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Multiple Myeloma progression in patient case study

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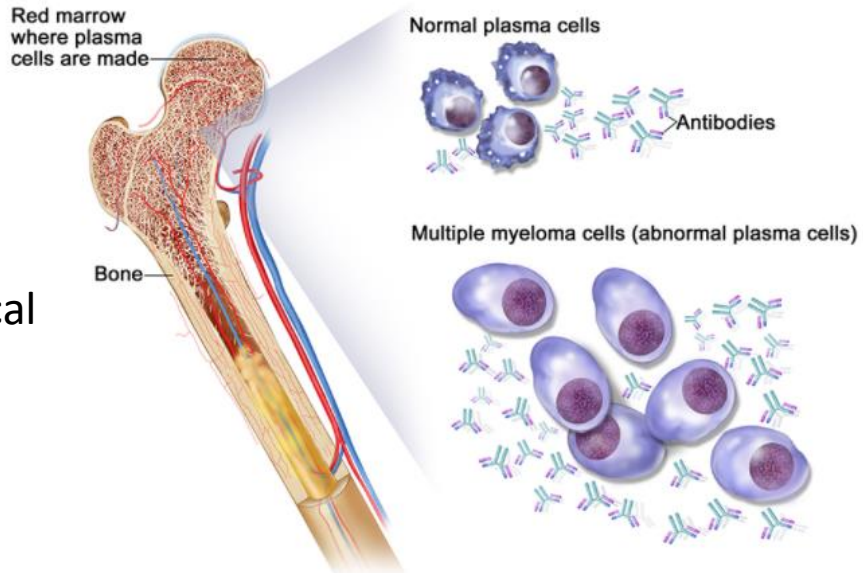
Multiple Myeloma



Cancer of plasma cells

(differentiated B-lymphocytes)

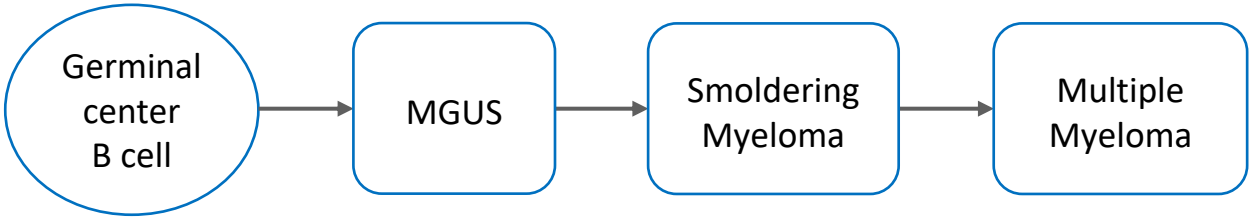
characterized by production of pathological immune globulin (parapotein)

Pre-clinical stages of Multiple Myeloma:

- Monoclonal gammopathy of undetermined significance (MGUS)
- Smoldering multiple myeloma (SMM)

https://www.cdc.gov/cancer/myeloma/index.htm

Genomic Alterations



Primary genomic events

- IGH translocations: *t(4;14), t(11;14), t(14;16), t(14;20), t(6;14)*
- Trisomies: *Odd-numbered chromosomes: 3; 5; 7; 9; 11; 15; 19 or 21*
- *13q loss*

Secondary genomic events

- Translocations affecting *MYC*
- Copy number variations: *gain (1q, 8q, or 11q); del (1p, 17p, 12p, 14q,16q)*
- Somatic mutations: *MAPK pathway (KRAS,NRAS,BRAF); NF-kB pathway (CYLD, TRAF3, NIK); DNA repair pathway (TP53,ATM,ATR)*

Patients are divided into groups:

- Standard
- High-Risk

Castañeda, O.S., & Baz, R. (2019). Multiple Myeloma Genomics - A Concise Review. *Acta medica academica, 48 1*, 57-67 .

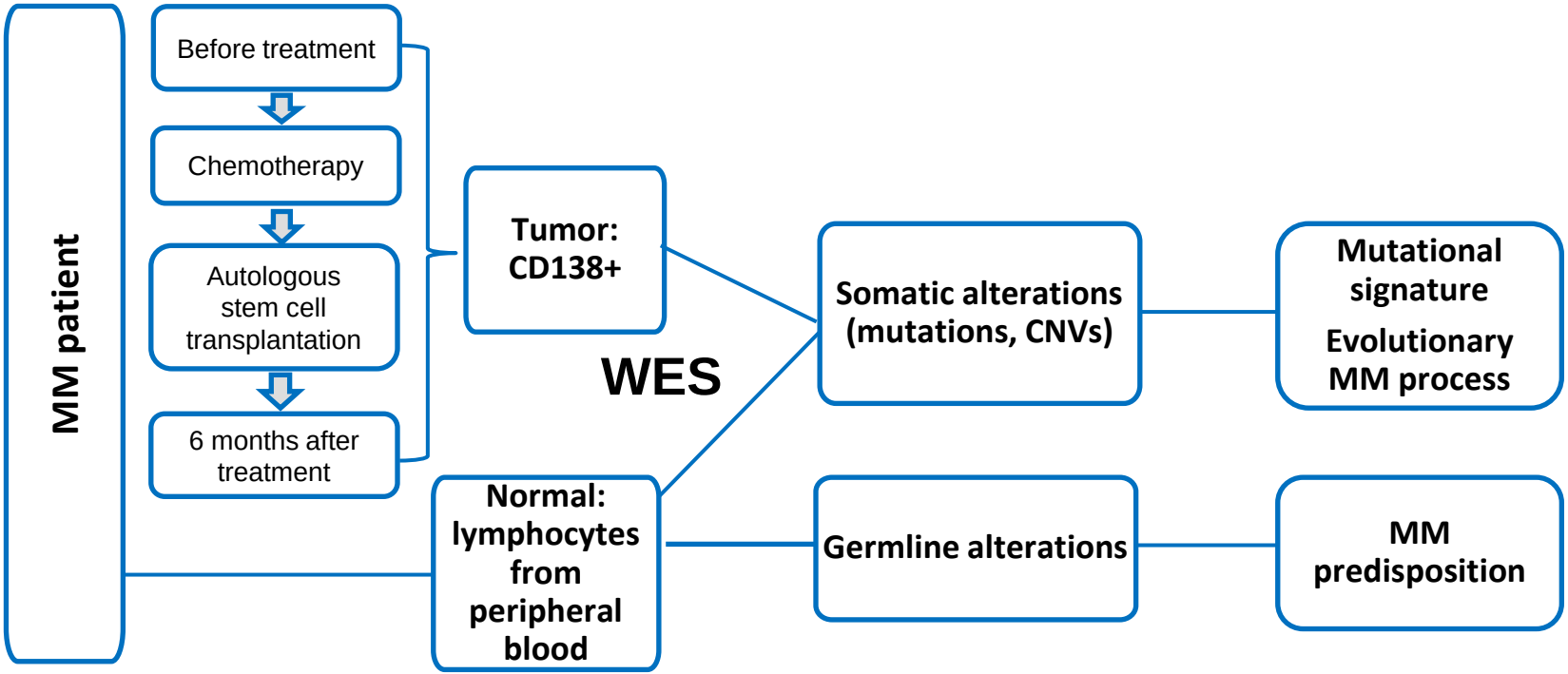
Study goal

Goal: analysis of tumor-normal pair samples for revealing of genetic alterations at different time points of disease

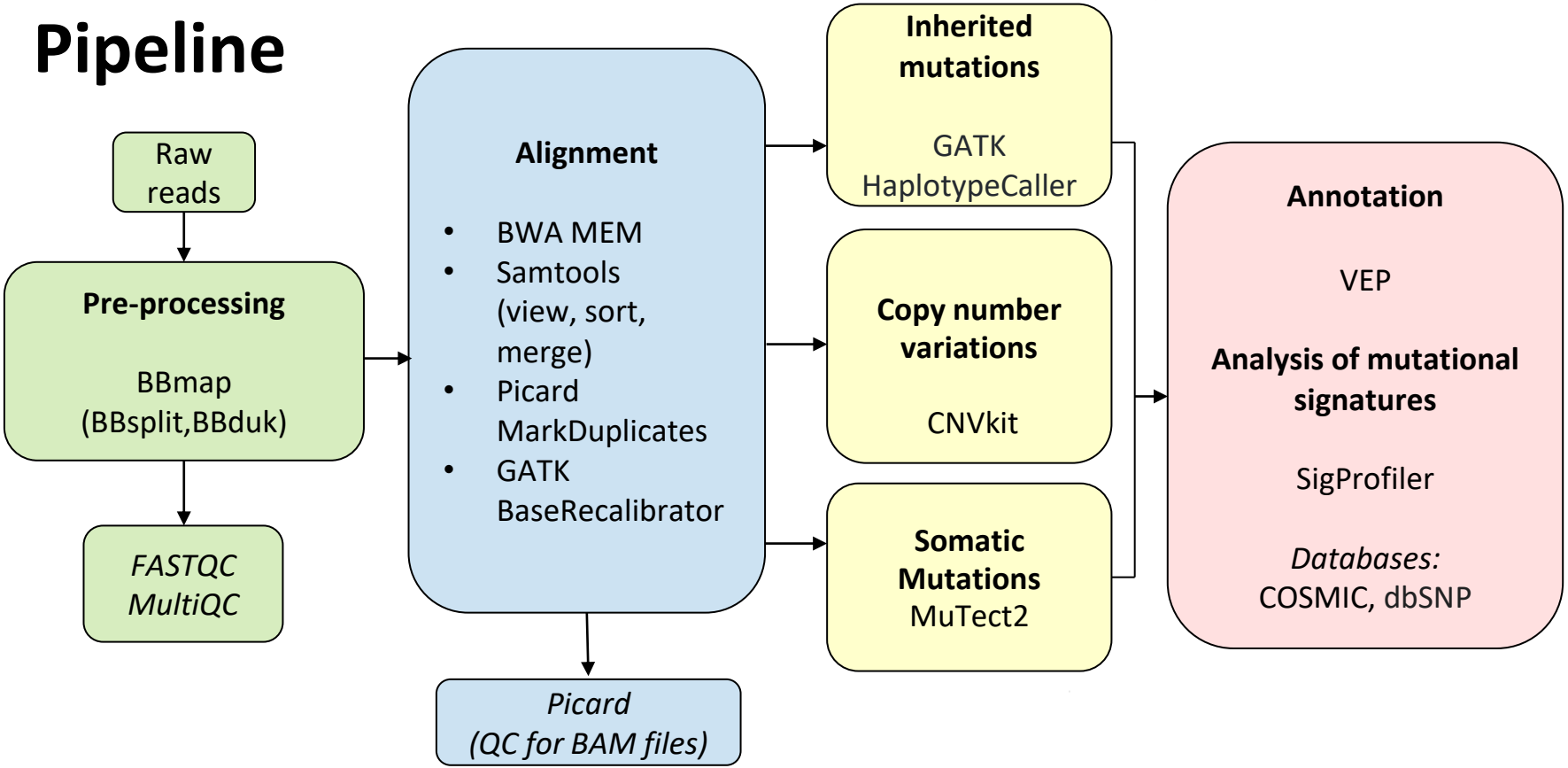
Aims: creation and realization of pipelines for detection and analysis:

- Somatic alterations
- Germline mutations
- Copy number variations

Study design

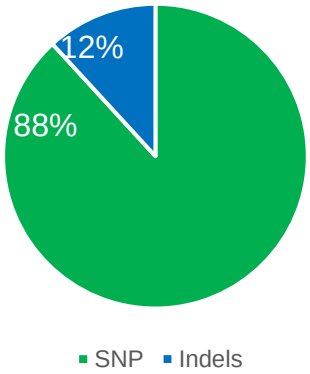


Pipeline


Raw reads
Pre-processing
BBmap (BBsplit,BBduk)
FASTQC
MultiQC
Alignment
BWA MEM
Samtools (view, sort, merge)
Picard MarkDuplicates
GATK BaseRecalibrator
Picard (QC for BAM files)
Inherited mutations
GATK HaplotypeCaller
Copy number variations
CNVkit
Somatic Mutations
MuTect2
Annotation
VEP
Analysis of mutational signatures
SigProfiler
Databases:
COSMIC, dbSNP

Results: Germline genetic variations associated with MM

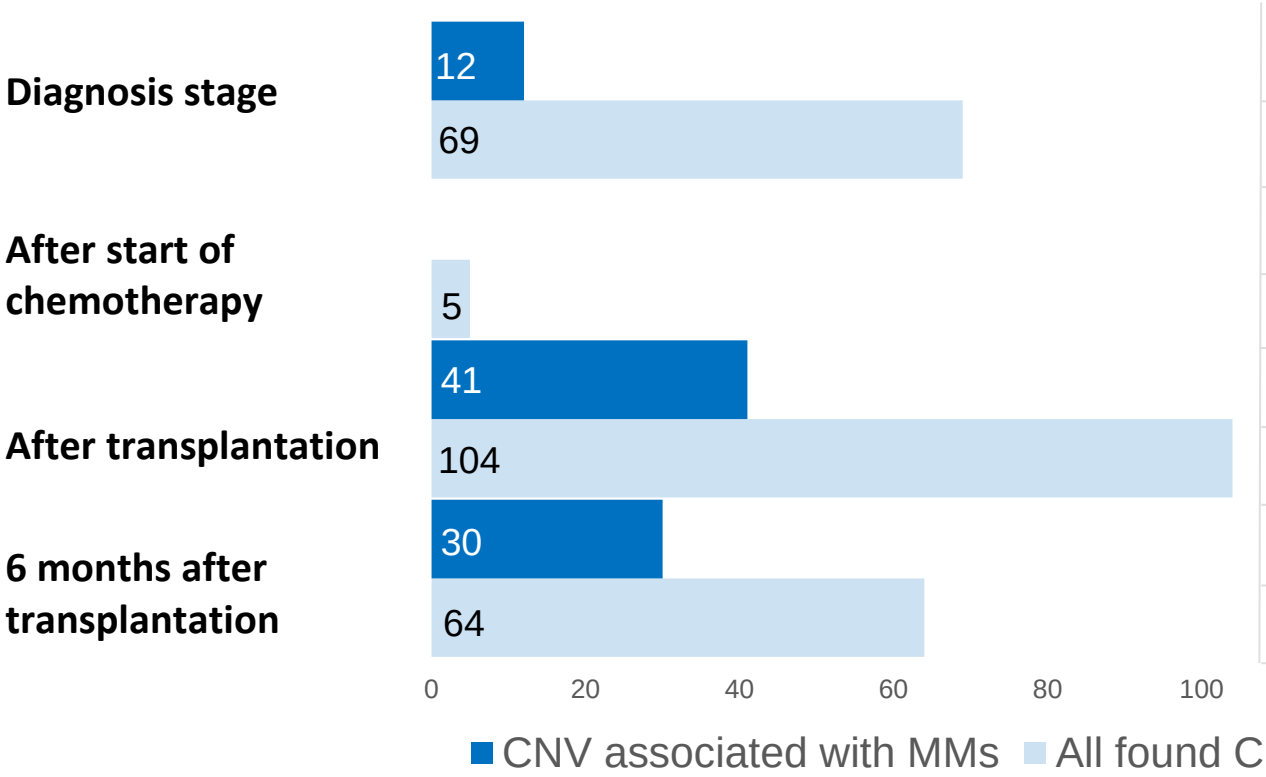
All variants found in normal sample by HaplotypeCaller



Variants associated with MM

Chromosome	Gene symbol	dbSNP ID
2	XRCC5	rs207906
2	XRCC5	rs2440
3	MYNN	rs10936599
3	LRRC34	rs10936600
3	LRRC34	rs6793295
3	LRRC31	rs9290375
5	ELL2	rs3815768
9	NDUFA8	rs3793616
14	XRCC3	rs861531
14	XRCC3	rs1799794
16	RFWD3	rs7193541
19	KLF2	rs11086029

Results: Copy number variations



	Duplications	Deletions
Diagnosis stage	4	8
	13	56
After start of chemotherapy	0	0
	3	2
After transplantation	26	15
	57	47
6 months after transplantation	17	13
	34	30

Results: Copy number variations associated with MM

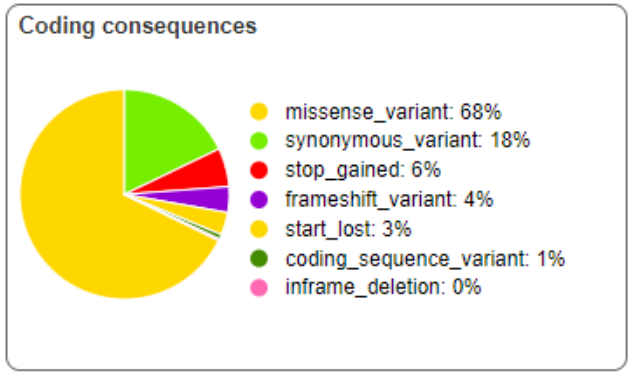
Timepoints		1	2	3	4
Chromosome gains	1q	■		■	■
	6p			■	
	8q			■	■
	11q			■	
Chromosome losses	1p				
	6q				
	8p			■	
	12p				
	13q	■			
	14q	■		■	■
	16q	■			
	17p	■		■	■

1 - Diagnosis stage
2 - After start of chemotherapy
3 - After transplantation
4 - 6 months after start of chemotherapy

1q (MCL1 and ILF2) and **17p** (TP53) – **markers of poor outcome**

Results: Somatic mutations before treatment

Variants processed 282



Most deleterious variants:

Chr	Gene
11	**NFRKB**
19	**CD22**
X	**DDX3X**

Variants associated with Myeloma

Chromosome	Gene symbol	Effect on protein function
1	EPHB2	+
2	CXCR2	+
2	HJURP	-
3	FANCD2	-
5	DNAH5	-
5	XRCC4	-
8	KHDRBS3	+
11	KMT2A	+
13	**DIS3**	+
14	**NFKBIA**	+
15	**TP53BP1**	+
16	CDH13	+
22	XBP1	+
X	HUWE1	+

+ severe effect - mild effect

Results: somatic mutations in samples during treatment

After chemotherapy

Chromosome	Gene symbol	Effect on protein function
8	ZFHX4	+
14	NFKBIA	+
15	TP53BP1	+

After autological stem cell transplantation

Chromosome	Gene symbol	Effect on protein function
3	ATP11B	+

6 months after stem cell transplantation

Chromosome	Gene symbol	Effect on protein function
3	FANCD2	-
15	TP53BP1	+
17	BRIP1	-

SNVs detected at few stages over period of treatment:

- TP53BP1 - encodes TP53-binding protein 1, related to tumor suppression
- NFKBIA - participates NF-κB signaling activation, one of key pathways in MM genesis
- FANCD2 – related to defective DNA-repair

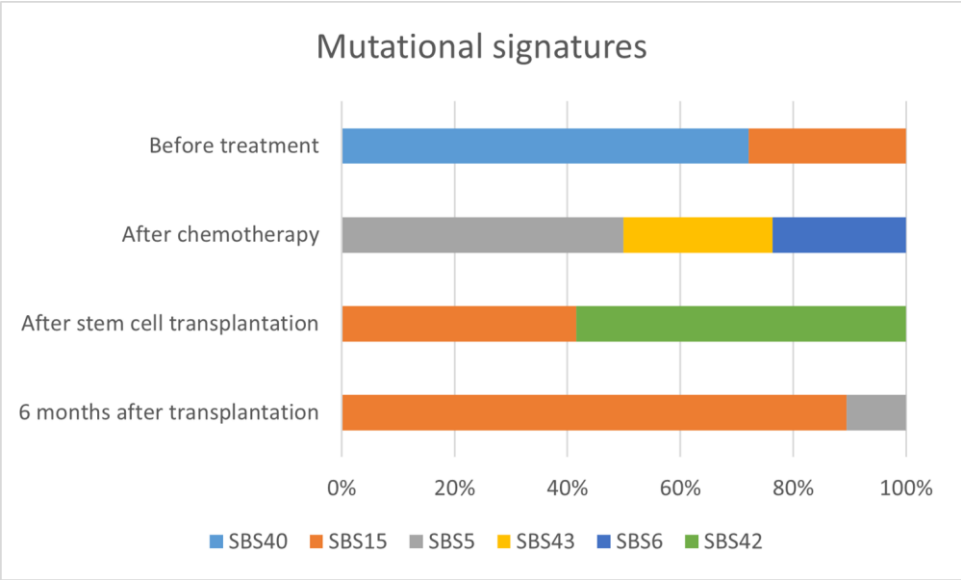
Mutational signatures

Signatures related to defective DNA damage repair

- SBS15
- SBS6

Age-linked signatures

- SBS5
- SBS40



Conclusion

- Number of alterations involving DNA repair processes
- Co-occurrence of gain(1q) and del(17p) – high-risk double-hit myeloma, associated with significantly poorer prognosis
- Therapy strategy can be corrected based on revealed genomic alterations

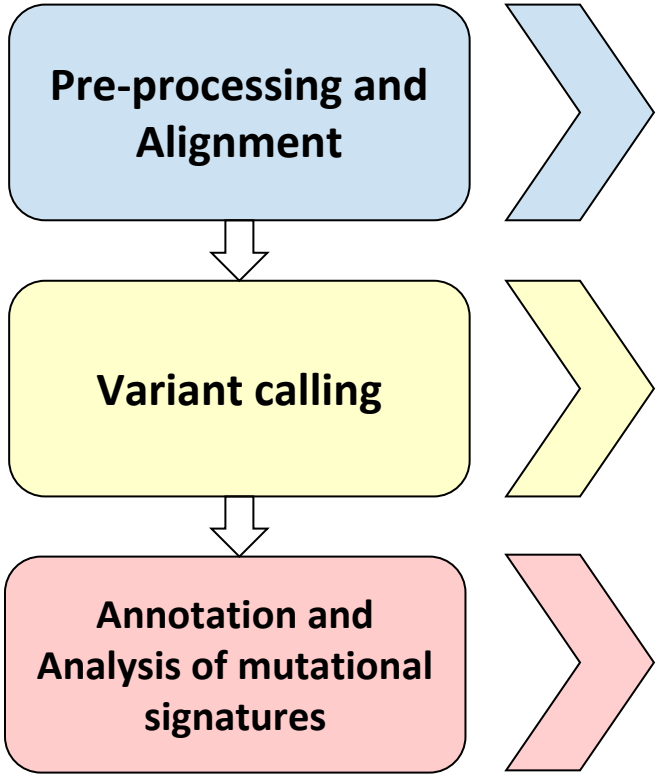
Table 1 Molecular cytogenetic classification and risk stratification of multiple myeloma (MM).

Cytogenetic abnormality	Gene/chromosome (s) affected	Risk stratification[a]
Secondary cytogenetic abnormality		
Gain (1q)	1q	High risk
Del (17p)	p53	High risk
p53 mutation	p53	High risk
Other		Variable

[a]Presence of any two high-risk cytogenetic abnormalities is considered double-hit MM. Presence of any three or more high-risk cytogenetic abnormalities is considered triple-hit MM.

Hassan H, Szalat R. Genetic Predictors of Mortality in Patients with Multiple Myeloma. Appl Clin Genet. 2021 Apr 29;14:241-254.
Rajkumar, S.V., Kumar, S. Multiple myeloma current treatment algorithms. Blood Cancer J. 10, 94 (2020).

Personal contribution



Split samples:

Svetlana – normal, after therapy
Yulia - diagnosis, transplantation, 6 months after transplantation

Split callers:

Svetlana – Copy number variations (CNVkit); Germline variations (HaplotypeCaller)
Yulia - Copy number variations (CNVkit), Somatic mutations variations (Mutect2)

Annotation

Svetlana – Germline Mutations; Copy number variations
Yulia - **Somatic Mutations**

Mutational signature analysis

Svetlana – transplantation, 6 months after transplantation
Yulia - diagnosis sample; after therapy sample

Thank you!

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