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Determining Pathogenicity & Interpretation of Results Challenging Cases from Cologne

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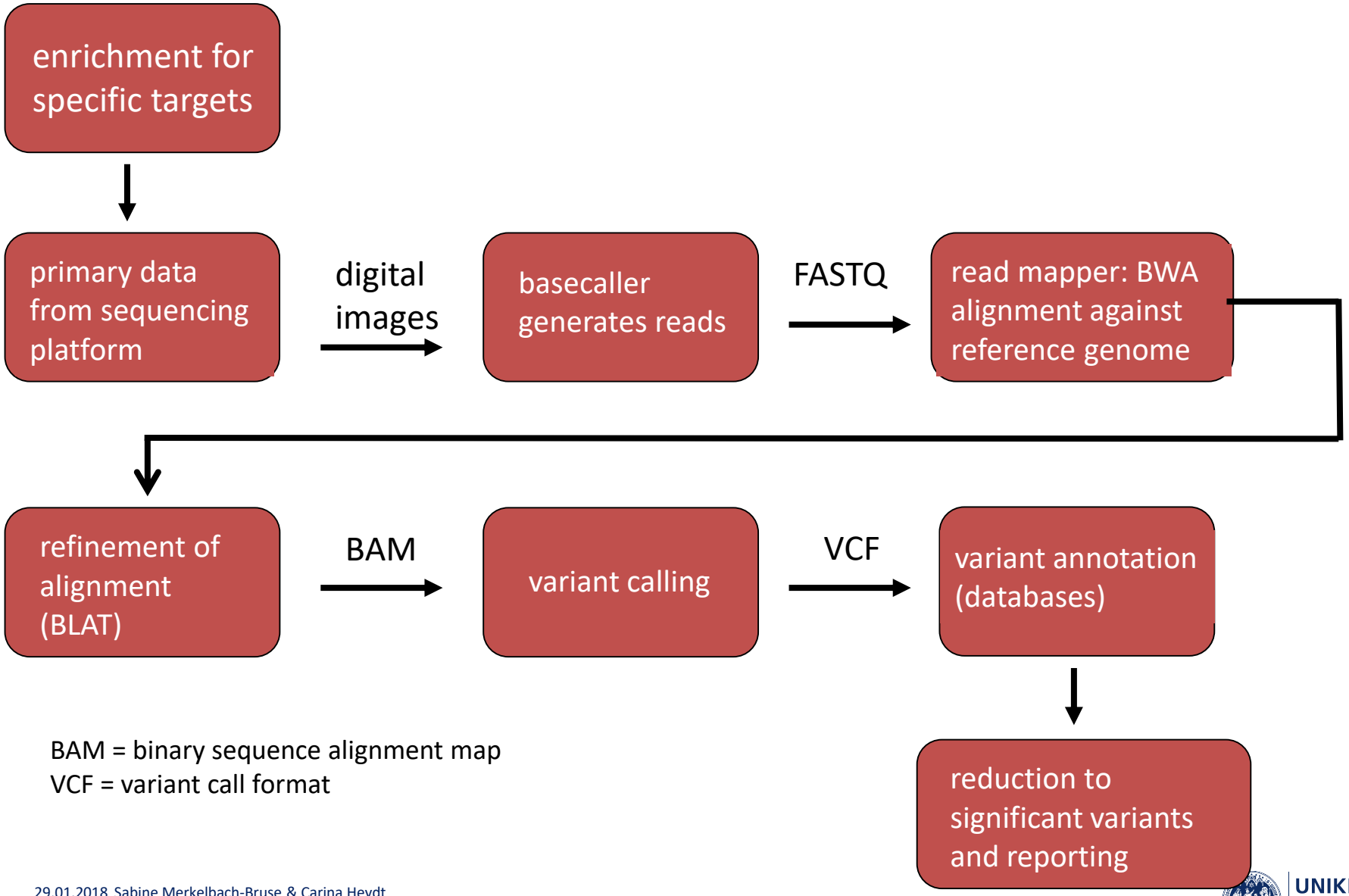
Bioinformatic pipeline and quality criteria

Which bioinformatic solutions are available?

- **Laboratory developed using open source software, e.g.**
  - BWA mapping
  - freebayes variant calling
- **Platform specific solutions, e.g.**
  - IonReporter (ThermoFisher)
  - BaseSpace (Illumina)
- **Commercial software packages, e.g.**
  - CLCbio
  - Nextgene
  - SEQNext JSI

…..and many others

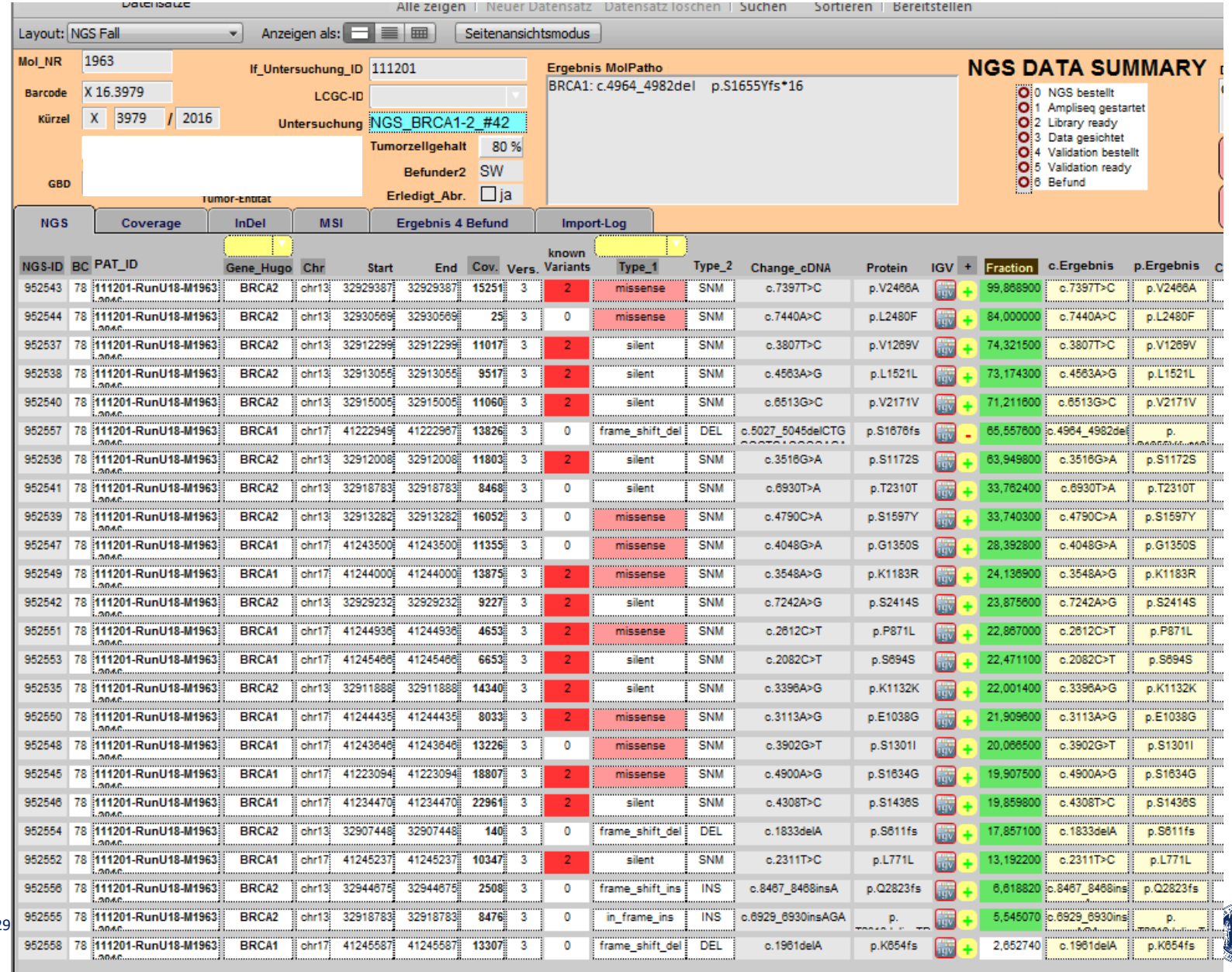
Bioinformatic pipeline and quality criteria



BAM = binary sequence alignment map
VCF = variant call format

Bioinformatic pipeline and quality criteria

- files are uploaded into the FileMaker data base for evaluation:



NGS-ID	BC	PAT_ID	Gene_Hugo	Chr	Start	End	Cov.	Vers.	known Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
952543	78	111201-RunU18-M1963	BRCA2	chr13	32929387	32929387	15251	3	2	missense	SNM	c.7397T>C	p.V2466A	IGV	+	99,868900	c.7397T>C	p.V2466A
952544	78	111201-RunU18-M1963	BRCA2	chr13	32930569	32930569	25	3	0	missense	SNM	c.7440A>C	p.L2480F	IGV	+	84,000000	c.7440A>C	p.L2480F
952537	78	111201-RunU18-M1963	BRCA2	chr13	32912299	32912299	11017	3	2	silent	SNM	c.3807T>C	p.V1269V	IGV	+	74,321500	c.3807T>C	p.V1269V
952538	78	111201-RunU18-M1963	BRCA2	chr13	32913055	32913055	9517	3	2	silent	SNM	c.4563A>G	p.L1521L	IGV	+	73,174300	c.4563A>G	p.L1521L
952540	78	111201-RunU18-M1963	BRCA2	chr13	32915005	32915005	11060	3	2	silent	SNM	c.6513G>C	p.V2171V	IGV	+	71,211600	c.6513G>C	p.V2171V
952557	78	111201-RunU18-M1963	BRCA1	chr17	41222945	41222967	13826	3	0	frame_shift_del	DEL	c.5027_5045delCTG	p.S1676fs	IGV	-	65,557600	c.4964_4982del	p.
952536	78	111201-RunU18-M1963	BRCA2	chr13	32912008	32912008	11803	3	2	silent	SNM	c.3516G>A	p.S1172S	IGV	+	63,949800	c.3516G>A	p.S1172S
952541	78	111201-RunU18-M1963	BRCA2	chr13	32918783	32918783	8468	3	0	silent	SNM	c.6930T>A	p.T2310T	IGV	+	33,762400	c.6930T>A	p.T2310T
952539	78	111201-RunU18-M1963	BRCA2	chr13	32913282	32913282	16052	3	0	missense	SNM	c.4790C>A	p.S1597Y	IGV	+	33,740300	c.4790C>A	p.S1597Y
952547	78	111201-RunU18-M1963	BRCA1	chr17	41243500	41243500	11355	3	0	missense	SNM	c.4048G>A	p.G1350S	IGV	+	28,392800	c.4048G>A	p.G1350S
952549	78	111201-RunU18-M1963	BRCA1	chr17	41244000	41244000	13875	3	2	missense	SNM	c.3548A>G	p.K1183R	IGV	+	24,136900	c.3548A>G	p.K1183R
952542	78	111201-RunU18-M1963	BRCA2	chr13	32929232	32929232	9227	3	2	silent	SNM	c.7242A>G	p.S2414S	IGV	+	23,875600	c.7242A>G	p.S2414S
952551	78	111201-RunU18-M1963	BRCA1	chr17	41244936	41244936	4653	3	2	missense	SNM	c.2612C>T	p.P871L	IGV	+	22,867000	c.2612C>T	p.P871L
952553	78	111201-RunU18-M1963	BRCA1	chr17	41245466	41245466	6653	3	2	silent	SNM	c.2082C>T	p.S694S	IGV	+	22,471100	c.2082C>T	p.S694S
952535	78	111201-RunU18-M1963	BRCA2	chr13	32911888	32911888	14340	3	2	silent	SNM	c.3396A>G	p.K1132K	IGV	+	22,001400	c.3396A>G	p.K1132K
952550	78	111201-RunU18-M1963	BRCA1	chr17	41244435	41244435	8033	3	2	missense	SNM	c.3113A>G	p.E1038G	IGV	+	21,909600	c.3113A>G	p.E1038G
952548	78	111201-RunU18-M1963	BRCA1	chr17	41243646	41243646	13226	3	0	missense	SNM	c.3902G>T	p.S1301I	IGV	+	20,066500	c.3902G>T	p.S1301I
952545	78	111201-RunU18-M1963	BRCA1	chr17	41223094	41223094	18807	3	2	missense	SNM	c.4900A>G	p.S1634G	IGV	+	19,907500	c.4900A>G	p.S1634G
952546	78	111201-RunU18-M1963	BRCA1	chr17	41234470	41234470	22961	3	2	silent	SNM	c.4308T>C	p.S1436S	IGV	+	19,859800	c.4308T>C	p.S1436S
952554	78	111201-RunU18-M1963	BRCA2	chr13	32907448	32907448	140	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	IGV	+	17,857100	c.1833delA	p.S611fs
952552	78	111201-RunU18-M1963	BRCA1	chr17	41245237	41245237	10347	3	2	silent	SNM	c.2311T>C	p.L771L	IGV	+	13,192200	c.2311T>C	p.L771L
952556	78	111201-RunU18-M1963	BRCA2	chr13	32944675	32944675	2508	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	IGV	+	6,618820	c.8467_8468ins	p.Q2823fs
952555	78	111201-RunU18-M1963	BRCA2	chr13	32918783	32918783	8476	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	IGV	+	5,545070	c.6929_6930ins	p.
952558	78	111201-RunU18-M1963	BRCA1	chr17	41245587	41245587	13307	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	IGV	+	2,652740	c.1961delA	p.K654fs

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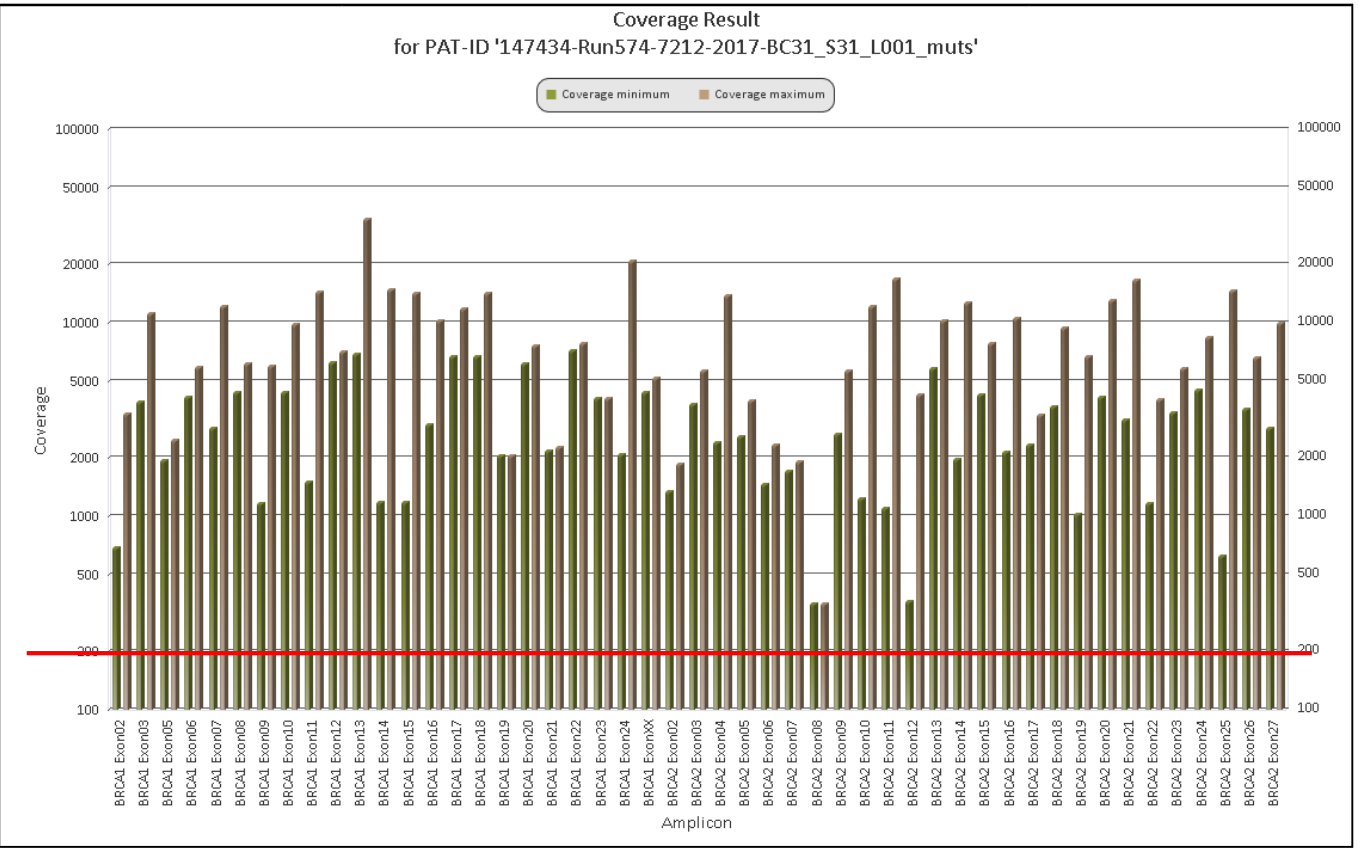
Bioinformatic pipeline and quality criteria

- Which variants are taken into account?

  - coverage of all amplicons **> 200**

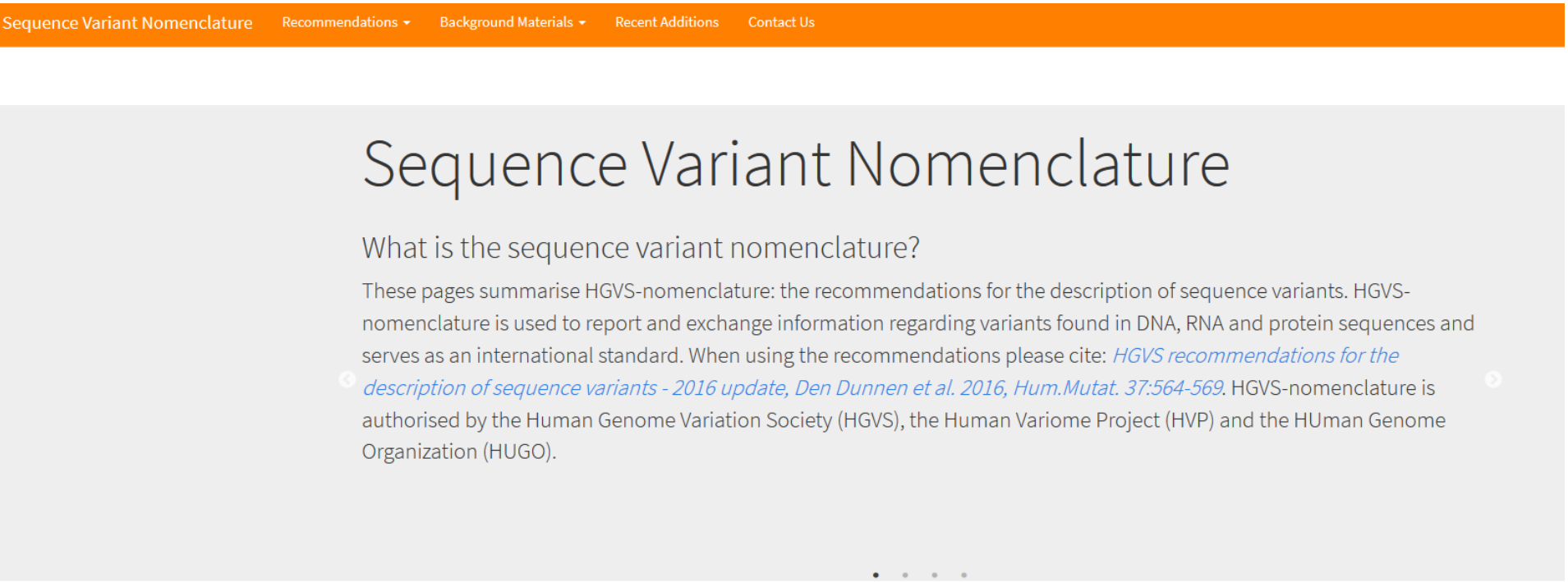
  - allele frequency **> 5%**



Fraction	c.Ergebnis	p.Ergebnis
99,874300	c.2612C>T	p.P871L
99,849600	c.2082C>T	p.S694S
99,752100	c.3548A>G	p.K1183R
99,622300	c.7397T>C	p.V2466A
99,558000	c.4308T>C	p.S1436S
99,396600	c.3113A>G	p.E1038G
99,340800	c.4900A>G	p.S1634G
98,340900	c.2077G>A	p.D693N
91,252800	c.4484+1G>C	c.4484+1G>C
54,727800	c.4563A>G	p.L1521L
44,943400	c.59A>G	p.N20S
35,010200	c.2311T>C	p.L771L
31,810700	c.6513G>C	p.V2171V
23,720900	c.1114A>C	p.N372H
22,917300	c.4048G>A	p.G1350S
21,300400	c.8467_8468ins	p.Q2823fs
14,870100	c.4643_4644del	p.E1548A
6,303500	c.9090_9092del	p.
4,199890	c.4538delG	p.G1513fs
3,633460	c.1961delA	p.K654fs

Nomenclature of Variants

- You need to be sure that you are looking at the right variant!!!



http://varnomen.hgvs.org/

Nomenclature of variants

1. "c" for coding DNA sequence
   „p“ for amino acid sequence

2. Change on **DNA level**
   in capital letters beginning with the first nucleotide affected:
   c.1799T>A

3. Change **on amino acid level**
   in capital letters beginning with the first amino acid affected
   p.V600E

➢ the amino acids affected can be described in one- or three-letter code, whereas the three letter code is preferred

Nomenclature of complex variants

1. Change on **DNA level**

- the position of the first nucleotide is given:
- c.2240
- "del" indicates a deletion of a nucleotide
- c.2240del
- "_" (underscore) indicates a range of affected residues, separating the first and the last residue affected
- c.2240_2254del

2. Change **on amino acid level**

- the first amino acid and its position are given
- p.L747
- "del" indicates a deletion of an amino acid
- p.L747del
- "_" (underscore) indicates a range of affected residues, separating the first and the last residue affected
- p.L747_T751

- "ins" indicates an insertion of nucleotide or amino acid
- "dup" indicates a duplication of nucleotide or amino acid
- "fs" indicates a frameshift

Nomenclature of variants

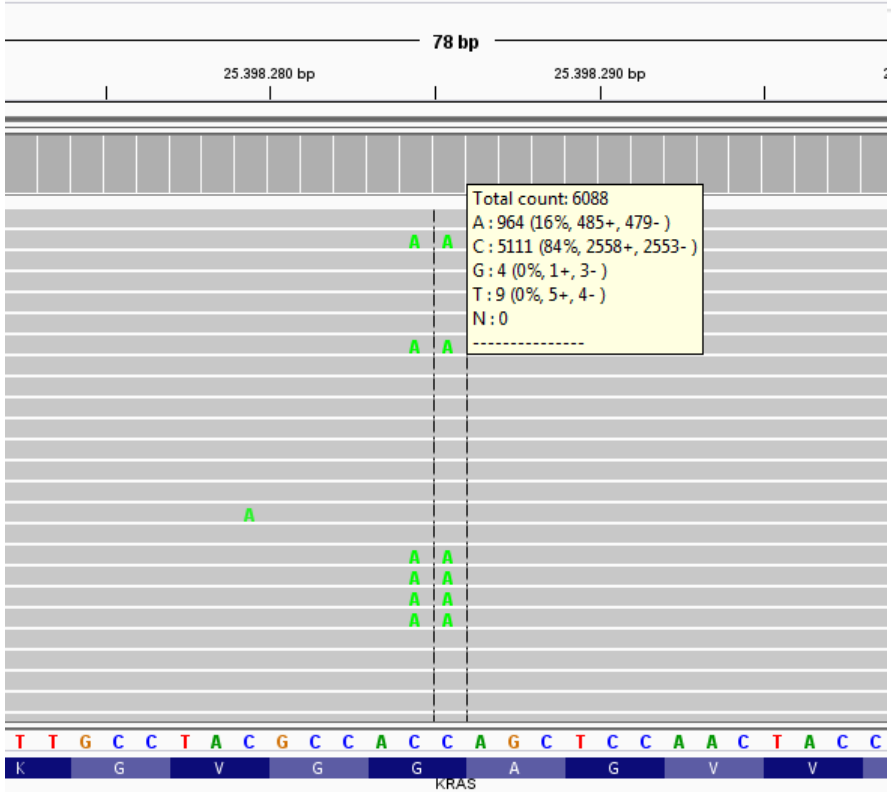
➢ some general rules – **indel/point mutation**

What is an **"indel"**?

An "indel", **deletion/insertion** in HGVS nomenclature, is a variant which is a combination of a deletion and an insertion. Based on existing nomeclature, the variant can be described as a deletion and insertion occuring at the same position, using the format g.112_117delinsTG.

Can I describe a GC to TG variant as a dinucleotide substitution (g.4GC>TG)?

No this is not allowed. By definition a substitution changes **one** nucleotide into **one** other nucleotide (*see Substitution*). The change TGTGCCA to TGTTGCA should be described as g.4_5delinsTG, i.e. a deletion/insertion (indel).



***KRAS* Exon 2:**

c.34_35delinsTT **not** c.34_35GG>TT

p.G12F

Nomenclature of variants

➢ some general rules - **duplication**

- **prefix** reference sequences accepted are g., m., c. and n. (genomic, mitochondrial, coding DNA and non-coding DNA).
- the "position" description should contain **two flanking nucleotides**, e.g. 123 and 124 but not 123 and 125.
- an insertion can not be described using one nucleotide position, like g.123insG
- for all descriptions the **most 3' position** possible of the reference sequence is arbitrarily assigned to have been changed (**3'rule**)
  - the 3'rule applies to ALL descriptions (genome, gene, transcript and protein) of a given variant
- tandem duplications are described as a duplication (g.123_456**dup**), not an insertion (g.456_457ins123_456)
  - **inverted duplications** are described as insertion (g.234_235ins123_234inv), not as a duplication (*see Inversion*)

➢ some general rules – **3'rule**

- **3'rule**: for all descriptions the most 3' position possible of the reference sequence is arbitrarily assigned to have been changed
  - the 3'rule also applies for changes in single residue stretches and tandem repeats (nucleotide or amino acid)
  - the 3'rule applies to ALL descriptions (genome, gene, transcript and protein) of a given variant

Nomenclature of variants – Reference sequences

- BRCA2: NM_000059
- BRCA1: NM_007300 is used for panel design (Qiagen!)

  NM_007294 is used by most databases (codons 1454-1475 are missing, from codon 1475, 21 amino acids have to be subtracted)

ROI				COVERED					
chr17	41197684	41197829	BRCA1	chr17	41197616	41197907	BRCA1	Exon 24	Exon 24
chr17	41199649	41199730	BRCA1	chr17	41199637	41199752	BRCA1	Exon 23	Exon 23
chr17	41201127	41201221	BRCA1	chr17	41201107	41201296	BRCA1	Exon 22	Exon 22
chr17	41203069	41203144	BRCA1	chr17	41203016	41203283	BRCA1	Exon 21	Exon 21
chr17	41209058	41209162	BRCA1	chr17	41209030	41209174	BRCA1	Exon 20	Exon 20
chr17	41215339	41215400	BRCA1	chr17	41215328	41215442	BRCA1	Exon 19	Exon 19
chr17	41215880	41215978	BRCA1	chr17	41215860	41216029	BRCA1	Exon 18	Exon 18
chr17	41219614	41219722	BRCA1	chr17	41219594	41219810	BRCA1	Exon 17	Exon 17
chr17	41222934	41223265	BRCA1	chr17	41222902	41223363	BRCA1	Exon 16	Exon 16
chr17	41226337	41226548	BRCA1	chr17	41226327	41226586	BRCA1	Exon 15	Exon 15
chr17	41228494	41228641	BRCA1	chr17	41228483	41228669	BRCA1	Exon 14	Exon 14
chr17	41231340	41231426	BRCA1	chr17	41231314	41231536	BRCA1	Exon 13	XX
chr17	41234410	41234602	BRCA1	chr17	41234399	41234680	BRCA1	Exon 12	Exon 13
chr17	41242950	41243059	BRCA1	chr17	41242891	41243091	BRCA1	Exon 11	Exon 12
chr17	41243441	41246887	BRCA1	chr17	41243410	41246955	BRCA1	Exon 10	Exon 11
chr17	41247852	41247949	BRCA1	chr17	41247838	41248041	BRCA1	Exon 9	Exon 10
chr17	41249250	41249316	BRCA1	chr17	41249201	41249393	BRCA1	Exon 8	Exon 9
chr17	41251781	41251907	BRCA1	chr17	41251719	41251991	BRCA1	Exon 7	Exon 8
chr17	41256128	41256288	BRCA1	chr17	41256045	41256353	BRCA1	Exon 6	Exon 7
chr17	41256874	41256983	BRCA1	chr17	41256854	41257069	BRCA1	Exon 5	Exon 6
chr17	41258462	41258560	BRCA1	chr17	41258405	41258643	BRCA1	Exon 4	Exon 5
chr17	41267732	41267806	BRCA1	chr17	41267681	41267906	BRCA1	Exon 3	Exon 3
chr17	41276023	41276123	BRCA1	chr17	41276008	41276226	BRCA1	Exon 2	Exon 2

CDS komplett (NM_007300, BRCA1-005)

Classification of Variants

1 (not pathogenic or of no clinical significance);

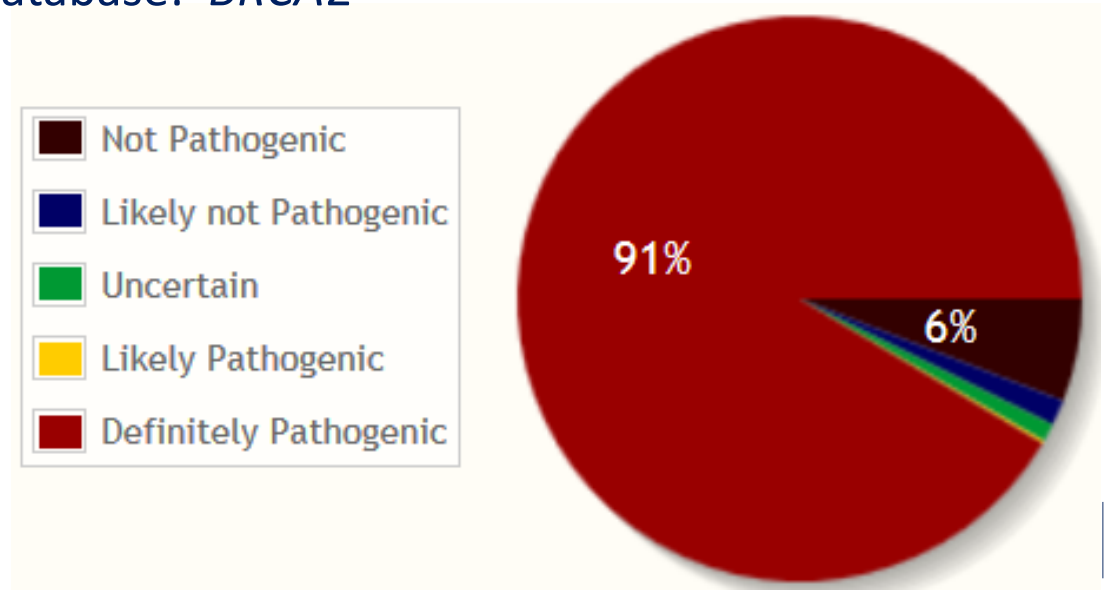
2 (likely not pathogenic or of little clinical significance);

3 (uncertain);

4 (likely pathogenic);

5 (definitely pathogenic)

- distribution according to ARUP database: *BRCA2*



Classification of Variants - Databases

Database name (website link if available)	Provider	Description
The DMuDB	Curated by the NGRL Manchester, UK	Established in 2005 by NGRL as a repository of diagnostic variant data, to support the diagnostic process in UK genetic testing laboratories Access to DMuDB now extended to non-UK laboratories through a partnership with EMQN Subscription charges are €275 per laboratory per year classification?
BIC	NHGRI, USA	International database of many BRCA1 and BRCA2 mutations including UV/VUS that have been voluntarily submitted by researchers, clinicians, counsellors and nurses Classifications results are shown when available Free to registered users
IARC database (brca.iarc.fr)	IARC, Lyon, France	Results of multifactorial analyses of unclassified variants classification not always available
UMD (http://www.umd.be)		Database updated for BRCA1/2 mutations every month from all French genetic laboratories (n=16) There is a manual check on every database each time a new variant is identified Includes breast and ovarian cancer; every family with a variant identified in France
HGMD (www.hgmd.cf.ac.uk),		Public database site (that at www.hgmd.cf.ac.uk), updated twice a year while the subscription version (HGMD Professional; available from, BIOBASE), is updated every three months Not synchronized to other databases Data are derived from scientific literature; consequently, all the data has been peer-reviewed Also includes details of functional analyses in the secondary referencing to provide more evidence of causation
ENIGMA	ENIGMA, a consortium of investigators focused on determining the involvement of all UV/VUS, in the BRCA1/2	New variants are constantly examined, as submitted by members As variants are classified, they are transferred to the BIC database (and others) Not publically available; consortium is a group of researchers who bring their separate expertise to the whole problem of unclassified variants. They work exclusively with germ-line variants that have been observed in samples referred to genetic testing because of personal or family history of breast and/or ovarian cancer

Capuluongo et al., Sem Oncol 2017

BIC, Breast Cancer Information Core; CIMBA, Consortium of Investigators of Modifiers of BRCA1/2; DMuDB, Diagnostic Mutation Database; EMQN, European Molecular Genetics Quality Network; ENIGMA, Evidence-based Network for the Interpretation of Germline Mutant Alleles; HGMD, The Human Gene Mutation Database; IARC, International Agency for Cancer Research; NGRL, National Genetics Reference Laboratory; NHGRI, National Human Genome Research Institute; UMD, Universal Mutation Database; UV: unknown variants; VUS: variants of unknown significance

Classification of Variants - Databases

Database name (website link if available)	Provider	Description
CIMBA (ccge.medschl.cam.ac.uk/consortia/cimba)	CIMBA, a collaborative group of researchers working on genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers	Database for research purposes Not synchronized with other databases All mutations in CIMBA are pathogenic mutations 1621 unique BRCA1 and 1640 unique BRCA2 mutations in CIMBA No subscription (yet)
BRCA Challenge (http://brcaexchange.org/).	Joint initiative of the Global Alliance for Genomic and Global Health and the Human Variome Project	Involves several hundred institutes and academic organizations around the world; aim is to pool available data on BRCA1/2 genetic variants from around the world to further the understanding of genetic variation in these genes and improve patient diagnoses and disease prevention under construction Collated information available via a public website which enables the user to search for available information on individual BRCA1/2 genetic variants and their current classification of benign or pathogenic
ClinVar: (http://www.ncbi.nlm.nih.gov/clinvar/)		Freely accessible, public archive of reports of the relationship between human genetic variations and phenotypes, with supporting evidence Submission are received from clinical testing laboratories, research laboratories, locus-specific databases, expert panels and professional societies ClinVar is an active partner of the ClinGen project, providing data for evaluation and archiving the results of interpretation by recognized expert panels and providers of practice guidelines When submitters update their records, the previous version is retained for review
ARUP http://arup.utah.edu/database/BRCA/index.php	University of Utah Huntsman Cancer Institute (HCI), and with the WHO International Agency for Research on Cancer (IARC), the University of Utah Department of Pathology and ARUP Laboratories	The purpose of this database is to provide information on *BRCA1* and *BRCA2* gene mutations and their impact on risk of developing breast cancer, ovarian cancer and certain other cancers. **Two types of databases** are provided. One is a list of mutations curated from critical review of literature and family studies. The other provides *in silico* prediction of risk to help understand variants of unknown significance. Free and functional relevance Two genes (***BRCA1*** and ***BRCA2***) are curated separately. The two databases mentioned above are available for both genes. Go to the landing pages for either gene with the buttons below.

modified from Capuluongo et al., Sem Oncol 2017

Classification of Variants - Reporting

Informations concerning the mutation:

- targets analyzed (ie, BRCA1 and/or BRCA2)
- the regions covered for each gene (eg, coding region only or intronic and exonic regions)
- overall results: either pathogenic or deleterious variants present or absent
- mutation details (when present): cDNA and amino acid change according to HGVS nomenclature
- reference sequence, including version used for annotation and HGVS nomenclature
- summary and interpretation: including pathogenic or likely pathogenic classification of the identified variants.
- non–pathogenic variants should not be reported, although the laboratory may keep a record of these. Variants of unknown significance should be reported separately and clearly indicate the lack of sufficient clinical or biological evidence. Information regarding the potential therapeutic implications should be included when possible.

Capoluongo et al., Sem Oncol 2017

Classification of Variants - Reporting

- additional informations in the report:

Table 4
Recommendations for information to be included when reporting tumor *BRCA1/2* results.

Information to include	Comments
Histology and grade of the tumor	If available and not previously performed
Percentage of neoplastic content in the analyzed sample and if macrodissection has been performed	Defined as the percentage of neoplastic cells out of the total nucleated cells in the area used for DNA extraction
Procedure used	eg, NGS or Sanger sequencing
Coverage of the analysis	Prevalent mutations vs the recommended whole coding regions including flanking intron sequences
Panel used	Commercial or on demand or multipanel gene (eg, panels including more than *BRCA1/BRCA2* genes)
Minimum coverage and guaranteed analytical sensitivity with the employed methodology	Any regions for which there was insufficient sequencing read coverage (eg, 33 reads, dependent on assay limits of detection) should be declared to avoid false negative assessment
If the analysis includes all types of alterations or just single point mutations or small indel alterations	eg, 'large rearrangements/deletions cannot be detected using this methodology'
Sequencer	Models include: MiSeq, NextSeq 500, PGM, IonProton
Bioinformatic tools employed	Freeware or commercial; indicate if they have been validated or not for this type of analysis. If the tools are web based, include the https address
Requested databases for grading of detected mutation	UMD and ClinVar-DB, etc.
Variant allele frequency	It is important to correlate with neoplastic tumor content and potential LOH, which is present in the vast majority of cases
Explanation of therapeutic consequence of mutations according to the classification of the variants	Mutation class 4 or 5 ~pathogen = possibility of therapy with PARPi; mutation class 1–3 or wild type = no therapeutic consequence and should not be reported (see text for VUS)
A recommendation of germline analysis and genetic counseling in cases of pathogenic mutations	Germline analysis should be always considered if not already performed
Accreditation of the laboratory	ISO15189, CAP, CLIA or equivalent
Participation in EQA	For example: EMQN, QuIP, UK NEQAS

Capoluongo et al., Sem Oncol 2017

Classification of Variants – Example report from Cologne

*written by the **molecular biologist***

Molekularpathologische Untersuchung:

Mutationsanalyse von *BRCA1* und *BRCA2* mittels Parallelsequenzierung (Next Generation Sequencing) von Multiplex PCR Amplikons. Die Generierung der Multiplex Amplikons erfolgte mit einem GeneRead DNASeq Mix-n-Match Panel V2 (Qiagen). Für die anschließende Library Erstellung wurden die GeneRead DNA Library I Core und GeneRead DNA I Amp Kits (Qiagen) sowie die NEXTflex-96 DNA Barcode Adapter (BIOO Scientific) verwendet. Die Sequenzierung wurde auf dem MiSeq (Illumina) durchgeführt.

description of the method

Ergebnis für Block 6A:

Gen	Exon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2,3,5-24	EX11: c.4065_4068delTCAA p.N1355Kfs*10	75,01	in der UMD Datenbank als pathogen (Kategorie 5) beschrieben	
BRCA2	2-27	Wildtyp			

/merk

result

*written by the **pathologist***

Statement:

….recommendation of ‘targeted therapy‘ if clinical indication is given and patient has a class 4 or 5 mutation

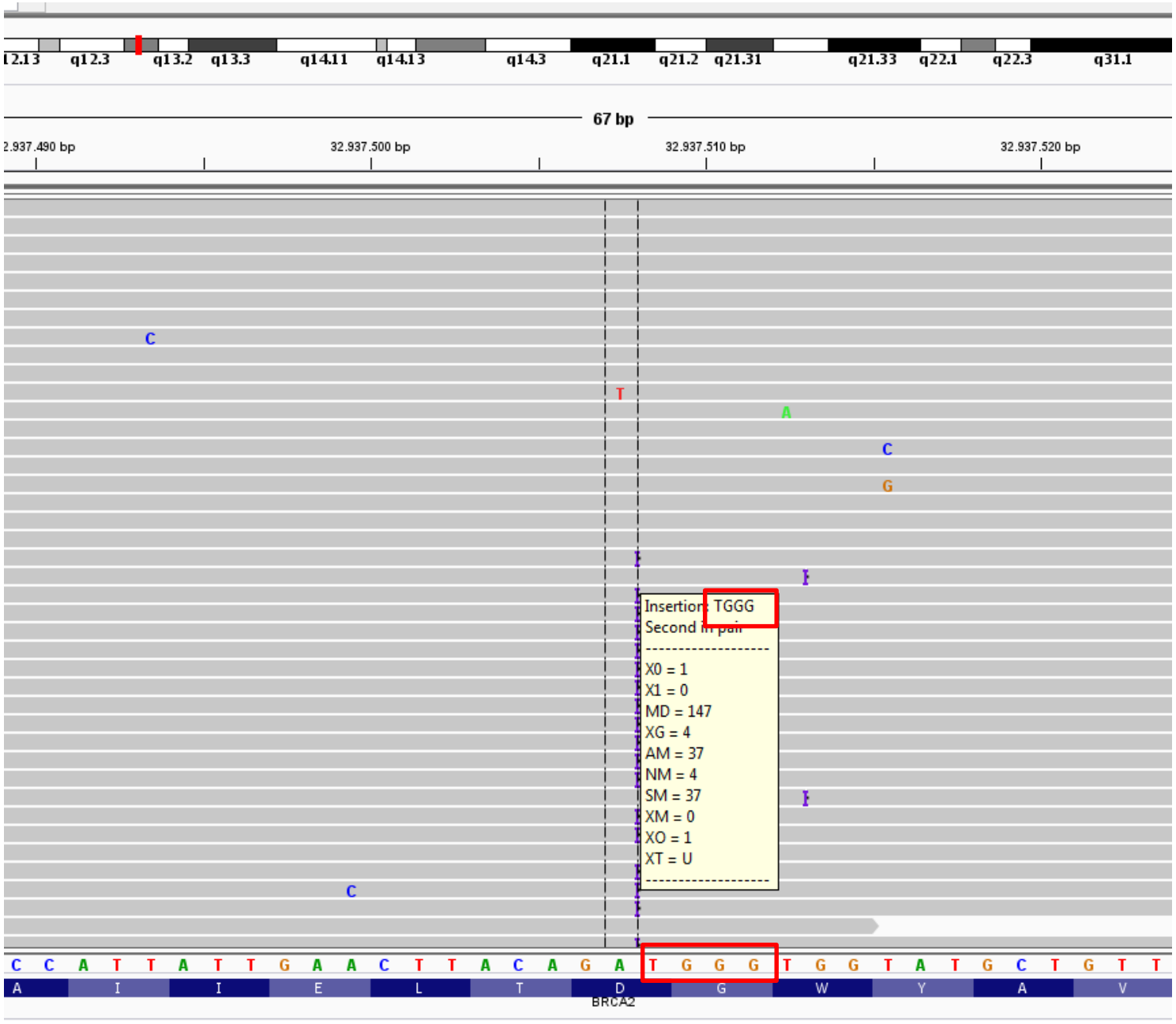
….if BRCA mutation is found in tumour and no germline data are available the report needs to clarify that there may be a germline mutation⇨ recommendation of genetic counseling

Challenging Cases – Case 1

- Nomenclature of variants: Variant Calling gives wrong description:
- BRCA2: ~~c.8168_8169insTGGG p.Asp2723fs~~ **c.8172_8175dup p.Tyr2726Valfs*5**

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32929387	32929387	7583	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,947300	c.7397T>C	p.V2466A
BRCA2	chr13	32913055	32913055	7083	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	66,116100	c.4563A>G	p.L1521L
BRCA2	chr13	32913136	32913136	9473	3	0	missense	SNM	c.4644A>T	p.E1548D	igv	+	48,210700	c.4644A>T	p.E1548D
BRCA2	chr13	32915005	32915005	11356	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	32,854900	c.6513G>C	p.V2171V
BRCA2	chr13	32930568	32930570	27	3	0	in_frame_del	DEL	c.7439_7441delTAA	p.	igv	+	22,222200	c.7439_7441del	p.
BRCA2	chr13	32930569	32930570	56	3	0	frame_shift_del	DEL	c.7440_7441delAA	p.L2480fs	igv	+	19,642900	c.7440_7441del	p.L2480fs
BRCA2	chr13	32937508	32937508	9044	3	0	frame_shift_ins	INS	c.8168_8169insTGG	p.D2723fs	igv	-	19,593100	c.8172_8175du	p.Y2726Vfs*5
BRCA2	chr13	32944675	32944675	2765	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	15,153700	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32907448	32907448	160	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	6,875000	c.1833delA	p.S611fs
BRCA2	chr13	32954023	32954023	5289	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	3,478920	c.9090delA	p.T3030fs
BRCA2	chr13	32918783	32918783	1123	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	igv	+	2,582370	c.6929_6930ins	p.
BRCA2	chr13	32968895	32968895	25620	3	0	missense	SNM	c.9326T>A	p.L3109H	igv	+	1,951600	c.9326T>A	p.L3109H
BRCA1	chr17	41244000	41244000	10737	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	+	53,245800	c.3548A>G	p.K1183R
BRCA1	chr17	41244936	41244936	3890	3	2	missense	SNM	c.2612C>T	p.P871L	igv	+	50,796900	c.2612C>T	p.P871L
BRCA1	chr17	41234470	41234470	54124	3	2	silent	SNM	c.4308T>C	p.S1436S	igv	+	49,931600	c.4308T>C	p.S1436S
BRCA1	chr17	41245466	41245466	5213	3	2	silent	SNM	c.2082C>T	p.S694S	igv	+	49,031300	c.2082C>T	p.S694S
BRCA1	chr17	41223094	41223094	18288	3	2	missense	SNM	c.4900A>G	p.S1634G	igv	+	48,567400	c.4900A>G	p.S1634G
BRCA1	chr17	41244435	41244435	4723	3	2	missense	SNM	c.3113A>G	p.E1038G	igv	+	48,020300	c.3113A>G	p.E1038G
BRCA1	chr17	41245237	41245237	14332	3	2	silent	SNM	c.2311T>C	p.L771L	igv	+	22,704400	c.2311T>C	p.L771L
BRCA1	chr17	41243500	41243500	13592	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	10,153000	c.4048G>A	p.G1350S
BRCA1	chr17	41249286	41249286	12134	3	0	missense	SNM	c.568A>C	p.T190P	igv	+	4,227790	c.568A>C	p.T190P
BRCA1	chr17	41245587	41245587	12781	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	3,872940	c.1961delA	p.K654fs
BRCA1	chr17	41231352	41231352	4231	3	0	silent	SNM	c.4422A>G	p.S1474S	igv	+	3,592530	c.4422A>G	p.S1474S

Challenging Cases – Case 1



- Duplication, not Insertion!!

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Challenging Cases – Case 1

- BRCA2: **c.8172_8175dup p.Tyr2726Valfs*5**
- within this region, only a duplication can be found in the databases:

ARUP:

Exon 18	Insertion	c.8172_8175dup4		5 - Definitely pathogenic	>0.99	Foretova (2004) Hum Mutat 23; 397
Exon 18	Indel	c.8174_8185del12insTT		5 - Definitely pathogenic	>0.99	Diez (2010) Breast Cancer Res Treat 123; 587
Exon 18	Nonsense	c.8175G>A	p.W2725*	5 - Definitely pathogenic	>0.99	Levanat (2012) Gene 498; 169

ClinVar:

NM_000059.3(**BRCA2**):c.8172_8175dupGTGG (p.Tyr2726Valfs) *GRCh37:* Chr13:32937511-32937514 *GRCh38:* Chr13:32363374-32363377	BRCA2	Breast-ovarian cancer, familial 2	Pathogenic (Sep 8, 2016)
NM_000059.3(**BRCA2**):c.8174_8185delGGTATGCTGTTAinsTT (p.Trp2725Phefs) *GRCh37:* Chr13:32937513-32937524 *GRCh38:* Chr13:32363376-32363387	BRCA2	Breast-ovarian cancer, familial 2, not provided	Pathogenic (Oct 18, 2016)
NM_000059.3(**BRCA2**):c.8195_8202delTAGATCCT (p.Leu2732Serfs) *GRCh37:* Chr13:32937534-32937541 *GRCh38:* Chr13:32363397-32363404	BRCA2	Breast-ovarian cancer, familial 2	Pathogenic (Oct 18, 2016)
NM_000059.3(**BRCA2**):c.8200_8209delCCTCCCCTCT (p.Pro2734Terfs) *GRCh37:* Chr13:32937539-32937548 *GRCh38:* Chr13:32363402-32363411	BRCA2	Breast-ovarian cancer, familial 2, not provided	Pathogenic (Sep 8, 2016)

Challenging Cases – Case 1

Übersandtes Material: Paraffinblock 5745/17.

Klinische Angaben: Adnexe rechts, links. Z.n. invasiv-duktalem Mamma-CA, BRCA-Trägerin, Bitte BRCA-Mutationsanalyse erbeten.

- *it was previously known that patient has a BRCA mutation*
- *blood sample not available*

Molekularpathologische Untersuchung:
Mutationsanalyse von ***BRCA1*** **und** ***BRCA2*** mittels Parallelsequenzierung (Next Generation Sequencing) von Multiplex PCR Amplikons. Die Generierung der Multiplex Amplikons erfolgte mit einem GeneRead DNASeq Mix-n-Match Panel V2 (Qiagen). Für die anschließende Library Erstellung wurden die GeneRead DNA Library I Core und GeneRead DNA I Amp Kits (Qiagen) sowie die NEXTflex-96 DNA Barcode Adapter (BIOO Scientific) verwendet. Die Sequenzierung wurde auf dem MiSeq (Illumina) durchgeführt.

Ergebnis für Block 1 + 2 (gepoolt):

Gen	Exon	Codon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2, 3, 5 - 24	1 - 1864	Wildtyp			
BRCA2	2 - 27	1 - 1185, 1253 - 3419	EX18: c.8172_8175dup p.Y2726Vfs*5	19,59	Kategorie 5 (ARUP-DB) Kategorie 5 (ClinVar-DB)	Therapieoption mit PARP-Inhibitor prüfen

- *result*

Bei der hier vorliegenden Mutation der Kategorie 5 im *BRCA2*-Gen ergibt sich die Möglichkeit einer Therapie mit einem PARP Inhibitor.

Beurteilung:
Im Normalgewebe der Adnexe beidseits zeigt sich eine Mutation im BRCA2-Gen, Exon 18, diese ist in die Kategorie 5 einzuordnen. /frie/zanf

Statement:

….normal tissue was analysed

….recommendation of 'targeted therapy'; patient has class 5 mutation

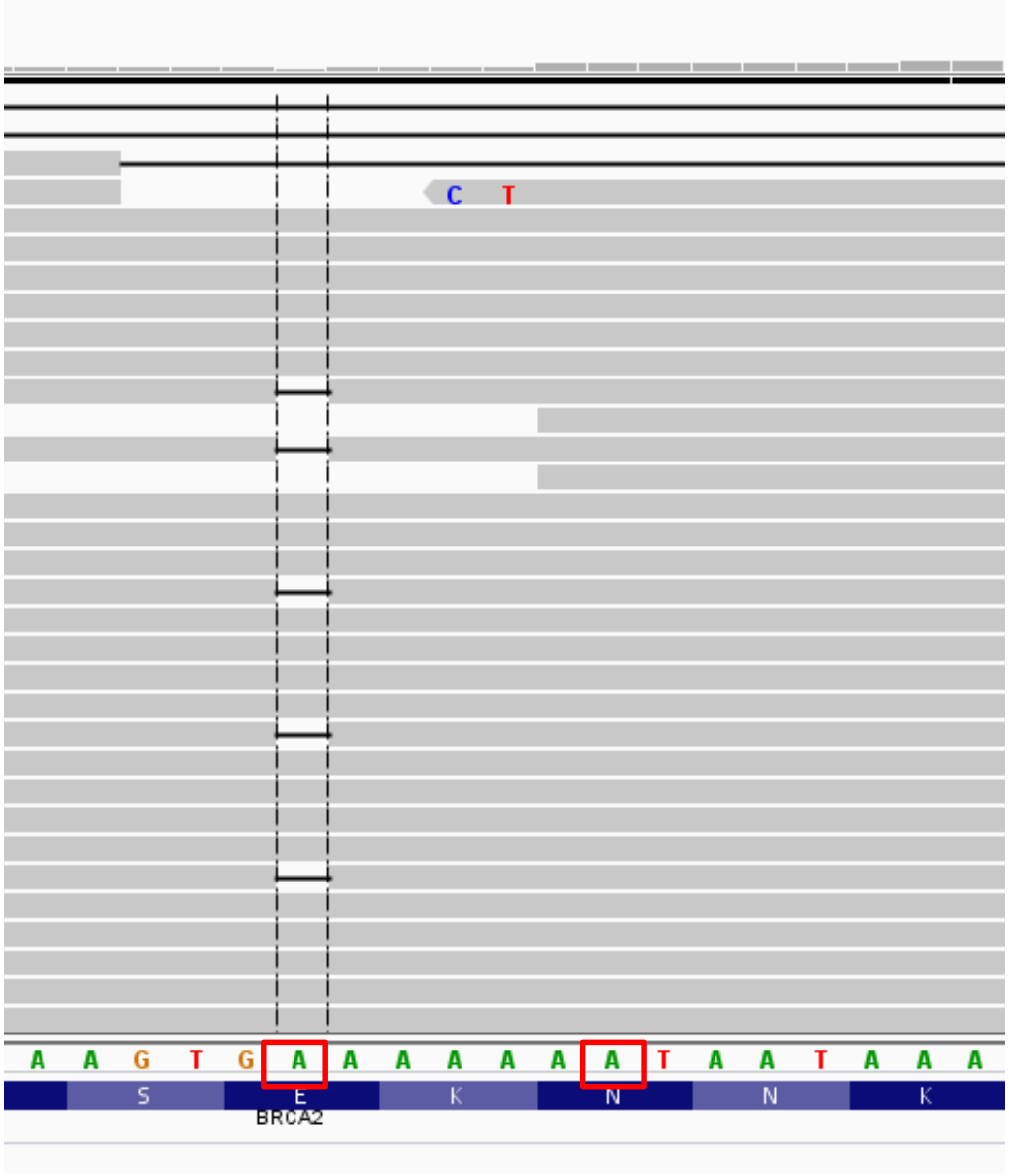
Challenging Cases – Case 2

- Nomenclature of variants: Variant Calling gives wrong description:
- BRCA2: ~~c.3854delA p.Glu1285fs~~ **c.3860delA p.Asn1287Ilefs*6**

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32929387	32929387	6784	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,941000	c.7397T>C	p.V2466A
BRCA2	chr13	32915005	32915005	8350	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	64,071900	c.6513G>C	p.V2171V
BRCA2	chr13	32911888	32911888	9427	3	2	silent	SNM	c.3396A>G	p.K1132K	igv	+	51,819200	c.3396A>G	p.K1132K
BRCA2	chr13	32913282	32913282	8961	3	0	missense	SNM	c.4790C>A	p.S1597Y	igv	+	47,918800	c.4790C>A	p.S1597Y
BRCA2	chr13	32929232	32929232	1784	3	2	silent	SNM	c.7242A>G	p.S2414S	igv	+	47,309400	c.7242A>G	p.S2414S
BRCA2	chr13	32913055	32913055	8948	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	46,502000	c.4563A>G	p.L1521L
BRCA2	chr13	32912346	32912346	1746	3	0	frame_shift_del	DEL	c.3854delA	p.E1285fs	igv	-	38,430700	c.3860delA	p.N1287Ifs*6
BRCA2	chr13	32913136	32913136	14104	3	0	missense	SNM	c.4644A>T	p.E1548D	igv	+	30,402700	c.4644A>T	p.E1548D
BRCA2	chr13	32944675	32944675	737	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	10,719100	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32907448	32907448	156	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	5,769230	c.1833delA	p.S611fs
BRCA2	chr13	32954023	32954023	4078	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	4,683670	c.9090delA	p.T3030fs
BRCA2	chr13	32914828	32914828	6765	3	0	silent	SNM	c.6336A>G	p.R2112R	igv	+	3,695490	c.6336A>G	p.R2112R
BRCA2	chr13	32968895	32968895	14738	3	0	missense	SNM	c.9326T>A	p.L3109H	igv	+	3,080470	c.9326T>A	p.L3109H
BRCA2	chr13	32913559	32913559	10214	3	0	frame_shift_del	DEL	c.5067delA	p.A1689fs	igv	+	2,242020	c.5067delA	p.A1689fs
BRCA1	chr17	41246481	41246481	4821	3	2	missense	SNM	c.1067A>G	p.Q356R	igv	+	48,330200	c.1067A>G	p.Q356R
BRCA1	chr17	41245587	41245587	8197	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	4,721240	c.1961delA	p.K654fs
BRCA1	chr17	41244697	41244697	777	3	0	frame_shift_del	DEL	c.2851delA	p.R951fs	igv	+	3,346200	c.2851delA	p.R951fs
BRCA1	chr17	41249286	41249286	7848	3	0	missense	SNM	c.568A>C	p.T190P	igv	+	2,268090	c.568A>C	p.T190P

Challenging Cases – Case 2

- 3‘ rule: deletion has to be moved forward in the direction of 3‘ end:



- first A in stretch: position **3854**
- last A in stretch: position **3860**

Challenging Cases – Case 2

- BRCA2: **c.3860delA p.Asn1287Ilefs*6**
- within this region only a deletion of a single „A“ in position 3860 can be found in databases

ARUP:

Exon 11	Deletion	c.3858_3860delAAA	p.K1286del	2 - Likely not pathogenic or of little clinical significance	>0.99	Malone (2000) Cancer 88; 1393
Exon 11	Deletion	c.3860delA		5 - Definitely pathogenic	>0.99	Llort (2002) Hum Mutat 19; 307
Exon 11	Deletion	c.3860_3863del4		5 - Definitely pathogenic	>0.99	Lecarpentier (2012) Breast Cancer Res 14; R99

ClinVar:

NM_000059.3(**BRCA2**):c.3849_3852delAAGT (p.Ser1284Lysfs) *GRCh37:* Chr13:32912341-32912344 *GRCh38:* Chr13:32338204-32338207	BRCA2	Breast-ovarian cancer, familial 2	Pathogenic (Oct 18, 2016)
NM_000059.3(**BRCA2**):c.3857_3860delAAAA (p.Lys1286Ilefs) *GRCh37:* Chr13:32912349-32912352 *GRCh38:* Chr13:32338212-32338215	BRCA2	Breast-ovarian cancer, familial 2	Pathogenic (Sep 8, 2016)
NM_000059.3(**BRCA2**):c.3859_3860delAA (p.Asn1287Terfs) *GRCh37:* Chr13:32912351-32912352 *GRCh38:* Chr13:32338214-32338215	BRCA2	Breast-ovarian cancer, familial 2, Hereditary breast and ovarian cancer syndrome, Hereditary cancer-predisposing syndrome	Pathogenic (Sep 8, 2016)
NM_000059.3(**BRCA2**):c.3860dupA (p.Asn1287Lysfs) *GRCh37:* Chr13:32912352 *GRCh38:* Chr13:32338215	BRCA2	Breast-ovarian cancer, familial 2, Hereditary breast and ovarian cancer syndrome, not provided	Pathogenic (Sep 8, 2016)
NM_000059.3(**BRCA2**):c.3860delA (p.Asn1287Ilefs) *GRCh37:* Chr13:32912352 *GRCh38:* Chr13:32338215	BRCA2	Familial cancer of breast, Breast-ovarian cancer, familial 2, Breast-ovarian cancer, familial 1,	Pathogenic (Sep 8, 2016)

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Challenging Cases – Case 2

1. Molekularpathologische Untersuchung:
Mutationsanalyse von ***BRCA1*** **und** ***BRCA2*** mittels Parallelsequenzierung (Next Generation Sequencing) von Multiplex PCR Amplikons. Die Generierung der Multiplex Amplikons erfolgte mit einem GeneRead DNASeq Mix-n-Match Panel V2 (Qiagen). Für die anschließende Library Erstellung wurden die GeneRead DNA Library I Core und GeneRead DNA I Amp Kits (Qiagen) sowie die NEXTflex-96 DNA Barcode Adapter (BIOO Scientific) verwendet. Die Sequenzierung wurde auf dem MiSeq (Illumina) durchgeführt.

Ergebnis für Block 3:
Tumorzellgehalt: 90%

Gen	Exon	Codon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2, 3, 5 - 24	1 - 1864	Wildtyp			
BRCA2	2 - 27	1 - 1185, 1253 - 3419	EX11: c.3860delA p.N1287Ifs*6	38,43	Kategorie 5 (UMD-DB) Kategorie 5 (ARUP-DB) Kategorie 5 (ClinVar-DB)	Therapieoption mit PARP-Inhibitor prüfen

Beurteilung:
Somit handelt es sich hier um ein **rezidiviertes, gering differenziertes Prostatakarzinom** (Gleason-Score 10 = 5 + 5) mit einer zweifelsfrei pathogenen Mutation in BRCA2 (Kategorie 5) und einer weiteren zweifelsfrei pathogenen Mutation in ATM (Kategorie 5) und einer p53-Mutation.

Dieser Patient wäre ein idealer Kandidat für eine Therapie mit Olaparib, da zwei essentielle Gene im BRCA-DNA-Reparaturweg defekt sind.

Daneben empfehlen wir auch klinischerseits eine weitere humangenetische Beratung mit der Frage, ob hier eine hereditäre BRCA2-Mutation vorliegen könnte. Ein Teil dieser Mutationen sind Keimbahnmutationen, sodass diese Möglichkeit prinzipiell besteht. /bütt/zanf

….prostate cancer with class 5 mutation and an additional pathogenic mutation in ATM

…..recommendation of ‘targeted therapy‘ if clinical indication is given

……genetic counseling is recommended

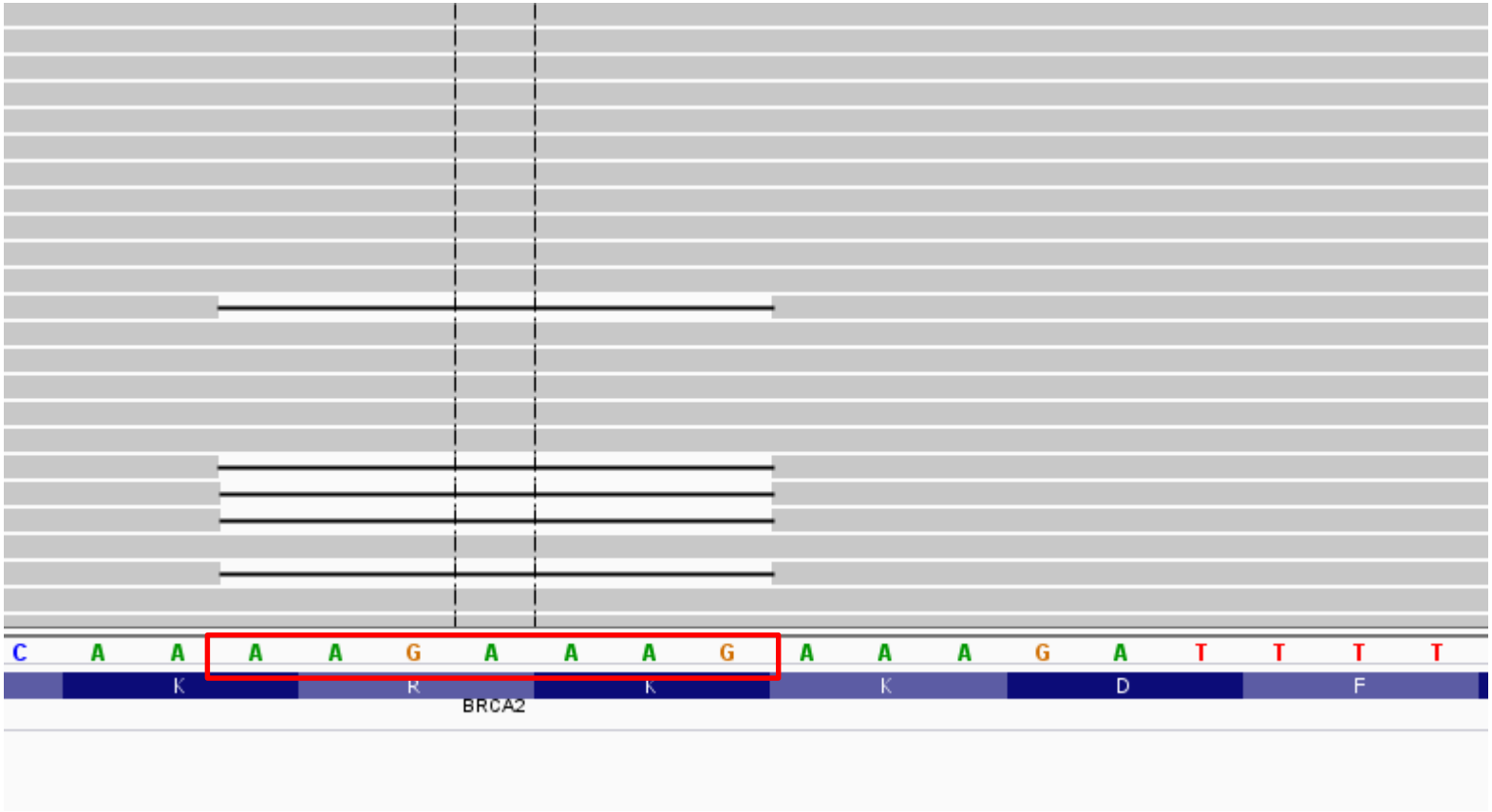
Challenging Cases – Case 3

- Nomenclature of variants: Variant Calling gives wrong description:
- BRCA2: ~~c.1302_1308del p.Lys434fs~~ **c.1304_1310del p.Arg435Lysfs*27**

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32929387	32929387	3560	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,859600	c.7397T>C	p.V2466A
BRCA2	chr13	32912299	32912299	1067	3	2	silent	SNM	c.3807T>C	p.V1269V	igv	+	79,006600	c.3807T>C	p.V1269V
BRCA2	chr13	32906917	32906923	1650	3	0	frame_shift_del	DEL	c.1302_1308delAAGA	p.K434fs	igv	-	45,030300	c.1304_1310del	p.R435Kfs*27
BRCA2	chr13	32915005	32915005	4390	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	39,886100	c.6513G>C	p.V2171V
BRCA2	chr13	32913055	32913055	6677	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	35,120600	c.4563A>G	p.L1521L
BRCA2	chr13	32930569	32930570	27	3	0	frame_shift_del	DEL	c.7440_7441delAA	p.L2480fs	igv	+	29,629600	c.7440_7441del	p.L2480fs
BRCA2	chr13	32907448	32907448	79	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	16,455700	c.1833delA	p.S611fs
BRCA2	chr13	32944675	32944675	680	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	8,382350	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32954023	32954023	1878	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	3,567630	c.9090delA	p.T3030fs
BRCA1	chr17	41243500	41243500	8657	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	19,764400	c.4048G>A	p.G1350S
BRCA1	chr17	41246481	41246481	6124	3	2	missense	SNM	c.1067A>G	p.Q356R	igv	+	18,125400	c.1067A>G	p.Q356R
BRCA1	chr17	41245587	41245587	8017	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	3,816890	c.1961delA	p.K654fs

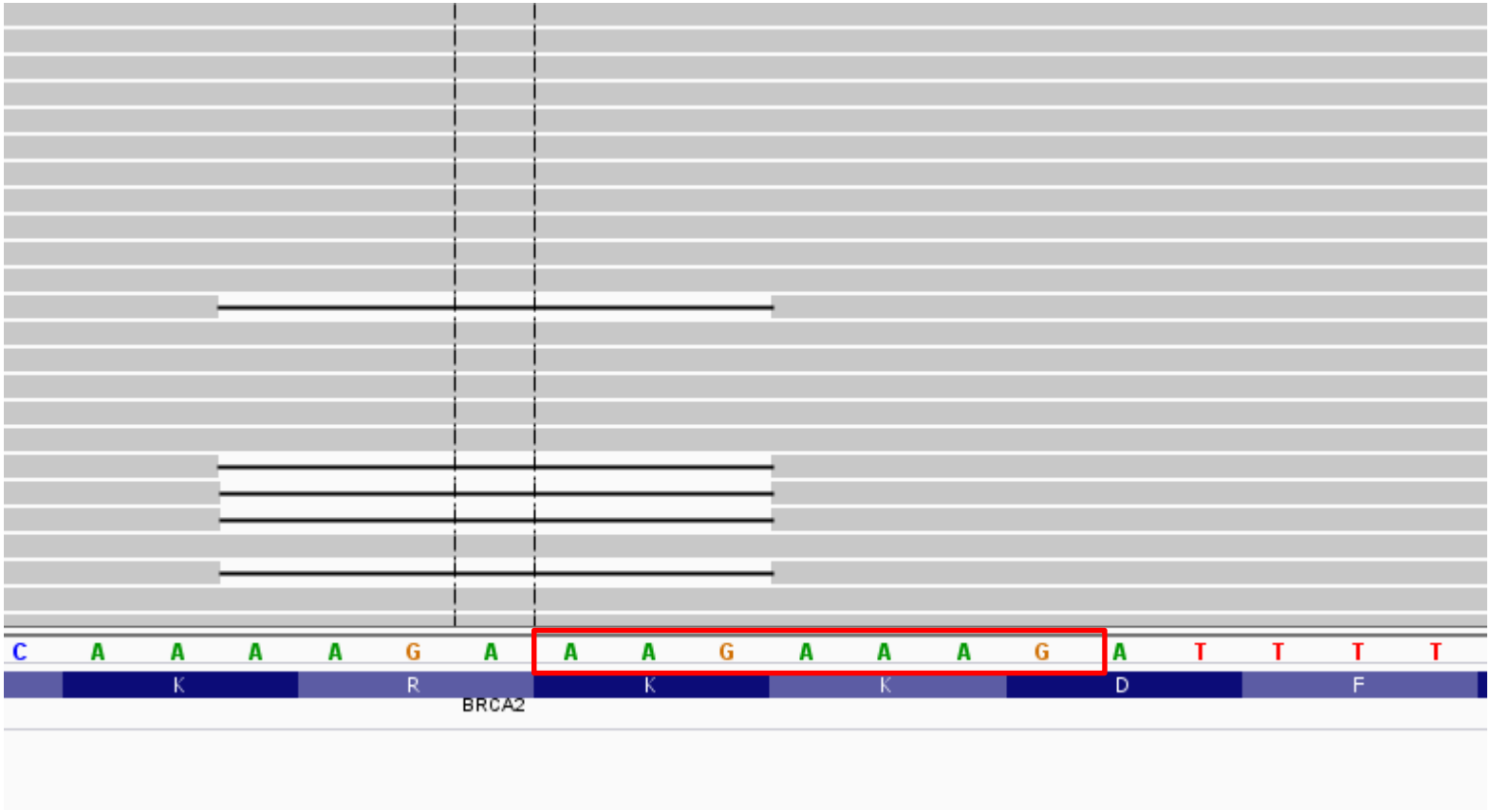
Challenging Cases – Case 3

- 3‘ rule: deletion has to be moved forward in the direction of 3‘ end:



Challenging Cases – Case 3

- 3‘ rule: deletion has to be moved forward in the direction of 3‘ end:



- Mutation not described in any of the databases
- How to classify?

Challenging Cases – Case 3

- BRCA2: **c.1304_1310del p.Arg435Lysfs*27**

- use of ENIGMA rules:

ENIGMA *BRCA1/2* Gene Variant Classification Criteria

ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) is an international consortium of investigators focused on determining the clinical significance of sequence variants in breast cancer genes. Information about the consortium purpose, membership criteria and operation can be found at http://www.enigmaconsortium.org/.

Table 2: Rationale for ENIGMA classification criteria

Class	Rationale for Criterion	Rationale/Summary of evidence stated for classification in ClinVar/other.
Class 5: pathogenic	IARC recommendation for Class 5 Pathogenic (Plon et al., 2008)	IARC class based on posterior probability from multifactorial likelihood analysis, thresholds for class as per Plon et al. 2008 (PMID: 18951446). Class 5 Pathogenic based on posterior probability = [insert posterior]
	Treated clinically as pathogenic	Variant allele predicted to encode a truncated non-functional protein.
	Treated clinically as pathogenic	Allele-specific assay on patient-derived mRNA demonstrated that the variant allele produces only predicted non-functional transcripts. Variant allele produces [insert r.#_#del] transcript(s).
	Treated clinically as pathogenic	Copy number deletion variant allele predicted to encode a non-functional protein.
	Treated clinically as pathogenic	Copy number duplication variant allele predicted to encode a non-functional protein.

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Challenging Cases – Case 3

Molekularpathologische Untersuchung:
Mutationsanalyse von ***BRCA1* und *BRCA2*** mittels Parallelsequenzierung (Next Generation Sequencing) von Multiplex PCR Amplikons. Die Generierung der Multiplex Amplikons erfolgte mit einem GeneRead DNASeq Mix-n-Match Panel V2 (Qiagen). Für die anschließende Library Erstellung wurden die GeneRead DNA Library I Core und GeneRead DNA I Amp Kits (Qiagen) sowie die NEXTflex-96 DNA Barcode Adapter (BIOO Scientific) verwendet. Die Sequenzierung wurde auf dem MiSeq (Illumina) durchgeführt.

Ergebnis:
Tumorzellgehalt: 70%

Gen	Exon	Codon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2, 3, 5 - 24	1 - 1864	Wildtyp			
BRCA2	2 - 27	1 - 1185, 1253 3419	EX10: c.1304_1310del p.R435Kfs*27	45,03	in keiner der bekannten Datenbanken zu finden, trunkierend, daher als pathogen (Kategorie 5) zu bewerten	

Bei der hier vorliegenden Mutation der Kategorie 5 *BRCA2*-Gen ergibt sich die Möglichkeit einer Therapie mit einem PARP Inhibitor.

Beurteilung:
Das vorliegende **Karzinom** zeigt eine trunkierende Mutation in BRCA2. Diese ist in den uns zugänglichen Datenbanken nicht zu finden, wird jedoch als pathogen eingeschätzt (Kategorie 5). Es ergibt sich somit die Möglichkeit einer Therapie mit PARP-Inhibitoren, falls klinisch indiziert.

Statement:

….truncating mutation, not found in databases, but classified as class 5

…..recommendation of ‘targeted therapy‘ if clinical indication is given

Challenging Cases – Case 4 (case from 1. German ring trial)

- Detection and nomenclature of variants can depend on panel design:
- BRCA1: Allele 1: c.1127delA p.Asn376Ilefs*18

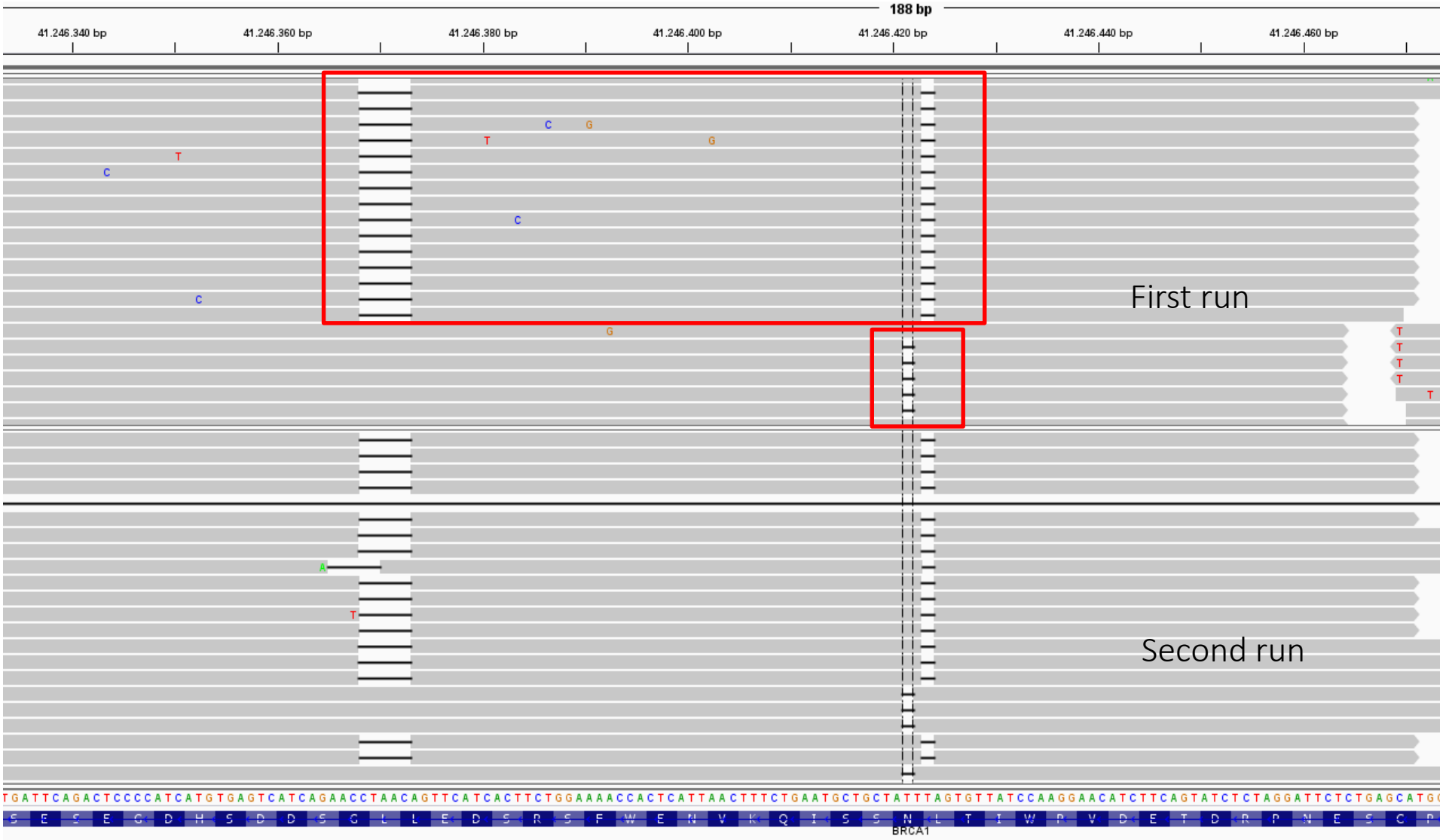
  Allele 2: c.1127delA and c.1179_1183delAGGTT

  or c.1127_1180delins48bp p.N376_G394delinsIAAFRKLMSGFPEVMNC
- both deletions must be covered by the same amplicon otherwise you can‘t decide whether they are located on the same allele

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32929387	32929387	3133	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	-	99,872300	c.7397T>C	p.V2466A
BRCA2	chr13	32912299	32912299	4626	3	2	silent	SNM	c.3807T>C	p.V1269V	igv	+	53,177700	c.3807T>C	p.V1269V
BRCA2	chr13	32913055	32913055	6386	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	52,959600	c.4563A>G	p.L1521L
BRCA2	chr13	32915005	32915005	5404	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	46,613600	c.6513G>C	p.V2171V
BRCA2	chr13	32930569	32930570	20	3	0	frame_shift_del	DEL	c.7440_7441delAA	p.L2480fs	igv	+	45,000000	c.7440_7441del	p.L2480fs
BRCA2	chr13	32907448	32907448	35	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	42,857100	c.1833delA	p.S611fs
BRCA2	chr13	32918783	32918783	2521	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	igv	+	16,660100	c.6929_6930ins	p.
BRCA2	chr13	32944675	32944675	1331	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	5,334340	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32914828	32914828	17780	3	0	silent	SNM	c.6336A>G	p.R2112R	igv	+	4,488190	c.6336A>G	p.R2112R
BRCA1	chr17	41246421	41246421	15876	3	0	frame_shift_del	DEL	c.1127delA	p.N376fs	igv	-	41,843000	c.1127delA	p.N376Ifs*18
BRCA1	chr17	41243500	41243500	10167	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	32,595700	c.4048G>A	p.G1350S
BRCA1	chr17	41246368	41246372	10873	3	0	frame_shift_del	DEL	c.1176_1180delGTTA	p.L392fs	igv	-	26,809500	c.1127_1180del	p.
BRCA1	chr17	41244000	41244000	13531	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	-	16,931500	c.3548A>G	p.K1183R
BRCA1	chr17	41246423	41246423	18025	3	0	frame_shift_del	DEL	c.1125delA	p.L375fs	igv	+	15,545100	c.1125delA	p.L375fs

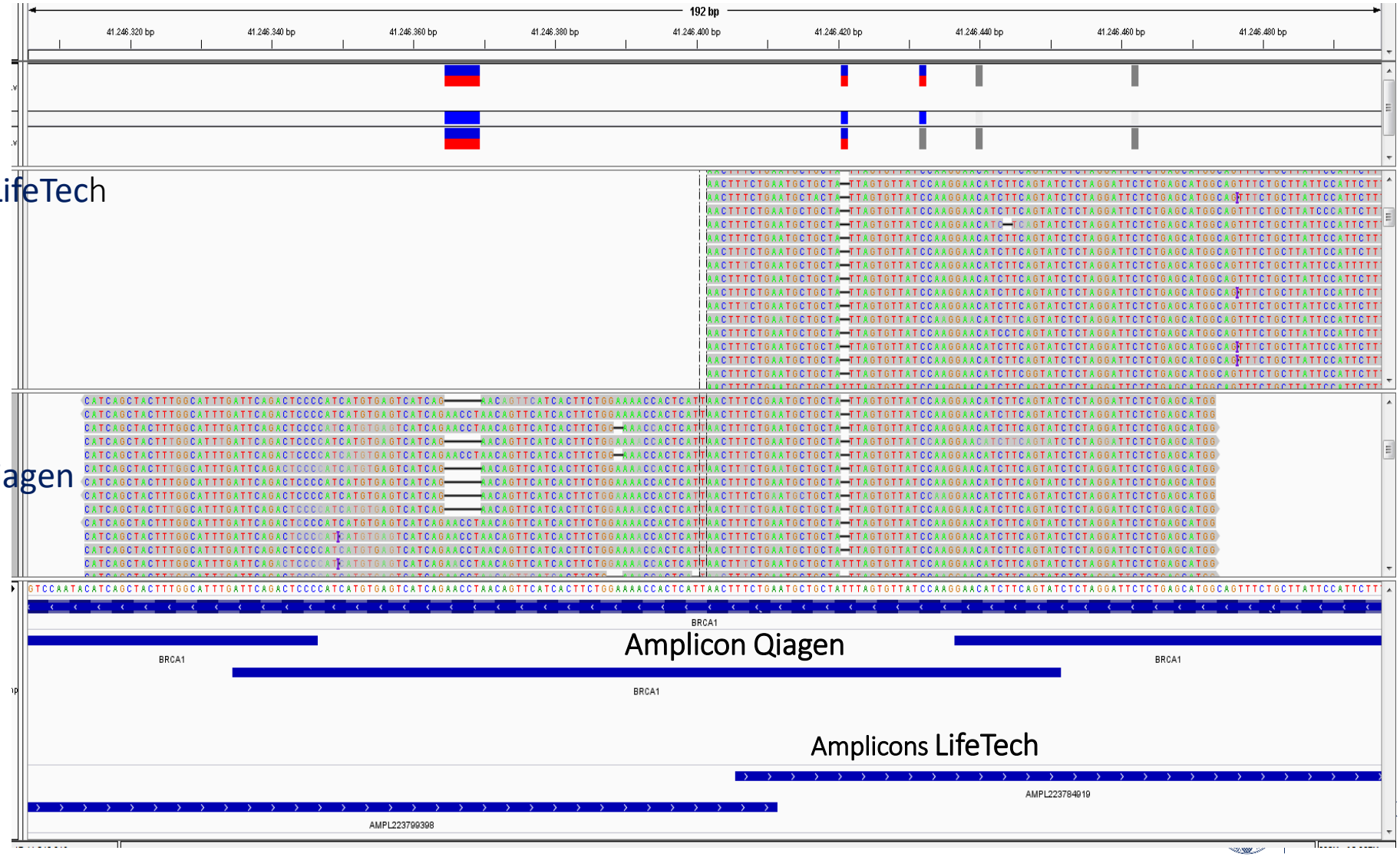
Challenging Cases – Case 4

- BAM file from Qiagen panel:



Challenging Cases – Case 4

- if the library is prepared with the LifeTech panel, both deletions are located on different amplicons and are detected separately



Challenging Cases – Case 4

- BRCA1: Allele 1: c.1127delA p.Asn376Ilefs*18

  Allele 2: c.1127_1180delins48bp p.N376_G394delinsIAAFRKLMSGFPEVMNC

- only the 1 bp deletion is found in the ARUP database, the complex deletion isn‘t described in any database

Exon 11	Deletion	c.1127delA		5 - Definitely pathogenic	>0.99	Arnold (1999) Hum Mutat 14; 333
Exon 11	Nonsense	c.1138C>T	p.Q380*	5 - Definitely pathogenic	>0.99	van der Hout (2006) Hum Mutat 27; 654
Exon 11	Nonsense	c.1141A>T	p.K381*	5 - Definitely pathogenic	>0.99	Garcia-Patino (1998) Acta Oncol 37; 299
Exon 11	Insertion	c.1152dupG		5 - Definitely pathogenic	>0.99	Ramus (2007) Hum Mutat 28; 1207
Exon 11	Deletion	c.1157_1170del14		5 - Definitely pathogenic	>0.99	Lecarpentier (2012) Breast Cancer Res 14; R99
Exon 11	Deletion	c.1158_1159delTT		5 - Definitely pathogenic	>0.99	Sauvan (2000) Hum Mutat 17; 154
Exon 11	Insertion	c.1159dupT		5 - Definitely pathogenic	>0.99	Yamashita (1999) Breast Cancer Res Treat 58; 11
Exon 11	Deletion	c.1166delG		5 - Definitely pathogenic	>0.99	Konecny (2007) Neoplasma 54; 137
Exon 11	Deletion	c.1175_1178del4		5 - Definitely pathogenic	>0.99	Martin (2001) J Clin Oncol 19; 2247
Exon 11	Deletion	c.1188delT		5 - Definitely pathogenic	>0.99	Gajalakshmi (2007) Breast Cancer Res Treat 101; 3

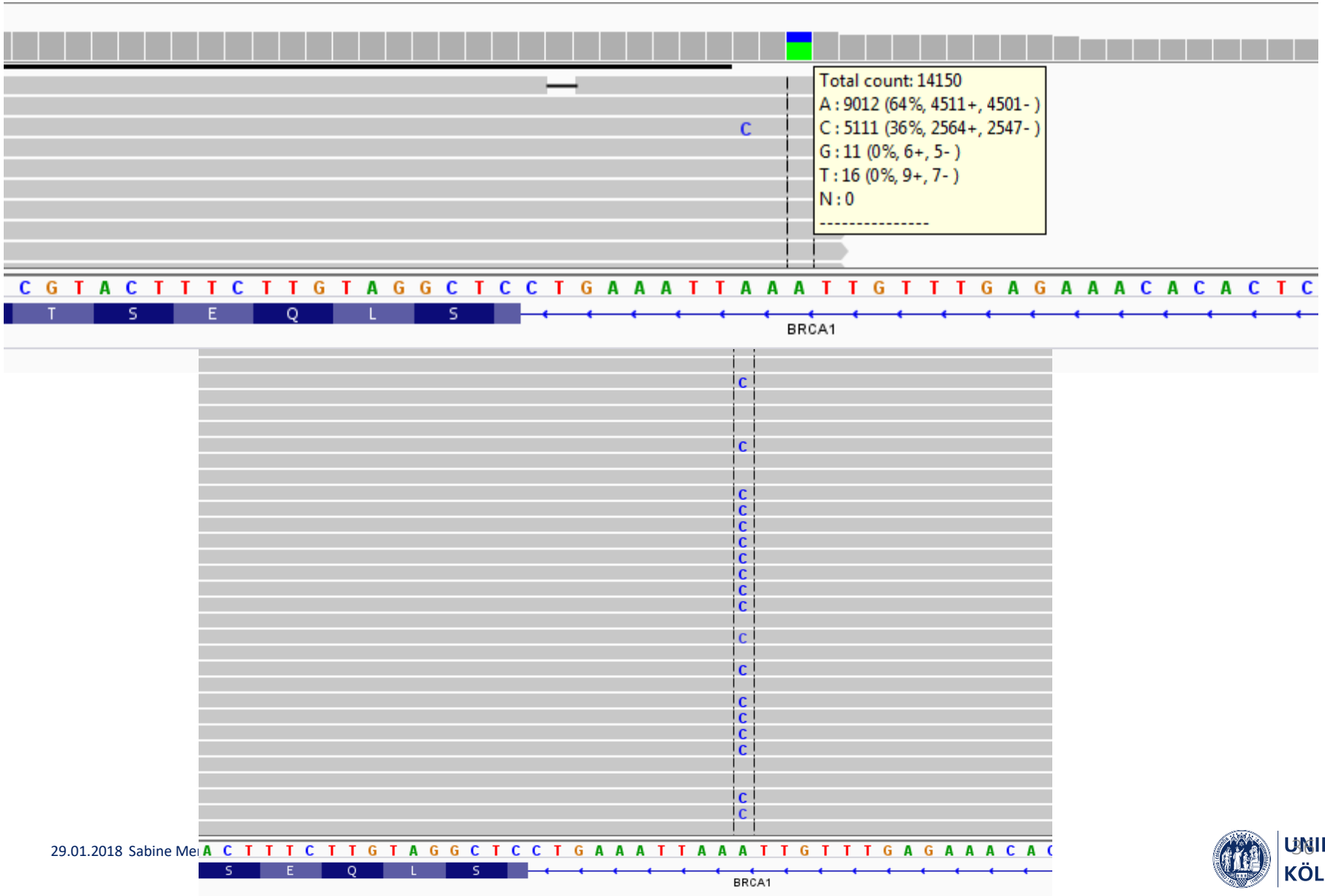
Challenging Cases – Case 5 (from the 1. European ring trial)

- Detection of variants depends on bioinformatics:
- BRCA1: c.213-11T>G
- region wasn‘t defined in our BED file, so we couldn‘t detect this variant in our first analysis

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32929387	32929387	10050	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,661700	c.7397T>C	p.V2466A
BRCA2	chr13	32915005	32915005	9519	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	69,944300	c.6513G>C	p.V2171V
BRCA2	chr13	32911888	32911888	13487	3	2	silent	SNM	c.3396A>G	p.K1132K	igv	+	51,479200	c.3396A>G	p.K1132K
BRCA2	chr13	32929232	32929232	4228	3	2	silent	SNM	c.7242A>G	p.S2414S	igv	+	50,993400	c.7242A>G	p.S2414S
BRCA2	chr13	32918783	32918783	4285	3	0	silent	SNM	c.6930T>A	p.T2310T	igv	+	50,175000	c.6930T>A	p.T2310T
BRCA2	chr13	32913136	32913136	16677	3	0	missense	SNM	c.4644A>T	p.E1548D	igv	+	39,839300	c.4644A>T	p.E1548D
BRCA2	chr13	32913055	32913055	7342	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	31,885000	c.4563A>G	p.L1521L
BRCA2	chr13	32918783	32918783	4287	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	igv	+	11,056700	c.6929_6930ins	p.
BRCA2	chr13	32944675	32944675	1337	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	4,637250	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32954023	32954023	8288	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	3,933400	c.9090delA	p.T3030fs
BRCA1	chr17	41244000	41244000	17659	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	+	53,898900	c.3548A>G	p.K1183R
BRCA1	chr17	41244936	41244936	1845	3	2	missense	SNM	c.2612C>T	p.P871L	igv	+	51,111100	c.2612C>T	p.P871L
BRCA1	chr17	41245471	41245471	6361	3	2	missense	SNM	c.2077G>A	p.D693N	igv	+	49,992100	c.2077G>A	p.D693N
BRCA1	chr17	41245466	41245466	6244	3	2	silent	SNM	c.2082C>T	p.S694S	igv	+	49,775800	c.2082C>T	p.S694S
BRCA1	chr17	41244435	41244435	4735	3	2	missense	SNM	c.3113A>G	p.E1038G	igv	+	49,503700	c.3113A>G	p.E1038G
BRCA1	chr17	41223094	41223094	15338	3	2	missense	SNM	c.4900A>G	p.S1634G	igv	+	49,348000	c.4900A>G	p.S1634G
BRCA1	chr17	41234470	41234470	30824	3	2	silent	SNM	c.4308T>C	p.S1436S	igv	+	48,154000	c.4308T>C	p.S1436S
BRCA1	chr17	41245237	41245237	9565	3	2	silent	SNM	c.2311T>C	p.L771L	igv	+	34,208100	c.2311T>C	p.L771L
BRCA1	chr17	41243500	41243500	8683	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	31,141300	c.4048G>A	p.G1350S
BRCA1	chr17	41243646	41243646	17694	3	0	missense	SNM	c.3902G>T	p.S1301I	igv	+	10,331200	c.3902G>T	p.S1301I
BRCA1	chr17	41245587	41245587	9224	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	4,379880	c.1961delA	p.K654fs

Challenging Cases – Case 5

- BRCA1: BRCA1: c.213-11T>G
- mutation can be identified in IGV with 36% allele frequency:



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Challenging Cases – Case 5

- BRCA1: BRCA1: c.213-11T>G
- mutation is classified as pathogenic in the ARUP database:

ARUP:

Intron 5	Splice Site	c.213-12A>G		5 - Definitely pathogenic	>0.99	Hoffman (1998) Am J Med Genet 80; 140
Intron 5	Splice Site	c.213-11T>G		5 - Definitely pathogenic	>0.99	Friedman (1994) Nat Genet 8; 399
Intron 5	Splice Site	c.213-2A>C		5 - Definitely pathogenic	>0.99	Caux-Moncoutier (2010) Hum Mutat 32; 325
Intron 5	Splice Site	c.213-2A>G		5 - Definitely pathogenic	>0.99	Lecarpentier (2012) Breast Cancer Res 14; R99
Intron 5	Splice Site	c.213-1G>A		5 - Definitely pathogenic	>0.99	van der Hout (2006) Hum Mutat 27; 654
Intron 5	Splice Site	c.213-15A>G		5 - Definitely pathogenic	>0.99	Houdayer (2012) Hum Mutat 33, 1228

Challenging Cases – Case 6

- Interpretation of variants of unknown significance:
- BRCA1: c.211A>T p.Arg71Trp
- not detected in any of the databases; discussed with department for Familiar Breast and Ovarian Cancer (FBZ) in Cologne

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32906729	32906729	13768	3	2	missense	SNM	c.1114A>C	p.N372H	igv	+	16,857900	c.1114A>C	p.N372H
BRCA2	chr13	32912299	32912299	9355	3	2	silent	SNM	c.3807T>C	p.V1269V	igv	+	74,441500	c.3807T>C	p.V1269V
BRCA2	chr13	32912922	32912922	2507	3	0	missense	SNM	c.4430T>A	p.I1477N	igv	+	3,031510	c.4430T>A	p.I1477N
BRCA2	chr13	32913055	32913055	10331	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	52,589300	c.4563A>G	p.L1521L
BRCA2	chr13	32913135	32913136	13025	3	0	missense	DNM	c.4643_4644delinsCT	p.E1548A	igv	+	22,914800	c.4643_4644del	p.E1548A
BRCA2	chr13	32915005	32915005	13630	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	60,410900	c.6513G>C	p.V2171V
BRCA2	chr13	32929387	32929387	14376	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,860900	c.7397T>C	p.V2466A
BRCA2	chr13	32968895	32968895	33434	3	0	missense	SNM	c.9326T>A	p.L3109H	igv	+	2,820480	c.9326T>A	p.L3109H
BRCA1	chr17	41223094	41223094	17263	3	2	missense	SNM	c.4900A>G	p.S1634G	igv	+	25,667600	c.4900A>G	p.S1634G
BRCA1	chr17	41234470	41234470	31042	3	2	silent	SNM	c.4308T>C	p.S1436S	igv	+	26,866800	c.4308T>C	p.S1436S
BRCA1	chr17	41243500	41243500	11001	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	21,498000	c.4048G>A	p.G1350S
BRCA1	chr17	41244000	41244000	17696	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	+	27,350800	c.3548A>G	p.K1183R
BRCA1	chr17	41244435	41244435	2944	3	2	missense	SNM	c.3113A>G	p.E1038G	igv	+	25,271700	c.3113A>G	p.E1038G
BRCA1	chr17	41244936	41244936	3572	3	2	missense	SNM	c.2612C>T	p.P871L	igv	+	27,827500	c.2612C>T	p.P871L
BRCA1	chr17	41245237	41245237	10543	3	2	silent	SNM	c.2311T>C	p.L771L	igv	+	14,919900	c.2311T>C	p.L771L
BRCA1	chr17	41245466	41245466	8799	3	2	silent	SNM	c.2082C>T	p.S694S	igv	+	27,241700	c.2082C>T	p.S694S
BRCA1	chr17	41258474	41258474	4389	3	1	missense	SNM	c.211A>T	p.R71W	igv	-	46,092500	c.211A>T	p.R71W
BRCA2	chr13	32907421	32907421	1028	3	0	frame_shift_del	DEL	c.1806delA	p.G602fs	igv	+	2,918290	c.1806delA	p.G602fs
BRCA2	chr13	32918783	32918783	5536	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	igv	+	6,105490	c.6929_6930ins	p.
BRCA2	chr13	32944675	32944675	3052	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	3,145480	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32954023	32954023	10985	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	2,994990	c.9090delA	p.T3030fs
BRCA1	chr17	41245587	41245587	12547	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	3,219890	c.1961delA	p.K654fs

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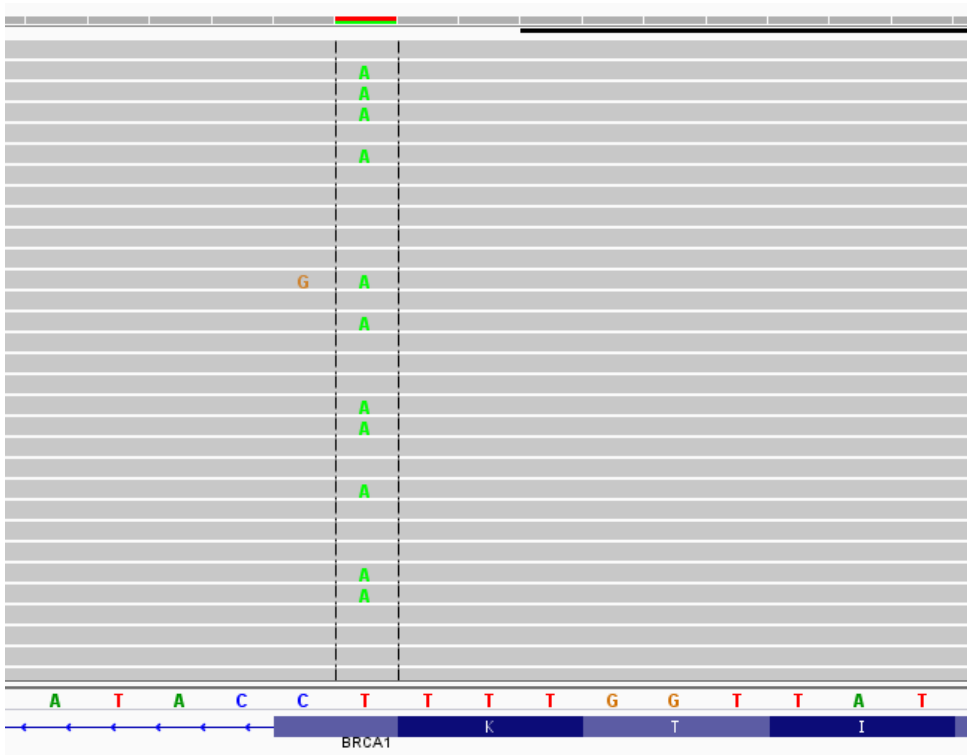
Challenging Cases – Case 6



- BRCA1: c.211A>T p.Arg71Trp
- not in databases
- ENIGMA criteria for class 4 didn‘t match

Variant at IVS±1 or IVS±2 or G>non-G at last base of exon when adjacent intronic sequence is not GTRRGT that is predicted to alter used of native donor/acceptor site
AND is untested for splicing aberrations using RNA assays on patient blood that assess allele-specific transcript expression,
AND is not predicted or known to alter production of (naturally occurring) in-frame RNA isoforms that may rescue gene functionality.

- first ENIGMA criterion for class 4
- not fulfilled

Challenging Cases – Case 6

A variant that encodes the same amino acid change as a previously established Class 5 pathogenic **missense** variant with a different underlying nucleotide change, is located in a known clinically important functional protein domain, with no evidence of mRNA aberration (splicing or expression) from in vitro mRNA assays on patient RNA, and the variant is absent from outbred control reference groups.

- second ENIGMA criterion for class 4
- not fulfilled

ARUP:

Exon 5	Splice Site	c.211A>G		5 - Definitely pathogenic	>0.99	Vega (2001) Hum Mutat 17; 520

ClinVar:

NM_007294.3(**BRCA1**):c.211A>G (p.Arg71Gly) *GRCh37:* Chr17:41258474 *GRCh38:* Chr17:43106457	BRCA1	Familial cancer of breast, Breast-ovarian cancer, familial 1, Hereditary breast and ovarian cancer syndrome, not specified, not provided, Hereditary cancer-predisposing syndrome	Pathogenic/Likely pathogenic (Jul 18, 2017)

- mutation case 6: **c.211A>T** **p.Arg71Trp**
- an exchange in the same position can be found but leads to another amino acid exchange which is classified as pathogenic

Challenging Cases – Case 6

- case was discussed with the FBZ and classified as **pathogenic**
- a splice site prediction algorithm was used and predicted a 45.7% probability of alternative splicing
- additionally the exchange is located within a functionally important domain of BRCA1 (ring domain, 1-101)
- pathogenicity was also predicted by SIFT, Mutationtaster and Polyphen

1. Statement:

- tumor shows wildtype sequence for *BRCA2* and VUS for *BRCA1*; until now no evidence for any therapy option, result will be discussed with FBZ and reported back

2. Statement:

- variant in *BRCA1* is classified as class 4
- targeted therapy is recommended if the clinical indication is given
- genetic counseling is recommended to clarify the family history and a potential germline mutation

Challenging Cases – Case 7

- Interpretation of variants of unknown significance:
- BRCA2: c.8420C>T p.Ser2807Leu
- classified as VUS in databases; agreement with FB

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Known Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32911888	32911888	86113	3	2	silent	SNM	c.3396A>G	p.K1132K	igv	+	11,156300	c.3396A>G	p.K1132K
BRCA2	chr13	32912299	32912299	26713	3	2	silent	SNM	c.3807T>C	p.V1269V	igv	+	90,368000	c.3807T>C	p.V1269V
BRCA2	chr13	32913055	32913055	47801	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	36,053600	c.4563A>G	p.L1521L
BRCA2	chr13	32913135	32913136	74887	3	0	missense	DNM	c.4643_4644delinsCT	p.E1548A	igv	+	17,446600	c.4643_4644del	p.E1548A
BRCA2	chr13	32913282	32913282	52470	3	0	missense	SNM	c.4790C>A	p.S1597Y	igv	+	46,702900	c.4790C>A	p.S1597Y
BRCA2	chr13	32915005	32915005	38804	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	62,403400	c.6513G>C	p.V2171V
BRCA2	chr13	32918783	32918783	12291	3	0	silent	SNM	c.6930T>A	p.T2310T	igv	+	39,988600	c.6930T>A	p.T2310T
BRCA2	chr13	32929387	32929387	30122	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,910400	c.7397T>C	p.V2466A
BRCA2	chr13	32944627	32944627	18434	3	1	missense	SNM	c.8420C>T	p.S2807L	igv	-	19,941400	c.8420C>T	p.S2807L
BRCA1	chr17	41243500	41243500	27296	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	24,380900	c.4048G>A	p.G1350S
BRCA1	chr17	41243646	41243646	42774	3	0	missense	SNM	c.3902G>T	p.S1301I	igv	+	12,841900	c.3902G>T	p.S1301I
BRCA2	chr13	32907448	32907448	1388	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	4,683000	c.1833delA	p.S611fs
BRCA2	chr13	32918783	32918783	12304	3	0	in_frame_ins	INS	c.6929_6930insAGA	p.	igv	+	6,680750	c.6929_6930ins	p.
BRCA2	chr13	32930569	32930570	182	3	0	frame_shift_del	DEL	c.7440_7441delAA	p.L2480fs	igv	+	8,791210	c.7440_7441del	p.L2480fs
BRCA2	chr13	32944675	32944675	4117	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	17,269900	c.8467_8468ins	p.Q2823fs

Challenging Cases – Case 7

NM_000059.3(BRCA2):c.8419T>C (p.Ser2807Pro) *GRCh37:* Chr13:32944626 *GRCh38:* Chr13:32370489	BRCA2	Hereditary cancer-predisposing syndrome	Uncertain significance (Aug 29, 2016)
NM_000059.3(BRCA2):c.8420C>T (p.Ser2807Leu) *GRCh37:* Chr13:32944627 *GRCh38:* Chr13:32370490	BRCA2	Breast-ovarian cancer, familial 2, Hereditary breast and ovarian cancer syndrome, not specified, Hereditary cancer-predisposing syndrome	Uncertain significance (Jul 21, 2017)
NM_000059.3(BRCA2):c.8422C>G (p.Leu2808Val) *GRCh37:* Chr13:32944629 *GRCh38:* Chr13:32370492	BRCA2	Hereditary cancer-predisposing syndrome	Uncertain significance (Nov 6, 2015)
NM_000059.3(BRCA2):c.8423T>C (p.Leu2808Pro) *GRCh37:* Chr13:32944630 *GRCh38:* Chr13:32370493	BRCA2	Breast-ovarian cancer, familial 2	Uncertain significance (Jul 24, 2014)

Domain/ Motif	AA start	AA end	AA alterations with Demonstrated Clinical Importance[a]	Classification of in-frame deletions targeting domain/motifs
DBD (DNA/DSS 1 binding domain - helical, OB1, OB2, OB3)	2481	3186	W2626C (c.7878G>C (p.Trp2626Cys)) I2627F(c.7879A>T (p.Ile2627Phe)) E2663V (c.7988A>T (p.Glu2663Val)) T2722R (c.8165C>G (p.Thr2722Arg)) D2723G (c.8168A>G (p.Asp2723Gly)) D2723H (c.8167G>C (p.Asp2723His)) G2748D (c.8243G>A (p.Gly2748Asp)) I2778_Q2829del (c.8332_8487del (p.Ile2778_Gln2829del)) R3052W (c.9154C>T (p.Arg3052Trp))	Class-5 if at least one clinically relevant residue (or all of AA 2778-2829) is removed. Class-3 otherwise

ENIGMA

Challenging Cases – Case 7

1. Statement:

- tumor shows wildtype sequence for *BRCA1* and VUS for *BRCA2*; until now no evidence for any therapy option, result will be discussed with FBZ and reported back
- genetic counseling is recommended

2. Statement:

- after discussion with the FBZ the variant in *BRCA2* is still classified as VUS
- no therapy option with PARP inhibitor

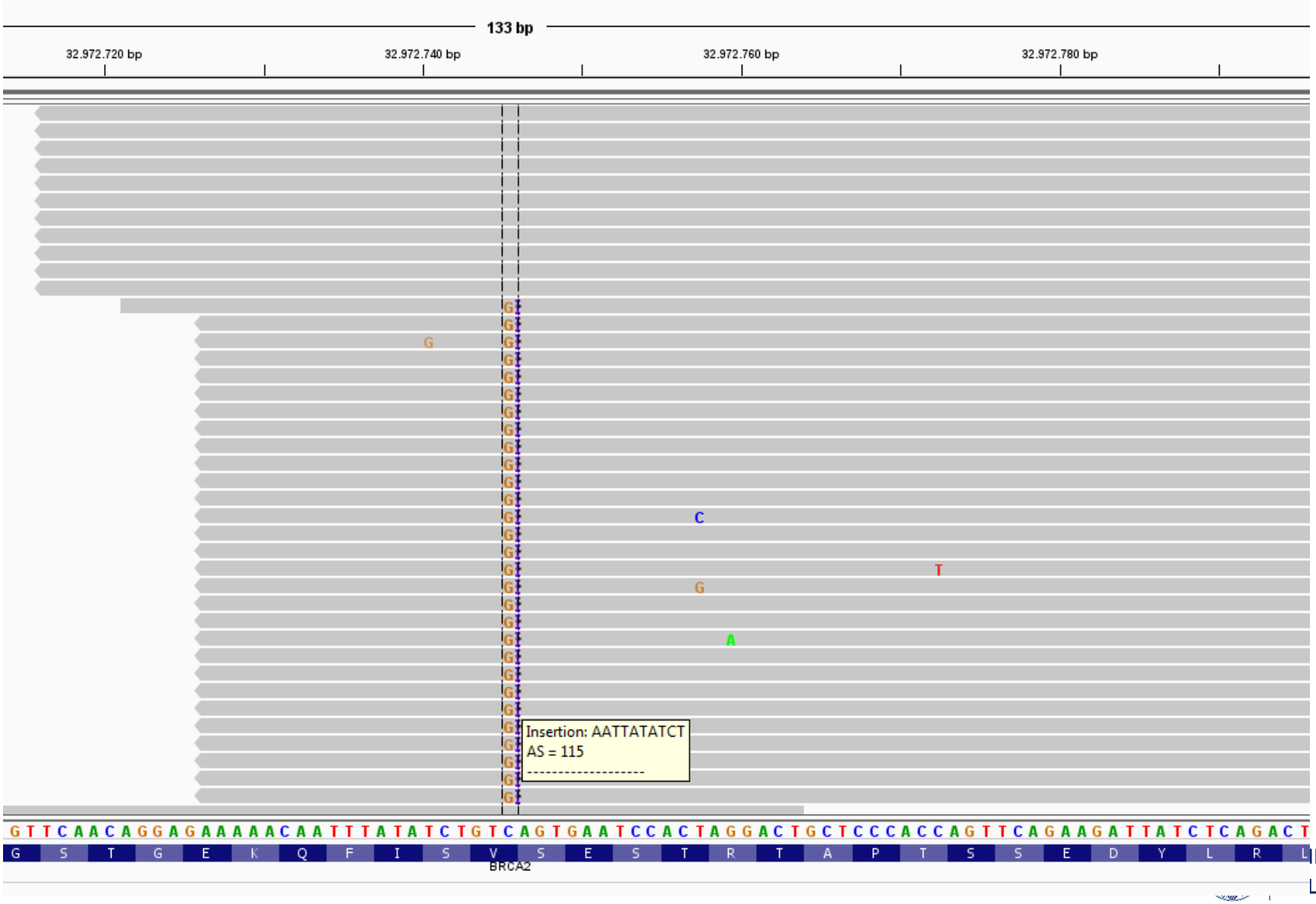
Challenging Cases – Case 8

- Interpretation of frame-shift variants:
- BRCA2: c.10095delinsGAATTATATCT p.Ser3366Asnfs*4
- not found in databases, according to ENIGMA criteria without relevance

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32911888	32911888	10860	3	2	silent	SNM	c.3396A>G	p.K1132K	igv	+	52,081000	c.3396A>G	p.K1132K
BRCA2	chr13	32913055	32913055	10687	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	39,636900	c.4563A>G	p.L1521L
BRCA2	chr13	32913136	32913136	18176	3	0	missense	SNM	c.4644A>T	p.E1548D	igv	+	31,134500	c.4644A>T	p.E1548D
BRCA2	chr13	32915005	32915005	12005	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	39,783400	c.6513G>C	p.V2171V
BRCA2	chr13	32918783	32918783	1715	3	0	silent	SNM	c.6930T>A	p.T2310T	igv	+	10,903800	c.6930T>A	p.T2310T
BRCA2	chr13	32929232	32929232	5082	3	2	silent	SNM	c.7242A>G	p.S2414S	igv	+	51,456100	c.7242A>G	p.S2414S
BRCA2	chr13	32929387	32929387	8585	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,848600	c.7397T>C	p.V2466A
BRCA2	chr13	32972745	32972745	4785	3	0	silent	SNM	c.10095C>G	p.V3365V	igv	+	17,199600	c.10095C>G	p.V3365V
BRCA1	chr17	41223094	41223094	22224	3	2	missense	SNM	c.4900A>G	p.S1634G	igv	+	99,761500	c.4900A>G	p.S1634G
BRCA1	chr17	41234470	41234470	44739	3	2	silent	SNM	c.4308T>C	p.S1436S	igv	+	99,369700	c.4308T>C	p.S1436S
BRCA1	chr17	41243500	41243500	12076	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	21,414400	c.4048G>A	p.G1350S
BRCA1	chr17	41244000	41244000	15266	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	+	99,862400	c.3548A>G	p.K1183R
BRCA1	chr17	41244435	41244435	8253	3	2	missense	SNM	c.3113A>G	p.E1038G	igv	+	99,733400	c.3113A>G	p.E1038G
BRCA1	chr17	41244936	41244936	3170	3	2	missense	SNM	c.2612C>T	p.P871L	igv	+	99,653000	c.2612C>T	p.P871L
BRCA1	chr17	41245237	41245237	11711	3	2	silent	SNM	c.2311T>C	p.L771L	igv	+	56,656100	c.2311T>C	p.L771L
BRCA1	chr17	41245466	41245466	4372	3	2	silent	SNM	c.2082C>T	p.S694S	igv	+	99,428200	c.2082C>T	p.S694S
BRCA1	chr17	41245471	41245471	4467	3	2	missense	SNM	c.2077G>A	p.D693N	igv	+	52,764700	c.2077G>A	p.D693N
BRCA2	chr13	32907448	32907448	325	3	0	frame_shift_del	DEL	c.1833delA	p.S611fs	igv	+	9,538460	c.1833delA	p.S611fs
BRCA2	chr13	32930569	32930570	26	3	0	frame_shift_del	DEL	c.7440_7441delAA	p.L2480fs	igv	+	23,076900	c.7440_7441del	p.L2480fs
BRCA2	chr13	32944675	32944675	1361	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	15,356400	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32954023	32954023	4491	3	0	frame_shift_del	DEL	c.9090delA	p.T3030fs	igv	+	3,273210	c.9090delA	p.T3030fs
BRCA2	chr13	32972746	32972746	4888	3	0	frame_shift_ins	INS	c.10095_10096insAA	p.S3366fs	igv	-	16,489400	c.10095delinsG	p.S3366Nfs*4

Challenging Cases – Case 8

- BRCA2: c.10095delinsGAATTATATCT p.Ser3366Asnfs*4



Challenging Cases – Case 8

- BRCA2: c.10095delinsGAATTATATCT p.Ser3366Asnfs*4

References and summary interpretation[a]
Case-control and frequency data indicate that BRCA2 c.9976A>T (p.Lys3326Ter) does not confer a high risk of cancer (OR 1.3-1.5, dependent on breast or ovarian cancer subtype (Meeks et al., 2016), demonstrating that residues at and downstream of 3327 are likely dispensable.
Position 3308 is implicated as clinically important by the observation that a nonsense variant c.9924C>G (p.Tyr3308Ter) is recognized as a high-risk pathogenic variant with known functional relevance ((Vallee et al., 2016); Bayes score 1122:1 from a single large kConFab family, Spurdle unpublished data). There is currently no publicly available clinical information to support pathogenicity of nonsense or frameshift variants located between positions 3309 and 3325.
These data combined suggest that the C-terminal border of the BRC-9 relevant to the clinical interpretation of sequence variants in exon 27 of BRCA2 lies between 3309 and 3325. That is, a variant predicted to disrupt expression only of protein sequence downstream of position 3325 would be considered unlikely to be clinically important. Further functional and clinical studies are underway to refine risk, if any, for predicted nonsense or frameshift variants downstream of position 3326.

ENIGMA

Challenging Cases – Case 8

Ergebnis:

Gen	Exon	Codon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2, 3, 5 - 24	1 - 1864	Wildtyp			
BRCA2	2 - 27		EX27: c.10095delinsGAATTATATCT p.S3366Nfs*4	16,49	benigne Veränderung	keine Therapierelevanz

/ merk

Zur Interpretation der detektierten Mutationen wurden folgende Datenbanken (DB) verwendet:
UMD-DB (http://www.umd.be/BRCA1/ bzw. http://www.umd.be/BRCA2/), ARUP-DB (http://arup.utah.edu/database/BRCA/), LOVD-DB (http://brca.iarc.fr/LOVD/home.php), ClinVar-DB (http://www.ncbi.nlm.nih.gov/clinvar/).
Die Mutationen werden dabei in folgende Kategorien unterteilt:
Kategorie 1: neutral, Kategorie 2: wahrscheinlich neutral, Kategorie 3: unklare Signifikanz, Kategorie 4: wahrscheinlich pathogen, Kategorie 5: pathogen.

Bei der hier vorliegenden Mutation der Kategorie 1 im *BRCA2*-Gen ergibt sich keine Möglichkeit einer Therapie mit einem PARP Inhibitor.

Beurteilung:

In der von Ihnen gewünschten molekularpathologischen Mutationsanalyse liegt das BRCA1-Gen in der Wildtyp-Sequenz vor. Bei der hier vorliegenden Mutation der Kategorie 1 im BRCA2-Gen ergibt sich keine Möglichkeit einer Therapie mit einem PARP-Inhibitor. /serc/zanf/PL2017.948:

Statement:

- tumor shows wildtype sequence for *BRCA1* and a benign mutation for *BRCA2*
- there is no evidence for a therapy option with a PARP inhibitor

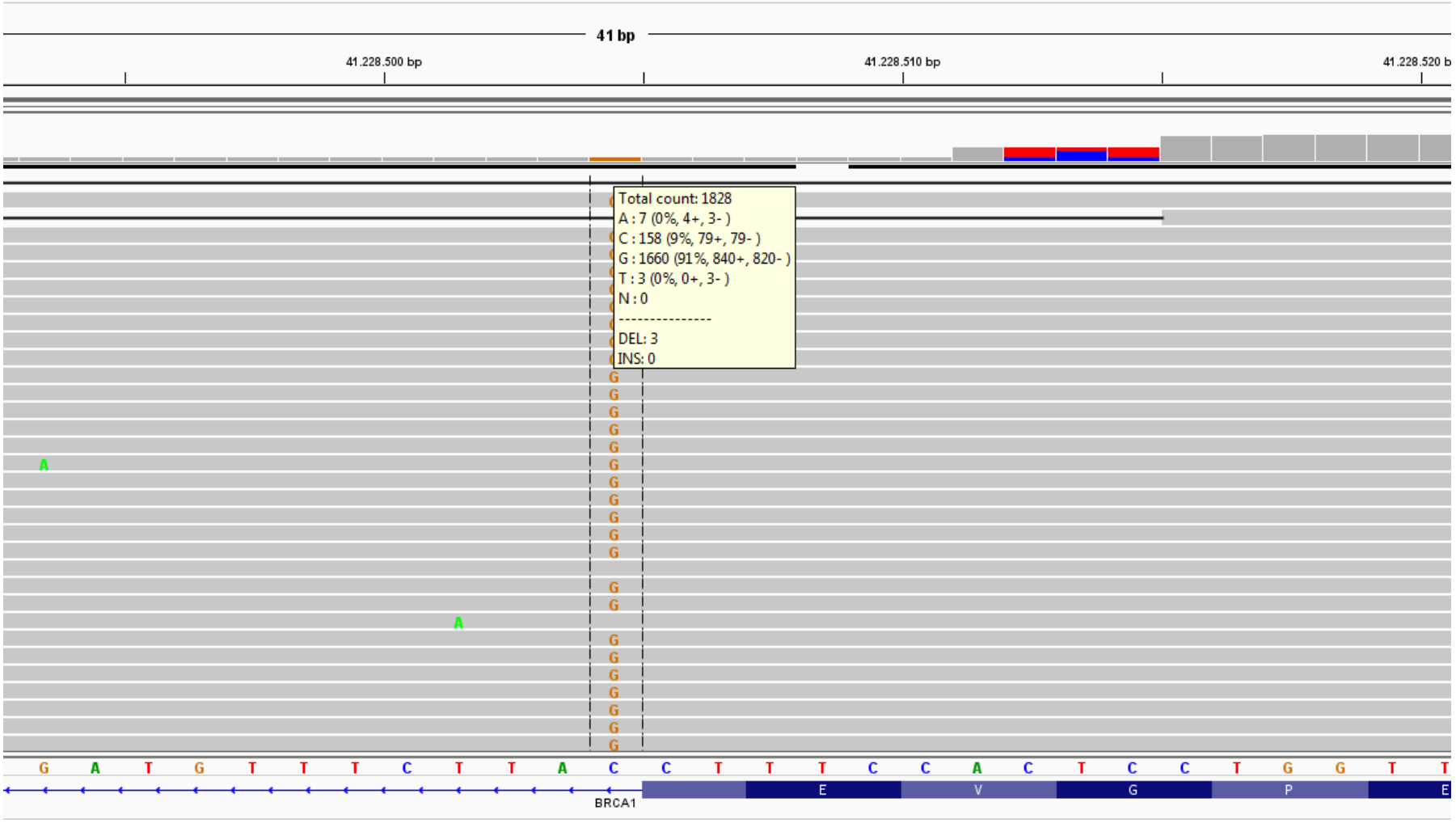
Challenging Cases – Case 9

- Interpretation of splice-site variants:
- **BRCA1: c.4484+1G>C** BRCA2: c.59A>G p.Asn20Ser
- BRCA2 mutation: VUS, BRCA1 mutation: class 4

Gene_Hugo	Chr	Start	End	Cov.	Vers.	Variants	Type_1	Type_2	Change_cDNA	Protein	IGV	+	Fraction	c.Ergebnis	p.Ergebnis
BRCA2	chr13	32890656	32890656	2472	3	0	missense	SNM	c.59A>G	p.N20S	igv	-	44,943400	c.59A>G	p.N20S
BRCA2	chr13	32906729	32906729	6606	3	2	missense	SNM	c.1114A>C	p.N372H	igv	+	23,720900	c.1114A>C	p.N372H
BRCA2	chr13	32913055	32913055	6832	3	2	silent	SNM	c.4563A>G	p.L1521L	igv	+	54,727800	c.4563A>G	p.L1521L
BRCA2	chr13	32913135	32913136	7337	3	0	missense	DNM	c.4643_4644delinsCT	p.E1548A	igv	+	14,870100	c.4643_4644del	p.E1548A
BRCA2	chr13	32915005	32915005	5451	3	2	silent	SNM	c.6513G>C	p.V2171V	igv	+	31,810700	c.6513G>C	p.V2171V
BRCA2	chr13	32929387	32929387	3707	3	2	missense	SNM	c.7397T>C	p.V2466A	igv	+	99,622300	c.7397T>C	p.V2466A
BRCA1	chr17	41223094	41223094	10619	3	2	missense	SNM	c.4900A>G	p.S1634G	igv	+	99,340800	c.4900A>G	p.S1634G
BRCA1	chr17	41228504	41228504	1772	3	1	splice	SNM	c.4547_splice	e14+1	igv	-	91,252800	c.4484+1G>C	c.4484+1G>C
BRCA1	chr17	41234470	41234470	21492	3	2	silent	SNM	c.4308T>C	p.S1436S	igv	+	99,558000	c.4308T>C	p.S1436S
BRCA1	chr17	41243500	41243500	6122	3	0	missense	SNM	c.4048G>A	p.G1350S	igv	+	22,917300	c.4048G>A	p.G1350S
BRCA1	chr17	41244000	41244000	5244	3	2	missense	SNM	c.3548A>G	p.K1183R	igv	+	99,752100	c.3548A>G	p.K1183R
BRCA1	chr17	41244435	41244435	1823	3	2	missense	SNM	c.3113A>G	p.E1038G	igv	+	99,396600	c.3113A>G	p.E1038G
BRCA1	chr17	41244936	41244936	1591	3	2	missense	SNM	c.2612C>T	p.P871L	igv	+	99,874300	c.2612C>T	p.P871L
BRCA1	chr17	41245237	41245237	3916	3	2	silent	SNM	c.2311T>C	p.L771L	igv	+	35,010200	c.2311T>C	p.L771L
BRCA1	chr17	41245466	41245466	665	3	2	silent	SNM	c.2082C>T	p.S694S	igv	+	99,849600	c.2082C>T	p.S694S
BRCA1	chr17	41245471	41245471	663	3	2	missense	SNM	c.2077G>A	p.D693N	igv	+	98,340900	c.2077G>A	p.D693N
BRCA2	chr13	32944675	32944675	446	3	0	frame_shift_ins	INS	c.8467_8468insA	p.Q2823fs	igv	+	21,300400	c.8467_8468ins	p.Q2823fs
BRCA2	chr13	32954023	32954025	1285	3	0	in_frame_del	DEL	c.9090_9092delAAA	p.	igv	+	6,303500	c.9090_9092del	p.
BRCA1	chr17	41228514	41228514	1881	3	0	frame_shift_del	DEL	c.4538delG	p.G1513fs	igv	+	4,199890	c.4538delG	p.G1513fs
BRCA1	chr17	41245587	41245587	5009	3	0	frame_shift_del	DEL	c.1961delA	p.K654fs	igv	+	3,633460	c.1961delA	p.K654fs

Challenging Cases – Case 9

- BRCA1: c.4484+1G>C



Challenging Cases – Case 9

- BRCA1: c.4484+1G>C
- other exchanges in the same position are described as pathogenic in the databases

Intron 14	Splice Site	c.4484+1G>A		5 - Definitely pathogenic	>0.99	Perkowska (2003) Hum Mutat 21; 553
Intron 14	Splice Site	c.4484+1G>T		5 - Definitely pathogenic	>0.99	Men,ndez (2012) Breast Cancer Res Treat 132; 979

BRCA1):c.4484+1G>T 17:41228504 17:43076487	BRCA1	Breast-ovarian cancer, familial 1	Pathogenic (Oct 2, 2015)
BRCA1):c.4484+1delG 17:41228504 17:43076487	BRCA1	Breast-ovarian cancer, familial 1	Pathogenic (Oct 2, 2015)
BRCA1):c.4484+1G>A 17:41228504 17:43076487	BRCA1	Breast-ovarian cancer, familial 1, Hereditary breast and ovarian cancer syndrome, not provided	Pathogenic (Aug 18, 2017)

Class	Criterion
Class 4: likely pathogenic	Variant at IVS±1 or IVS±2 or G>non-G at last base of exon when adjacent intronic sequence is not GTRRGT that is predicted to alter used of native donor/acceptor site AND is untested for splicing aberrations using RNA assays on patient blood that assess allele-specific transcript expression, AND is not predicted or known to alter production of (naturally occurring) in-frame RNA isoforms that may rescue gene functionality.

ENIGMA criteria classify the mutation as likely pathogenic

Challenging Cases – Case 9

Ergebnis:

Tumorzellgehalt: 80%

Gen	Exon	Codon	Mutationsstatus	Freq. %	Interpretation	Therapieoption
BRCA1	2, 3, 5 - 24	1 - 1864	EX14: c.4484+1G>C*	91,25	Kategorie 4 (ENIGMA)	
BRCA2	2 - 27	1 - 1185, 1253 - 3419	EX2: c.59A>G p.N20S	44,94	Veränderung unklarer Signifikanz, keine Therapierelevanz	

Bei der hier vorliegenden Mutation der Kategorie 4 im *BRCA1*-Gen ergibt sich die Möglichkeit einer Therapie mit einem PARP Inhibitor.

Beurteilung:

Das auswärts diagnostizierte **Ovarkarzinom** zeigt eine wahrscheinlich pathogene Mutation in BRCA1 (Kategorie 4). Es ergibt sich hieraus die Möglichkeit einer Therapie mit PARP-Inhibitoren, falls klinisch indiziert.

Bei nachgewiesener pathogener Mutation eines BRCA-Gens, bitten wir die Familienanamnese weiter abzuklären und ggf. um Vorstellung im Zentrum für familiären Brust- und Eierstockkrebs und Klärung einer möglichen Keimbahnmutation mit ggf. entsprechender familiärer Belastung. /mark/zan8

Statement:

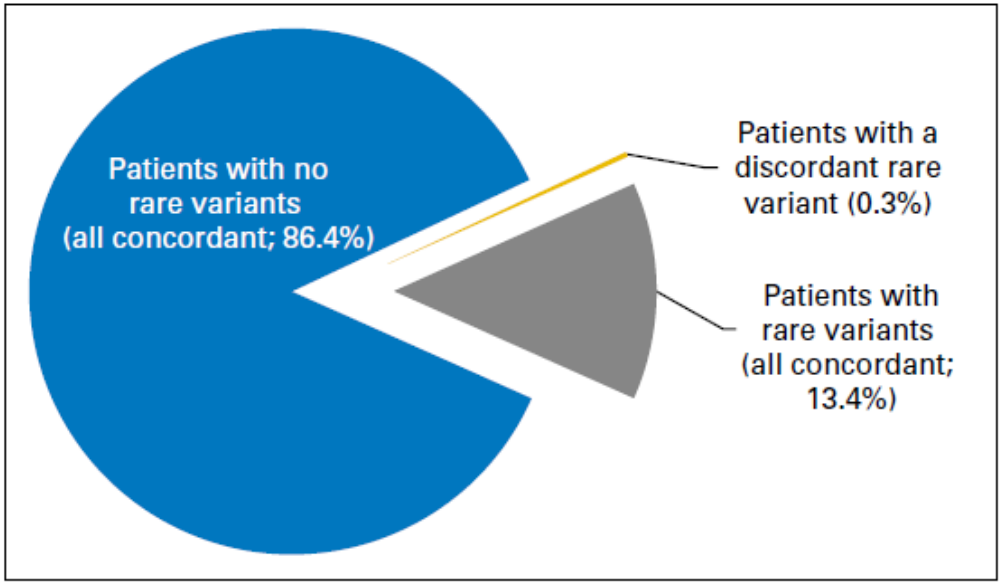
- variant in *BRCA1* is classified as class 4
- targeted therapy is recommended if the clinical indication is given
- genetic counseling is recommended to clarify the family history and a potential germline mutation

Challenging Cases – Data from Literature

Consistency of *BRCA1* and *BRCA2* Variant Classifications Among Clinical Diagnostic Laboratories

Lincoln et al., JCO PO 2017

ClinVar Submitter	No. Classified Variants
Ambry	2,792
SCRP/Myriad Genetics	2,327
Invitae	1,998
GeneDx	1,216
Counsyl	272
CHEO	257
Emory	203
Total	5,124



- discordance was only found for patients with rare variants 0,3% of analysed cases

Clinical Variant Classification: A Comparison of Public Databases and a Commercial Testing Laboratory

Comparison of ClinVar and Myriad

Table 2. Case examples and evidence of pathogenicity for variants with discordant laboratory and database classification

	Laboratory	Database
BRCA1 c.427G>A (p.Glu143Lys)		
Classification	Benign	Benign/Likely Benign
Evidence	• Phenotypic evidence based on family history weighting algorithm [22] • *in trans* observation of the variant	• Literature: Lindor et al. [18] • Link to variant in HCI Breast Cancer Genes Prior Probabilities database
Classification	–	VUS
Evidence	–	• Literature: Lindor et al. [18]; Harte et al. [19]; Towler et al. [20]; Wei et al. [21]
Notes	Literature was reviewed by the reference laboratory, but not used for classification. The same literature is cited without additional evidence for the Benign and VUS entries in ClinVar.	
BRCA1 c.65T>C		
Classification	VUS	Pathogenic/Likely Pathogenic
Evidence	• Literature: Lindor et al. [18]; Sweet et al. [24]	• Literature: Lindor et al. [18]; Katagiri et al. [23]; Sweet et al. [24]; Vallee et al. [25]; Whiley et al. [26] • Link to variant in HCI Breast Cancer Genes Prior Probabilities database
Classification	–	VUS
Evidence	–	• None
Notes	Literature is considered insufficient for classification by reference laboratory due to limited clinical information in multifactorial probability analysis.	

Gradishar et al., The Oncologist 2017

Clinical Variant Classification: A Comparison of Public Databases and a Commercial Testing Laboratory

Comparison of ClinVar and Myriad

BRCA1 c.2286A>T (p.Arg762Ser)		
Classification	Likely Benign	VUS
Evidence	• Phenotypic evidence based on family history weighting algorithm [22]	• Literature: Cao et al. [27]; Sadr-Nabavi et al. [28]; Suter et al. [29]; Thirthagiri et al. [30]; Toh et al. [31]; Yu et al. [32]; Zhang et al. [33]; Zhong et al. [34]
Notes	Literature evidence report the observation of this variant without any evidence of pathogenicity.	
BRCA1 c.670 + 1G>T		
Classification	VUS	Pathogenic/Likely Pathogenic
Evidence	• Columbo et al. [36]; Dosil et al. [37]; Miki et al. [38]	• Steffensen et al. [35]
Notes	–	
BRCA1 c.301 + 1G>A		
Classification	Likely Benign	Pathogenic/Likely Pathogenic
Evidence	• Literature: Thomassen et al. [39] • Phenotypic evidence based on family history weighting algorithm [22]	• None
Notes	Direct evidence of pathogenicity was not referenced in the database, but may be based on the location of the variant at a canonical splice site [12].	

Abbreviations: –, not applicable; HCI, huntsman cancer institute; VUS, variant of uncertain significance

Gradishar et al., The Oncologist 2017

Thank you for your attention!!

Challenging Cases – Case 7 – IGV muss zu diesem Fall nicht gezeigt werden