Genetisk diagnostik
Riskgruppering-prognosfaktorer

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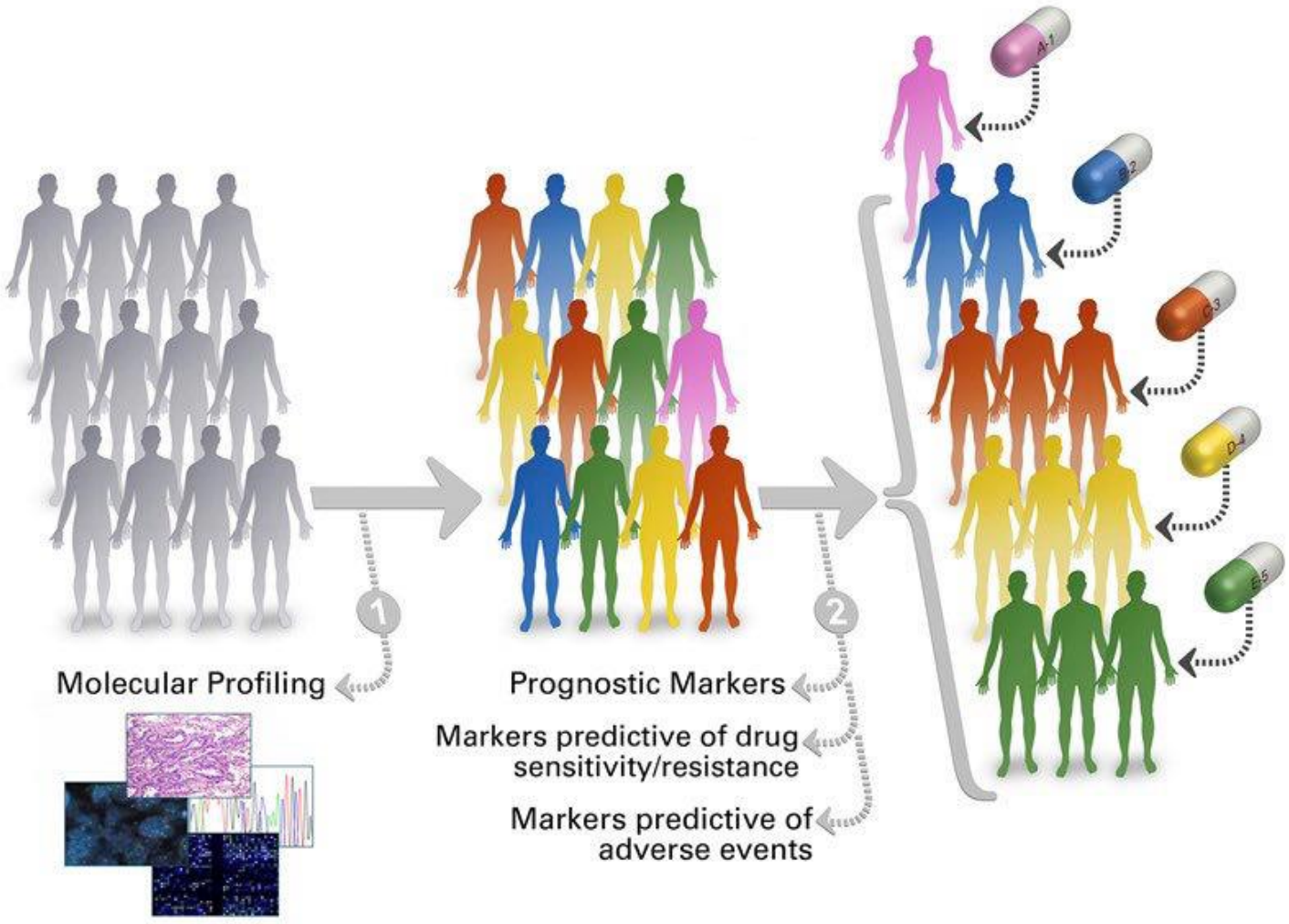
SciLifeLab

AKADEMISKA SJUKHUSET

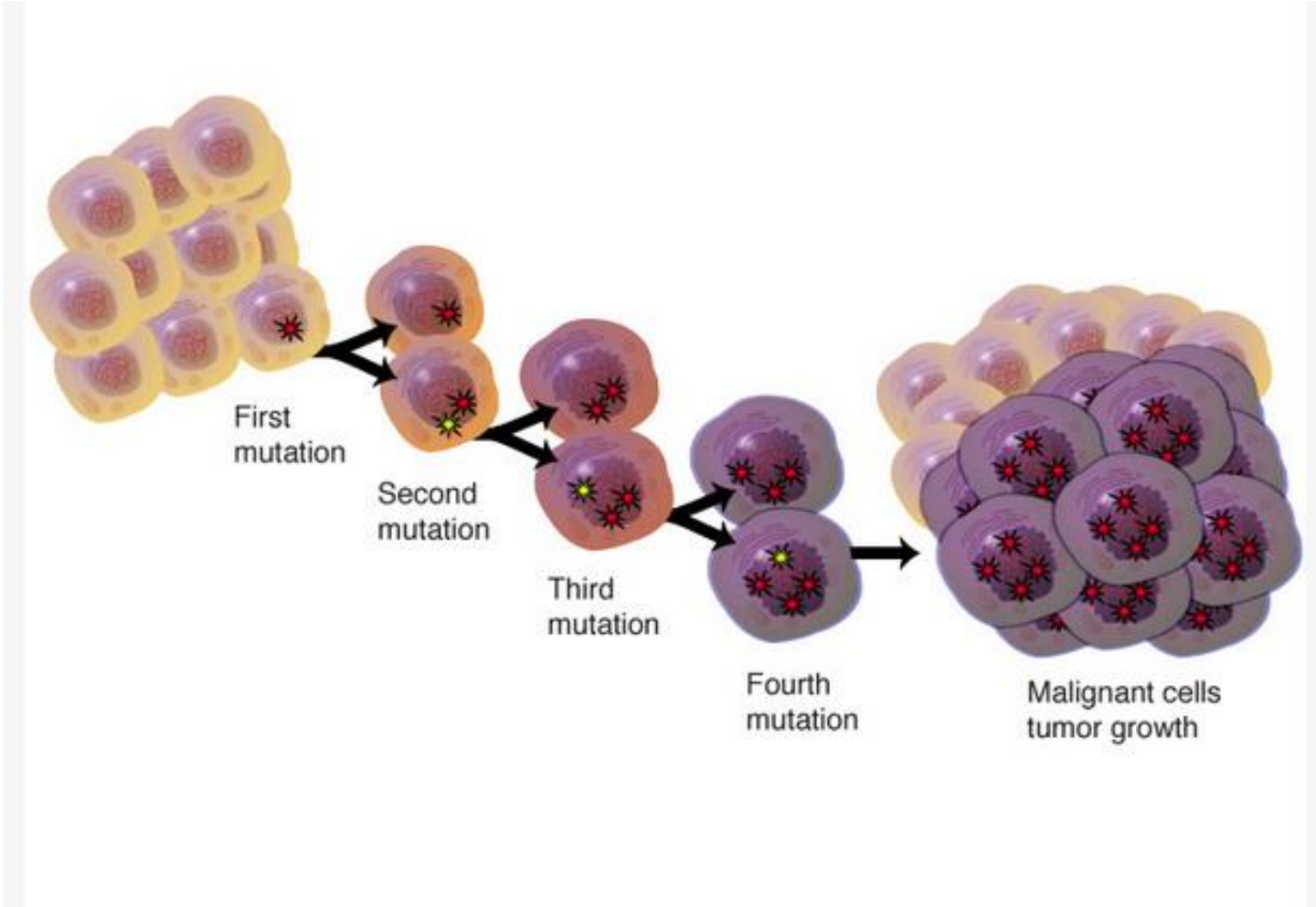
The concept of ”Precision Medicine”

matching patient profiles with treatments



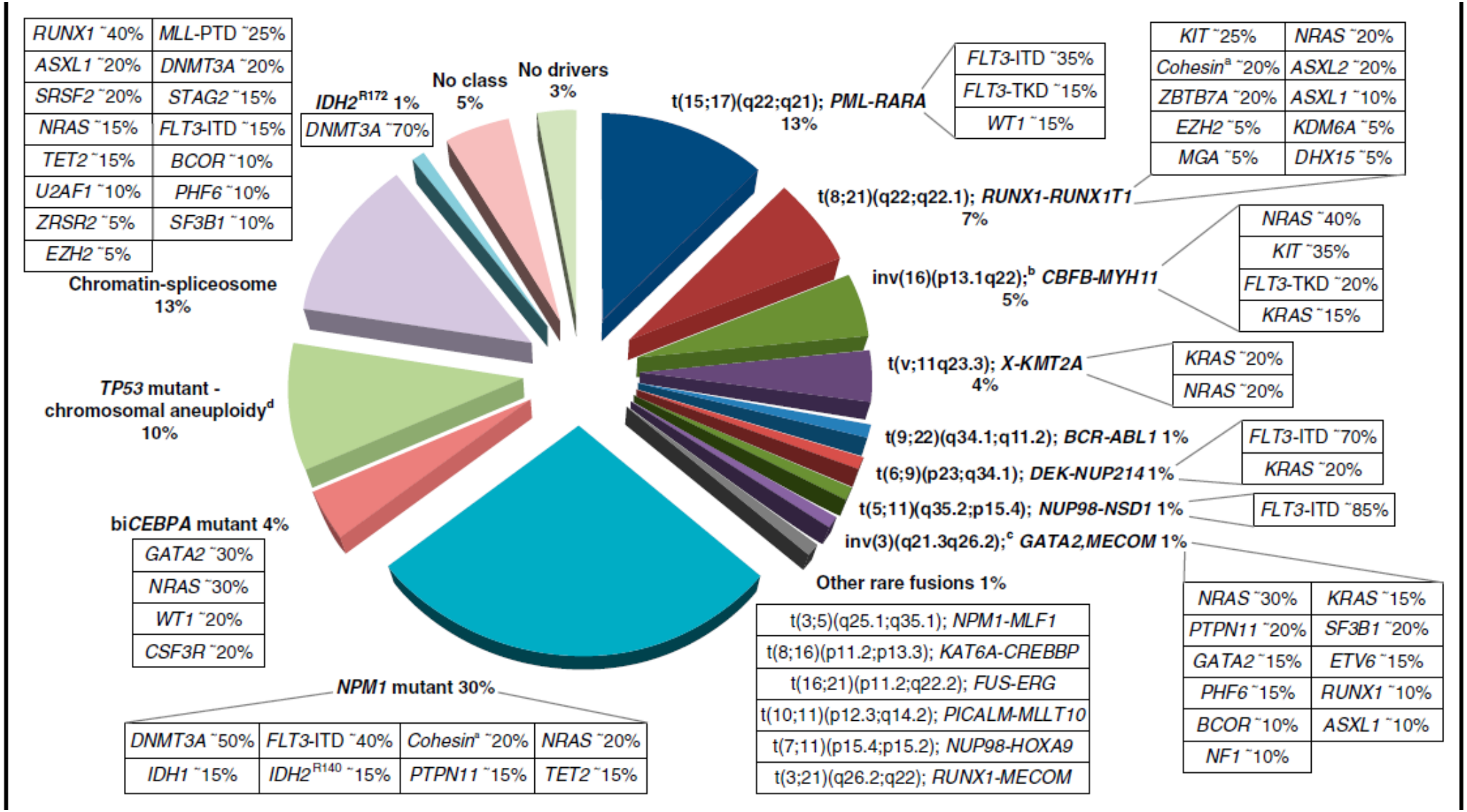
How to identify individuals/groups with distinct disease profiles?

Genetics may be the key



Senapathy K. Genetic Literacy Project

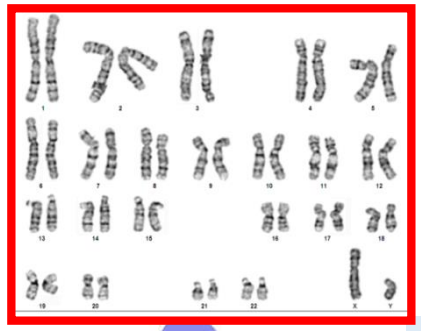
AML: a disease with a complex genetic background



Layout

- Methods
- Classification (WHO)
- Risk-stratification (ELN)
- Other prognostic parameters
- Cases

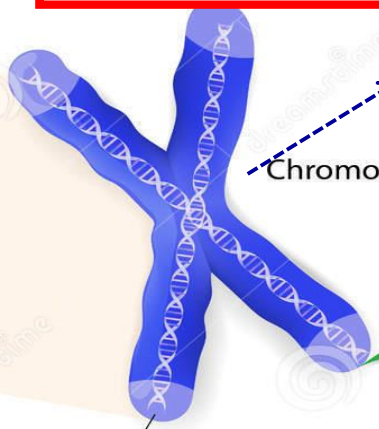
Differents methodologies: one goal



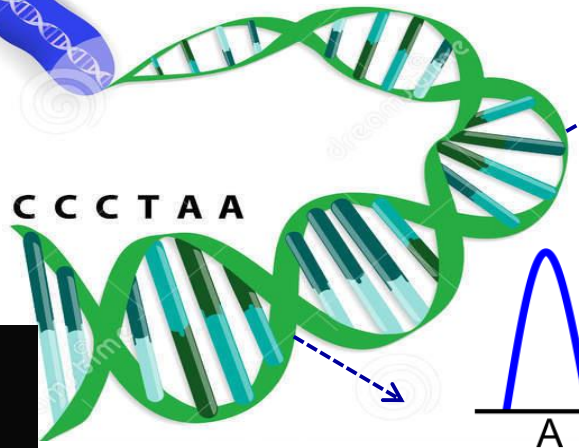
Heparin-rör

CBA

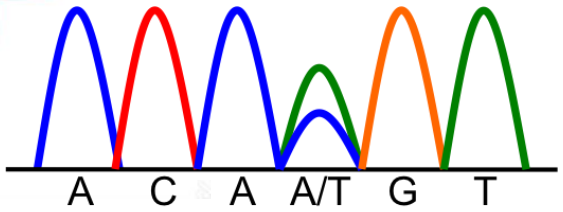
EDTA-rör



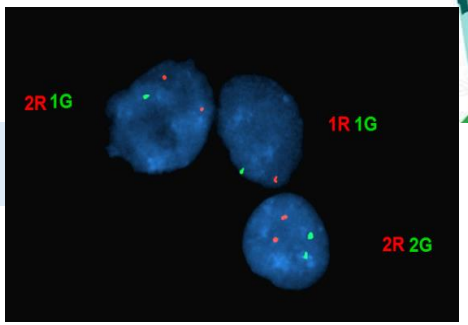
ARRAYS



Fragment MLPA



FISH



NGS

Sanger

Cytogenetics-Chromosome banding analysis (CBA)

practical information

Source*[1]	Preparation*[2]	TAT *[3]	Target*[4]	Aberrations*[5]
Heparine BM (PB?)	1-3 days culture	10 days	Whole genome	Structural and numerical Chromosomal aberrations

*[1]: BM is the standard source

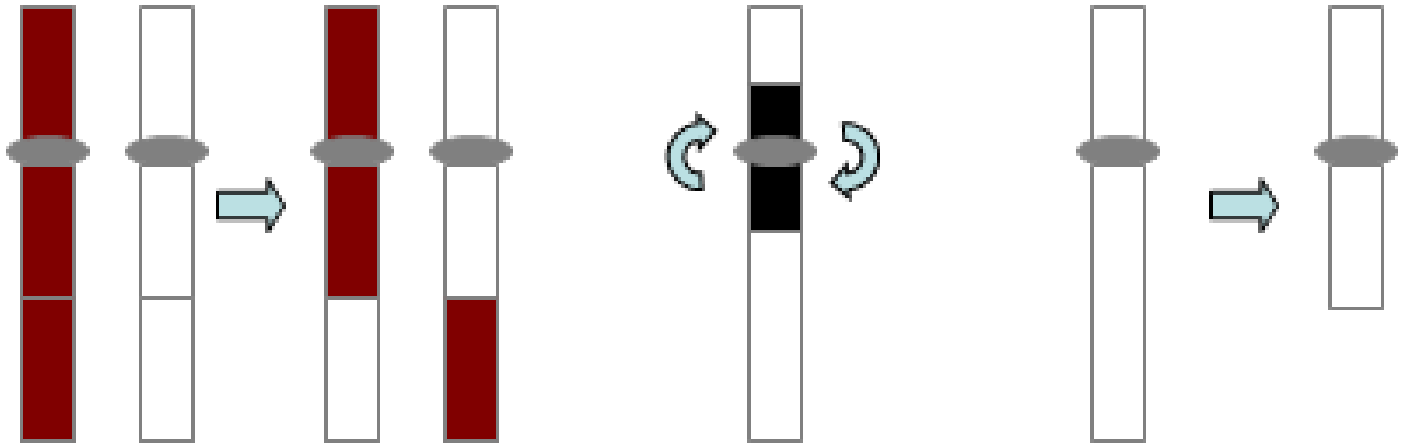
*[2]: The optimal protocoll includes 1-2 days of culture. Obs! If the sample arrives on a Friday, we proceed with direct-prep plus 3 days culture which decreases the possibility of detecting clonal aberrations

*[3]: We prioritize depending on the age of the pts

*[4]: The only WG method that is validated and reproducible in the clinical setting

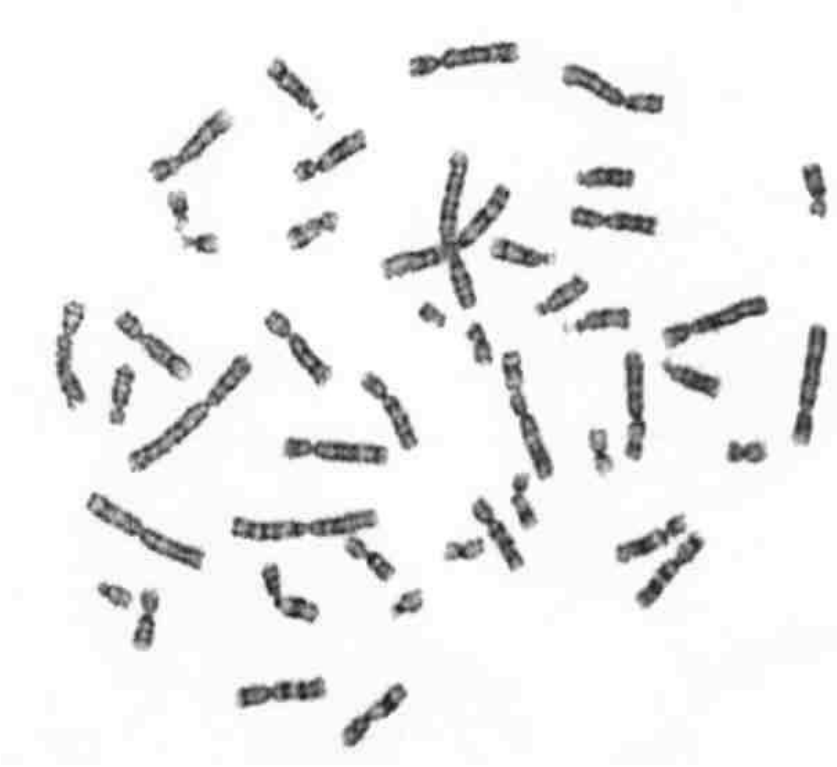
*[5]: hypodiploidy, hyperdyploidy, translocations, deletions, inversions etc. Aberrations with at least 5-10MB length

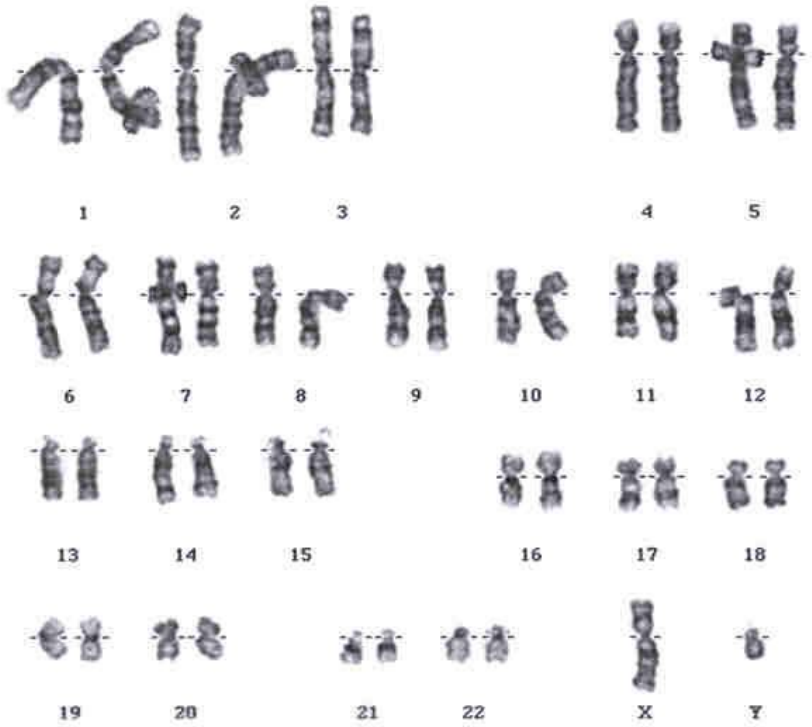
Structural aberrations



Translocation Inversion Deletion

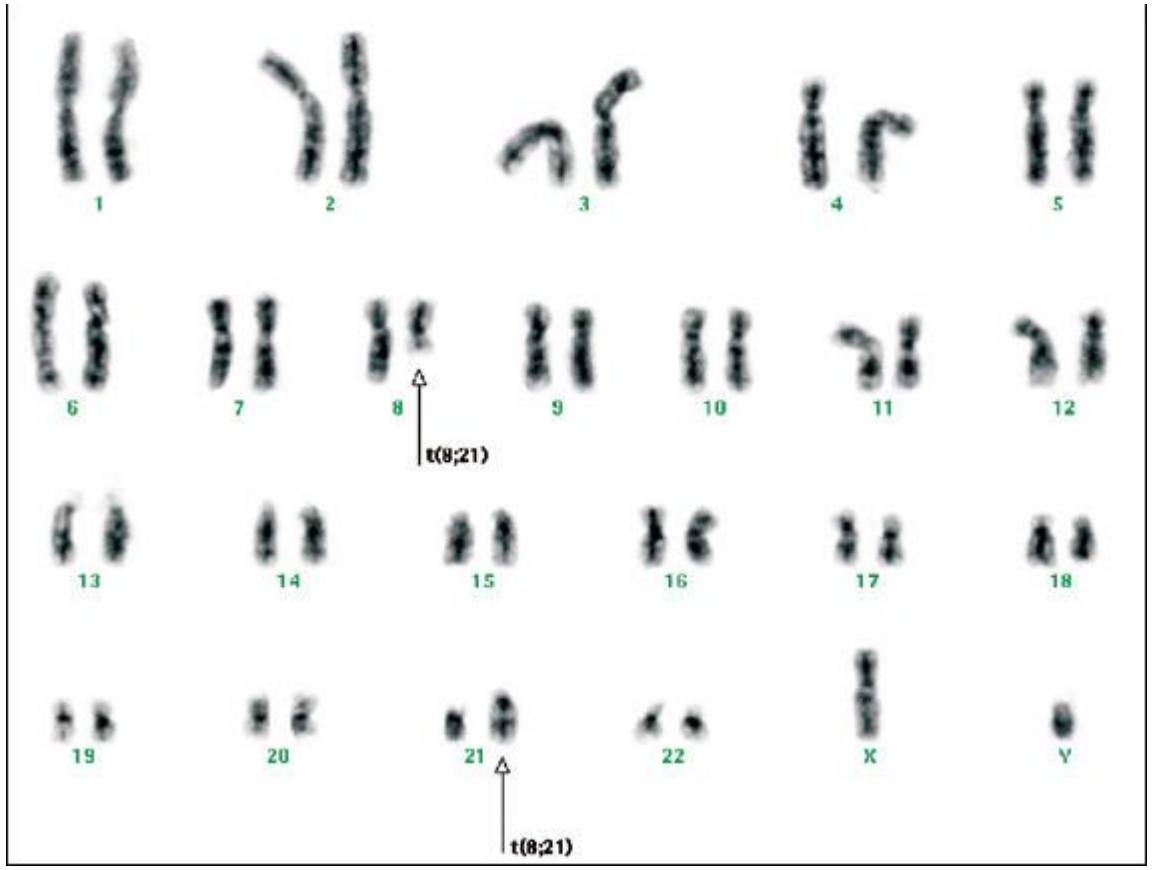
G-banding





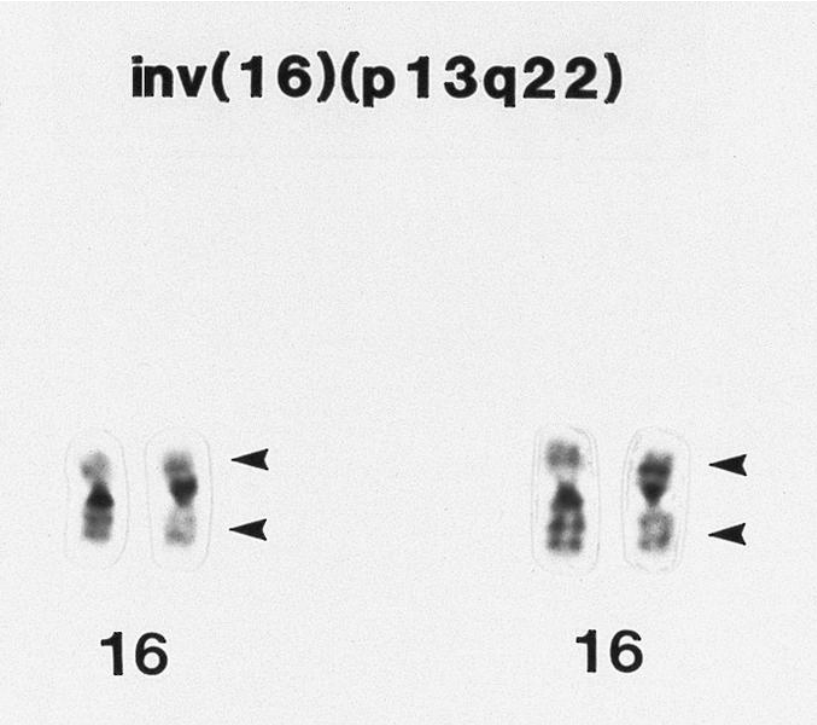
Metaphase chromosomes

Translocation

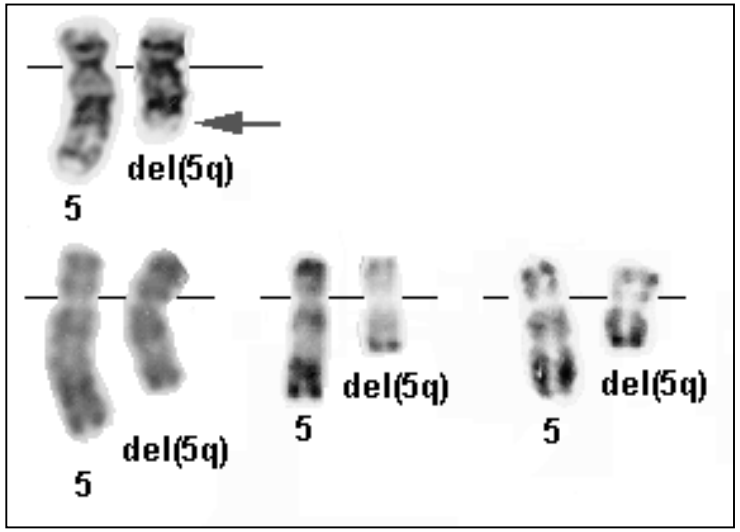


46,XY,t(8;21)(q22;q22)[19]/46,XY[1]

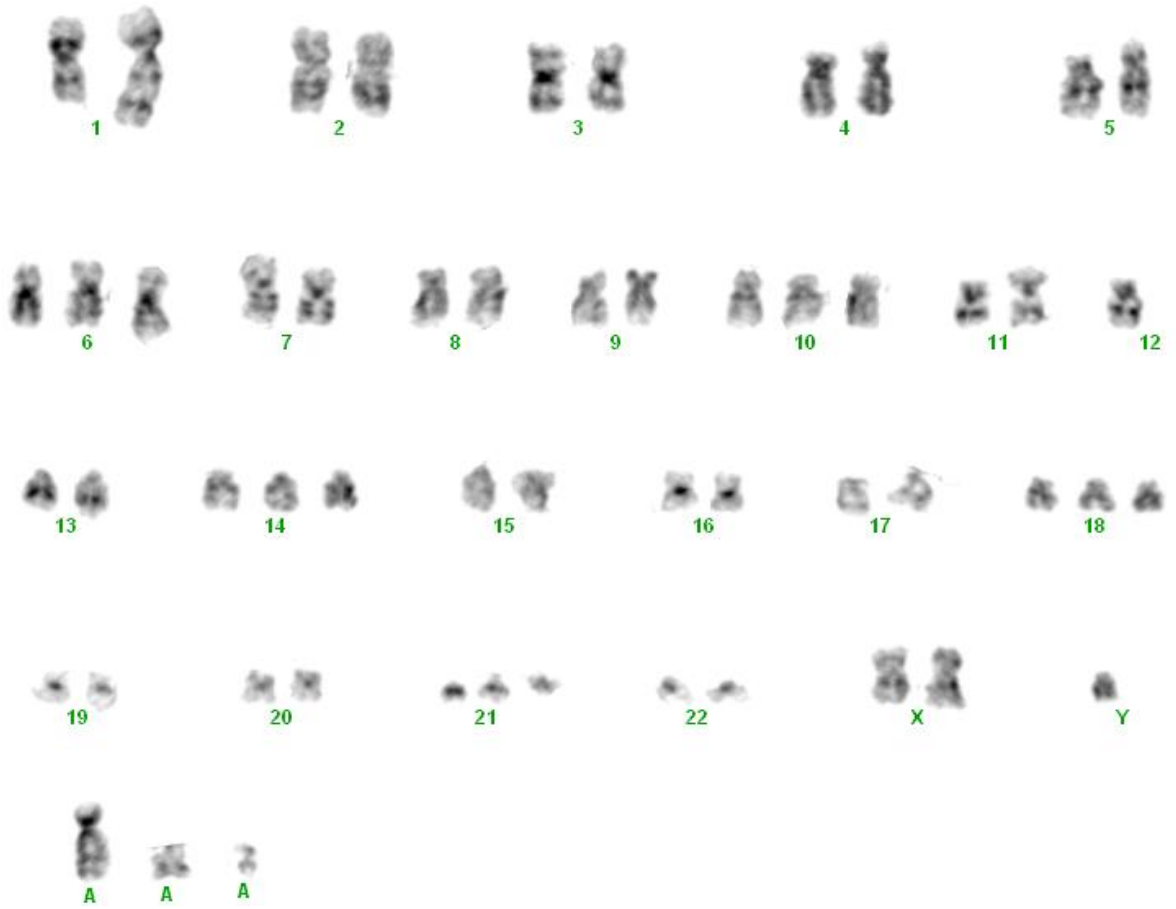
Inversion



Deletion



Myeloid malignancies
numerical and structural aberrations



54-55,XY,dup(1)(q21q32),+6,+10,+14,+18,+21,+3-7mar,inc[cp6]/46,XY[18]

Cytogenetics-Fluorescense In Situ Hybridization (FISH)

practical information

Source*[1]	Preparation*[2]	TAT *[3]	Target*[4]	Aberrations*[5]
Heparine EDTA? BM (PB?)	4-8 hours	2-3 days	Specific parts of the genome (targeted analysis)	Structural and numerical chromosomal aberrations

*[1]: BM is the standard source. EDTA can be used if the sample is fresh

*[2]: 4 hours for specific protocolls. For metaphase-FISH culturing according to CBA-protocol is needed.

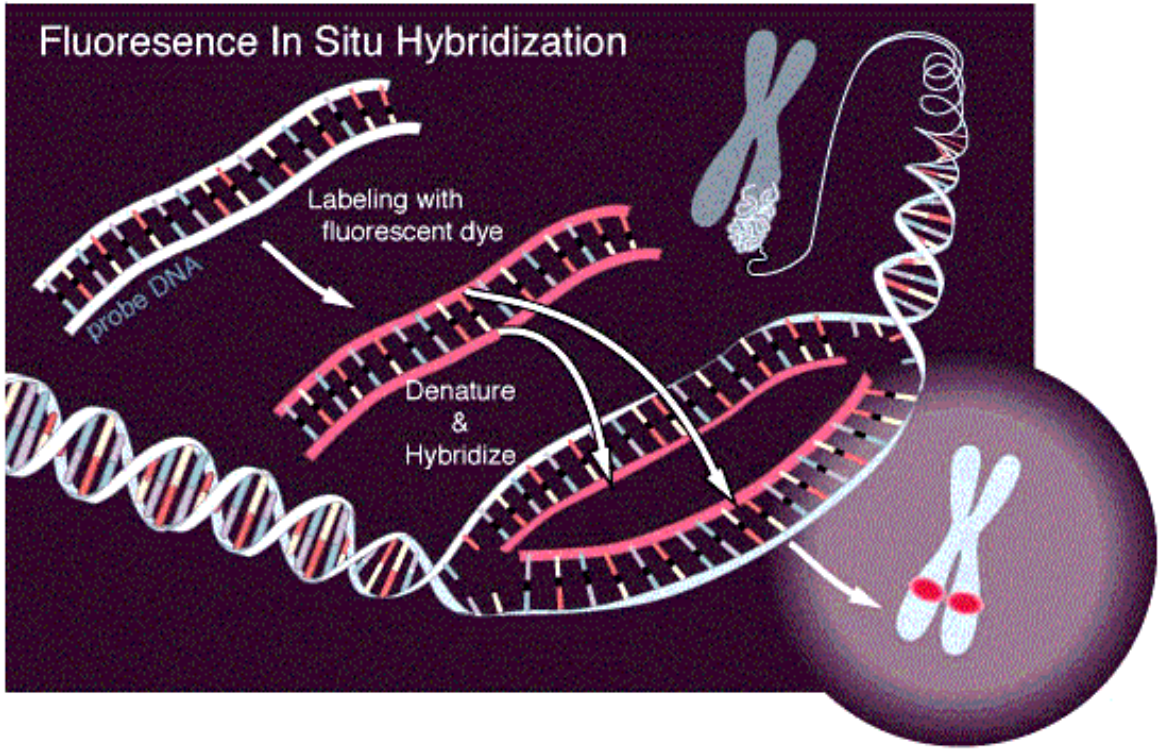
*[3]: we prioritize depending on the age of the pts

*[4]: no information on the remaining genome

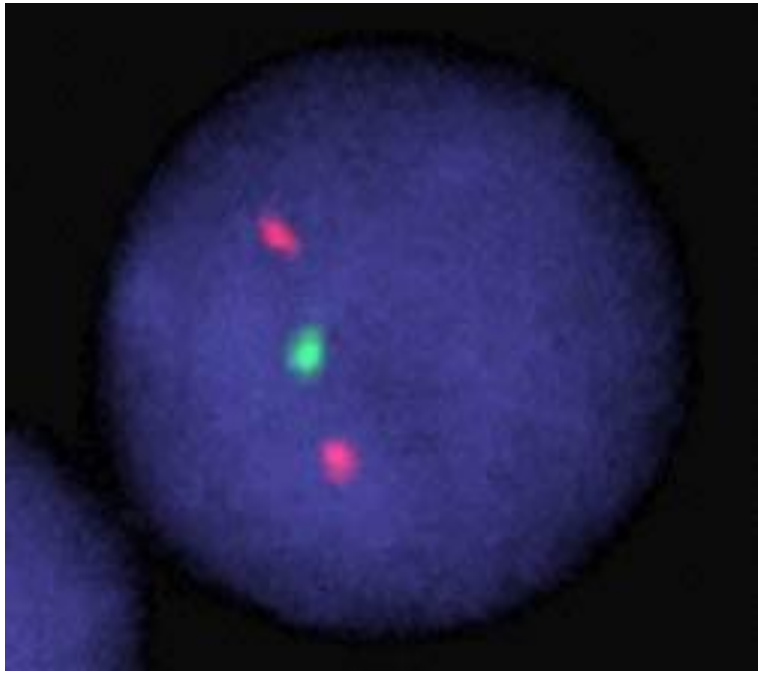
*[5]: hypodiploidy, hyperdyploidy, translocations, deletions, inversions etc. Aberrations with size down to kb level

Cytogenetics-Fluorescense In Situ Hybridization (FISH)

practical information



del17p



NGS

practical information

Source*1	Preparation*2	TAT *3	Target*4	Aberrations*5
EDTA BM (PB?)	3-4 days	1-2 weeks	Specific parts of the genome (targeted analysis)	SNVs, small CNVs

*1: BM is the standard source. Blood can also be used

*2: TWIST-panel

*3: we prioritize acute leukemias over MDS

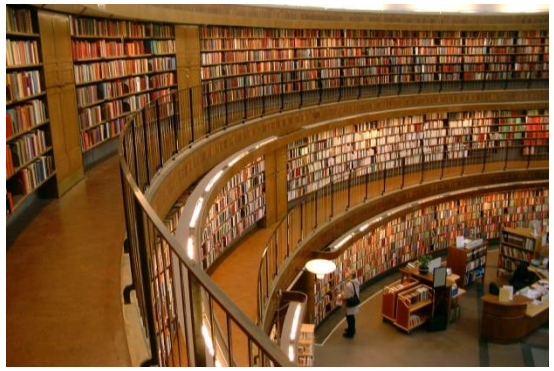
*4: no information on the remaining genome

*5: following the standrad pipeline

How much of the genome can we read?

From Sanger to Next Generation Sequencing (NGS)

Library



Whole genome

3 000 000 000 bp
30X coverage

Book

Gene

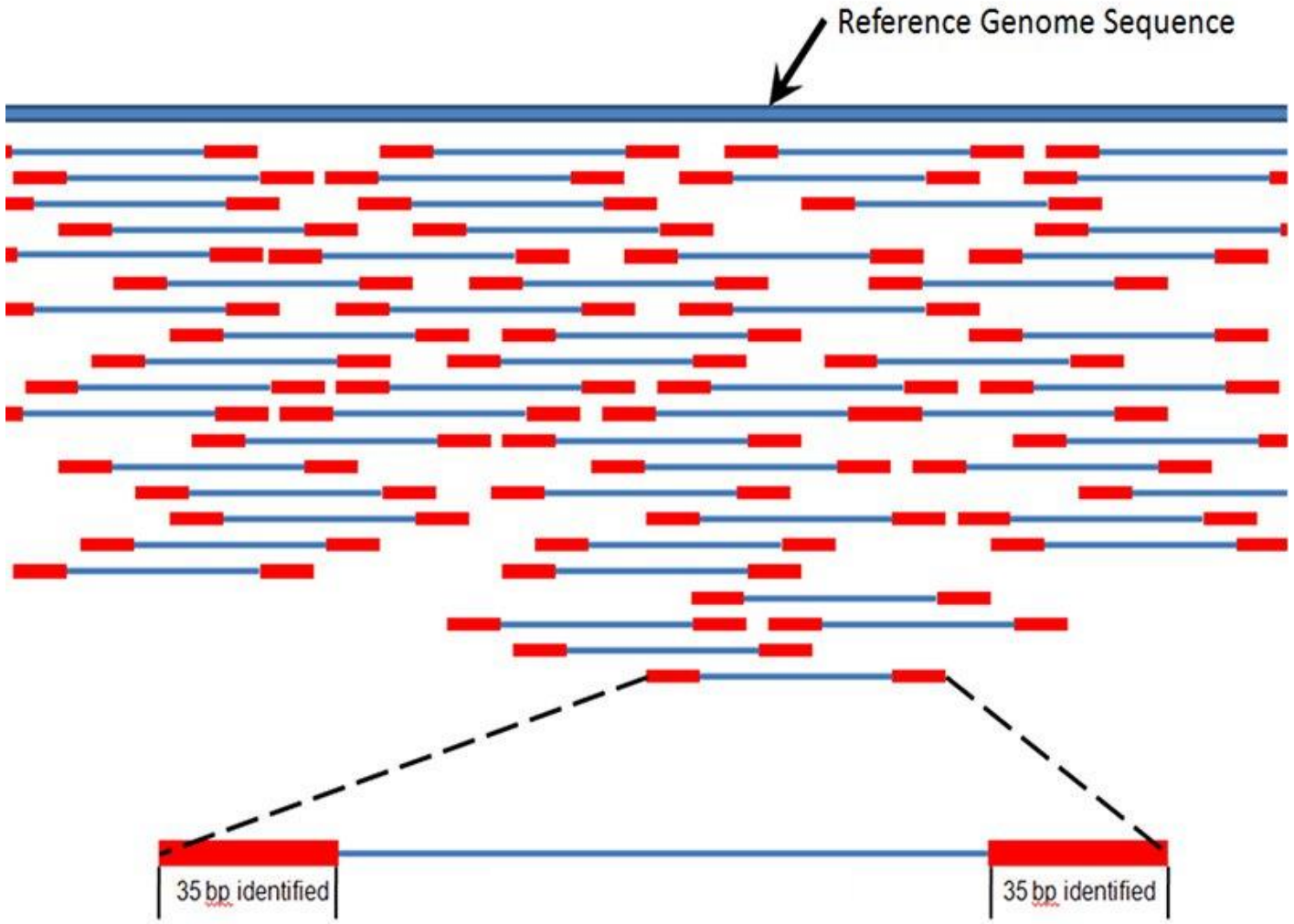
20 000 genes
100X coverage

Chapter

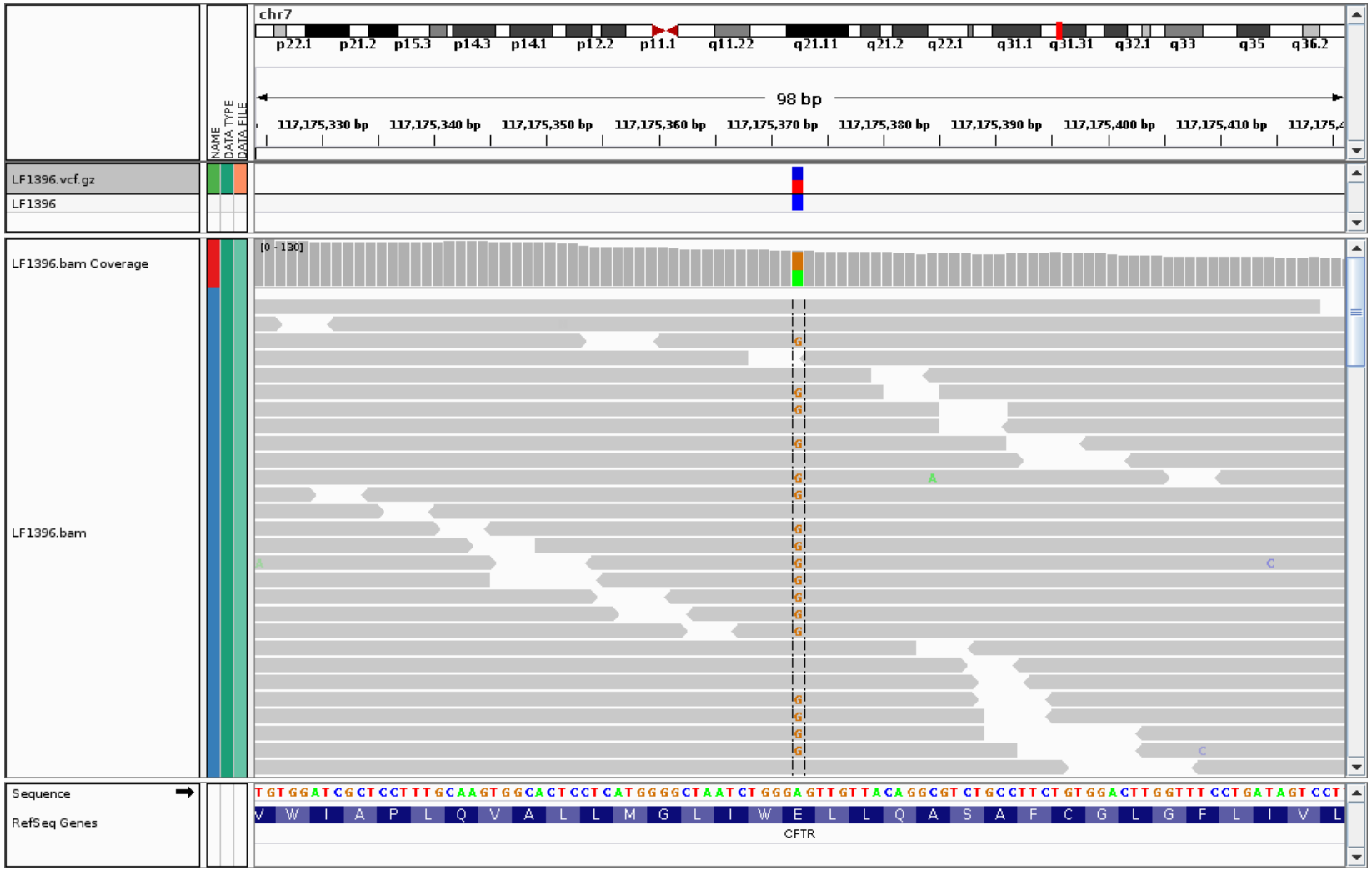
Exon

<100 genes
>1000X coverage

NGS basic principles

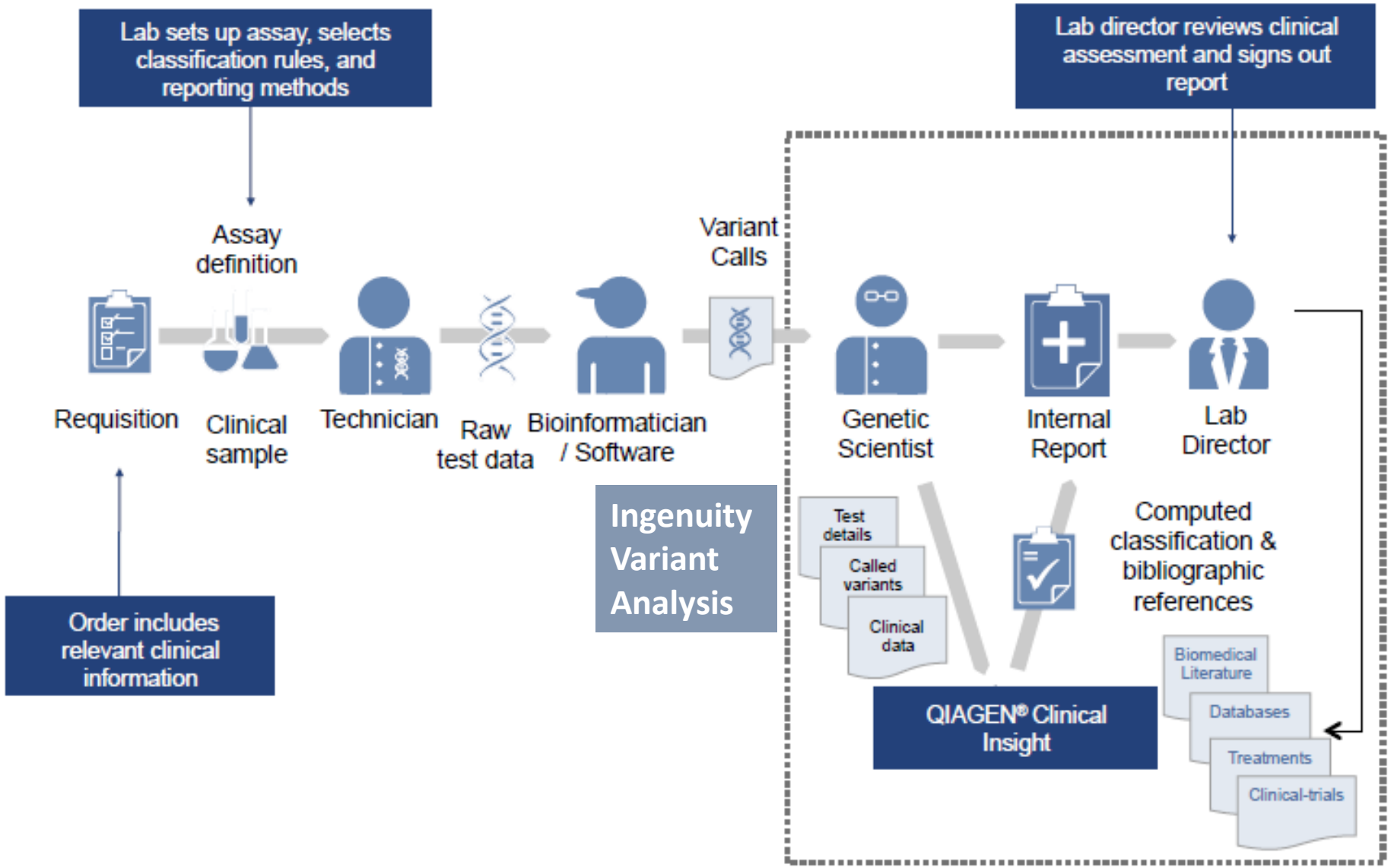


NGS – coverage and VAF*



*: variant allele frequency

Scalability of annotation, filtration, classification, and reporting



NGS report

Indication
Result
Variant description
Conclusion

Methodoly-limitations:

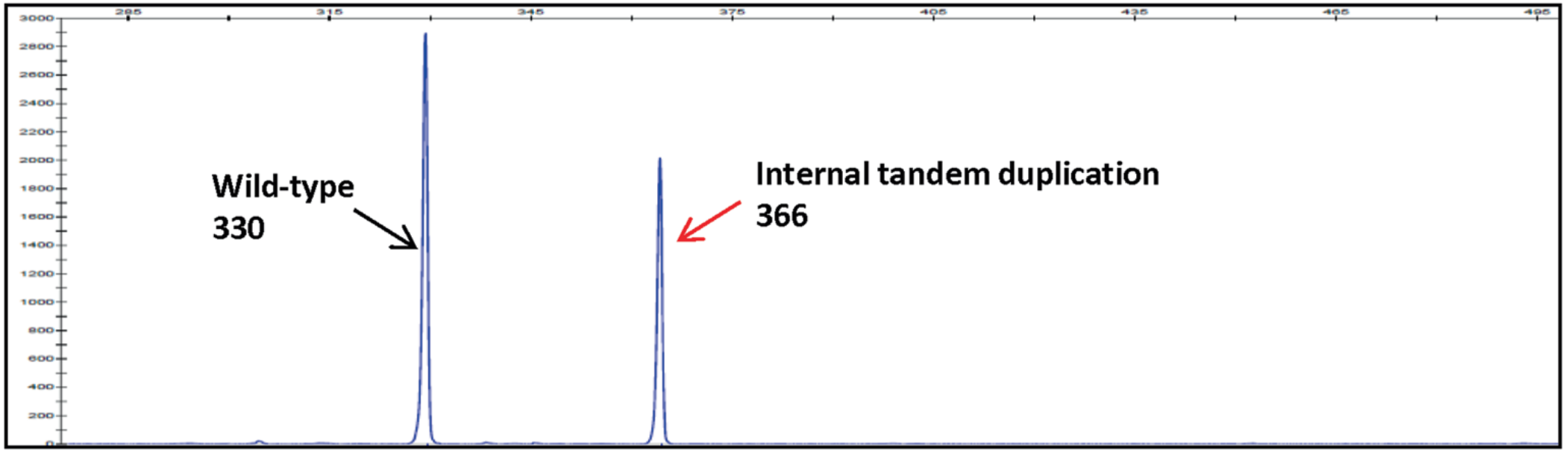
200 reads per position (hotspots)
Variant allele frequency (VAF) >10% (**even 5-10% is reported**)
Deletion/duplications cannot be excluded

It is not a germline analysis

NGS in AML- Open issues

- Variants of unknown clinical significance
- Clonal hematopoiesis
- VAF-threshold for reporting in the clinical setting (background-noise)
- Incidental finding
  - Patient
  - donor

FLT3: when the modern methods are not enough



R he etal. Modern pathology 2020

Genomic characterization of AML in the clinical setting

- Vid diagnos bör man göra både cytogenetisk analys (kromosomanalys) och NGS-baserad myeloisk genpanelanalys på alla patienter, förutom vid planerad palliativ behandling.

Utredningen vid misstänkt eller bekräftad AML syftar även till att kategorisera sjukdomen genetiskt, vilket har stor betydelse för prognosen och den fortsatta behandlingen. Det kompletta provtagnings- och utredningsprogrammet nedan bör följas när det gäller patienter för vilka vårdplanen innebär behandling syftande till långvarig remission med eller utan överväganden om allo-hSCT. Kromosomanalys och molekylärgenetiska analyser ger ofta värdefull information vid behandlingsbeslut även hos de äldre patienter där man tvekar mellan remissionssyftande och mer palliativt inriktad behandling [26].

National AML guidelines

AML: classification

Traditional

FAB type	Cytology	Incidence
M0	Undifferentiated myeloblastic	Uncommon
M1	Acute myeloblastic leukemia (without differentiation)	50% of all cases
M2	Acute myeloblastic leukemia (with differentiation)	
M3	Acute promyelocytic leukemia	Uncommon
M4	Acute myelomonoblastic leukemia	30% of all cases
M5	Acute monocytic leukemia	Uncommon
M6	Acute erythroblastic leukemia	Uncommon
M7	Acute megakaryocytic leukemia	Uncommon

Updated WHO classification 2016

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

Updated AML WHO classification

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Provisional entity: AML with mutated RUNX1

Arber D et al. Blood 2016

AML with mutated *NPM1*

- 30-35% of all AML
- Multilineage dyspasia is common but it is not associated with unfavorable prognosis
- Usually associated with normal karyotype (80-85%)
- In case of high-risk cytogenetics the prognosis is beased on the cytogenetic profile

AML with BCR-ABL

- <1% of all AML
- De novo AML- not CML in blast crisis
- Mainly major BCR-ABL transcript
- Extra chromosomal aberrations (monosomy 7, complex karyotype) are common
- Loss of IKZF1 and CDKN2A-deletions of IGH and TRG genes
- Dismal clinical outcome

AML with mutated *RUNX1*

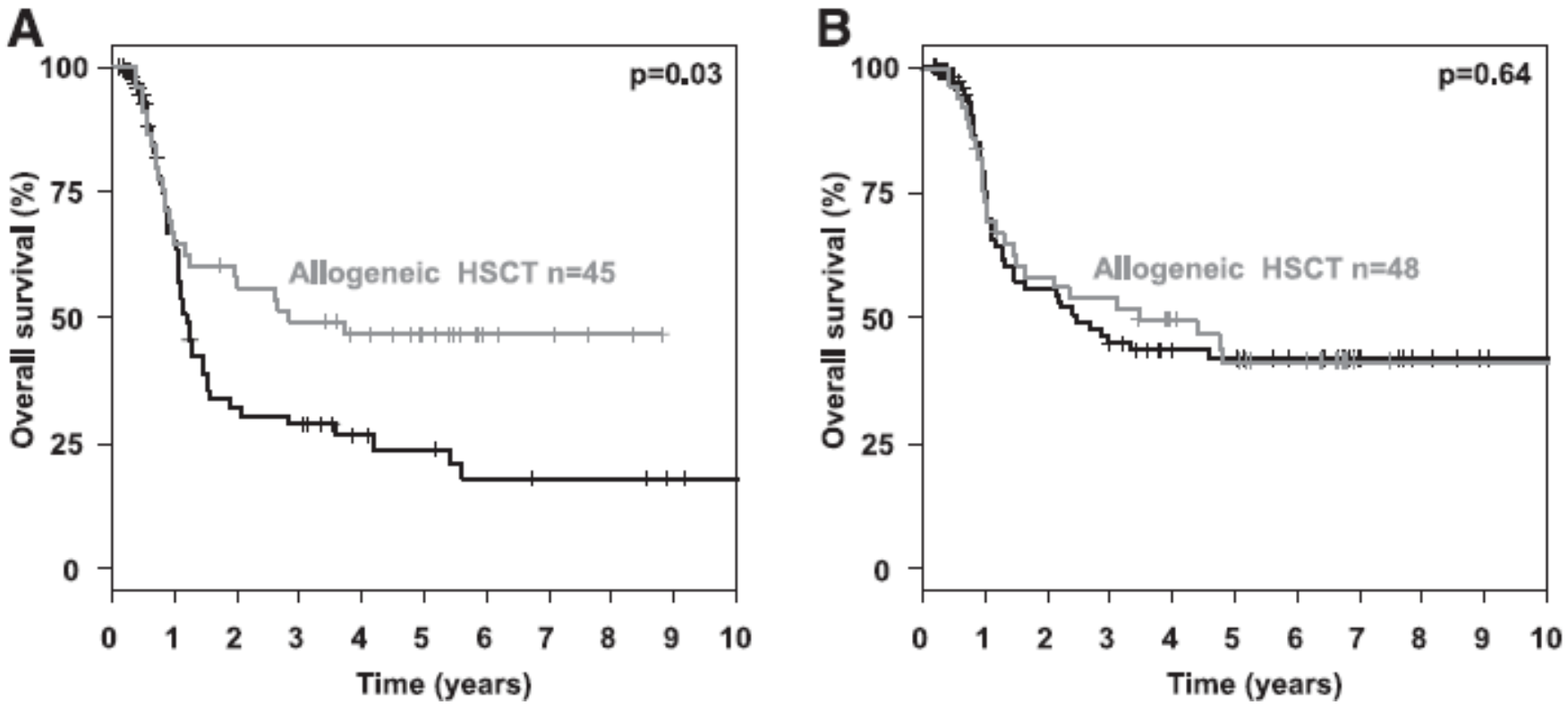
- 5-15%
- De novo- absence of myelodysplasia
- Absence of other recurrent genetic abnormalities
- May be indicative of a germline background
- Unfavorable clinical outcome

AML risk-stratification according to European Leukemia Net (ELN)

Risk Category[b]	Genetic Abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD$^{high(c)}$ Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD$^{low(c)}$ (w/o adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[d] Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,[e] monosomal karyotype[f] Wild type *NPM1* and *FLT3*-ITD$^{high(c)}$ Mutated *RUNX1*[g] Mutated *ASXL1*[g] Mutated *TP53*[h]

Döhner H et al. Blood 2017

FLT3^{high} vs FLT3^{low}



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	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*#

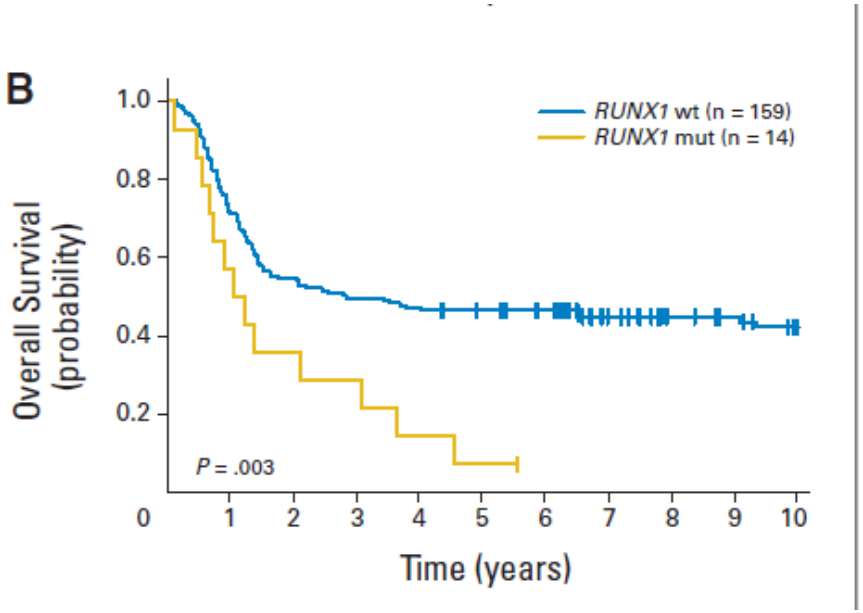
¶These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

#*TP53* mutations are significantly associated with AML with complex and monosomal karyotype.[37,66-69]

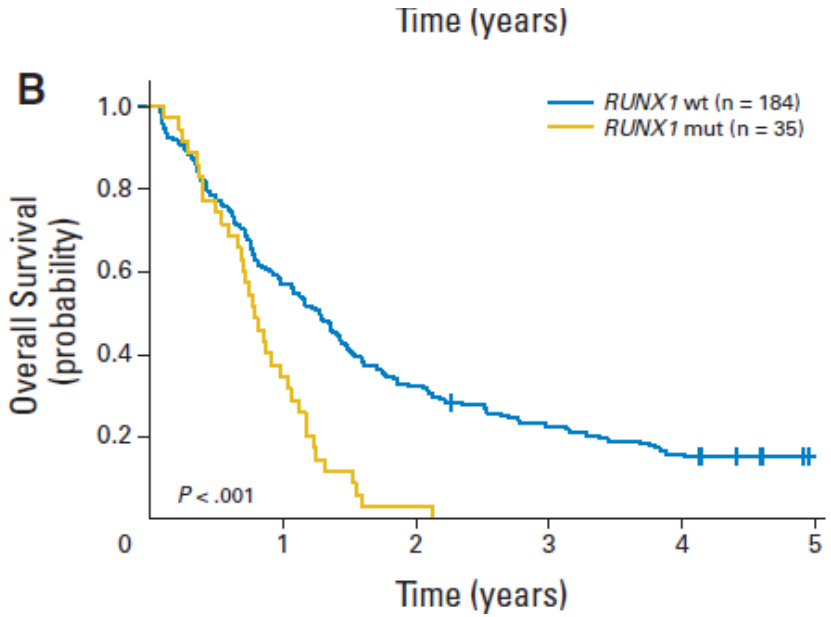
Döhner H et al. Blood 2017

*TP53, **RUNX1**, ASXL1*

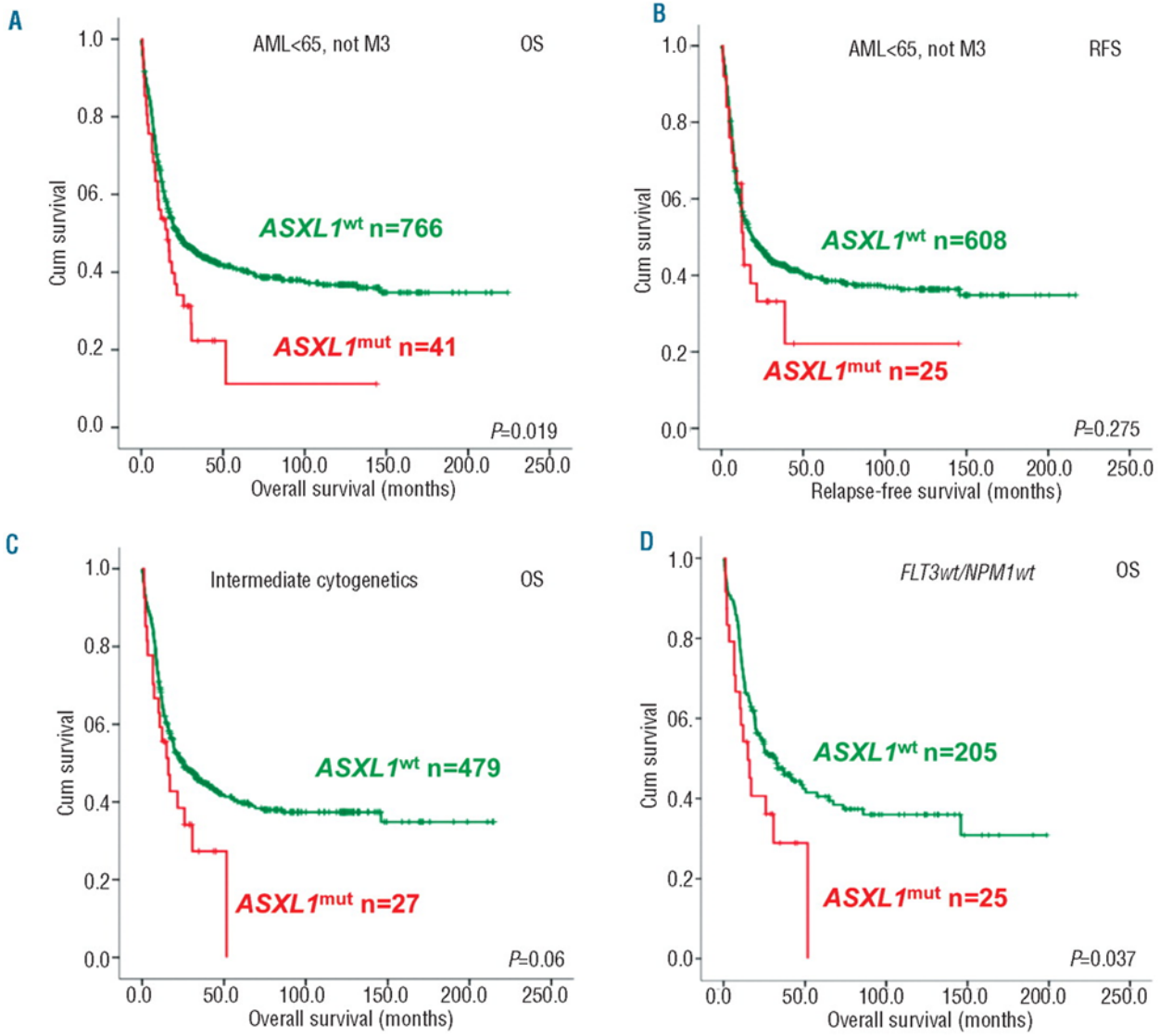
<60 år



>60 år



TP53, RUNX1, ***ASXL1***



AML: It not only about mutations

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Acute panmyelosis with myelofibrosis
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Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

AML with myelodysplasia related changes

- AML arising from previous MDS or MDS/MPN
- AML with multilineage dysplasia
- AML with MDS-related abnormality

Myelodyspasia defining cytogenetic aberrations*

Unbalanced abnormalities	Balanced abnormalities
−7 or del(7q)	t(11;16)(q23;p13.3)
−5 or del(5q)	t(3;21)(q26.2;q22.1)
i(17q) or t(17p)	t(1;3)(p36.3;q21.1)
−13 or del(13q)	t(2;11)(p21;q23)
del(11q)	inv(3)(q21q26.2)
del(12p) or t(12p)	t(6;9)(p23;q34)
del(9q)	
idic(X)(q13)	

Complex karyotype (3 or more chromosomal abnormalities) involving one or more of the above abnormalities.

*: OBS!! In the absence of AML recurrent aberrations

Arber DA et al. Blood 2016

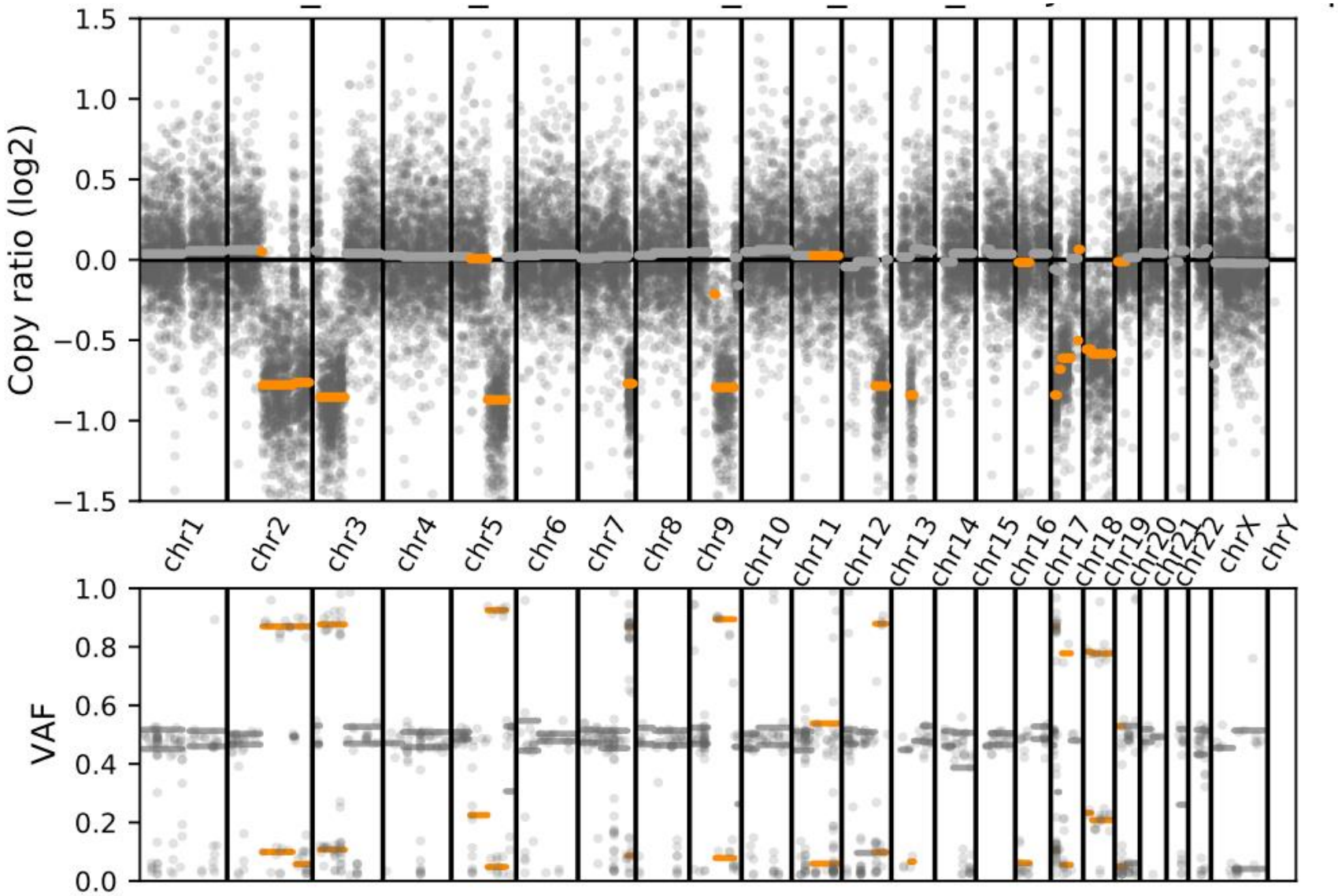
AML: It is not only about genetics

Other prognosticators

- Therapy related AML (t-AML)
- Hyperleucocytosis (LPK>100x10^9/L
- Extramedullary disease
- Age
- Comorbidities
- Response to treatment (MRD status)

- Number of mutations?

What is new in NGS?



Kromosomanalys: 45,XX,-3,-5,add(19)(q13),+mar[6]/42~44,idem,-add(19)(q13),-2,-9,-12,-12,-13,-17,-18,+5~7mar [cp18]/46,XX

ABL1
ANKRD26
ASXL1
ATRX
BCOR
BCORL1
BCL2
BTK
BRAF
CALR
CBL
CBLB
CDKN2A
CEBPA
CSF3R
CUX1
CXCR4
DDX41
DNMT3A
ETV6/TEL
EZH2
PHF6
FBXW7
AKT1
ALK
MLL3

ARHGEF10
ARID1A
ARID2
ASXL2
ATM
BAP1
BCL10
BCL11B
BRCC3
BTG1
CCND3
CDK4
CDKN1B
CDKN2B
CDKN2C
CHD4
CHEK2
CNOT3
CREBBP
CRLF2
CSF1R
RPL22
RPL5
RRAS
ZMYM3
ZNF318

FLT3
GATA1
GATA2
GNAS
HRAS
IDH1
IDH2
IKZF1
JAK2
JAK3
KDM6A
KIT
KRAS
KMT2A
MPL
MYD88
NF1
NOTCH1
NPM1
NRAS
PDGFRA
PLCG2
ZBTB33
ZBTB7A
CSF2RB
CSNK1A1

CXCR4
DCC
DCK
DDX23
DDX3X
DDX4
DDX54
DHX15
DHX33
DICER1
DNM2
DNMT3B
DPYD
EBF1
EED
EGFR
EP300
ERCC2
ETNK1
FAM175A
FGFR2
GFI1
GIGYF2
GNB1
GSTP1
YLPM1

PPM1D
PTEN
PTPN11
RAD21
RUNX1
SAMD9
SAMD9L
SBDS
SETBP1
SF3B1
SMC1A
SMC3
SRSF2
SRP72
STAG2
STAT3
STAT5B
TERT
TET2
TP53
U2AF1
WT1
ZRSR2
ZBTB33
ZBTB7A
ROBO2
RPL10

H3F3A
H3F3B
HIPK2
IL7R
INO80
IRF1
IRF4
IRF8
JAK1
JARID2
KDM5C
LEF1
LUC7L2
MDM2
MED12
MGA
MIR-516A
MIR-516B
MIR142
MIRN1267
MIRN632
MIRN891A
MLL2
CTCF
CTNNB1
ROBO1
ZEB2

MYB
MYC
MYCN
NF2
NFE2
NFE2L2
NIPBL
NOD2
NOTCH2
NT5C2
NXF1
PAX5
PHIP
PIK3CA
PIK3CD
PIK3R1
PRPF40A
PRPF40B
PRPF8
PTPRF
RAC1
RAD50
RAD51
RASGRF1
RB1
RHOA
RIT1

SAMHD1
SETD2
SETDB1
SF1
SF3A1
SH2B3
SLC29A1
SMARCA4
SMG1
SPRED2
SRCAP
STAG1
SUZ12
TBL1XR1
TOX
TPMT
TRRAP
TYMS
U2AF2
UBA2
UGT1A1
USH2A
USP7
USP9X
WHSC1
XPC
XRCC3

Genes reported in TWIST panel

Mars 2021-Clinical genetics Uppsala

ABL1
ANKRD26 (inklusive 5’-UTR)
ASXL1
ATRX
BCOR
BCORL1
BCL2
BTK
BRAF
CALR
CBL
CBLB
CDKN2A
CEBPA
CSF3R
CUX1
CXCR4
DDX41
DNMT3A
ETV6/TEL
EZH2
PHF6
FBXW7
FLT3
GATA1
GATA2 (inklusive intron 4)
GNAS
HRAS
IDH1
IDH2
IKZF1
JAK2
JAK3
KDM6A
KIT
KRAS
KMT2A
MPL
MYD88
NF1
NOTCH1
NPM1
NRAS
PDGFRA
PLCG2
PPM1D
PTEN
PTPN11
RAD21
RUNX1
SAMD9
SAMD9L
SBDS
SETBP1
SF3B1
SMC1A
SMC3
SRSF2
SRP72
STAG2
STAT3
STAT5B
TERT
TET2
TP53
U2AF1
WT1
ZRSR2

Genes reported in TWIST panel
Mars 2021-Clinical genetics Uppsala

ABL1
ANKRD26 (inklusive 5'-UTR)
ASXL1
ATRX
BCOR
BCORL1
BCL2
BTK
BRAF
CALR
CBL
CBLB
CDKN2A
CEBPA
CSF3R
CUX1
CXCR4
DDX41
DNMT3A
ETV6/TEL
EZH2
PHF6
FBXW7
FLT3
GATA1
GATA2 (inklusive intron 4)
GNAS
HRAS
IDH1
IDH2
IKZF1
JAK2
JAK3
KDM6A
KIT
KRAS
KMT2A
MPL
MYD88
NF1
NOTCH1
NPM1
NRAS
PDGFRA
PLCG2
PPM1D
PTEN
PTPN11
RAD21
RUNX1
SAMD9
SAMD9L
SBDS
SETBP1
SF3B1
SMC1A
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SRSF2
SRP72
STAG2
STAT3
STAT5B
TERT
TET2
TP53
U2AF1
WT1
ZRSR2

Genes reported in TWIST panel
Mars 2021-Clinical genetics Uppsala

ABL1
ANKRD26 (inklusive 5'-UTR)
ASXL1
ATRX
BCOR
BCORL1
BCL2
BTK
BRAF
CALR
CBL
CBLB
CDKN2A
CEBPA
CSF3R
CUX1
CXCR4
DDX41
DNMT3A
ETV6/TEL
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GATA1
GATA2 (inklusive intron 4)
GNAS
HRAS
IDH1
IDH2
IKZF1
JAK2
JAK3
KDM6A
KIT
KRAS
KMT2A
MPL
MYD88
NF1
NOTCH1
NPM1
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SAMD9
SAMD9L
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SF3B1
SMC1A
SMC3
SRSF2
SRP72
STAG2
STAT3
STAT5B
TERT
TET2
TP53
U2AF1
WT1
ZRSR2

Thank you for your attention

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