - AMR:0.017% - ASJ:0.058% - EAS:0.015% - SAS:0.033% - NFE:0.038% - FIN:0.056% - OTH:0.014%

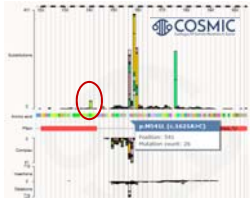
Oncogene, pathogenic (gain-of-function) mutation included in multiple population databases (commonly mutated genes in hematological malignancies can also be found in the blood of otherwise healthy individuals)

Radboudumc

KIT (NM_000222.2): c.1621A>C (p.(Met541Leu))

Moderate evidence of pathogenicity

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation



- Common germline benign variant:

gnomAD: ALL:7.65% - AFR:5.94% - AMR:4.91% - ASJ:9.71% - EAS:4.61% - SAS:7.59% - NFE:9.69% - FIN:4.13% - OTH:8.50%

- But also included in database for somatic mutations (COSMIC, 26x)

Oncogene, benign variant, included in somatic mutation in cancer databases (loosely controlled sources of submitted variants)

Radboudumc

De uitzonderingen…

Premature stop in tumor suppressor (klasse 1, benigne)

Premature stop in oncogen (klasse 4, waarschijnlijk pathogeen)

Bekende pathogene mutatie in populatie database

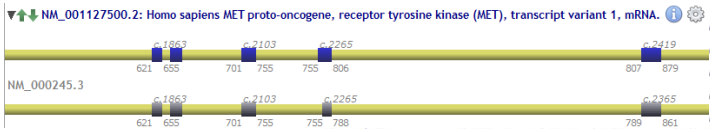
Bekende benigne variant uit populatie in somatische tumormutatie database

Radboudumc

MET (NM_001127500.2): c.2962C>T, p.(Arg988Cys) alias p.(R988C)

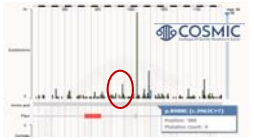
a.k.a.: MET (NM_000245.3) exon 14: 2908C>T, p.(Arg970Cys) alias p.(R970C)

Genomic position (DNA):	Chr7(GRCh37):g.116411923C>T
Inferred coding position in transcript (RNA):	c.2962C>T or 2908C>T
Inferred amino acid position in protein:	p.(Arg988Cys) or p.(Arg970Cys)



Radboudumc

MET (NM_001127500.2): c.2962C>T (p.(Arg988Cys))



- MET exon 14: no predicted effect on splicing
- Included in cancer somatic mutation & population databases
- gnomAD: ALL:0.28% - AFR:0.091% - AMR:0.12% - ASJ:0.16% - EAS:0.026% - SAS:0.065% - NFE:0.50% - FIN:0.088% - OTH:0.32%; Dutch: T=1.10%
- In vitro data conflicting

Oncogene, included in somatic mutation & population databases, conflicting in vitro data

Radboudumc

Strong evidence of pathogenicity

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established

Gene Variant Descriptions: MET R988C (corresponds to R970C in the canonical isoform) lies within the juxtamembrane domain of the Met protein (PMID: 14559814, UniProt.org). R988C does not increase Met phosphorylation, but results in increased phosphorylation of cellular proteins, and increased proliferation and migration of cultured cells (PMID: 14559814, PMID: 20670955, PMID: 22972954, PMID: 31004003).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4468339/

Conflicting studies for effect.
But, MET gain-of-function is normally mutually exclusive with RAS/RAF gain of function

Variant classification

Also take into consideration:

- Variant allele frequency (VAF) vs tumorload (%)
- Other pathogenic (driver) variants
- TMB
- Clinical context

Radboudumc

From variant list to clinical report

- Sequencing tumors, some numbers
- Variant classification in 5 classes
- **'Classification by similarity'**
- From variant list to clinical report

Radboudumc

Variant classification?

Variant is not described in any database

The Beatles

Radboudumc

Variant classification by similarity

PVS1	Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease	Gene level
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease	
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation	Amino acid level
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	

Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Richards et al., 2015

Radboudumc

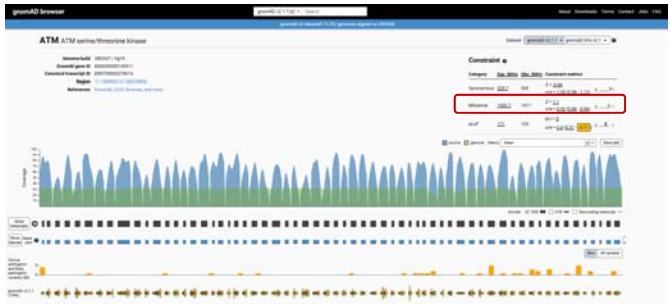
Variant classification by similarity

PVS1	Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease	Gene level
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease	
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	

→ ATM vs PTEN

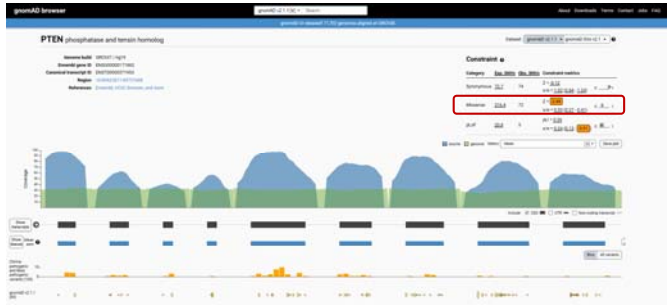
Radboudumc

ATM: number of missense variants in population is as expected

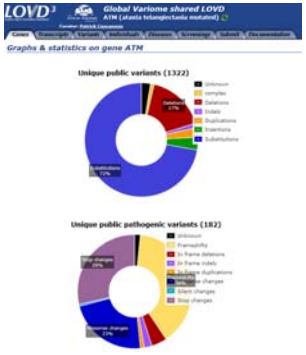


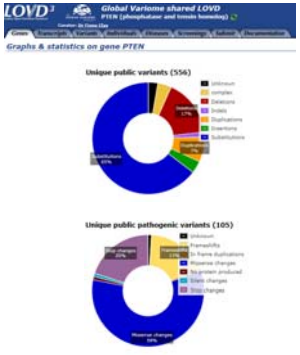
Radboudumc

PTEN: number of missense variants in population is lower than expected



Radboudumc





Radboudumc

Variant classification by similarity

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation

PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

} Domain/Amino acid level

Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

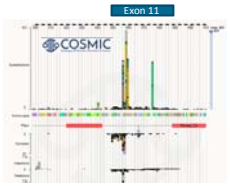
Richards et al., 2015

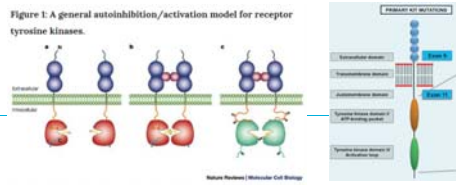
Radboudumc

KIT (NM_000222.2): c.1678_1738delinsCCTTATGATA

p.(Val560_His580delinsProTyrAspAsn)

- delins, *in frame*
- Not in any database
- But: in frequently mutated region (in exon 11) in regulatory juxtamembrane region





Juxtamembrane autoinhibition in receptor tyrosine kinases, 2004
Therapeutic Advances in Medical Oncology, 2014

Radboudumc

CXCR4 (NM-001008540.2): c.1024dup (p.(Ser342fs))

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Geen referentie in literatuur voor deze specifieke mutatie

Oncogen, maar premature stops/frameshifts beschreven → verhoogde expressie

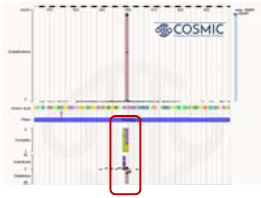
Bekend in Waldenström macroglobulinemia (WM)

KMBP-dag: Classification 3-5

Radboudumc

BRAF (NM_004333.4): c.1795_1800delinsTACAGTGAA

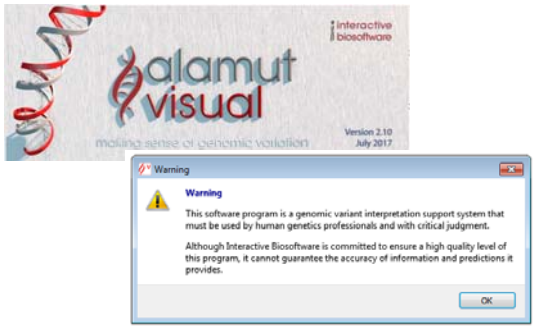
(p.(Thr599_Val600delinsTyrSerGlu))



- delins, *in frame*
- near p.V600 hotspot
- no literature, but comparable mutations ->
- Tyr599-Val600 becomes Tyr599-Ser600-Glu601

KMBP-dag: Classification 3-5

Radboudumc



Radboudumc

From variant list to clinical report

- Sequencing tumors, some numbers
- Variant classification in 5 classes
- 'Classification by similarity'
- **From variant list to clinical report**

Radboudumc

From variant list to clinical report

1: Benign

2: Likely Benign

3: Variant of Unknown Significance (VUS)

4: Likely pathogenic

5: Defenitely pathogenic

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Richards et al., 2015

Most Dutch laboratories use his system

Radboudumc

From variant list to clinical report

National guidelines required:

1). Which variants to include?

2). What variant information to include?

3). Clinical significance?

Radboudumc

From variant list to clinical report

National guidelines required :

1). Which variants to include?

2). What variant information to include?

3). Clinical significance?

Radboudumc

From variant list to clinical report

1: Benign
2: Likely Benign
→ Do not report

3: Variant of Unknown Significance (VUS) → ?

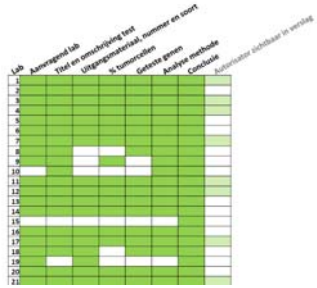
4: Likely pathogenic
(activating oncogene, inactivating TSG)
5: Defenitely pathogenic
(activating oncogene, inactivating TSG)
→ Report

→ Werkgroep: Update richtlijn Moleculaire Verslaglegging (dd 2012)

Radboudumc

Huidig gebruik richtlijn Moleculaire Verslaglegging

"Deze richtlijn geeft aan welke onderdelen in elk geval in verslagen moeten worden weergegeven. Het verdient aanbeveling deze items puntsgewijs en compact weer te geven."



https://pathology.nl/wp-content/uploads/PDF/kwaliteit/richtlijnen/WMDP_richtlijn_verslaglegging_moleculaire_diagnostiek_20120601.pdf

→ Werkgroep: Update richtlijn Moleculaire Verslaglegging (dd 2012)

Radboudumc

Which variants to include?

Recent example:

- Premature stop in *EGFR* exon 21. Very likely a 'benign' passenger mutation.
- Was included in report.
- In summary it was included as "*exon 21, not targetable".*
- Personal communication with clinician: *"I consider therapy targeting EGFR"*

Radboudumc

Which variants to include?


Genome Medicine

DATABASE Open Access

Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations

"Most mutations affecting cancer genes are of uncertain significance"
(88% in cohort of 44601 protein affecting mutations)

With increased panel size:
The number of VUS can outnumber class 4 and 5 variants in the reports

Radboudumc

Relevance of VUS (class 3 variants)?

Lessons from Genetics (Hereditary cancer predisposition testing)

Variants are frequently reclassified and usually downgraded over time

"Similar to recent publications on variant reclassifications [4–6], we determined that the vast majority of unique variant reclassifications were downgrades (90.3%) with a much smaller fraction (9.7%) being upgraded. In particular, only 7.5% of variants reclassified from the VUS category were upgraded."

"The disproportionate number of downgrades from the VUS category seen in our cohort, in addition to data from other recent publications [4, 6], shows the aggressive usage of the VUS category by laboratories during variant annotation. If the VUS category was used impartially, the number of upgraded and downgraded reclassifications from this category would be more evenly balanced. This overuse of VUS classification is becoming an even greater issue because VUS rates track with the number of genes tested [7] and the use of multigene panels has rapidly expanded in standard of care hereditary cancer predisposition testing [8]."

Oncotarget
Open Access Impact Journal

The effects of genomic germline variant reclassification on clinical cancer care

Radboudumc

Relevance of VUS (class 3 variants)?

Cancer genomes tolerate many coding mutations

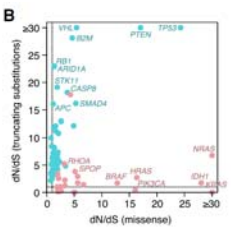

Article

Cell

Universal Patterns of Selection in Cancer and Somatic Tissues

Highlights

- Unlike the germline, somatic cells evolve predominantly by positive selection
- Nearly all (~99%) coding mutations are tolerated and escape negative selection
- Exome-wide estimates of the total number of driver coding mutations per tumor
- Half of the coding driver mutations occur outside of known cancer genes



Positive selection missense primarily in oncogenes, TP53 en PTEN

Radboudumc

From variant list to clinical report

		Gen versus indicatie	
Mutatie classificatie		klinisch relevant	onduidelijke klinische relevantie
	1: Benigne	-	-
	2: Waarschijnlijk benigne	-	-
	3: VUS	v (mits..., opmerking in verslag en/of bespreken in MTB)	x (tenzij..., uitgezonderd LUMC, UMCU, AvL)
	4: Waarschijnlijk pathogeen/ activerend oncogen/ inactiverend tumorsuppressorgen	v	v
	5: Pathogeen/ activerend oncogen/ inactiverend tumorsuppressorgen	v	v

→ Werkgroep: Update richtlijn Moleculaire Verslaglegging (dd 2012)

Radboudumc

From variant list to clinical report

National guidelines required :

1). Which variants to include?

2). What variant information to include?

3). Clinical significance?

Radboudumc

The clinical report: variant information

Variant details (inventory of prefered transcript numbers)

MET (NM_001127500.2): c.2962C>T, p.(Arg988Cys) alias p.(R988C)

a.k.a.: MET (NM_000245.3) exon 14: 2908C>T, p.(Arg970Cys) alias p.(R970C)

'Category of variant'

Clinician: "KIT exon 11?"

Conclusie:
Biopt ventrale zijde rectum: Beeld passend bij een gastro-intestinale stromacelтumor (GIST).

Next generation sequencing: in het weefsel wordt een mutatie aangetoond in KIT (c.1669_1674del (p.Trp557_Lys558del)). In het weefsel wordt geen tweede mutatie aangetroffen. Specifieke mutaties in KIT hebben gevolg voor de selectie en dosering van targeted therapie (imatinib, sunitinib) in patienten met GIST.

Radboudumc

From variant list to clinical report

National guidelines required:

1). Which variants to include?

2). What variant information to include?

3). Clinical significance?

Radboudumc

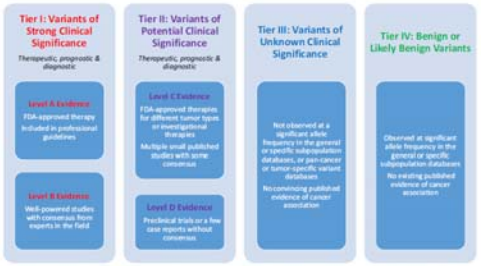


Figure 2 Evidence-based variant categorization. Somatic variants are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics. Variants in tier I are of strongest clinical significance, and variants in tier IV are benign or likely benign variants. FDA, Food and Drug Administration.

SPECIAL ARTICLE

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

→ Variant vs clinical significance

Radboudumc

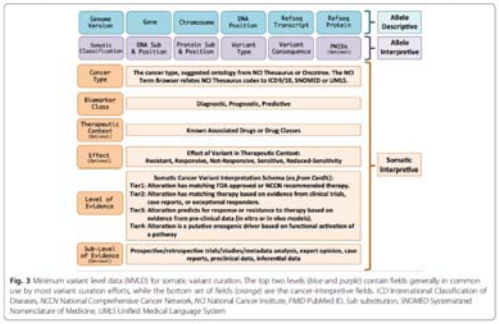


Fig. 3 Minimum variant level data (MVLD) for somatic variant curation. The top two levels (blue and purple) contain fields generally in common use by most variant curation efforts, while the bottom set of fields (orange) are the cancer-interpretive fields. ICD International Classification of Diseases, NCCN National Comprehensive Cancer Network, NCI National Cancer Institute, PMID PubMed ID, Sub substitution, SNOMED Systematized Nomenclature of Medicine, UMLS Unified Medical Language System

Genome Medicine

RESEARCH Open Access

Somatic cancer variant curation and harmonization through consensus minimum variant level data

→ Variant vs clinical significance

Radboudumc

Rapportage klinische relevantie van varianten?

"Alleen binnen richtlijn"

"Verantwoordelijkheid van behandelend arts"

"Altijd"

"Verantwoordelijkheid ligt ook bij KMBP"

"Nooit"

"KMBP is geen arts, kan klinische relevantie niet volledig overzien"

"In algemene termen"

"Arts begrijpt niet altijd de resultaten"

Mijn samenvatting: gedeelde verantwoordelijkheid.
KMBP heeft geen volledig klinisch overzicht, maar arts kan moleculair resultaat niet altijd goed duiden.

Suggestie: "Mogelijk aanknopingspunten voor therapiekeuze:
TMB 15; EGFR activerende mutatie (exon 19)."

Radboudumc

Molecular Tumor Boards

Platform to discuss potentially clinically relevant variants

Shared responsibility in the interpretation of clinical relevance for therapy

(Nuance might be lost in written reports)

Radboudumc

From variant list to clinical report

- Sequencing tumors, some numbers
- Variant classification in 5 classes
- ‘Classification by similarity’
- From variant list to clinical report

Radboudumc