# MLL 2.0 – from see behind to go beyond

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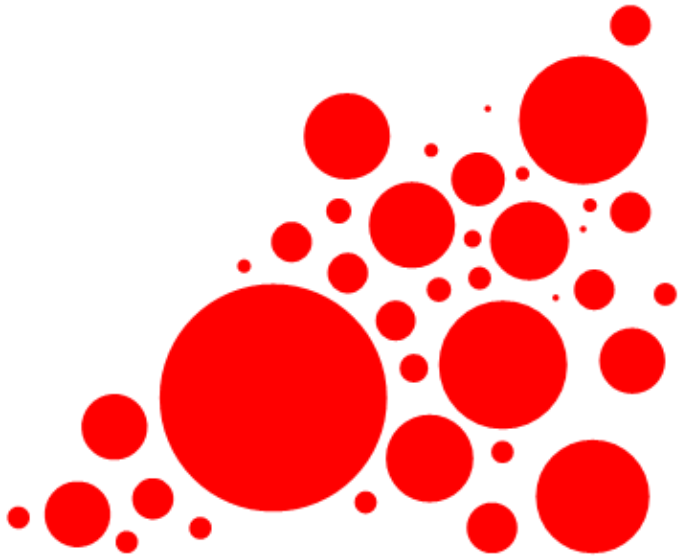
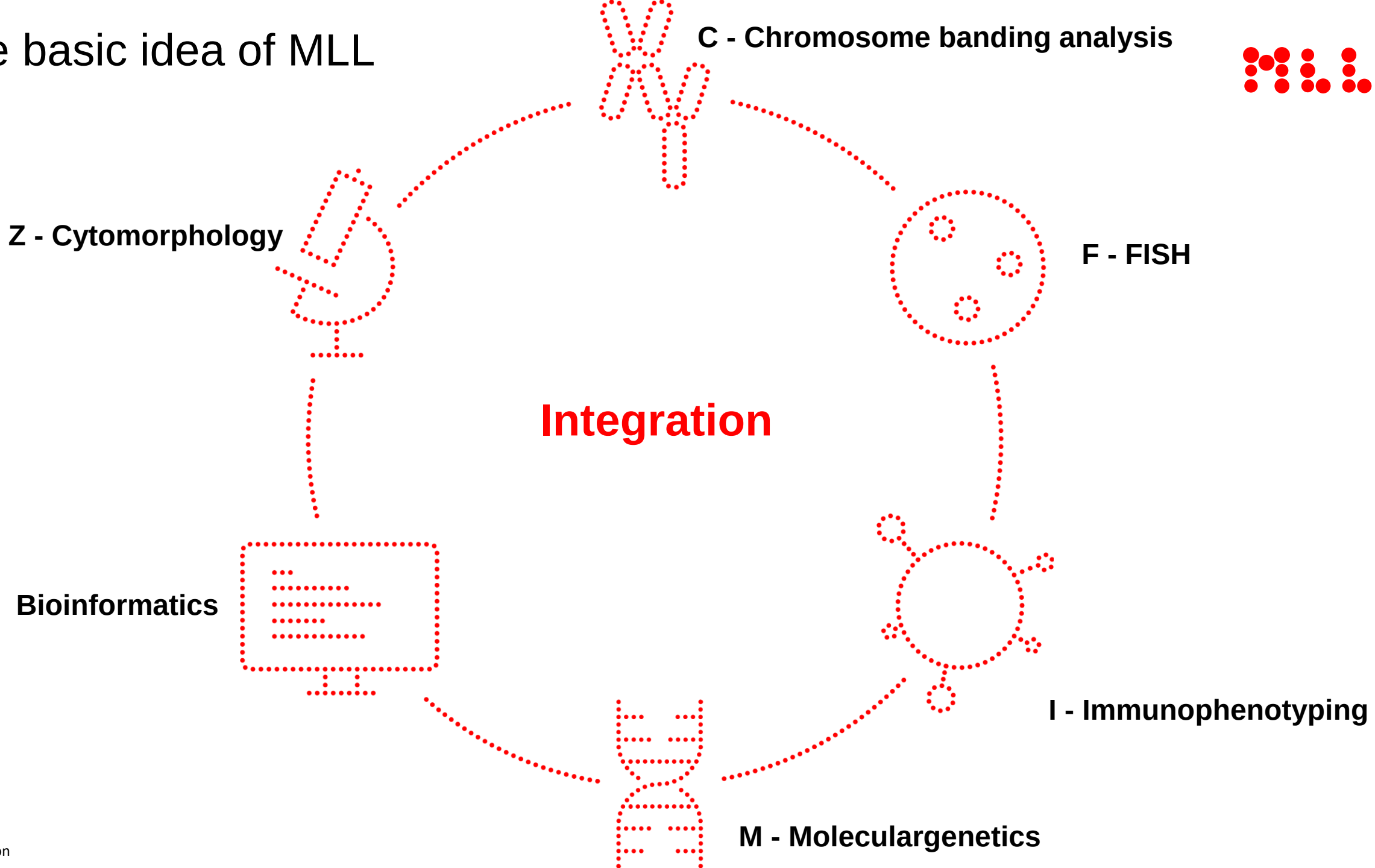
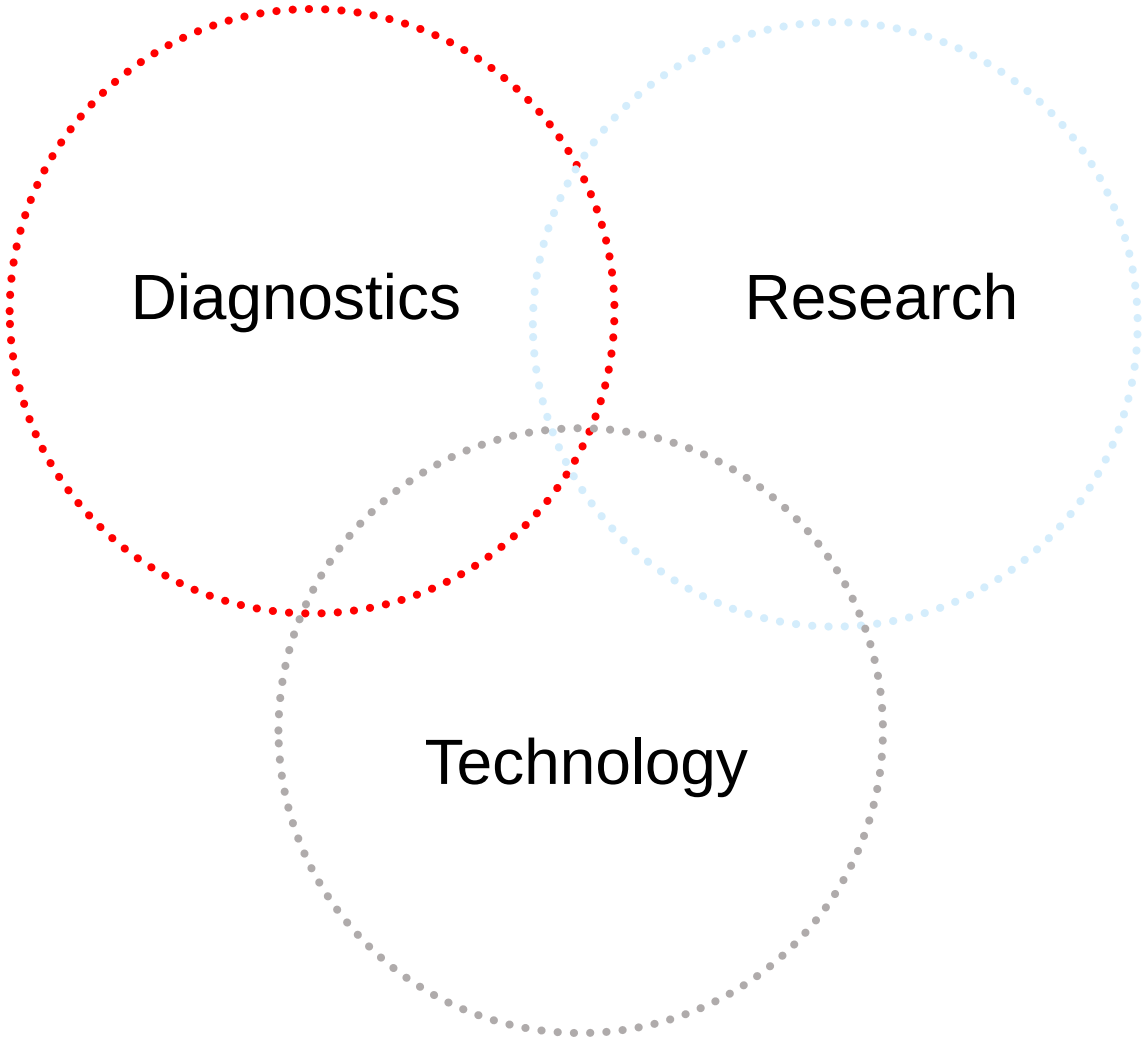
MLL Munich Leukemia Laboratory



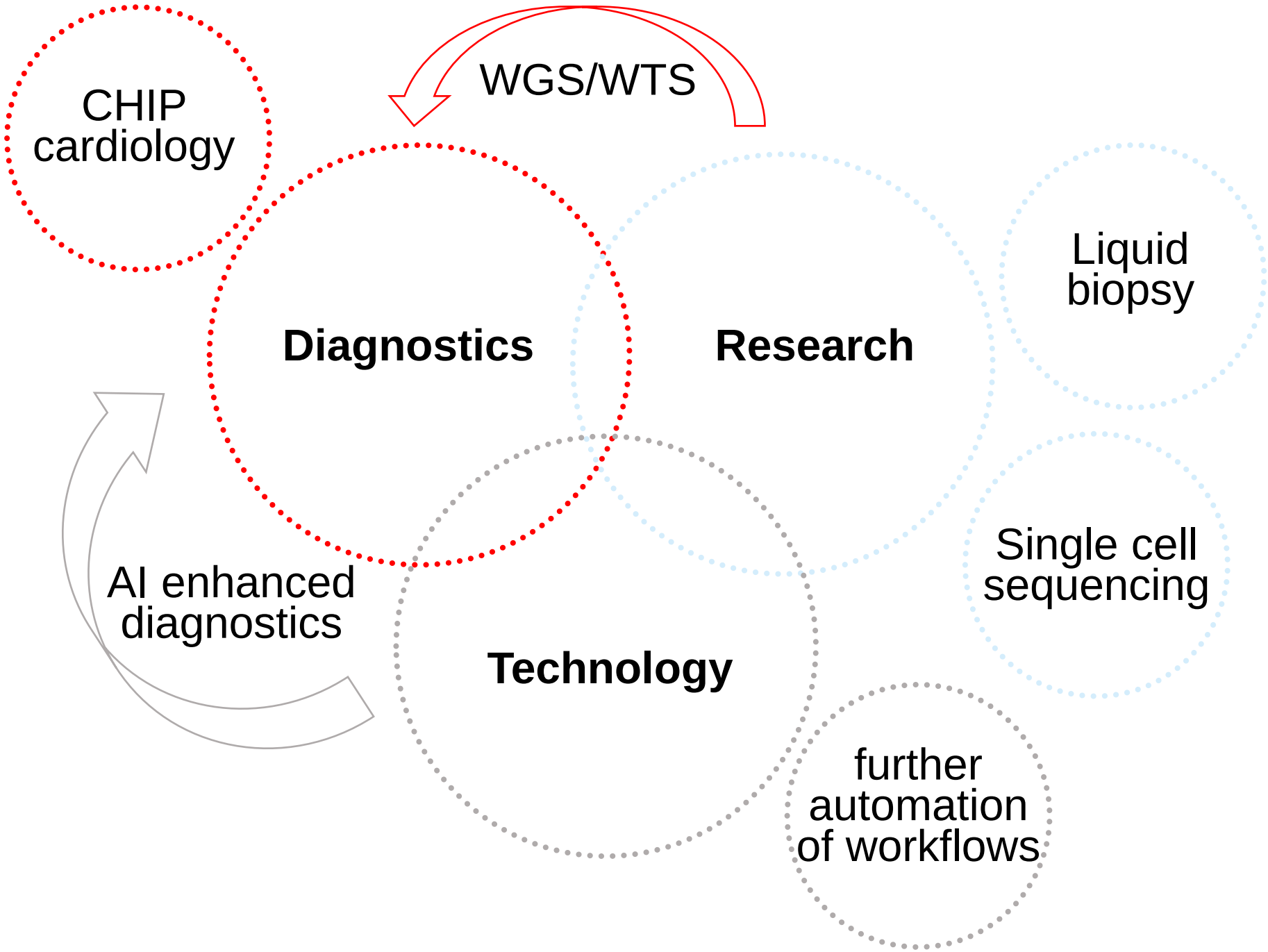
# The basic idea of MLL



# MLL



MLL



# Chromosome banding analysis

Cell counting

Preparation of smears

Check of cultures

Labeling culture tubes

Preparation of culture tubes

Add patient sample

Incubation

Colcemid

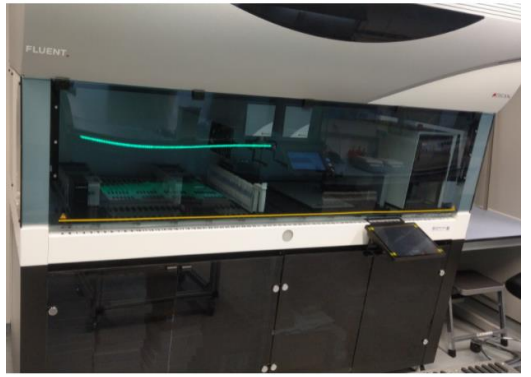
# Chromosome banding analysis

Harvesting

Cultures -> slides

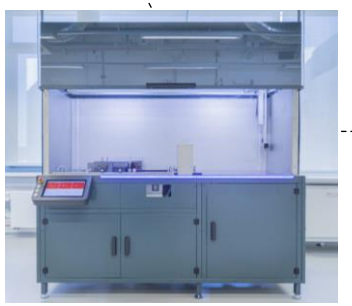
Preparation of slides

Tubes for storage

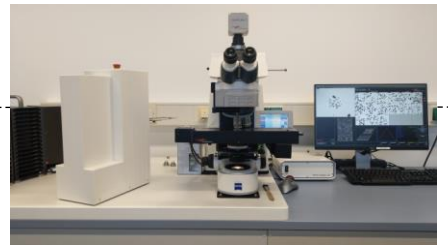


Labeling of slides

Banding & staining

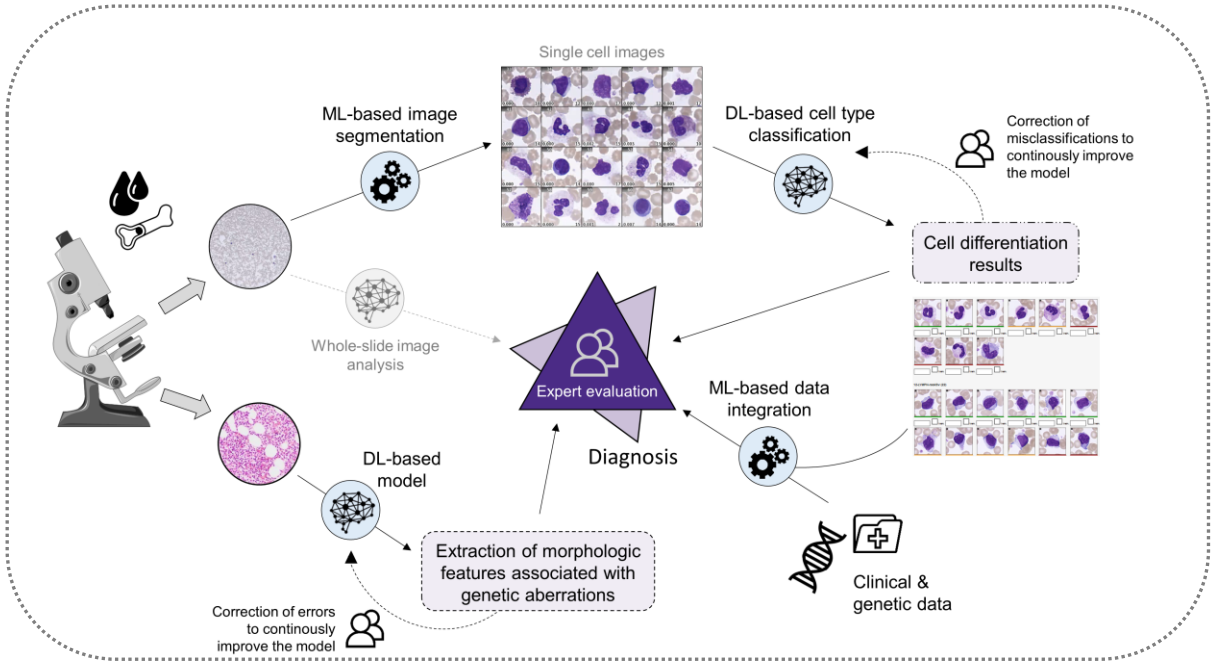


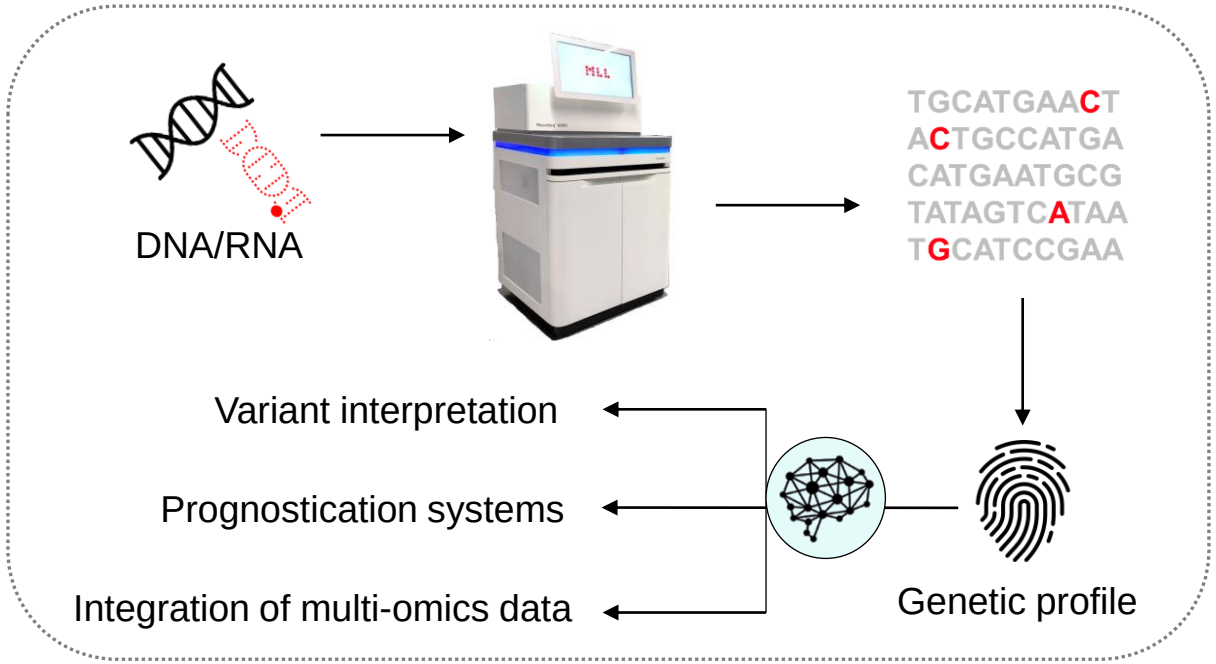
Metaphase capturing

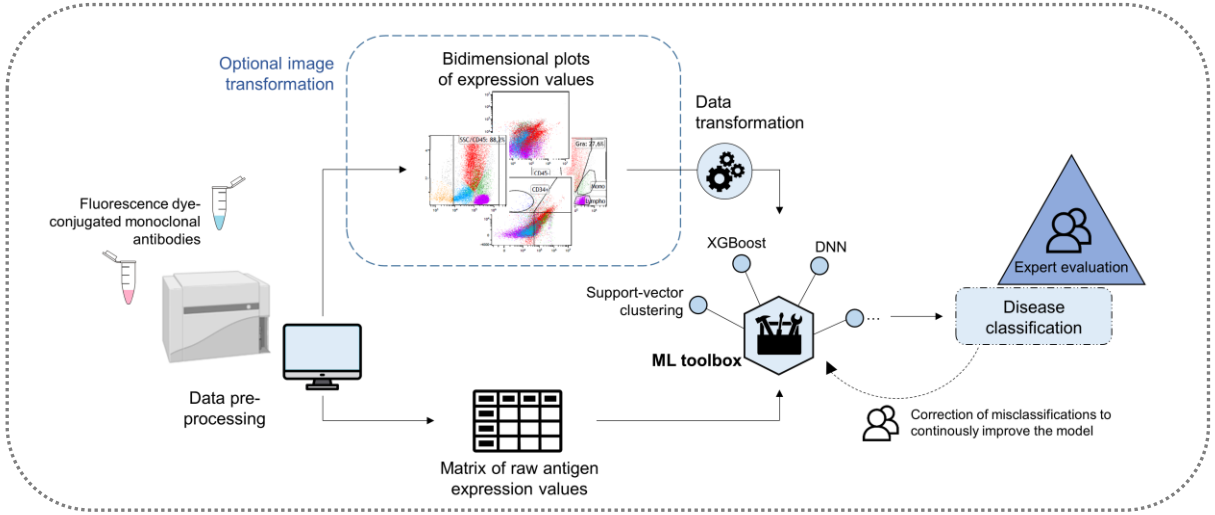


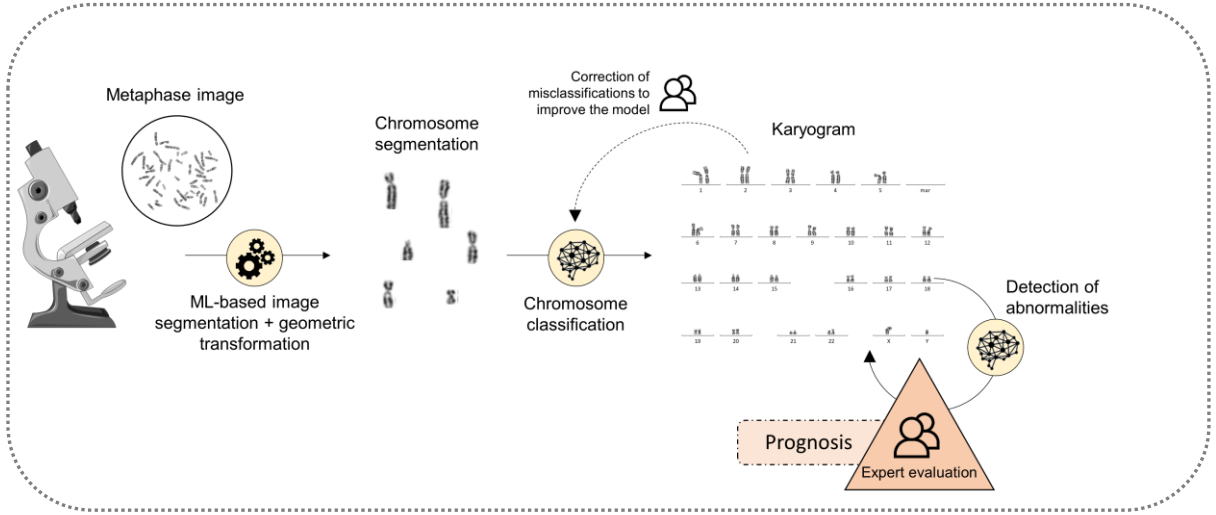
Slide archiving

# AI projects at MLL to enhance diagnostics



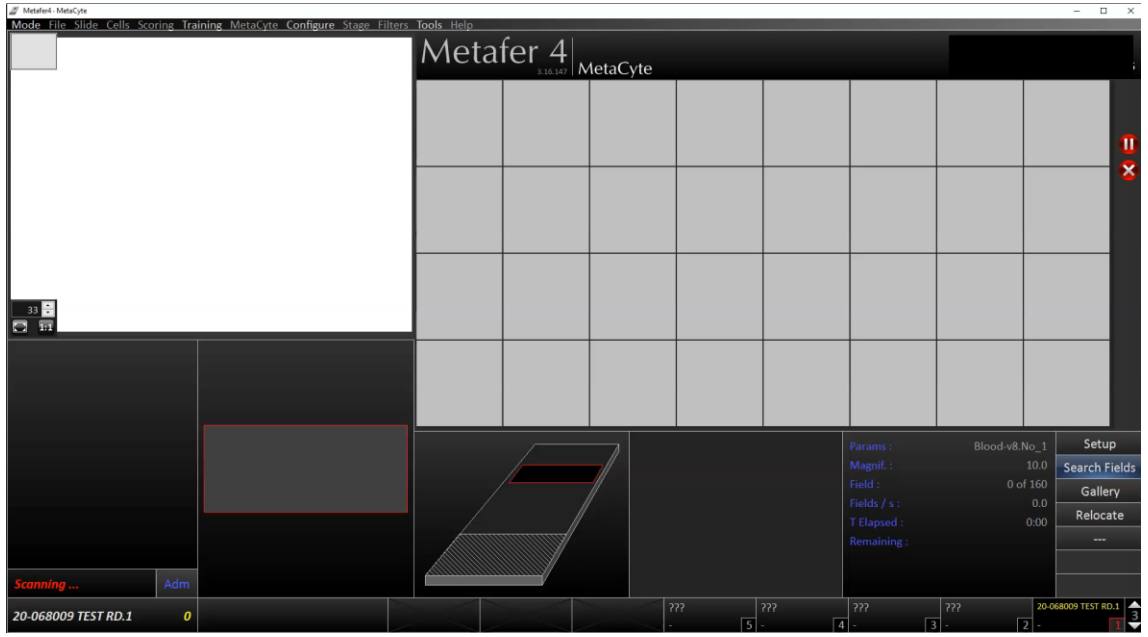



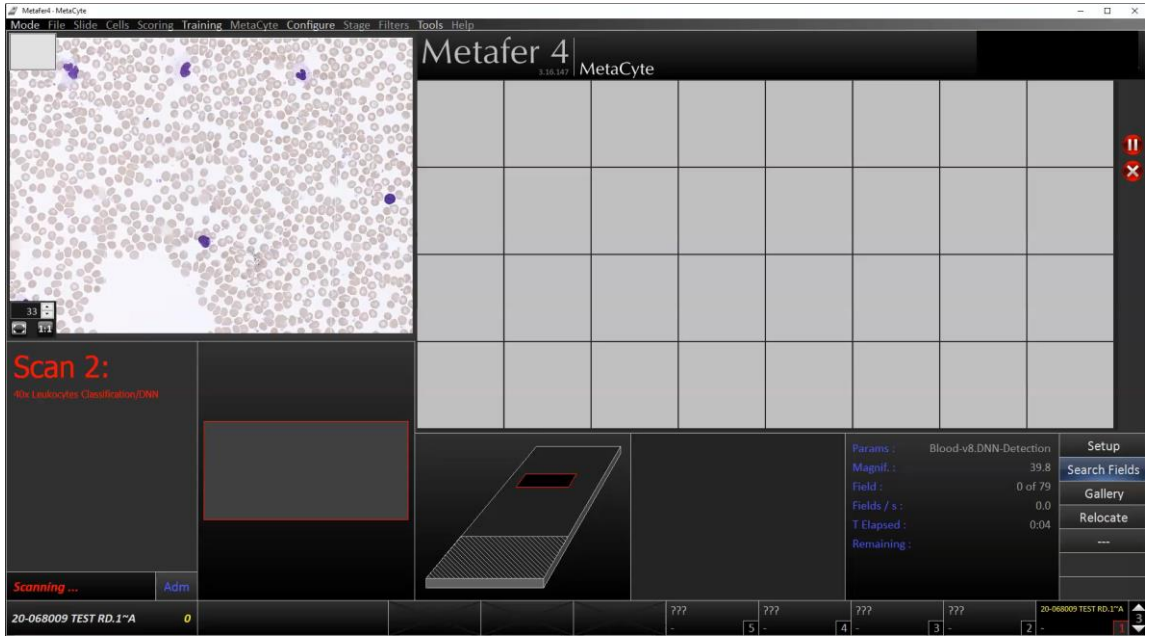




W. Walter et al. Blood Reviews 2022

# AI enhanced cytomorphology – peripheral blood




1. 10x 2.„Close-Up“ of single cells (400x oil): 300-500 cells/smear (~ 4:00 min)

**BELUGA Study („Better LeUkemia diaGnostics through AI“)** (Clinicaltrials.gov, NCT04466059)

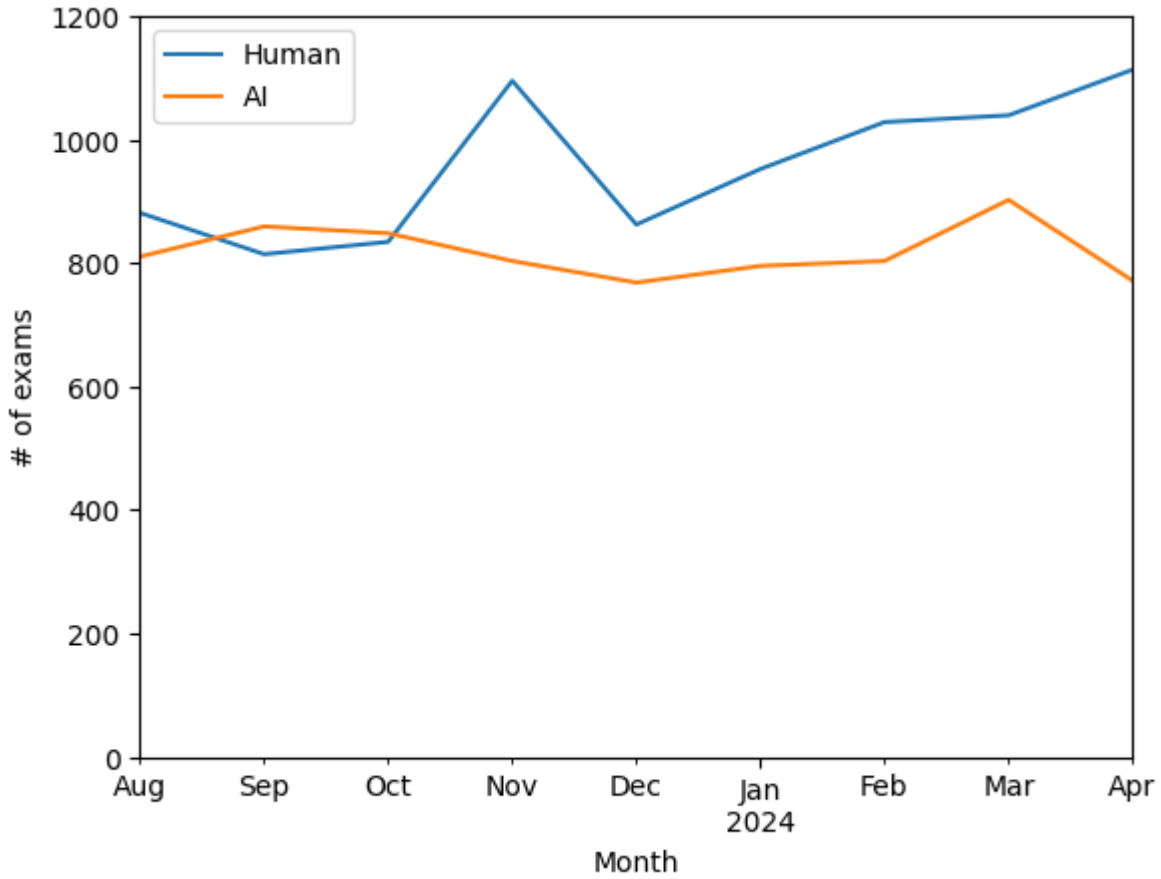
29,119 patient samples (Jan 2021 – Jul 2022)

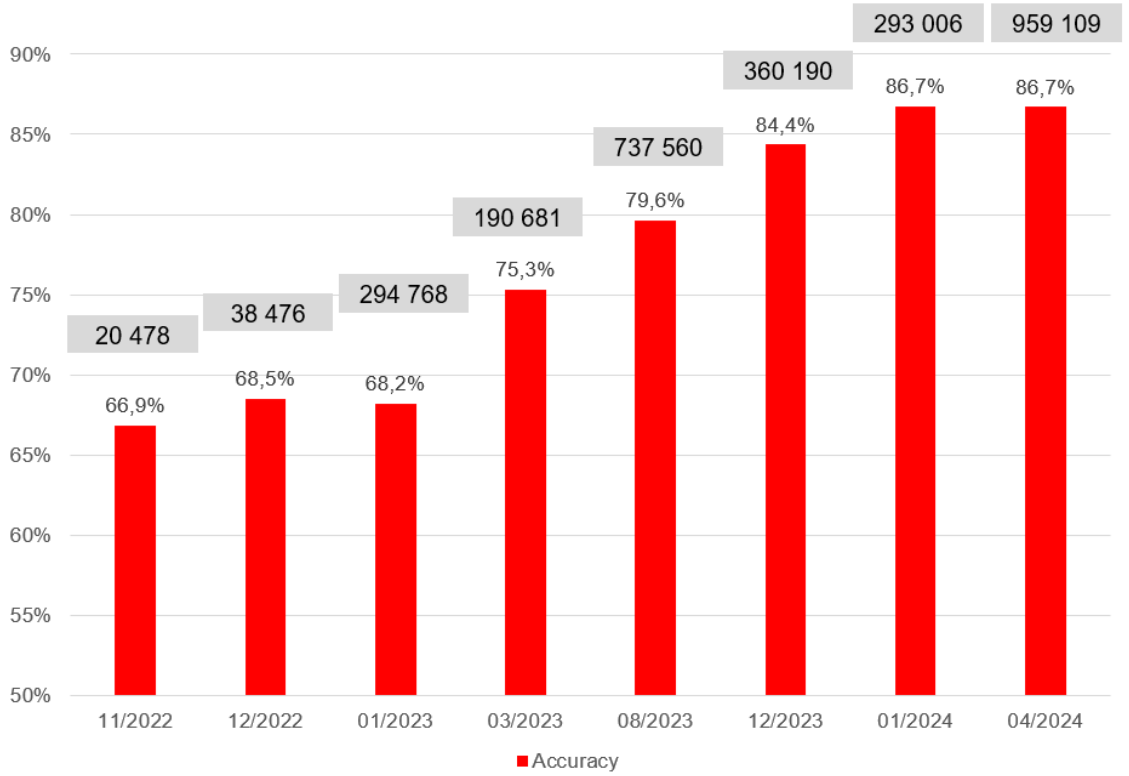
Concordance 94.5% for malignant/critical cells

→

Implemented into routine workflow

# Cytomorphology – AI workflows



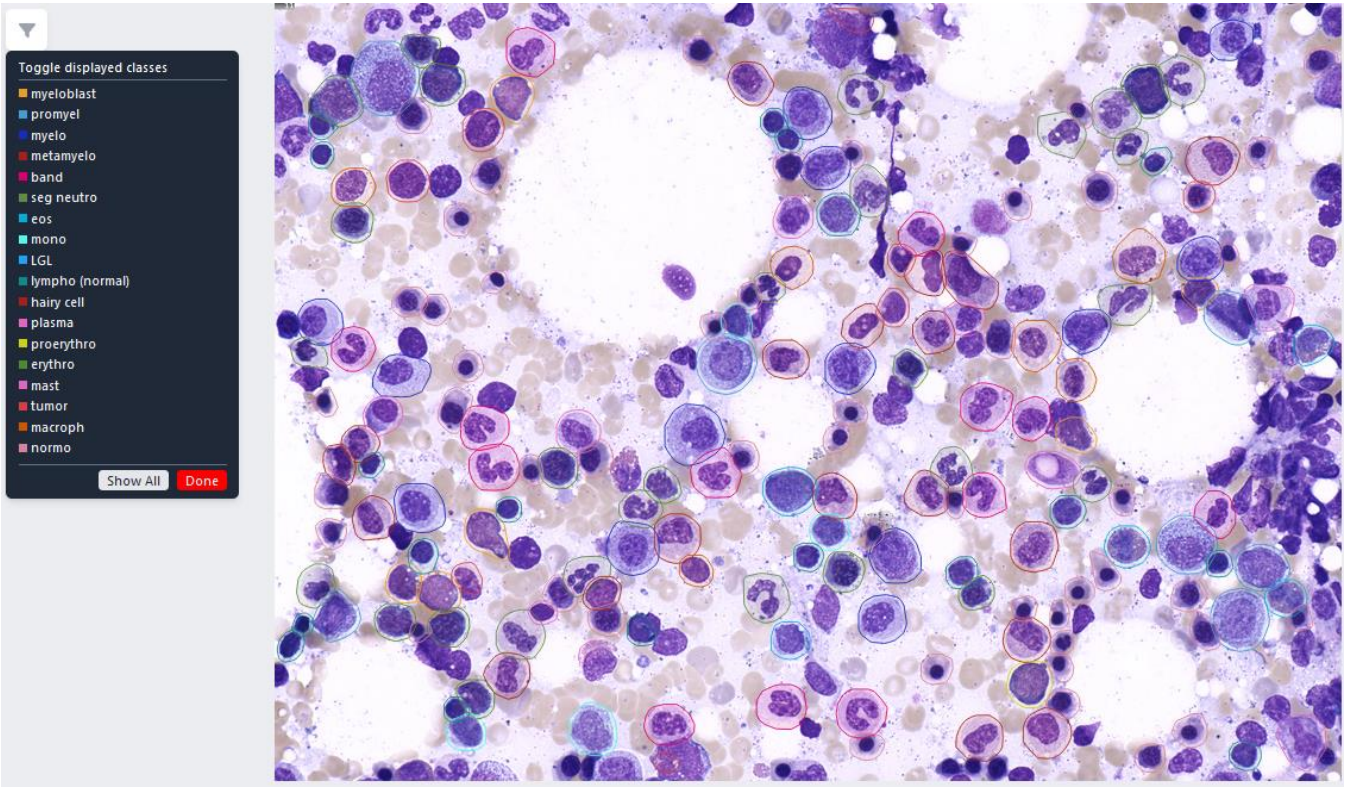



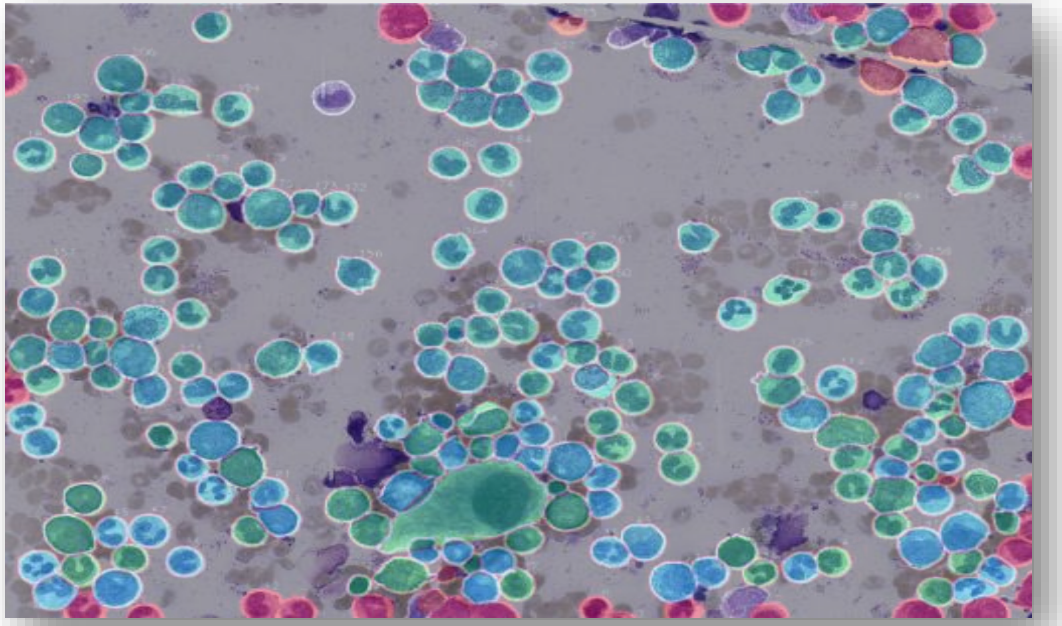

Number of cells evaluated

(n=15977, n AI =7359, n Human = 8618), rund 46,1% AI

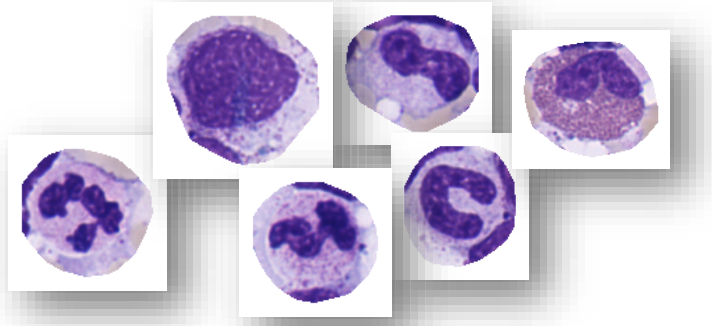
# AI enhanced cytomorphology – bone marrow

## Object Detection



Raw FOV Image



Isolated Single Cell Images

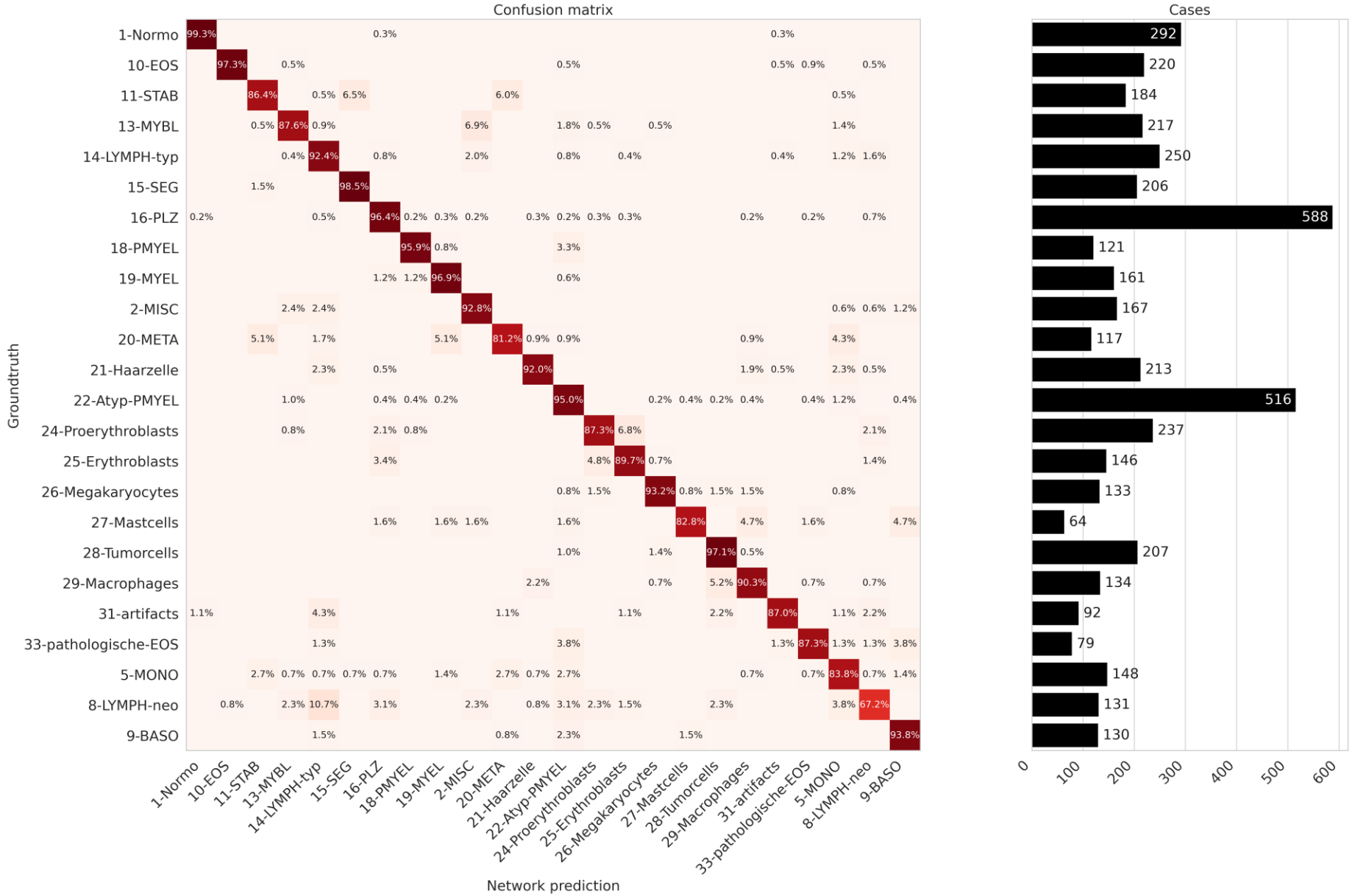
**C. Pohlkamp et al. ASH 2022 #4813**

# Single-cell classification accuracy in bone marrow

**92.22%**

overall accuracy
on clean data

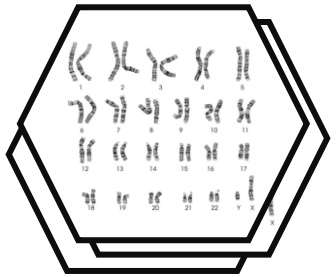
9.98% increase over
previous
model version

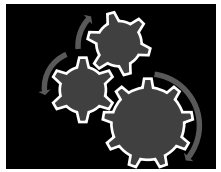


# AI-based karyotyping - current state in routine diagnostics

(88% of chromosomes correct)

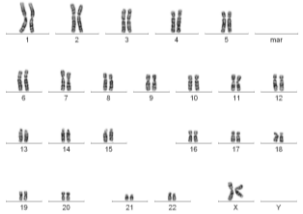
training data set
(100,000 normal karyograms)

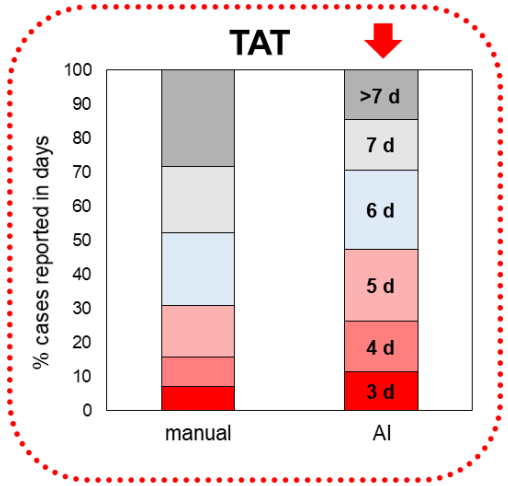


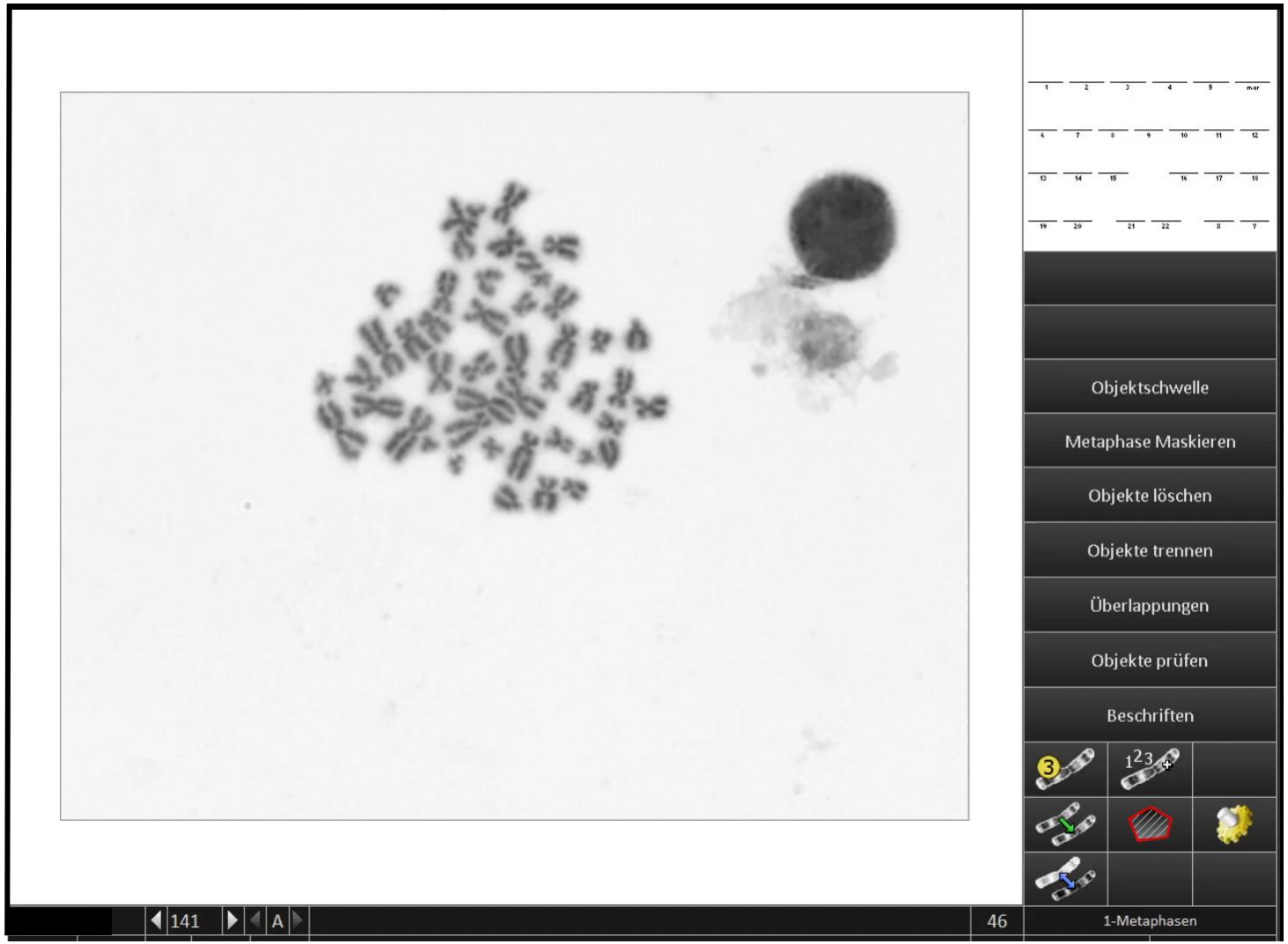


AI

classifier for normal chromosomes





# Classifiers currently in routine diagnostics

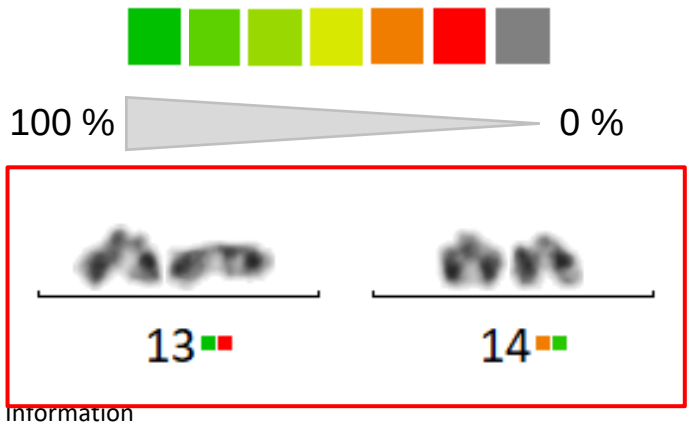
Classification of metaphase quality – score 1 to 10

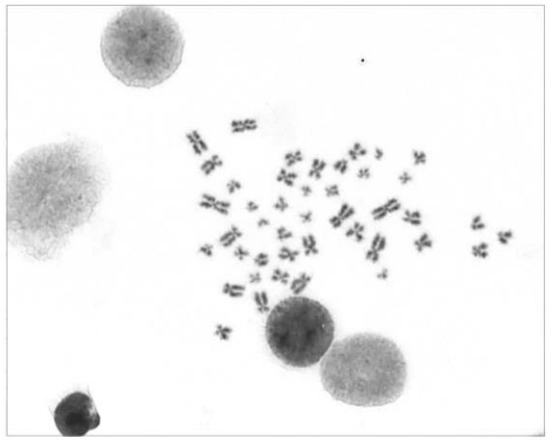
Object recognition & segmentation in unprocessed metaphase images

Object classification (24 classes: normal chromosomes)

Result:
Sorted karyogram
Probability score for each chromosome

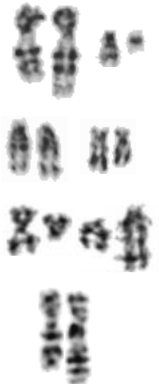
Batch processing implemented



# Training of aberrant chromosome classes

| Aberration | Short Name | # Training metaphases |
|---|---|---|
| t(9;22)(q34;q11) | T0922 | 14,334 |
| t(15;17)(q24;q21) | T1517 | 4,656 |
| t(11;14)(q13;q32) | T1114 | 1,532 |
| der(1;7)(q10;q10) | D0107 | 2,098 |



| No | Name | Chromosome class |
|---|---|---|
| 1A | der(9)t(9;22)(q34;q11) | 25 |
| 1B | der(22)t(9;22)(q34;q11) | 26 |
| 2A | der(15)t(15;17)(q24;q21) | 27 |
| 2B | der(17)t(15;17)(q24;q21) | 28 |
| 3A | der(11)t(11;14)(q13;q32) | 29 |
| 3B | der(14)t(11;14)(q13;q32) | 30 |
| 4 | der(7)der(1;7)(q10;p10) | 31 |

31 chromosome classes (24 normal, 7 aberrant)

# Classifier for chromosome abnormalities

Object recognition & segmentation in unprocessed metaphase images

Object classification (31 classes: 24 normal, 7 aberrant)

Output: probability matrix for classification of chromosomes sorted by surface area

Classification of metaphase based on probability matrix (5 classes: normal, t(9;22), t(15;17), t(11;14), der(1;7)

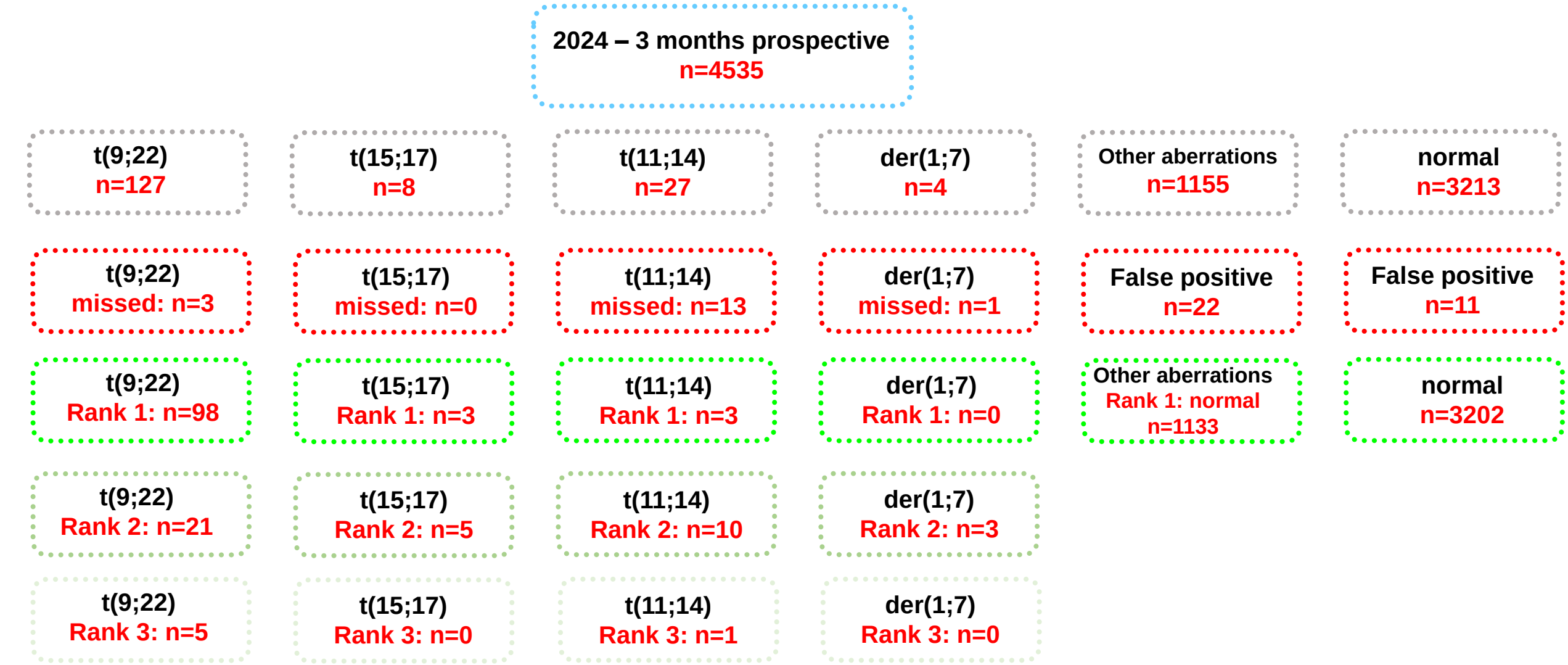
Majority decision on class for each case based on metaphases

Results in test set

Cases with ≥5 metaphases

| Aberration | N Cases | Rank 1 | Rank 1 [%] | Rank 2 | Rank 2 [%] |
|---|---|---|---|---|---|
| Normal | 113 | 112 | 99.1 | 1 | 0.9 |
| der(1;7) | 167 | 166 | 99.4 | 1 | 0.6 |
| t(9;22) | 120 | 119 | 99.2 | 1 | 0.8 |
| t(11;14) | 130 | 130 | 100.0 | 0 | 0.0 |
| t(15;17) | 143 | 136 | 95.1 | 7 | 4.9 |

# Classifier for chromosome abnormalities – real world data



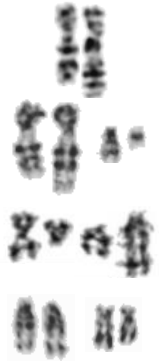
# Chromosome banding analysis using AI – 2024+

**Combination of 3 DNN**

Segmentation of chromosomes in metaphase using unprocessed images

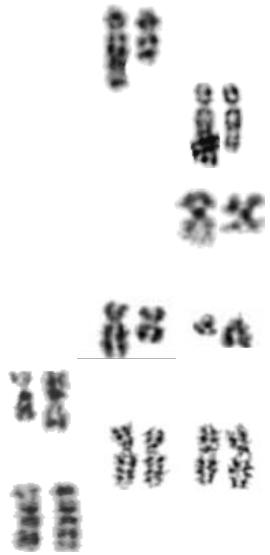
Classification of chromosomes in chromosome classes (normal chromosomes + certain aberrant chromosomes)

Classification of cases as normal karyotype or aberrant karyotype with distinct abnormality



| Aberration | N Cases | Rank 1 | Rank 1 % | Rank 2 | Rank 2 % |
|---|---|---|---|---|---|
| Normal | 113 | 112 | 99,1% | 1 | 0,9% |
| der(1;7) | 167 | 166 | 99,4% | 1 | 0,6% |
| t(9;22) | 120 | 119 | 99,2% | 1 | 0,8% |
| t(11;14) | 130 | 130 | 100,0% | 0 | 0,0% |
| t(15;17) | 143 | 136 | 95,1% | 7 | 4,9% |

Next step



del(5)(q14q34)
del(5)(q13q34)
del(5)(q21q34)
del(20)(q11q13)

t(8;21)
inv(16)
t(9;11)
inv(3)

# Chromosomal aberrations in (suspected) MDS at MLL in 2023

**MDS?**
**n=5.316**

| MDS, n=3,027 | | |
|---|---|---|
| normal karyotype: | 58.3% | 67.0% |
| trisomies: | 7.0% | |
| monosomy 7: | 1.7% | |
| del(5q): | 6.4% | 9.4% |
| del(20q): | 3.0% | |
| other abnormalities: | 16.5% | 23.6% |
| complex karyotype: | 7.1% | |

| MDS possible, n=863 | | |
|---|---|---|
| normal karyotype: | 80.0% | 84.0% |
| trisomies: | 3.0% | |
| monosomy 7: | 1.0% | |
| del(5q): | 1.9% | 3.0% |
| del(20q): | 1.2% | |
| other abnormalities: | 10.5% | 13.0% |
| complex karyotype: | 2.4% | |

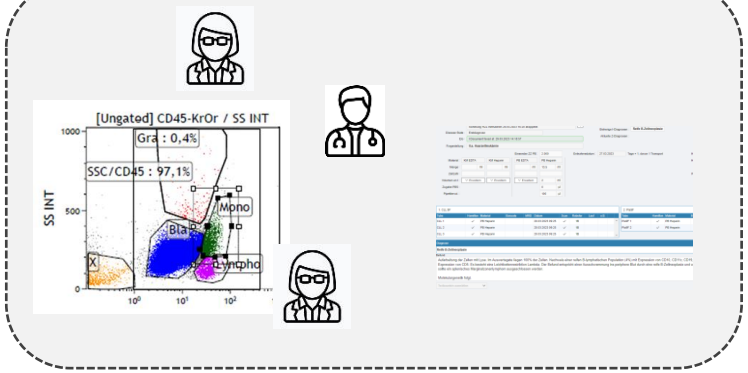
| MDS not confirmed, n=1,426 | | |
|---|---|---|
| normal karyotype: | 88.8% | 91.0% |
| trisomies: | 1.8% | |
| monosomy 7: | 0.3% | |
| del(5q): | 0.8% | 1.3% |
| del(20q): | 0.5% | |
| other abnormalities: | 6.0% | 7.7% |
| complex karyotype: | 1.8% | |

# Immunophenotyping - Roadmap

Current Process

Digital Transformation

(FCS files)




Future AI Process

Automated Gating + Report Generation

→ Q3 2023

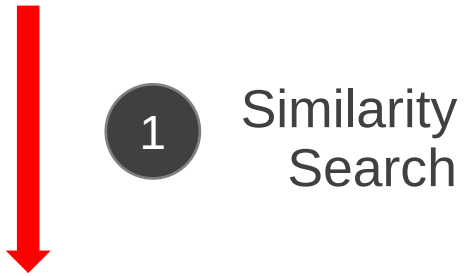
*Diagnosis*

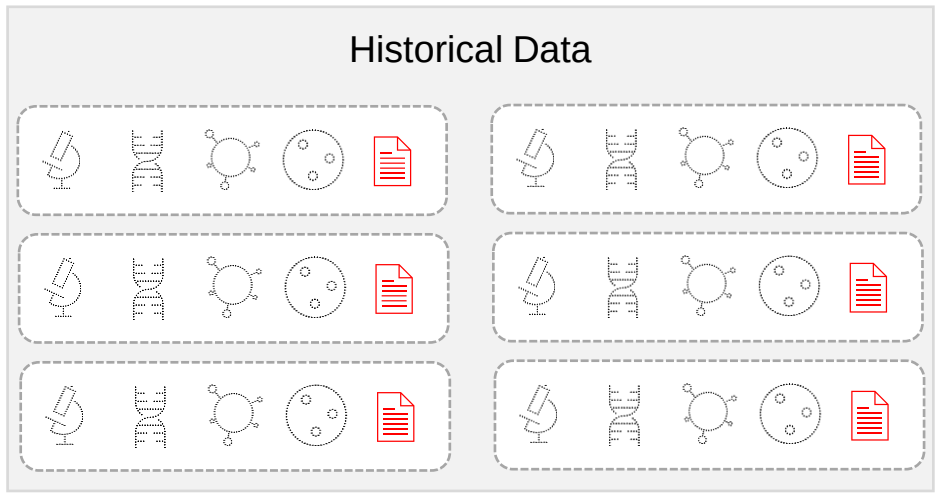
- AML
- ALL
- CLL
- MDS
- MM
- T-NHL

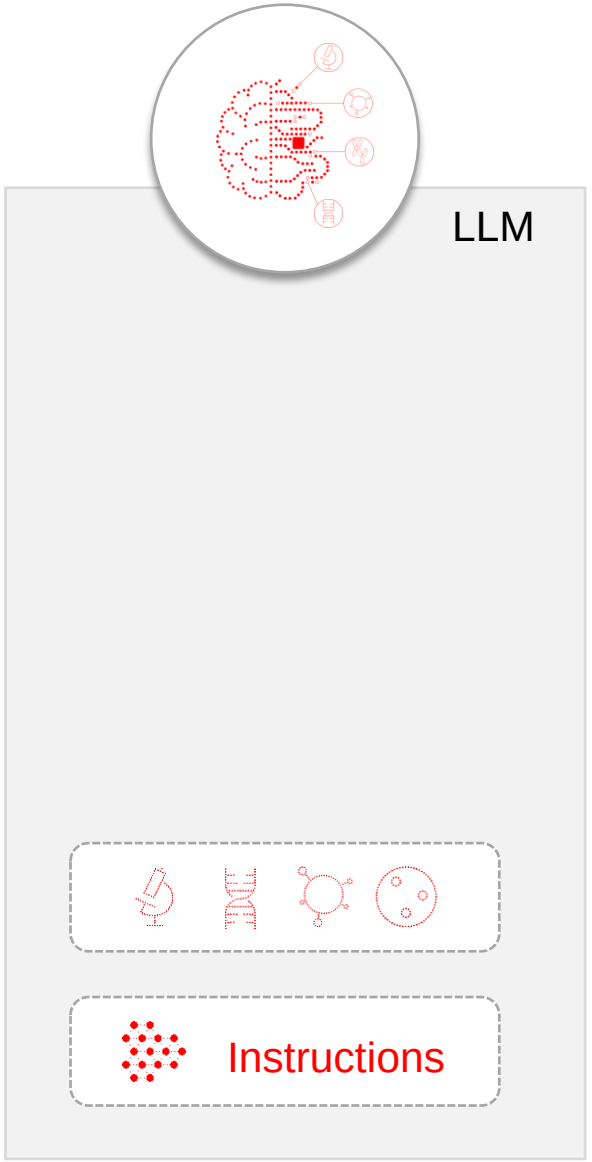
*MRD*

- AML
- CLL
- MM

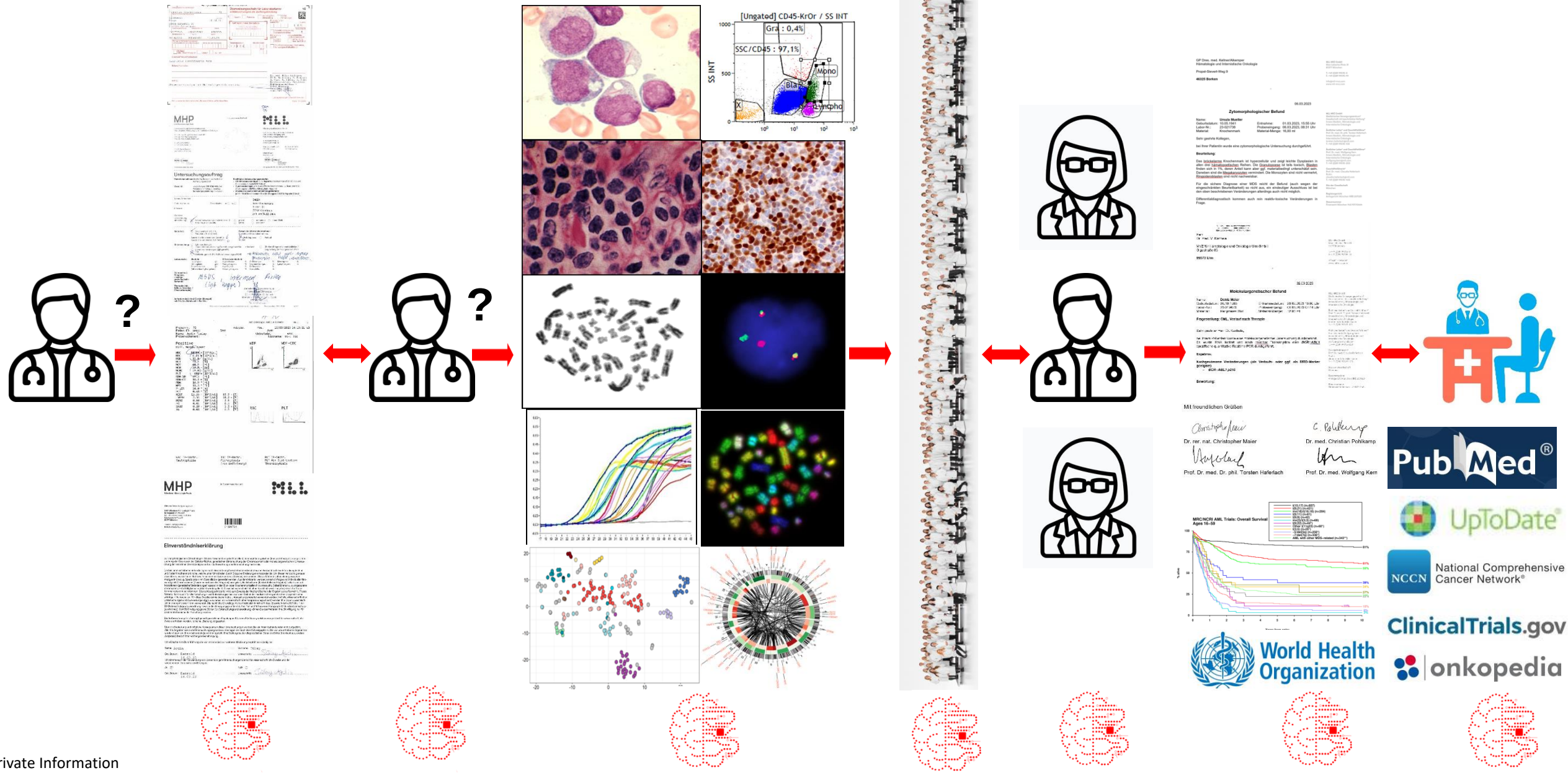
# Automated diagnosis with Large Language Models (LLM)

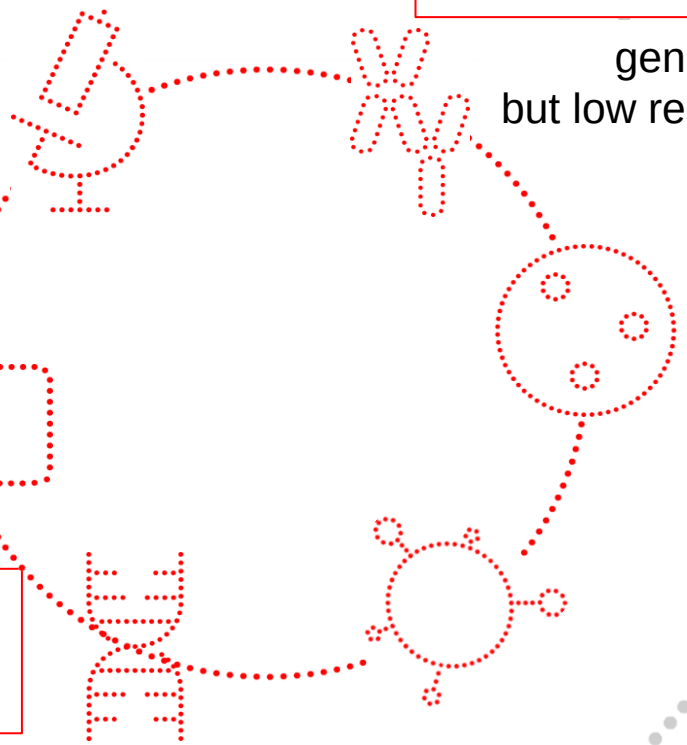

# AI driven diagnostics (based on > 1.2 Mio. cases) and treatment advice



# Leukemia Diagnostics today and tomorrow

copy number alterations
structural variants

genome wide
but low resolution 5-10MB



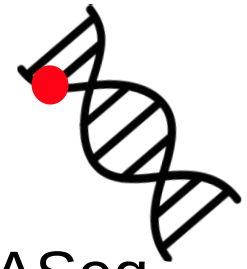
mutations
gene fusions

very high resolution
but only targeted

copy number alterations
structural variants

WGS

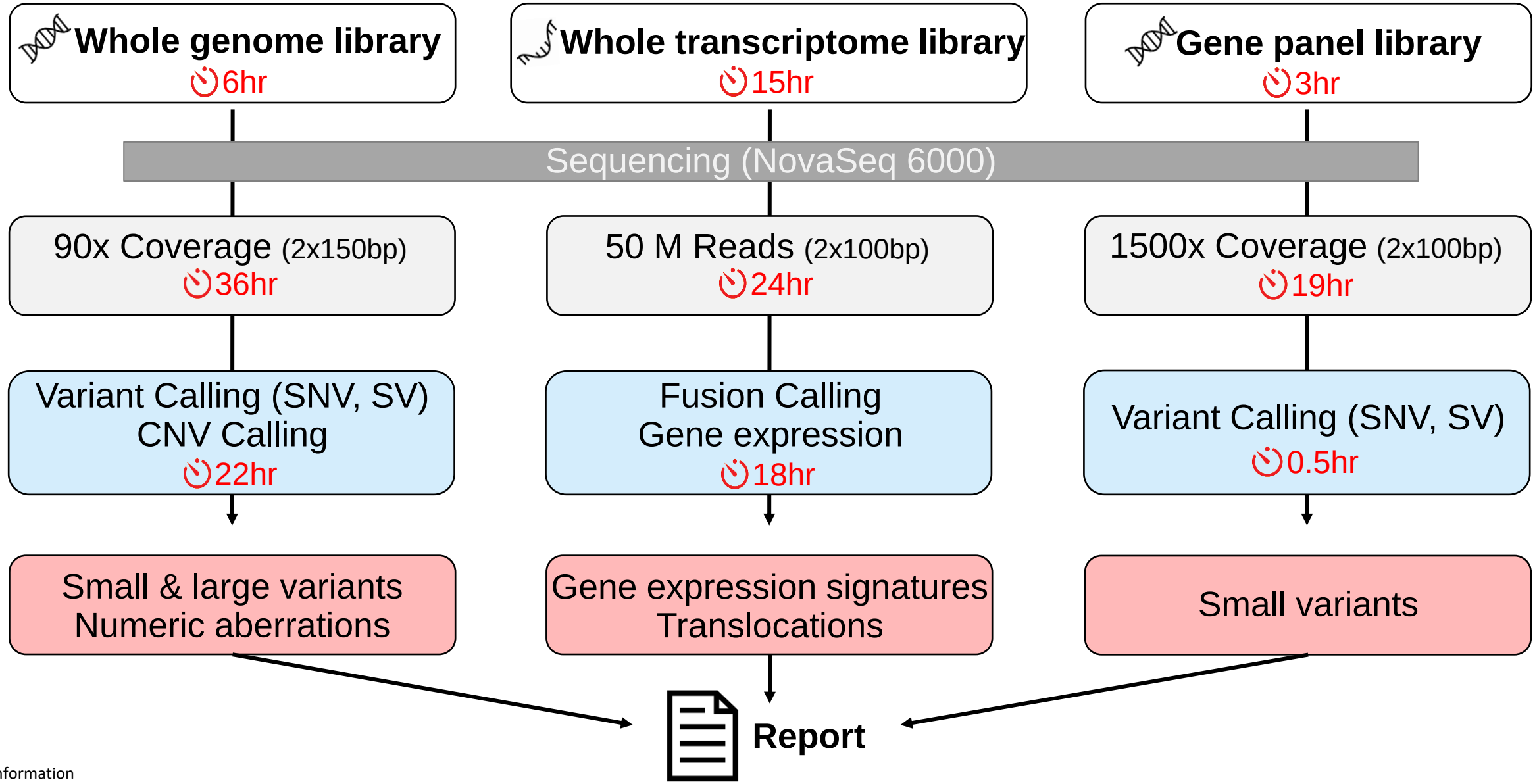
genome wide
and highest resolution



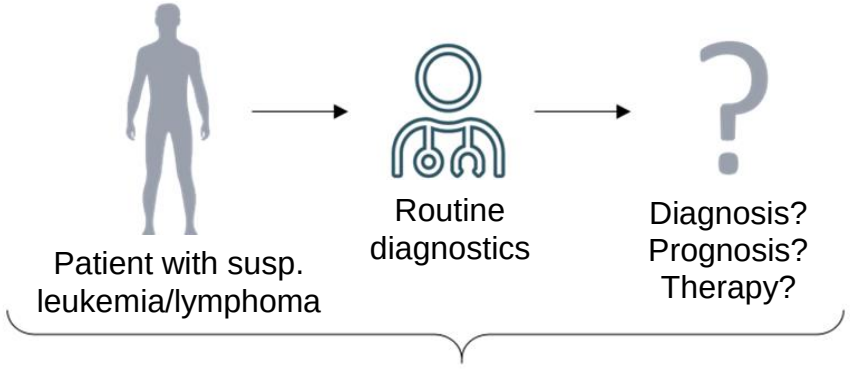
RNASeq

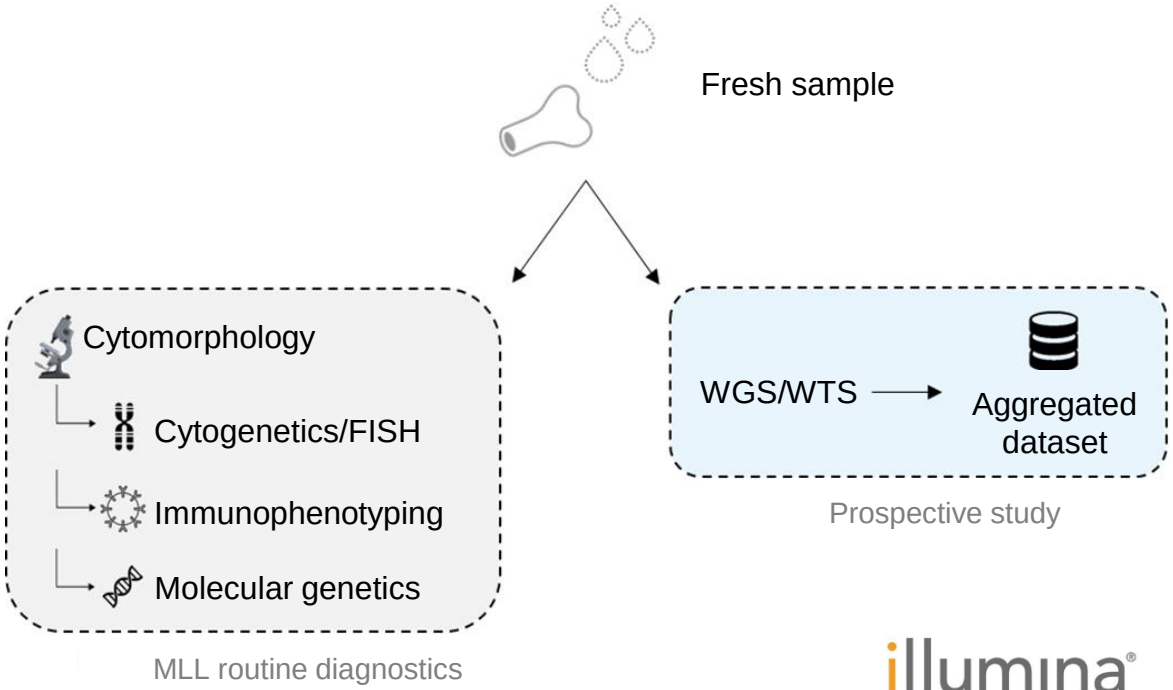
mutations
gene fusions

# Molecular genetics workflows



# „The difficult hematological case“




illumina®

NIH U.S. National Library of Medicine

ClinicalTrials.gov

## Solving Riddles Through Sequencing (SIRIUS)

ClinicalTrials.gov Identifier: NCT05046444

### Results

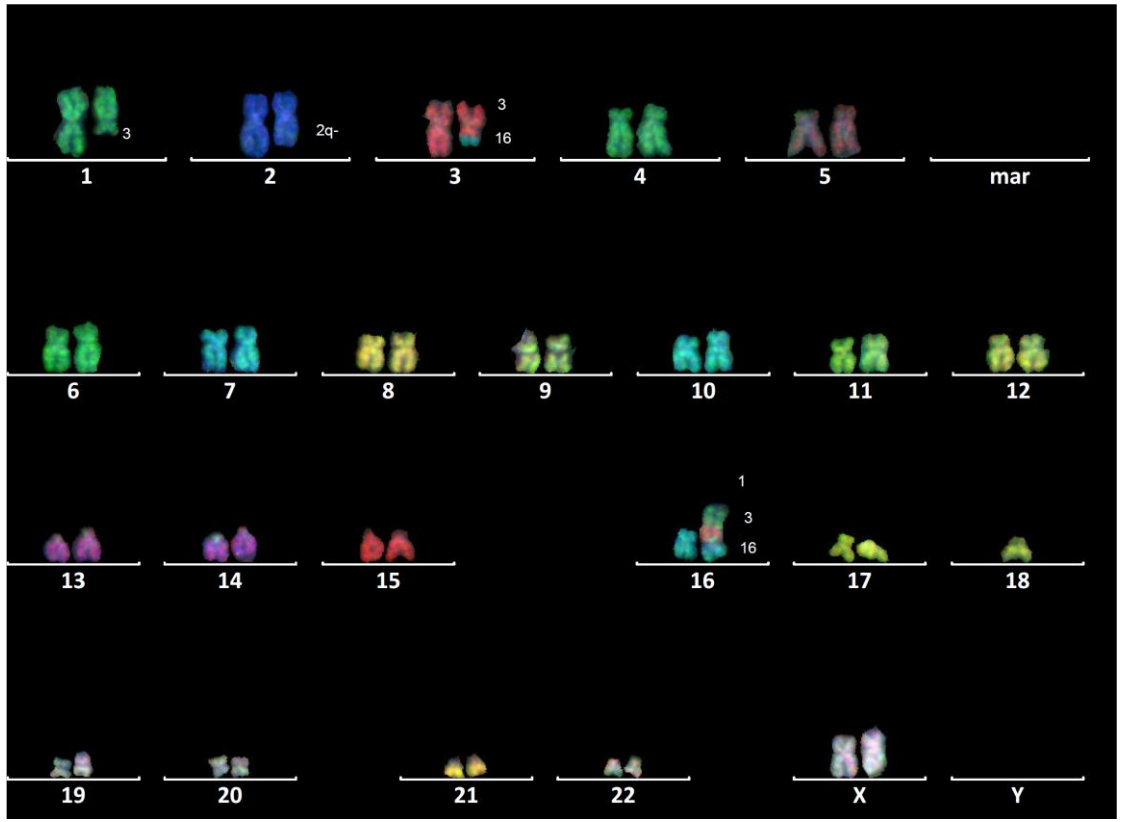
- Diagnosis
- Prognosis
- Therapy

### TAT

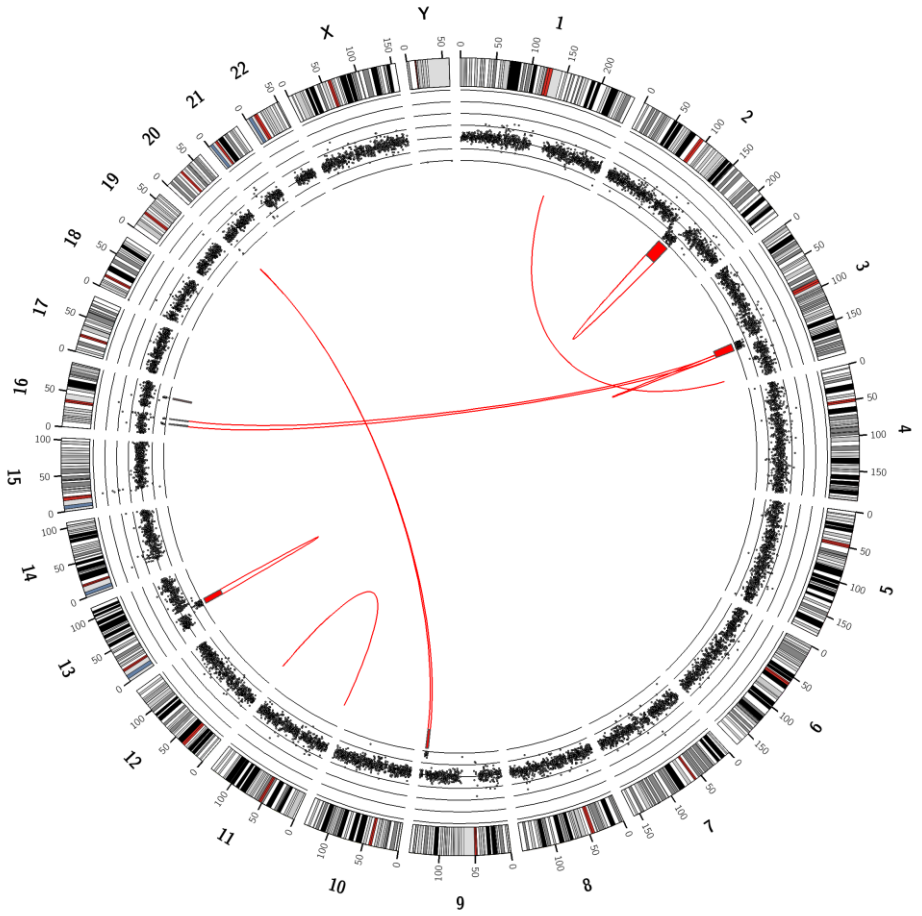
### Costs

# Acute megakaryoblastic leukemia

46,XX,der(1)t(1;3)(q21;q27),del(2)(q22q31),der(3)t(3;16)(q21;p11),
der(16)(1qter->1q21::3q27->3q21::16p11->16qter)




46,XX,der(1)t(1;3)(q21;q27),del(2)(q22q31),der(3)t(3;16)(q21;p11),
der(9)t(9;20)(q34.1;p13),t(11;12)(p15.4;p13.3),del(13)(q14.1q14.3),
der(16)(1qter->1q21::3q27->3q21::16p11->16qter),
der(20)t(9;20)(q34.2;p13)

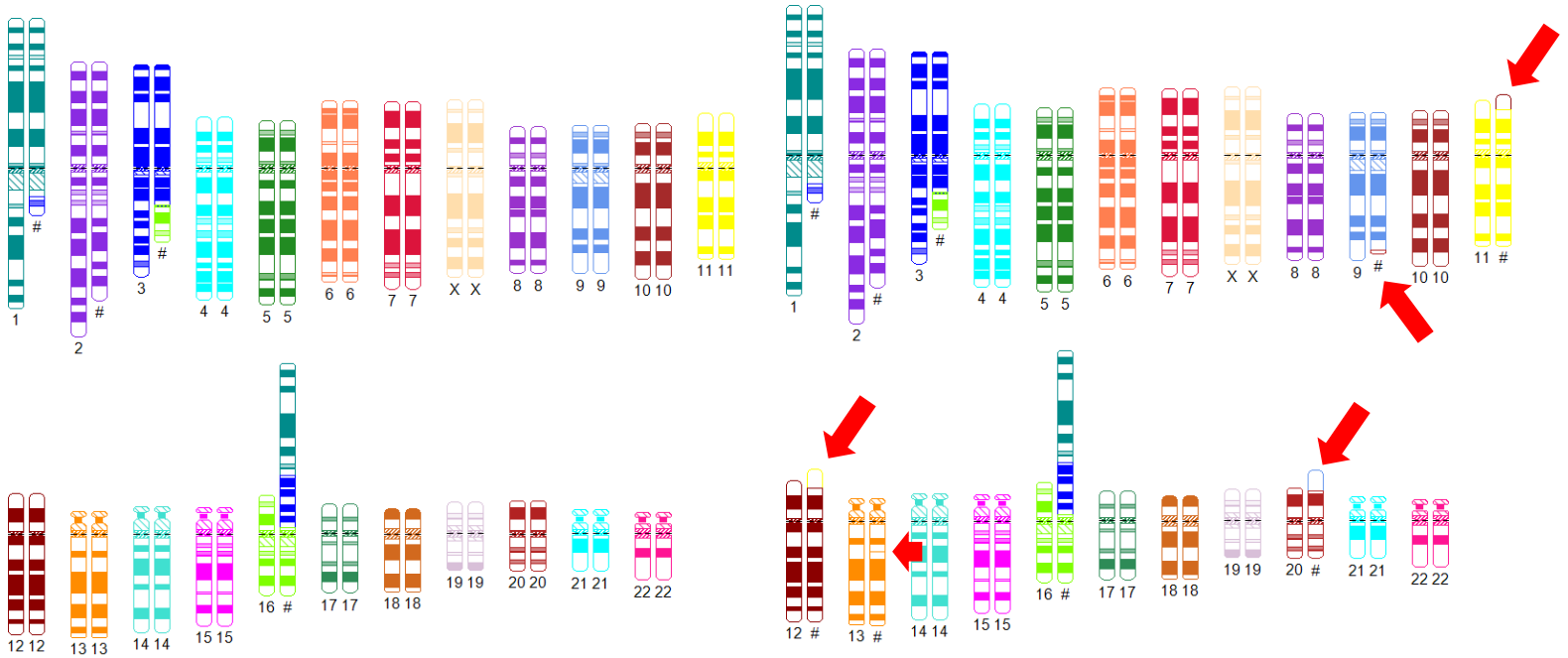


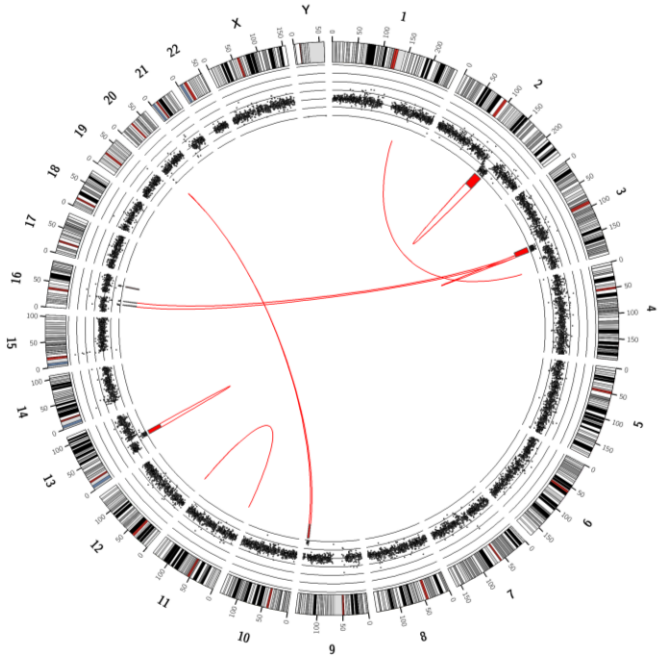
# Detection of additional abnormalities including a *NUP98-KDM5A* fusion by WGS

Chromosome banding analysis:

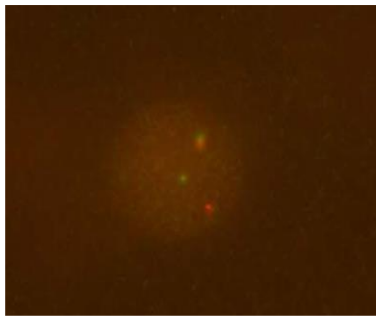
46,XX,der(1)t(1;3)(q21;q27),del(2)(q22q31),
der(3)t(3;16)(q21;p11),
der(16)(1qter->1q21::3q27->3q21::16p11->16qter)

WGS:
46,XX,der(1)t(1;3)(q21;q27),del(2)(q22.3q24.3),der(3)t(3;16)(q21;p11),
der(9)t(9;20)(q34.1;p13),t(11;12)(p15.4;p13.3),del(13)(q14.1q14.3),
der(16)(1qter->1q21::3q27->3q21::16p11->16qter),
der(20)t(9;20)(q34.2;p13)

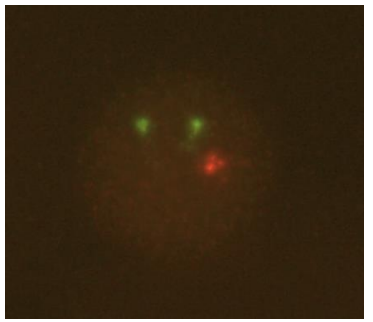




chr12_KDM5A_404739::3752808_NUP98_chr11
chr11_NUP98_3756420::402334_KDM5A_chr12



NUP98 BA



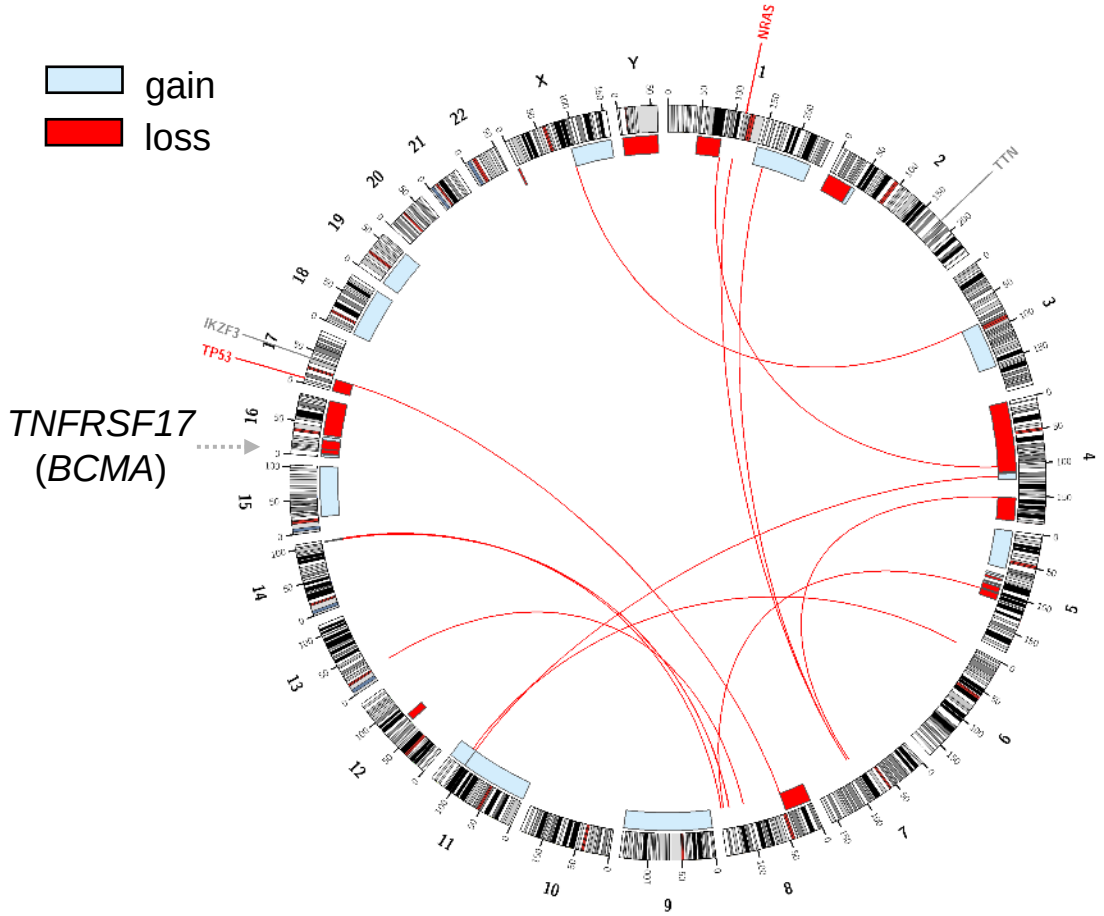
BCR-ABL1

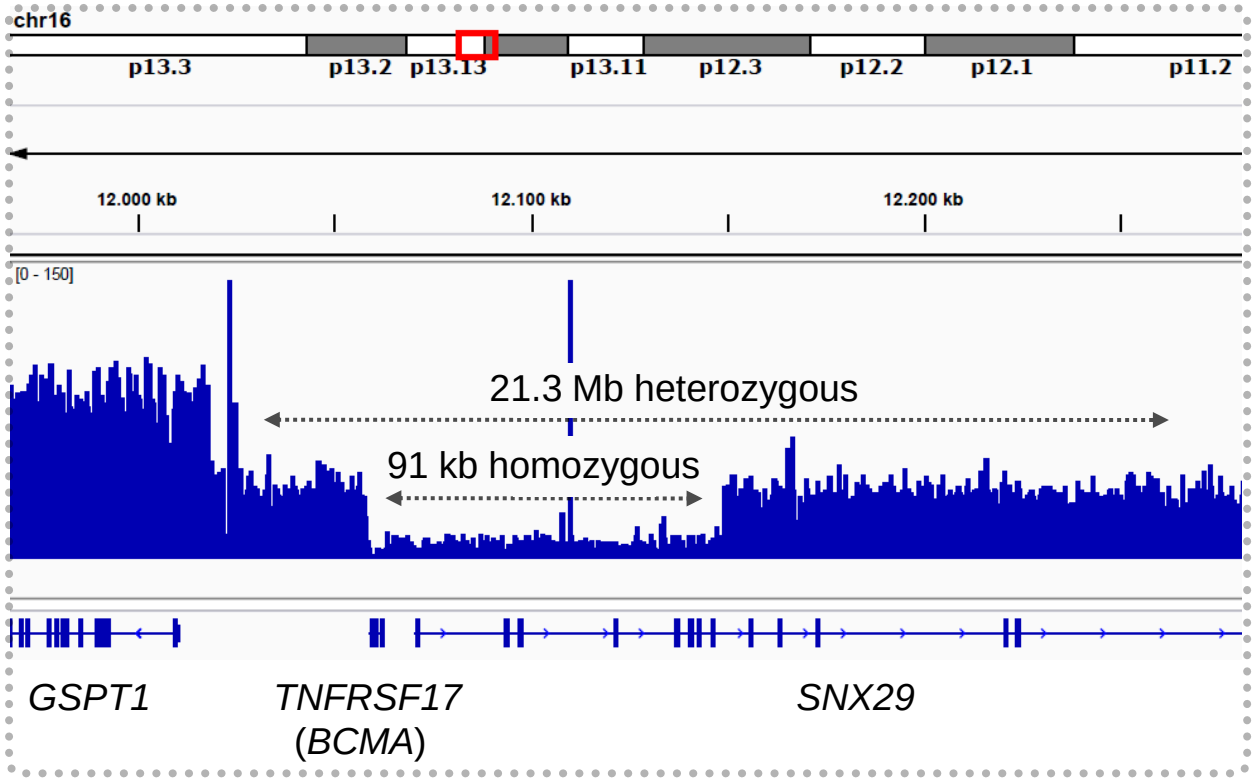
# Diagnostics of tomorrow – WGS

## Detection of biallelic events of therapeutic relevance

71-year-old patient with RRMM, relapse 5 months after BCMA targeting CAR T cell therapy

⇨ WGS: Identification of a homozygous 16p13.13 deletion, incl. *TNFRSF17* (*BCMA*)

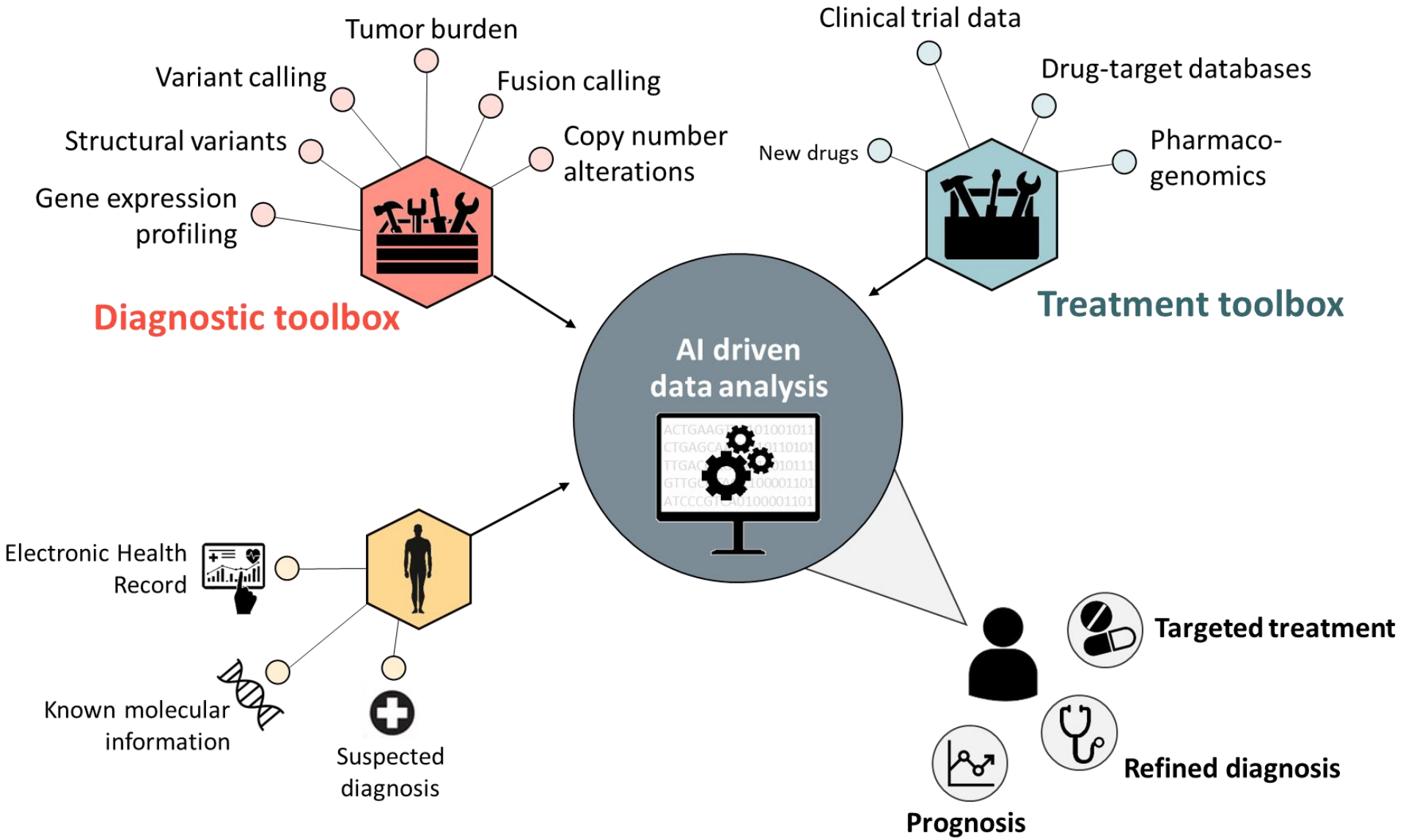





Da Và M *et al.*, Nature Medicine, 27(4), 616-19, 2021

# The vision for tomorrow



# Classification – Prognostication – Prediction

Group diseases according to biological features

## Disease classification

- **Unique parameters** with defined values reflecting the **biology of the disease**
- **Stable**, until new scientific findings are incorporated
- Result: clearly defined **diagnosis**
- Classification is **independent of the therapy**

Group patients according to **risk of progression/death**

## Prognostication

- Includes, in addition to parameters reflecting the biology of the disease, **individual patient parameters** such as age and progression markers, which are weighted against each other
- **Various endpoints**: e.g. overall survival, time to therapy, time to transformation, etc.
- CAVE: the prognosis of the individual patient **depends on the therapy administered**
  → a prognosis score is only valid for therapeutic situations under which it is developed/validated

Group patients according to **response to a specific therapy**

## Response prediction

- **Foresee the response** of a patient to a specific (often targeted) therapy
- Permit the identification of patients with a **higher probability of responding** or not to a specific treatment
- Guide the development of **tailored therapies**

# The Future

**Biomarkers for:**

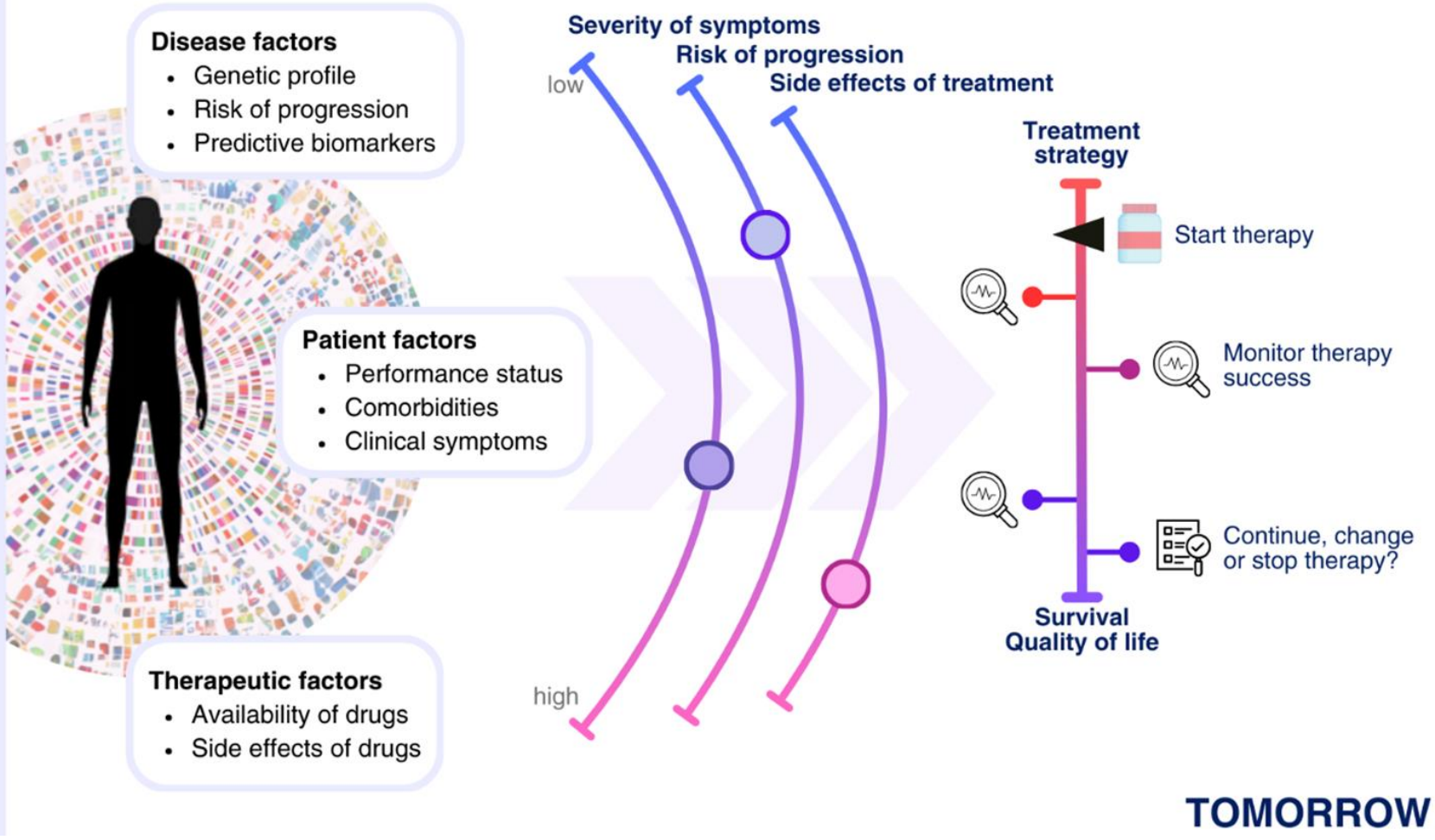
- **Classification**
  - AML
  - MDS
  - CHIP
  - ...

defines use of

- **Prognostic model**
  - ELN 2022
  - IPSS-M
  - ...

- **Targeted therapy**

**TODAY**




**TOMORROW**

# PERSONALIZED MEDICINE