Optical genome mapping refines cytogenetic diagnostics, prognostic stratification and provides new molecular insights in adult MDS/AML patients

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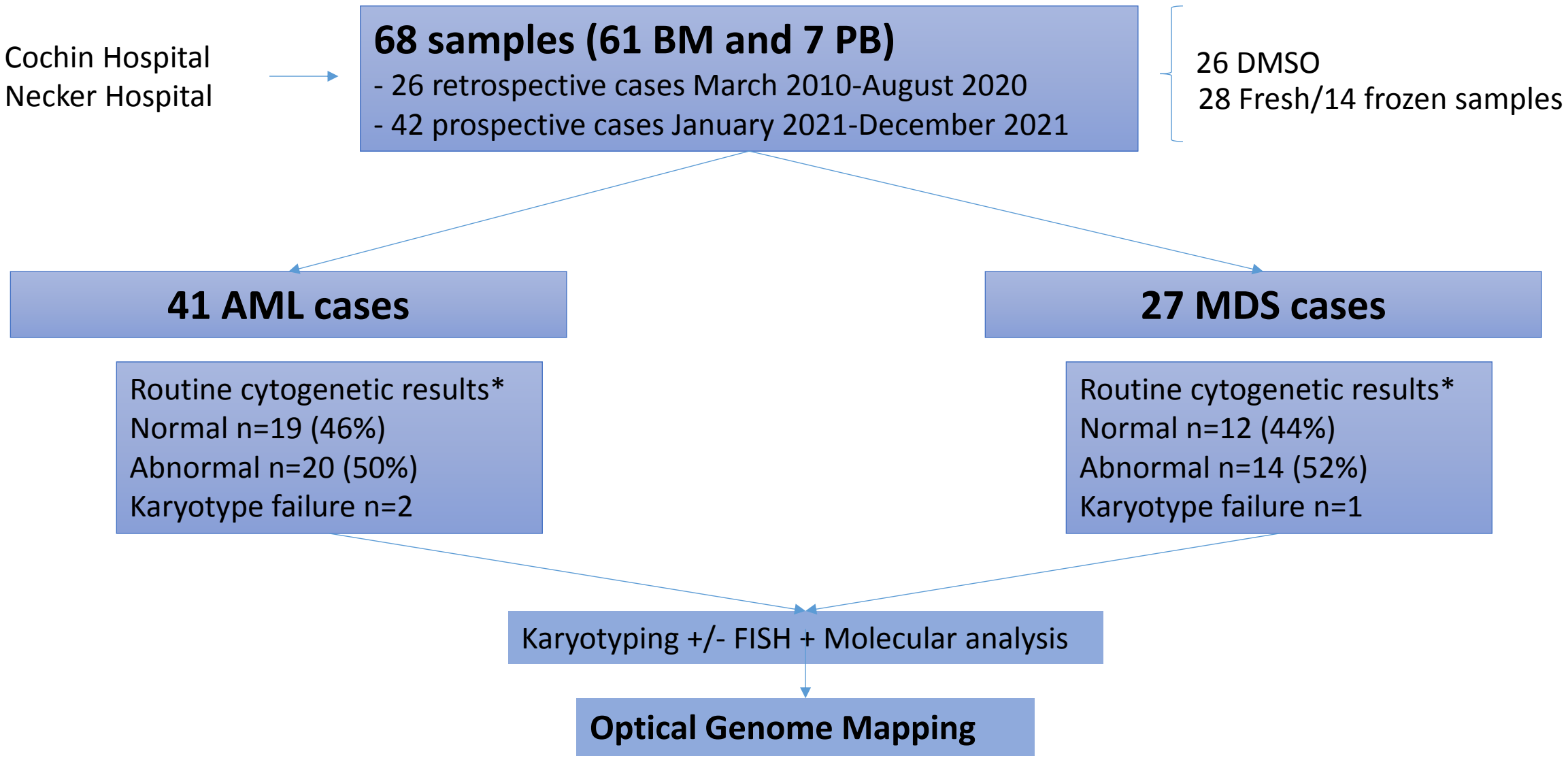
Patients and samples



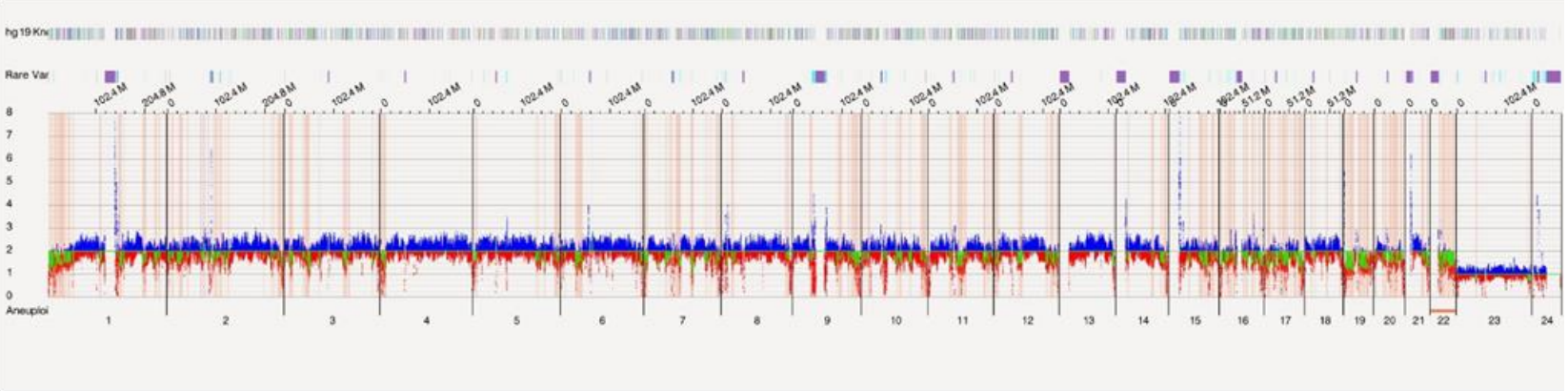
*Adult MDS/AML Patients were selected based on their cytogenetic profile to include a roughly equivalent number of patients with normal or abnormal karyotype in each entity

Purpose of the study

- Evaluation of the performances of OGM in the detection of somatic cytogenetic abnormalities in MDS/AML
- Assessment of the clinical utility of OGM in the risk stratification based on the established international prognostic risk scores in MDS/AML
- Identification of new candidates in MDS/AML pathogenesis

OGM quality data

- Average coverage: 386X (124-581X)
- 12/68 samples: fragmented DNA samples
- For 6 of these cases: background noise made the interpretation of the CNV tool impossible



➢ Artefactual CNVs due to low quality DNA, N50(>150Kb)=0.21 (Patient 19).

➢ **Examples of artefactual abnormalities rendered by OGM**

Data interpretation

Step 1: Prefiltration according to the Bionano Genomics recommended criteria

- Confidence scores: insertion, 0; deletion, 0; inversion, 0.7; duplication, -1; intra-translocation, 0.3; inter-translocation, 0.65, and copy number, 0.99 (low stringency, filter set to 0)
- Size cutoff: 5Kb for insertions/deletions detected by the SV tool, and 500Kbp for the CNV tool
- CNV fractional analysis: <1.8 for deletions > 2.2 for duplications

Step 2: Exclusion of artefactual and polymorphic variants

- Variants supported by less than 5 self molecules
- Variants detected in healthy individuals by comparison to the Bionano Genomics database of 200 human control samples and to the Database of Genomic Variants (DGV)
- Variants overlapping with difficult-to-map regions by comparison to the Bionano Genomics database of masked genomic regions
- Translocations with an incorrect mapping or close to difficult-to-map regions

Step 3: Inclusion of relevant SV and CNVs

- All CNVs with size > 500Kb
- All translocations not considered as artefactual in step 2
- All variants regardless of their size if they overlap one of the genes defined as relevant in malignant hematological diseases

➢ **Flowchart for filtering OGM variants**

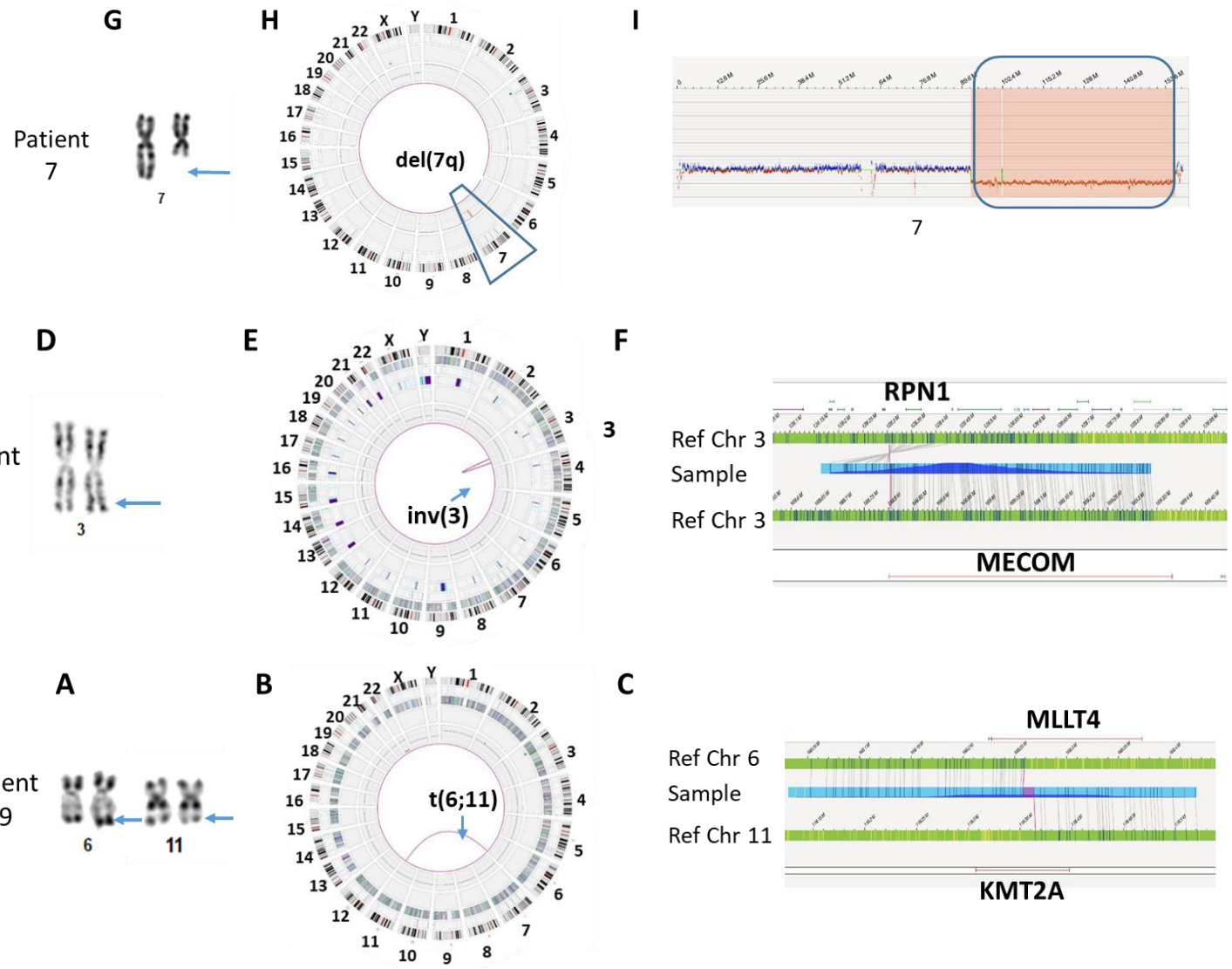
OGM precisely detects most of the significant cytogenetic abnormalities observed by routine cytogenetics

Concordance rate = 95% (53/56)*

*Calculated on cytogenetic abnormalities influencing the MDS and AML risk scores.

Discordant results :
-Y n=1
+8 n=2

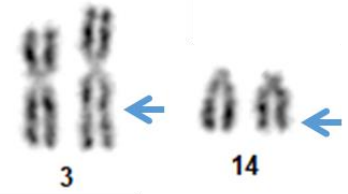
➢ Examples of variants detected by OGM

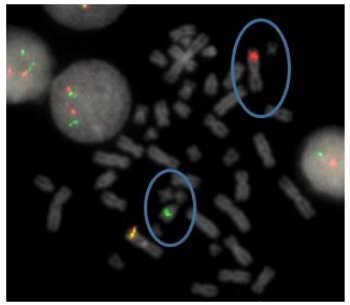


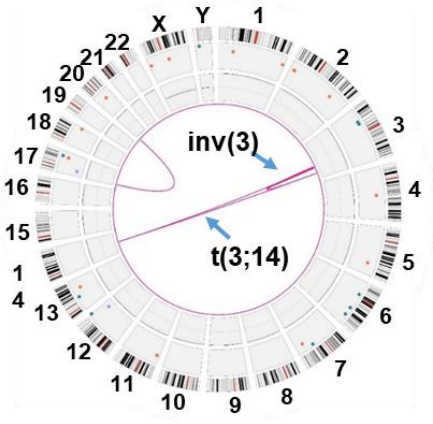
Abnormalities missed by OGM analysis

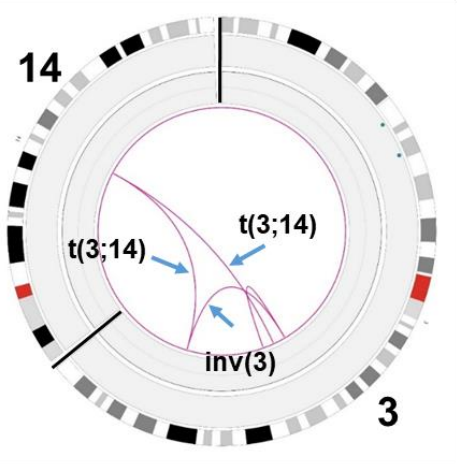
Patient ID	Diagnosis	Karyotype results*	Probable cause for missing the cytogenetic abnormalities
Low subclonal CNVs involving a whole chromosome			
1	AML	47,XX,add(1)(p31),del(6)(q14q22),+8,add(15)(q?),add(18)(p11),add(22)(q21)[12]/47,idem,add(8)(p?21)[6]/48,idem,+5[2]	Selective advantage of tumor sub-clone under culture
25	AML	47,XY,+8[4]/92,XXYY[7]/46,XY[14]	Below the limit of detection of OGM
112	AML	47,XY,+8[18]/48,idem,+13[1]/46,XY[1]	Below the limit of detection of OGM
222	MDS	46,XX,der(21)t(?1;21)(?q12;p11)[12]/47,idem,+8[3]/46,XX[6]	Below the limit of detection of OGM
234	MDS	45,X,-Y[4]/46,XY[16]	Below the limit of detection of OGM
Clone with a gain of a whole batch of chromosomes			
25	AML	47,XY,+8[4]/92,XXYY[7]/46,XY[14]	Tetraploidy not currently detected by OGM
145	MDS	44,XX,add(4)(q32),-7,del(9)(p12),-18[5]/44,idem,del(5)(q13q34)[5]/75,idemx2,-X,-X,-3,-3,-5,-5,-11,-11,-12,-12,-14,-14,-16,-16[cp4]/46,XX[3]	Triploidy not currently detected by OGM
Low subclones			
58	MDS	45~49,XY,t(4;6)(q2?;q2?),del(5)(q11),del(12)(p11p13),-21,+2~4mar[8]/46,XY,add(1)(q31)[2]/46,XY[4]	Below the limit of detection of OGM
59	AML	43,XY,-5,del(6)(q21q25),-7,-17,-18,+mar[16]/42,idem,-6,add(12)(q24),-13,-14,-16,+3mar[3]/46,XY[1]	Below the limit of detection of OGM
SVs which breakpoints located in poorly covered areas			
130	MDS	46,XY,del(5)(q15q34),del(7)(q22q36),add(14)(p10)[5]/46,XY[2]	Breakpoint localized in a non-covered area with the OGM
198	AML	54,XY,+1,del(5)(q21q34),+8,+8,+9,+10,add(14)(p11),+21,+22[6]/46,idem,+i(11q10)[5]	Breakpoint localized in a non-covered area with the OGM
222	MDS	46,XX,der(21)t(?1;21)(?q12;p11)[12]/47,idem,+8[3]/46,XX[6]	Breakpoint localized in a non-covered area with the OGM

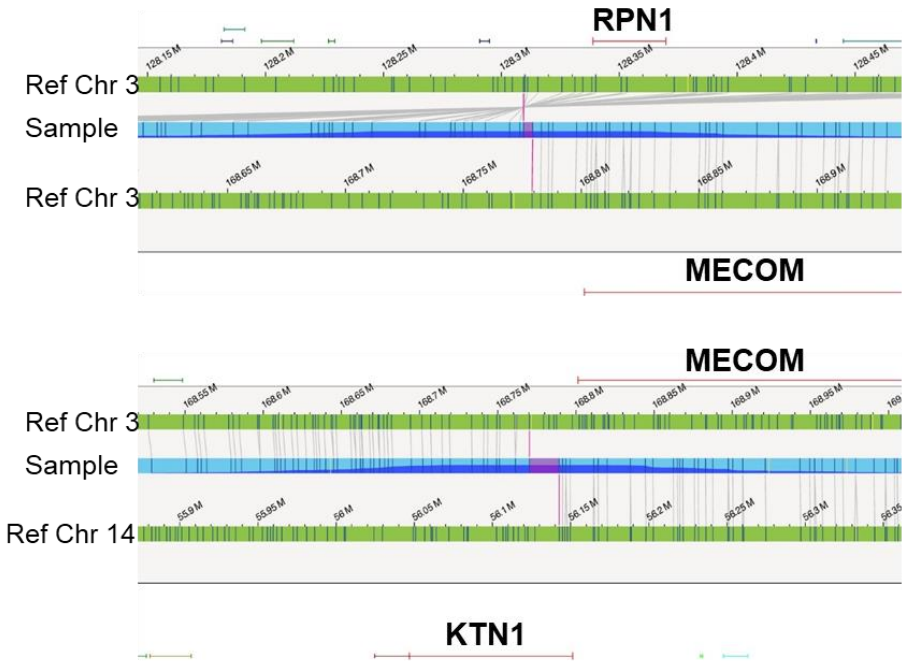
OGM reveals unexpected complexity of some cytogenetic abnormalities







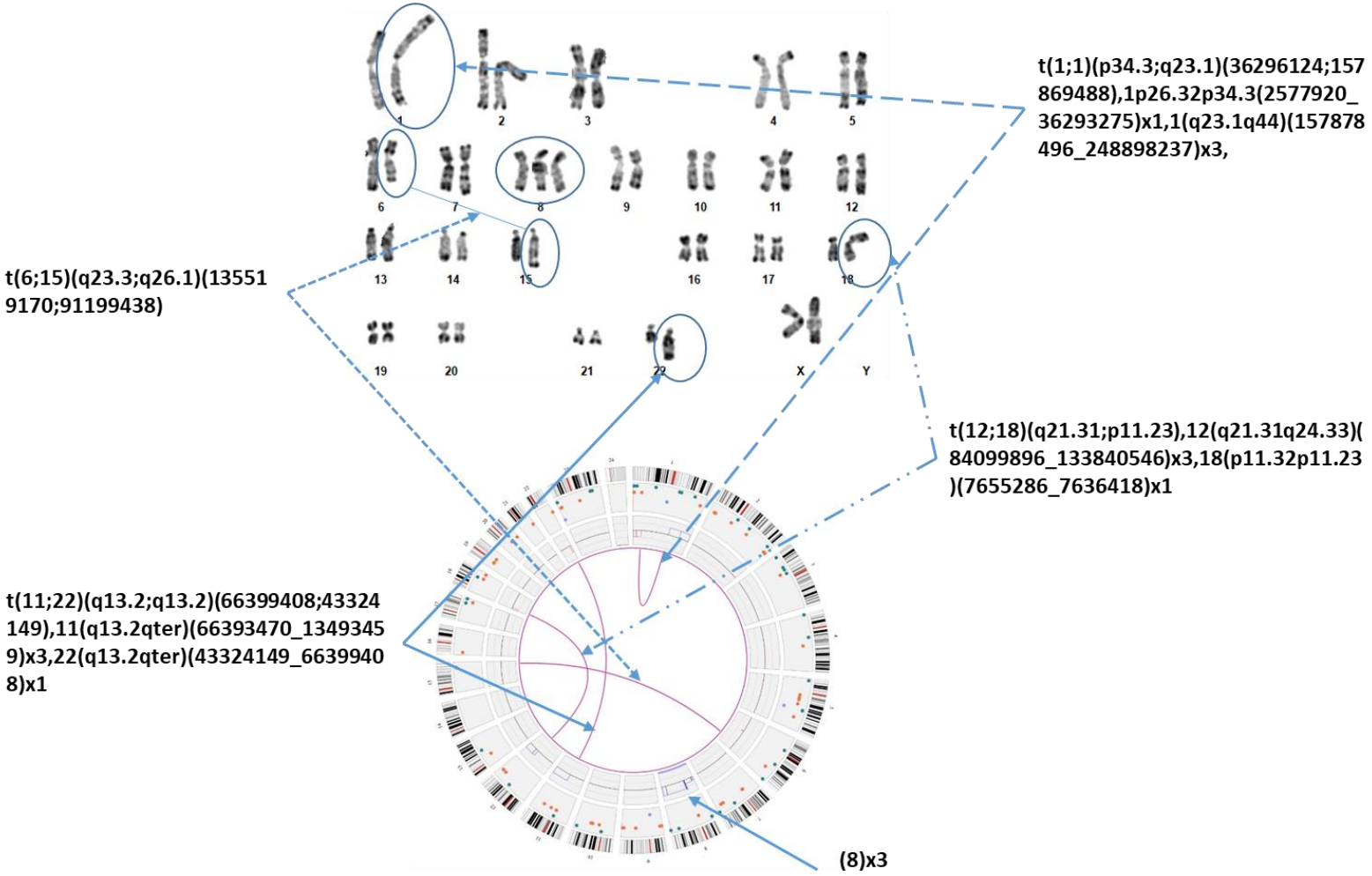




➢ **Example of complex SV affecting *MECOM* gene revealed by OGM (Patient 122)**

OGM resolves chromosomal abnormalities not identifiable by karyotype

47,XX,add(1)(p31),del(6)(q14q22),+8,add(15)(q?),add(18)(p11),add(22)(q21)

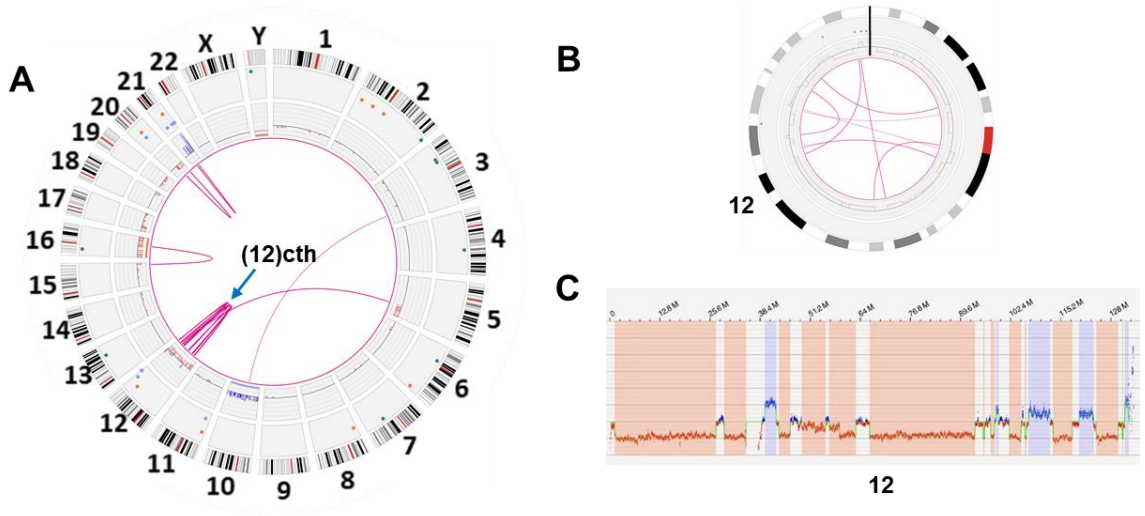


➢ **Example of complex karyotype analyzed by OGM in Patient 1**

OGM identifies recurrent complex rearrangements in complex karyotype

➢ Complex rearrangements involving chromosome 12
n=3/13 pts with complex karyotype

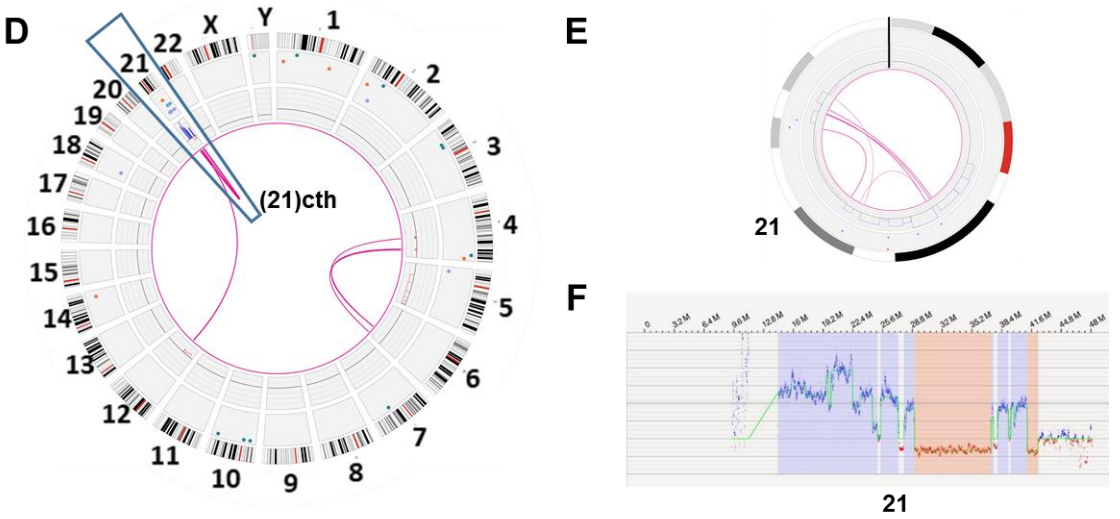
UPN 20



ETV6 deletion: 3/3

➢ Complex rearrangements involving chromosome 21
n=4/13 pts with complex karyotype

UPN 58



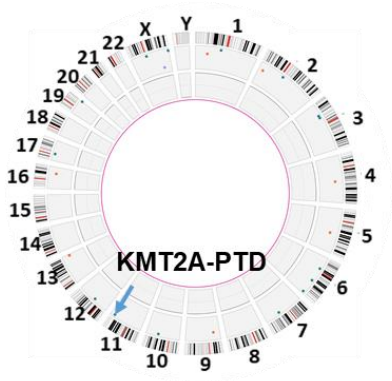
ERG amplification: 4/4 pts
RUNX1 amplification: 3/4 cases

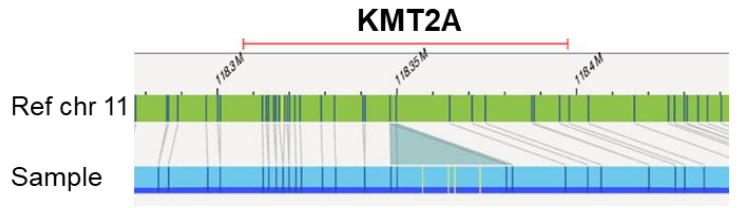
OGM identifies other relevant cytogenetic abnormalities not seen at karyotype

	AML n=41	MDS n=27
Normal karyotype	47.3% (9/19)	16.7% (2/12)
Simple abnormal karyotype (<3 abn)	50% (6/12)	22.2% (2/9)
Complex karyotype (≥3 abn)	87,5% 7/8	100% (5/5)

- KMT2A-PTD n=7/41 AML cases

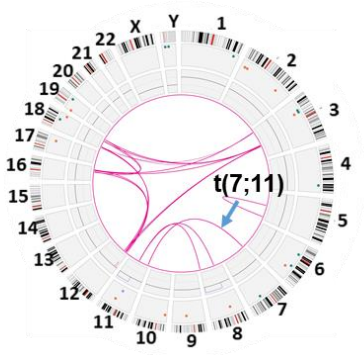
UPN 17

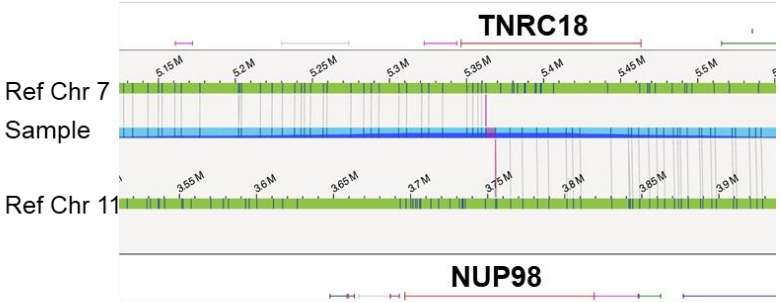




- NUP98 rearrangements n=2/41 AML cases

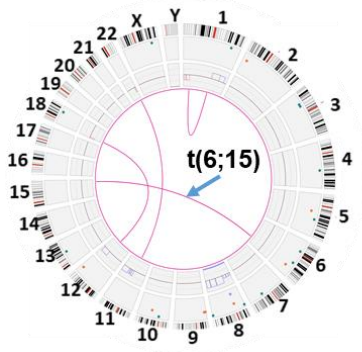
UPN 157

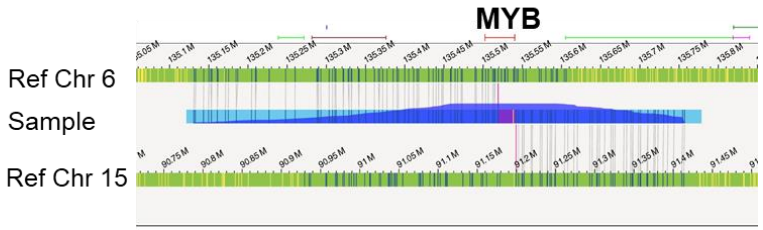




- MYB alterations n=3/41 AML cases

UPN 1





OGM successfully predicts risk score as karyotype in most AML/MDS cases and refines it in a subset of patients

MDS

Cytogenetics
(Karyotype vs OGM)

↓

IPSS-R risk score

Concordance: 21/27

Favorable → Poor n=2
Intermediate → very poor n=1
Intermediate → Poor n=1
Intermediate → Favorable n=1
Very Favorable → Favorable n=1

AML

Mutational data
+
Cytogenetics
(Karyotype vs OGM)

↓

ELN risk score

Concordance: 39/41

Adverse → Intermediate n=1
Favorable → Intermediate n= 1

Discussion

- Technical advantages of OGM
  - Minimum technical delay for OGM results is 6-7 days
  - Useful in case of karyotype failures (1,5 million cells, non-cultured cells)

- Technical limitations of OGM
  - Dependent on the quality of the DNA
  - Difficulty to map abnormalities in poorly covered areas of the genome
  - No detection of low subclonal CNVs involving whole chromosomes
  - No detection of independent clones

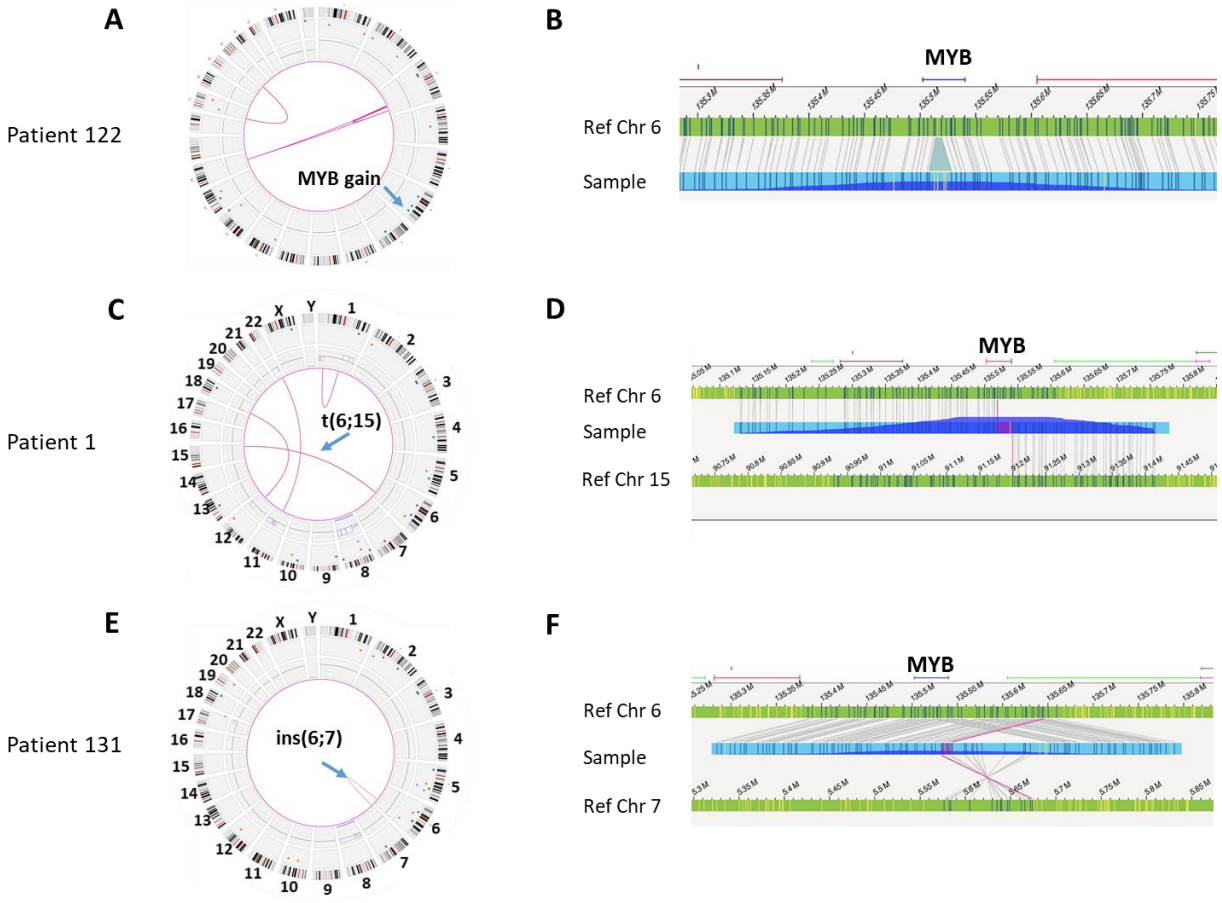
Discussion

- Biological advantages of OGM
  - detection of balanced cytogenetic abnormalities, unlike CGH/SNP array technology.
  - detection of unbalanced cytogenetic abnormalities, with a higher sensitivity than CGH/SNP array analysis.

  →Concordance rate = 95% (53/56)
  - elucidation of poorly identified or unidentified karyotype abnormalities due to poor karyotype quality and/or complex nature of abnormalities.
    - e.g. complex rearrangement and chromothripsis of chromosome 12 (n=3) or 21 (n=4)
  - detection of cryptical balanced and unbalanced cytogenetic abnormalities not observed in the karyotype.
    - Detection of cytogenetic abnormalities not seen at routine cytogenetics in 33% (9/27) and 53% (22/41) of the MDS and AML respectively.
    - Detection of recurrent pathogenic SVs such as *NUP98* rearrangement, KMT2A-PTD, and MYB cytogenetic abnormalities.

Discussion

- Detection of new candidates as MYB gene



RESEARCH LETTER

blood advances

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TO THE EDITOR:

Myb drives B-cell neoplasms and myeloid malignancies in vivo

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Perspectives

- Identification of new cytogenetic abnormalities
  - Significance
  - Prognosis
  - Interpretation of variants of unknown significance

- Integration of OGM in the diagnostic workup of hematological diseases
  - International rules for OGM interpretation and nomenclature
  - Definition of complexity for OGM vs karyotype
  - New prognostic risk scores integrating OGM and mutational data
  - Place of OGM in the diagnostic work-up of AML and MDS samples

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