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NGS Benchmarking trial in (hemato-)oncology

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Overview

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Methods

Results

- Wet and dry lab methods used
- Assessed variants and criteria
- Unexpected results:
  - Horizon artificial variants
  - Endogenous variants
- Variant description (Nomenclature)
- Clinical/biological interpretations

Conclusions

Next steps and perspectives

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Assess the quality of NGS tests carried out by the participating laboratories:

Examine the ability of the validated/accredited NGS assays to accurately and reproducibly detect mutations

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Methods: Participants

Participation was open to all Belgian laboratories which meet the actual legal obligations:

- Licensed (pathology, clinical biology, centre of human genetics)
- Accredited or in process to be accredited for NGS testing (ISO 15189)

First Benchmarking trial focused on solid tumors

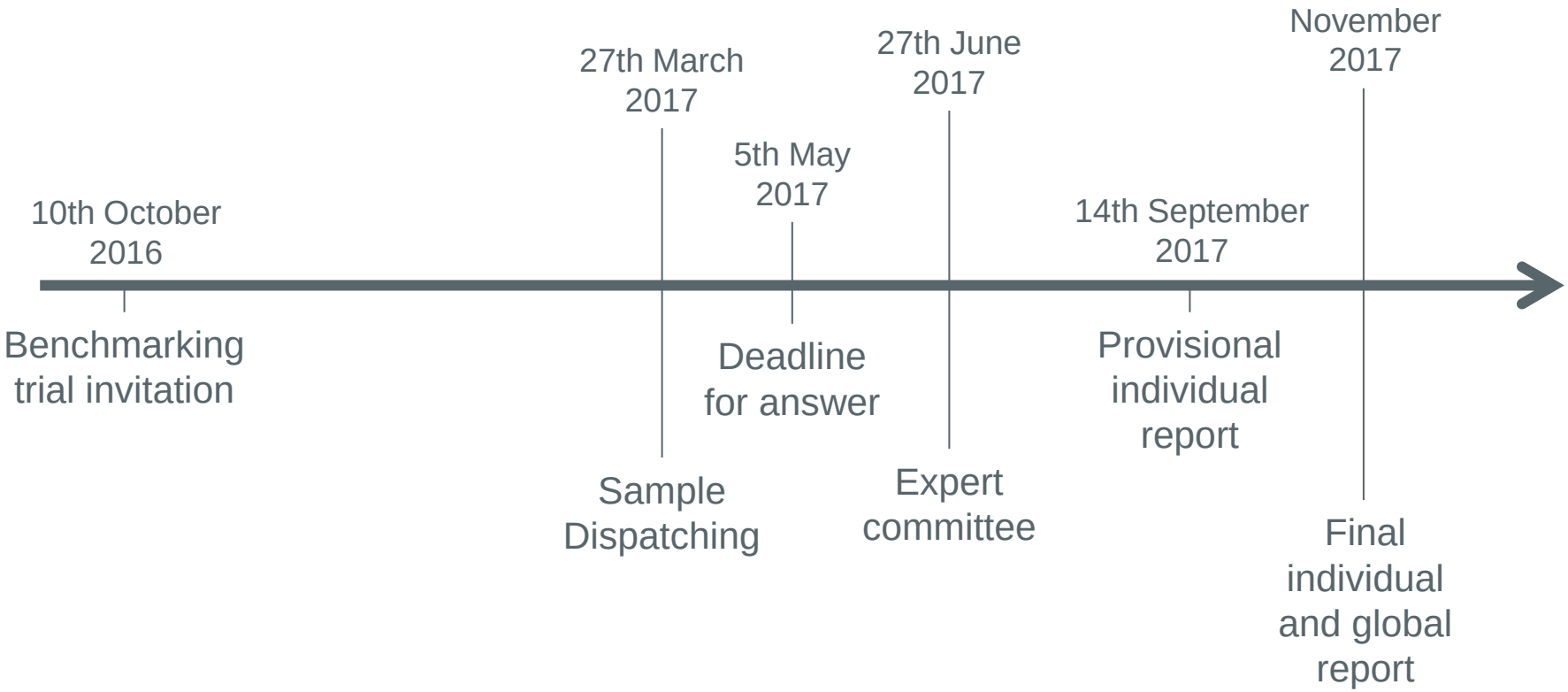
Methods: Participants

16 laboratories participated to the NGS benchmarking trial performed on solid tumors

Regions	N
Flemish region	10
Region of Brussels	4
Walloon region	2
Total	16

Laboratories	N
Pathology	10
Clinical biology	4
Center of Human Genetics	2
Total	16

Methods: Timeline



Methods: reference samples

Well-characterized genomic DNA from tumor cell lines:

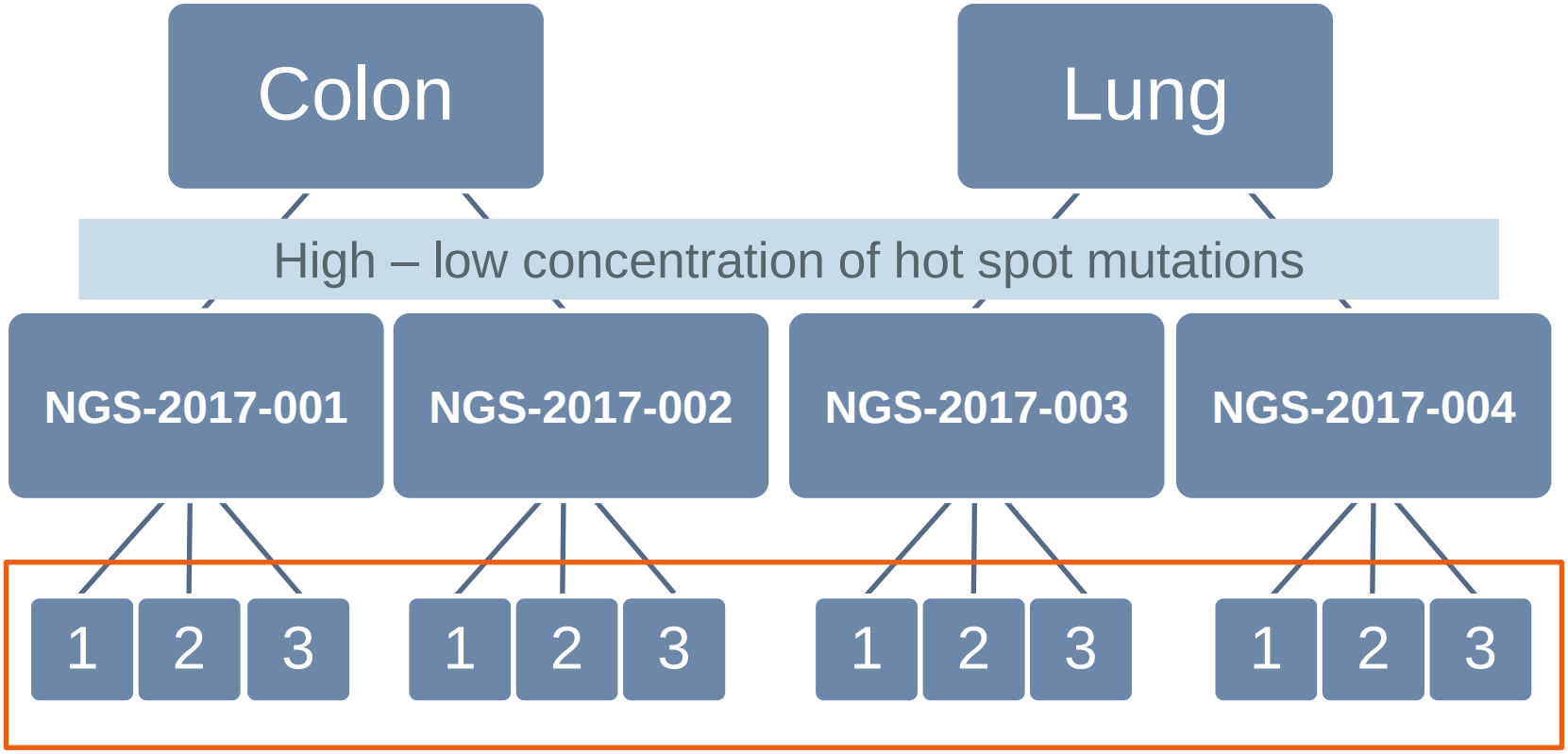
⟶ mixture of different cell lines

Reference samples produced externally according to our custom design and validated by ddPCR (HORIZON) :

- Type and number of variants/sample
- Allele frequency between 3 and 50%
- Clinically relevant mutations

⟶ ComPerMed discussions

Methods: reference samples



Total of 12 analyses

Methods: request

The laboratories had to analyze samples by NGS as in routine, using their standard methods

Requests for Benchmarking trial:

- Wet lab and dry lab methods
- Variant identification and their biologic and/or clinical classification
- Raw data files (fastq, bam and vcf files)
- Clinical reports

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Results: Wet and dry lab methods

Sequencer company and platform:

Company	N	Platform	N
Illumina	14	MiSeq	14
		NextSeq	3
		HiSeq	1
IonTorrent	2	PGM	2

→ Some labs have access to more than one sequencer

Results: Wet and dry lab methods

Bioinformatics pipelines:

Variant calling and annotating programs	N
Variant studio (Illumina)	6
Miseq Reporter (Illumina)	6
SeqNext (JSI)	5
NextGene (Softgenetics)	2
Sophia DDM (Sophia Genetics)	2
Torrent suite + TVC (Ion Torrent)	1
Genome Browser (Golden Helix)	1
BWA+GATK + Annovar (*open source*)	1

Results: Wet and dry lab methods

Assay Reads Characteristics:

Single/paired	N
Single end	2 (IonTorrent)
Paired end	14 (Illumina)

Minimum sequencing depth	N
1000X	7
500X	7
300X	2

Reads length	N
75bp	1
100bp	1
120bp	4
150bp	10
250bp	1

Results: Wet and dry lab methods

Which categories of somatic variants are detected by the assay?	N
SNV	16
Indels (<50bp)	16
Indels (50bp – 1kb)	0
CNV	0
Translocations	0

SNV lower limit of detection (%)	N
1%	3
2.5%	1
3%	1
4%	1
5%	10

indels < 50b Lower limit of detection (%)	N
1%	2
2.5%	2
3%	1
4%	1
5%	10

Results: Wet and dry lab methods

Which specimen types do laboratories test for somatic variant detection?	N
FFPE tissues	16
Frozen tissues	1
Fresh tissues	2
Cytological liquids	3
Blood	1
Swabs	1
Circulating tumor cell DNA	1

Do laboratories perform sequencing on tumor-normal paired specimens?	N
Yes	0
No	16

Results: Wet and dry lab methods

Required quantity of purified genomic DNA for the NGS assay	N
0-50ng	10
51-100ng	2
101-200ng	2
201-300ng	1
301-500ng	0
501-1000ng	0
>1000ng	0

→ One laboratory has 2 thresholds depending on the gene panel used

→ Two laboratories don't have a pre-set threshold but validate via PCR

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Results: 16 assessed variants

Sample	Gene	Mutation	Proportion of laboratories that identified the mutation	Median allele frequency (%)	Standard Deviation
NGS-2017-001	**BRAF**	**p.(Val600Glu)**	16/16	13.21	0.60
NGS-2017-001	**KRAS**	**p.(Gly13Asp)**	16/16	32.94	0.64
NGS-2017-001	**NRAS**	**p.(Gln61Lys)**	16/16	21.55	1.17
NGS-2017-002	**BRAF**	**p.(Val600Arg)**	16/16	11.26	1.13
NGS-2017-002	**KRAS**	**p.(Ala146Thr)**	**15/16**	20.07	2.31
NGS-2017-002	**NRAS**	**p.(Gly12Asp)**	16/16	19.42	2.24
NGS-2017-003	**BRAF**	**p.(Val600Lys)**	16/16	48.50	2.95
NGS-2017-003	**EGFR**	**p.(Glu746-Ala750del)**	**15/16**	35.70	2.89
NGS-2017-003	**EGFR**	**p.(Gly719Ser)**	**15/16**	11.10	1.38
NGS-2017-003	**KRAS**	**p.(Gly12Ala)**	**15/16**	18.24	1.36
NGS-2017-004	**BRAF**	**p.(Val600Met)**	16/16	19.73	0.83
NGS-2017-004	**EGFR**	**p.(Gly719Ser)**	**12/16**	3.73	0.50
NGS-2017-004	**EGFR**	**p.(Leu858Arg)**	16/16	38.13	0.96
NGS-2017-004	**EGFR**	**p.(Thr790Met)**	16/16	38.00	1.10
NGS-2017-004	**KRAS**	**p.(Gly12Cys)**	**15/16**	5.16	0.42
NGS-2017-004	**KRAS**	**p.(Gly13Asp)**	16/16	29.07	0.95

→ **Success criteria: Detection of all clinically relevant variants (only qualitative criteria)**

Results: Success rate

Success rate (% of identified variants/16)	N
16/16 (100%)	9
15/16 (93.75%)	5
14/16 (87.50%)	2
Total success rate : 247/256 (96.48%)	16

⟶ **In total, 9 unexpected results**

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Unexpected results

Sample	Gene	Mutation	Proportion of laboratories that identified the mutation	Median allele frequency (%)	Standard Deviation
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NGS-2017-002	**KRAS**	**p.(Ala146Thr)**	**15/16**	**20.07**	2.31
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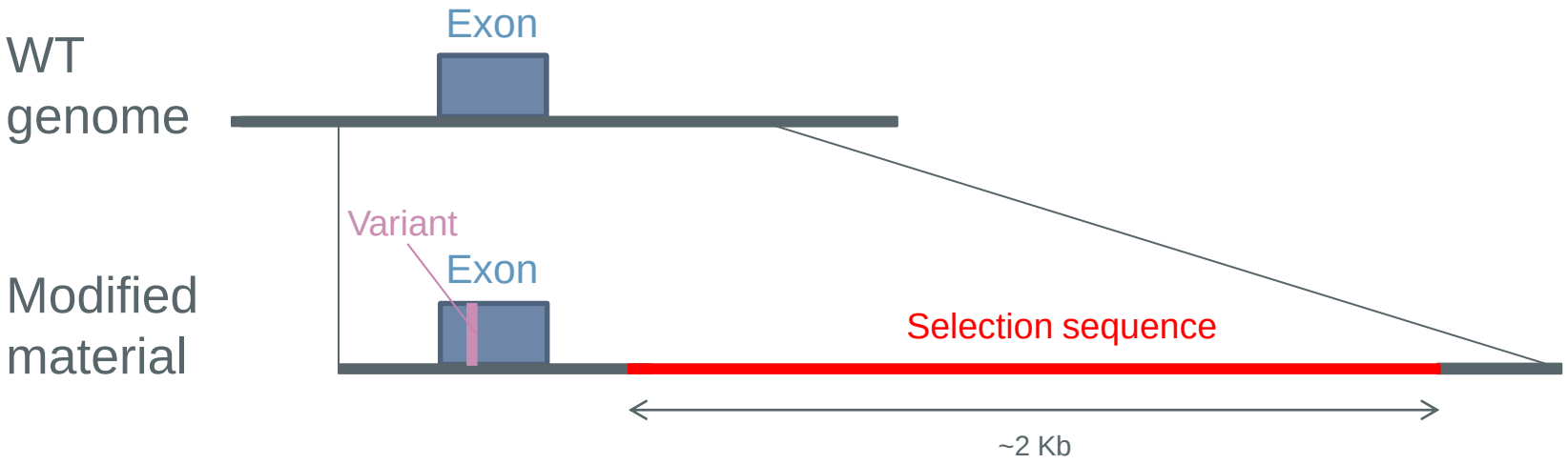
→ **3 Horizon artificial variants**

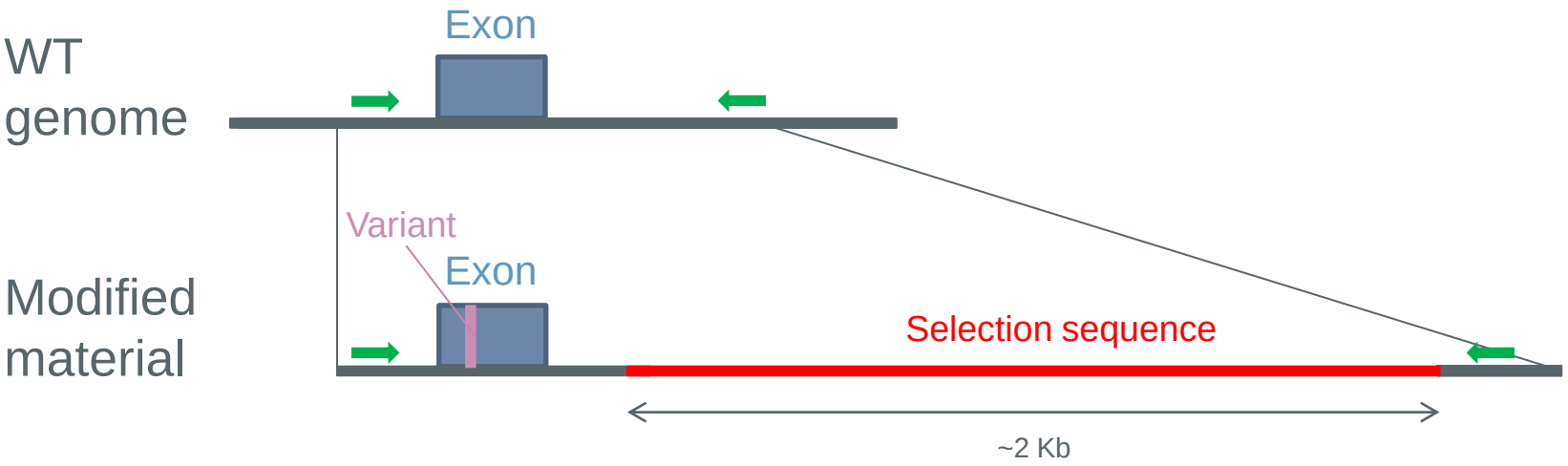
Unexpected results: Horizon artificial variants

- Horizon artificial variant : Homologous recombination



Unexpected results: Horizon artificial variants

- Library construction can cause enrichment bias



Unexpected results: Horizon artificial variants

- Library construction can cause enrichment bias

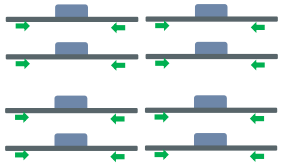
Initial mix:

WT (50) / Mutant (50)

PCR amplification

Resulting Amplicons:

WT (100) / Mutant (0)



→ **These variants have not been evaluated for the 2 concerned laboratories.**

Unexpected results: Horizon artificial variants

New success rate without the 3 Horizon artificial variants

success rate	N
100%	**11**
15/16 (93.75%)	**4**
14/16 (87.50%)	**1**
Total success rate : 247/253 (**97.63%**)	16

⟶ **In total, 6 unexpected results in endogenous variants**

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Unexpected results: endogenous variants

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→ **6 unexpected results concerning 3 endogenous variants**

Unexpected results: endogenous variants

1. NGS-2017-003 : **EGFR p.(Glu746-Ala750del)**

- One laboratory reported the EGFR p.(Glu746_Ala750delinsIlePro) variant instead of the EGFR p.(Glu746-Ala750del) variant.
- Presence of the correctly identified mutation in the raw data and the vcf file.
- **<u>Conclusion</u>:** Transcription error from the software to the transmitted protocol.

Unexpected results: endogenous variants

2. NGS-2017-003 : **EGFR p.(Gly719Ser)**

- One laboratory did not identify the EGFR p.(Gly719Ser) variant with a median allele frequency of 11.10%.
- **Conclusion**: Problem of identification of this variant with the laboratory method.

Unexpected results: endogenous variants

3. NGS-2017-004 : **EGFR p.(Gly719Ser)**

- 4 laboratories did not report the EGFR p.(Gly719Ser) variant with a median allele frequency of 3.73%.
- 3/4 laboratories have a lower limit of detection for SNV of 5%. **Conclusion**: The allele frequency of this mutation is below their detection threshold.
- 1/4 has a lower limit of detection for SNV of 3%. **Conclusion**: Problem of identification of this variant with the laboratory method.

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Results: variant description

No uniformity between laboratories for annotation of sequence variants:

In protein, cDNA and chromosomal position levels

Example:

- p.V600R
- p.Val600Arg
- **p.(Val600Arg)**
- p.Val600delinsArg
- p.V600delinsR

→ In the future, standardization required for Healthdata database (via the ComPerMed)

Results: variant description

HGVS-nomenclature: http://varnomen.hgvs.org/

- Recommendations for the description of sequence variants serves as an international standard

SPECIAL ARTICLE

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

HGVS Recommendations for the Description of Sequence Variants: 2016 Update

Johan T. den Dunnen,[1]* Raymond Dalgleish,[2] Donna R. Maglott,[3] Reece K. Hart,[4] Marc S. Greenblatt,[5] Jean McGowan-Jordan,[6] Anne-Francoise Roux,[7] Timothy Smith,[8] Stylianos E. Antonarakis,[9] and Peter E.M. Taschner[10] on behalf of the Human Genome Variation Society (HGVS), the Human Variome Project (HVP), and the Human Genome Organisation (HUGO)

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Results: clinical/biological interpretations

Example:

KRAS p.(Gly13Asp) Sample NGS-2017-001
Pred/prog in (other) tumor type
pathogene variant/COSMIC, My Cancer Genome, MD Anderson, Li et al. (2017)
pathogeen
pathogénique / impact clinique avéré
sterk klinisch significant
Ras mutatie positief. Geen indicatie voor anti-EGFR TKI behandeling bij darmtumoren (Douillard JY et al. 2013 NEJM & Berlin J. et al. 2013 NEJM & Bokemeyer C et al. JCO 2010).
Cette mutation confère une sensibilité réduite aux anticorps anti-EGFR
pathogeen/resistent aan therapie
pathogeen / De aanwezigheid van een activerende mutatie in KRAS wordt in het algemeen geassocieerd met een slechte respons op anti-EGFR monoklonale antilichaam behandeling.

→ **No uniformity between laboratories for interpretation of variant**

Results: clinical/biological interpretations

KRAS p.(Gly13Asp) Sample NGS-2017-001
Pred/prog in (other) tumor type
pathogene variant/COSMIC, My Cancer Genome, MD Anderson, Li et al. (2017)
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pathogeen / De aanwezigheid van een activerende mutatie in KRAS wordt in het algemeen geassocieerd met een slechte respons op anti-EGFR monoklonale antilichaam behandeling.

→In the future, discussion of standardization during the ComPerMed meetings

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- Numerous differences observed between NGS methods used in Belgium.
- Good global success rate (247/253): only 6 unexpected results with 3 linked to the limit of detection.
- No uniformity in sequence annotation and interpretation of the variants : ComPerMed meeting needed.

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- ✓ Benchmarking trial on hematological tumors:

  Timeline:

  - Benchmarking trial invitation: 5th July 2017
  - Sample dispatching: 9th October 2017
  - Deadline for answer: 17th November 2017
- ✓ Improvement of the methodology:
  - FFPE reference samples
  - Adaptation of the success criteria
- ✓ Development of a national EQA program

Thank you!

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