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Case 1

Monthly Multi-Institutional Hematopathology Interesting Case Conference

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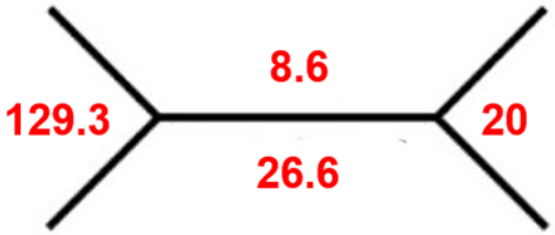
PGY-3

Clinical Information

- A 3-year-old male with no significant history presented for one-week of joint pain, difficulty walking, and refusal to move, and accompanied by fatigue, bruising, and fever.
- CBC showed marked leukocytosis with increased blasts as follows: WBC: 129.3 k/ul, Hemoglobin: 8.6 g/dl; Platelets: 20 k/ul; 44% blasts.
- Absolute neutrophil counts were elevated: 30.9 k/uL and 16.8 k/uL on repeat testing.

- Labs:



Parameter	Result	Neutrophils (%)	10
Red Blood Cell Count	3.51	Bands (%)	3
Mean Corpuscular Volume	75.8	Lymphocytes (%)	35
Mean Corpuscular Hemoglobin	24.5	Monocytes (%)	9
Mean Corpuscular Hemoglobin Conc.	32.3	Eosinophils (%)	1
RDW-CV	20.8	Basophils (%)	0
RDW-SD	55.4	Blasts (%)	44
Platelet Count	20	Granulocytes, Immature (%)	2
		Promyelocytes (%)	2

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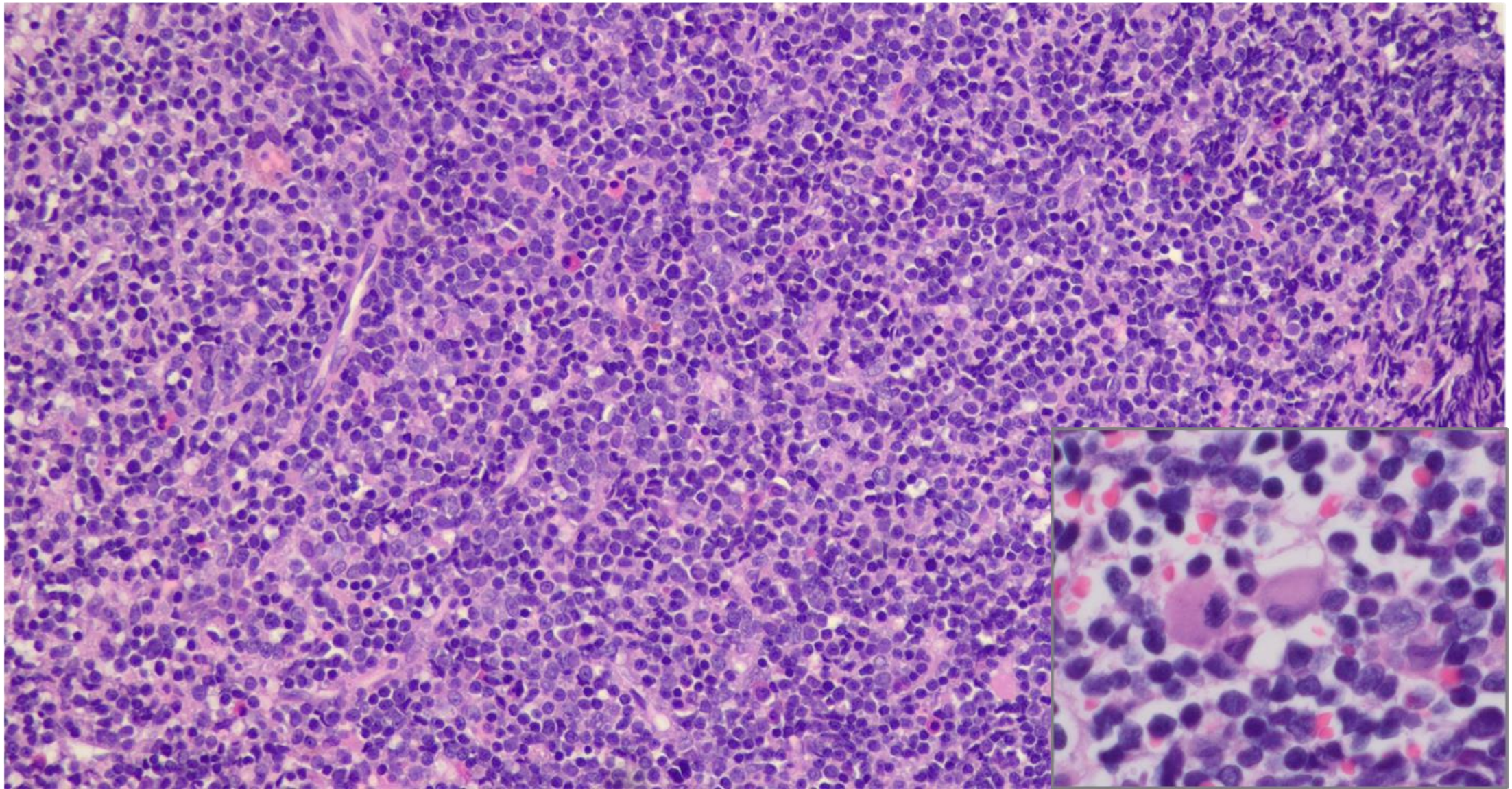
Microscopic Findings: Bone Marrow Core Bx

H&E, x200



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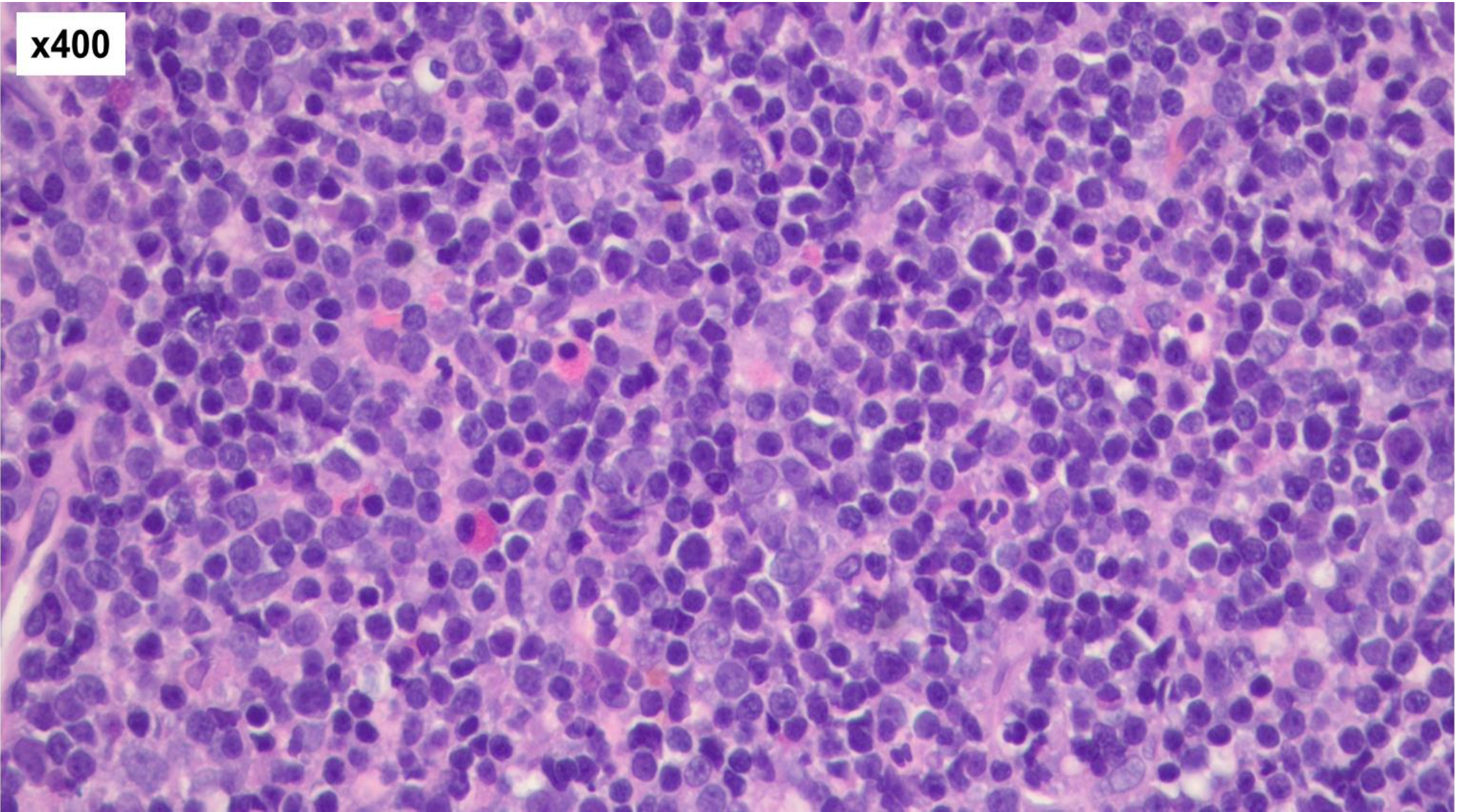
Microscopic Findings: Bone Marrow Core Bx



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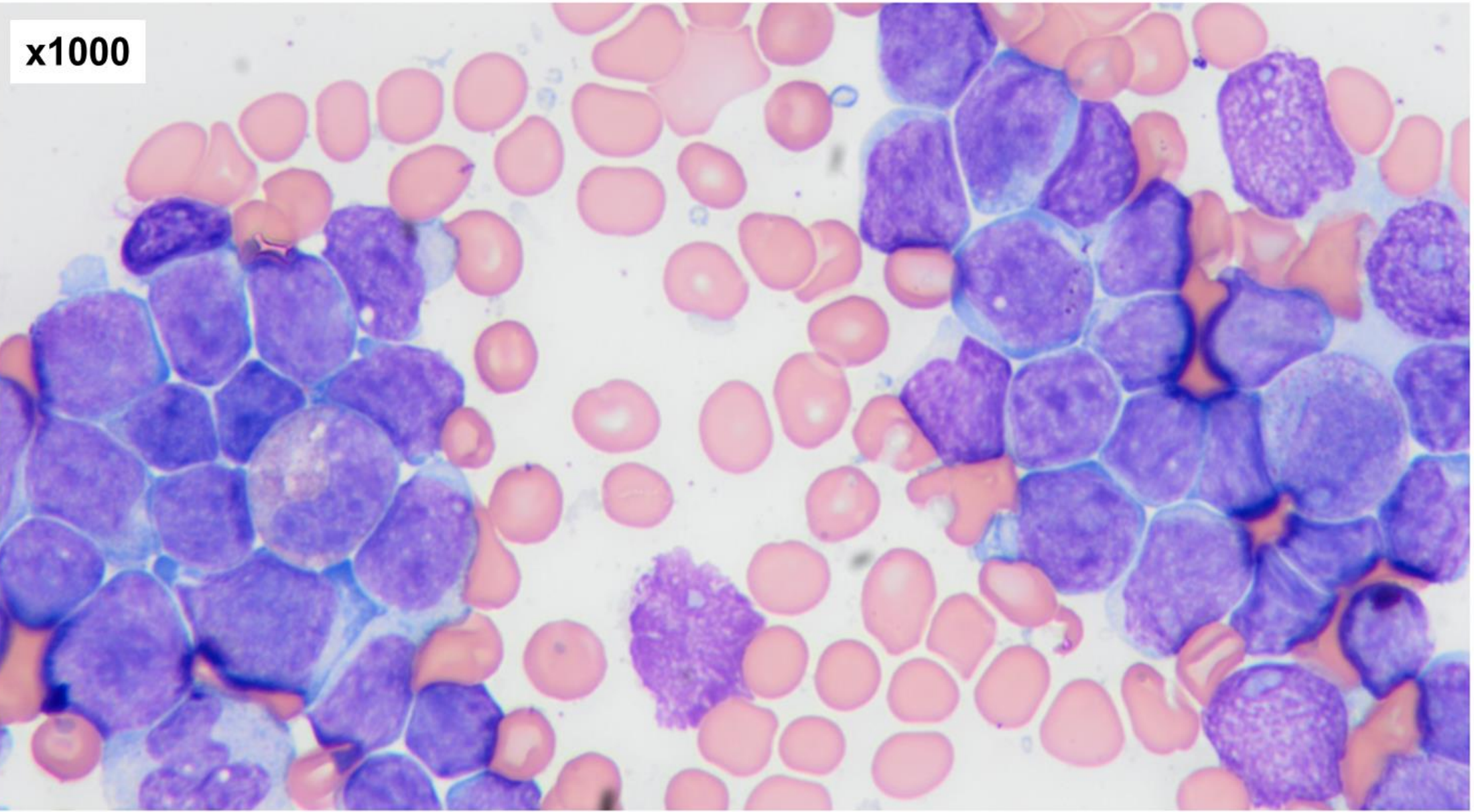
Microscopic Findings: Smear



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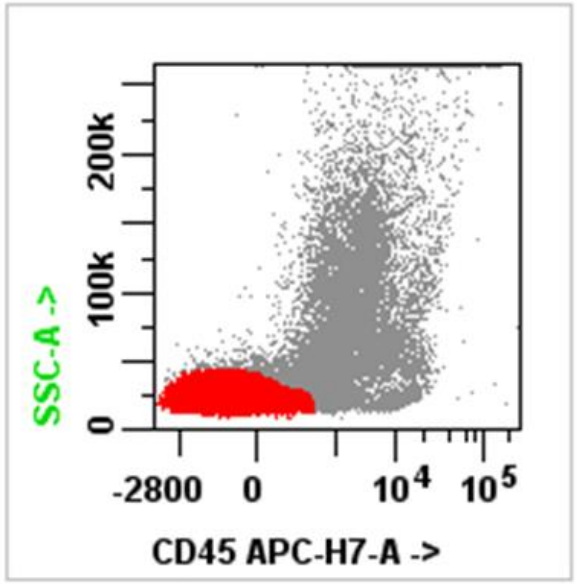
Immunophenotype



Markers	Expression
CD45	Largely negative
CD19	Positive
CD20	Few positive (3.8%)
CD10	Bright positive
CD34	Positive
CD13	Negative
CD33	Negative
CD38	Partial dim positive
HLA-DR	Positive
MPO	Negative
TdT	Positive
Surface Kappa/Lambda	Negative
s/cCD3	Negative

Cell Population	Percentage
Blasts	67%
Maturing Granulocytes	12%
Monocytes	2.80%
Small T Lymphocytes	1.10%

- MRD flow cytometric analysis detected 0.20% blasts after induction therapy (AALL1732).

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Cytogenetics

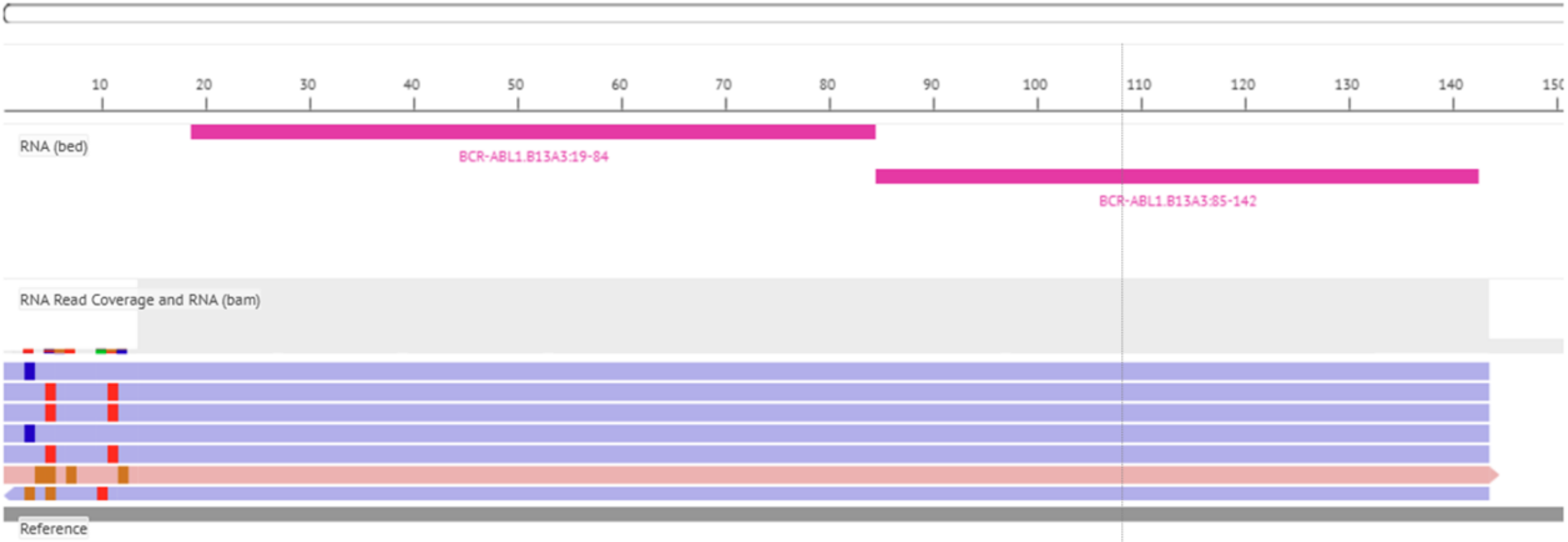
- Karyotype: 46,XY,t(9;22)(q34;q11.2)[16]/47,idem,+der(22)t(9;22)[2]/46,XY[4]

- FISH detected BCR-ABL1 fusion in 96.5% of cells and one copy of TCF3 in 10.5% of cells. Other Genes: Normal signal patterns for CRLF2, PBX1/TCF3, chromosomes 4, 10, 17, IKZF1, CDKN2A, KMT2A, ETV6/RUNX1, and IGH.

- FISH detected t(9;22) BCR::ABL1 in 41% of **flow sorted granulocytes**.

Molecular Studies

- MyeloSEQer NGS: BCR/ABL1 (chr22:23631808-chr9:133730188) fusion (**b2a3/e13a3**)



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BCR exon	*ABL1* exon	*BCR::ABL1* transcript type	Protein size (kDa)	Typical and atypical breakpoints and transcripts { 15703785 ; 29285010 }	Frequency in CML (overall and among the non-atypical major breakpoint cluster region[a]) { 29974949 ; 30675008 }	Frequency in *BCR::ABL1*+ B-ALL { 18055996 ; 26942999 ; 31595038 ; 32237084 }
e13 (b2)	a2	e13a2 (b2a2)	p210	Major breakpoint cluster region	~37.9%	17.50%[b]
e14 (b3)	a2	e14a2 (b3a2)	p210	Major breakpoint cluster region	~62.1%	12.5%[b]
e1	a2	e1a2	p190	Minor breakpoint cluster region	< 1%, 16.9%	~70%
e19	a2	e19a2	p230	Micro breakpoint cluster region	< 1%, 39.8%	Not available
e1	a3	e1a3	p190	Atypical/variant transcript	< 1%, 1.2%	< 1%
e13	a3	e13a3 (b2a3)	p203	Atypical/variant transcript	< 1%, 7.2%	< 1%
e14	a3	e14a3 (b3a3)	p203	Atypical/variant transcript	< 1%, 13.3%	< 1%
e6	a2	e6a2	p195	Atypical/variant transcript	< 1%, 3.6%	< 1%
e8	a2	e8a2	p200	Atypical/variant transcript	< 1%, 8.4%	Not available
e12	a2	e12a2	Not available	Atypical/variant transcript	< 1%, 1.2%	Not available
e1	a8	e1a8	p200	Atypical/variant transcript	Not available	< 1%

Molecular Studies (continued)

- Foundation one heme: BCR(NM_004327)-ABL1(NM_005157) fusion (B14*; A3) and IKZF1 loss exons 2-3
- Clonal immunoglobulin and T-Cell Receptor (TCR) gene rearrangements detected by PCR.
- Quantitative ddPCR test using e1a2 primer for BCR::ABL1 transcript (p190) was negative.
- After induction therapy: residual sequences detected estimated MRD value: 1,101 residual clonal cells per million nucleated cells (Range: 728 - 1,629).

Diagnosis?

- I.C.C. 2022:
  - B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2)/*BCR::ABL1* and multilineage involvement.

- W.H.O. 2022:
  - Chronic myeloid leukemia, blast phase (B-lymphoblastic leukemia).

The International Consensus Classification of B-ALL

B-ALL with (9;22)(q34.1;q11.2)/BCR::ABL1
with lymphoid only involvement
with multilineage involvement

B-ALL with t(11;19)(q23.3)/KMT2A rearranged
B-ALL with t(12;21)(p13.2;q22.1)/ETV6::RUNX1
B-ALL, hyperdiploid
B-ALL, low hypodiploid
B-ALL, near haploid
B-ALL with t(5;14)(q31.1;q32.3)/IL3::IGH
B-ALL with t(1;19)(q23.3;p13.3)/TCF3::PBX1
B-ALL, BCR::ABL1-like, ABL1 class rearranged
B-ALL, BCR::ABL1-like, JAK-STAT activated
B-ALL, BCR::ABL1-like, NOS
B-ALL with iAMP21

B-ALL with MYC rearrangement
B-ALL with DUX4 rearrangement
B-ALL with MEF2D rearrangement

B-ALL with ZNF384(362) rearrangement

B-ALL with NUTM1 rearrangement
B-ALL with HLF rearrangement
B-ALL with UBTF::ATXN7L3/PAN3, CDX2 ("CDX2/UBTF")
B-ALL with mutated IKZF1 N159Y
B-ALL with mutated PAX5 P80R

Provisional entities:
B-ALL, ETV6::RUNX1-like
B-ALL, with PAX5 alteration
B-ALL, with mutated ZEB2 (p.H1038R)/IGH::CEBPE
B-ALL, ZNF384 rearranged-like
B-ALL, KMT2A rearranged-like B-ALL, NOS

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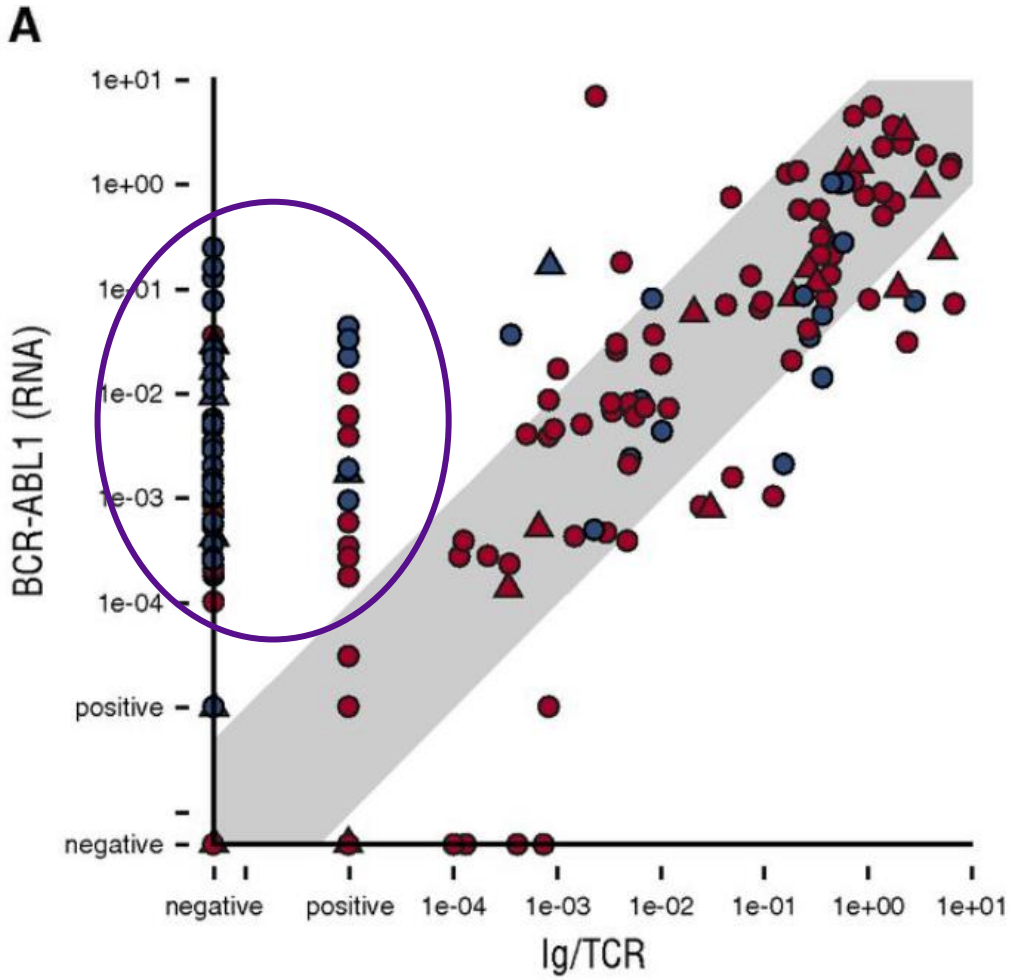
Monitoring of childhood ALL using *BCR-ABL1* genomic breakpoints identifies a subgroup with CML-like biology

(Blood, 2017;129:2771)

Lenka Hovorkova,[1,2] Marketa Zaliova,[1-3] Nicola C. Venn,[4] Kirsten Bleckmann,[5] Marie Trkova,[6] Eliska Potuckova,[1,2] Martina Vaskova,[1,2] Jana Linhartova,[7] Katerina Machova Polakova,[7] Eva Fronkova,[1,2] Walter Muskovic,[4] Jodie E. Giles,[4] Peter J. Shaw,[8] Gunnar Cario,[5] Rosemary Sutton,[4,9] Jan Stary,[2,3] Jan Trka,[1-3] and Jan Zuna[1-3]



MRD Discordance

- **Many cases are negative (or slightly positive) by IgH/TCR PCR**
- **BUT have high levels of BCR::ABL1 transcript**

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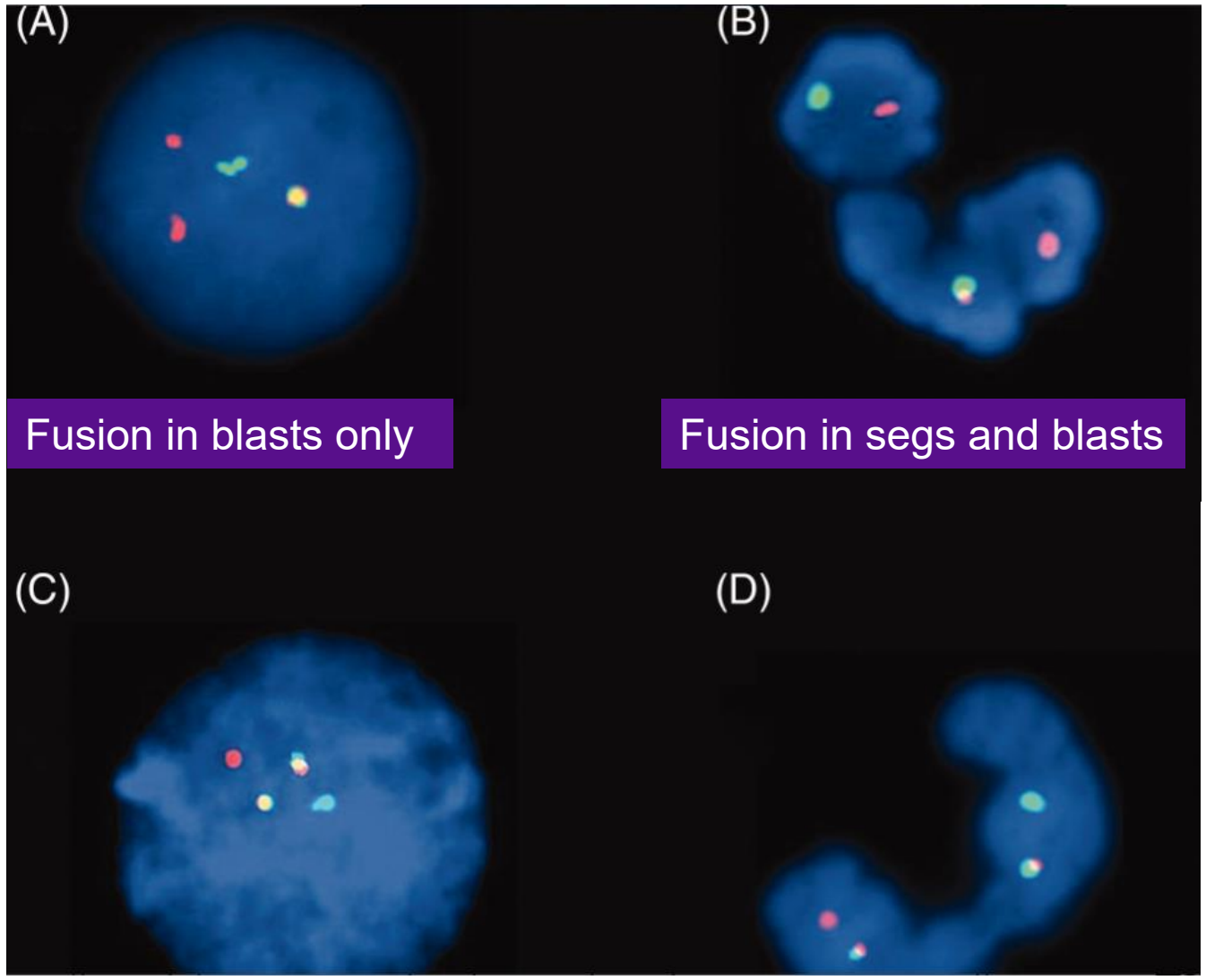
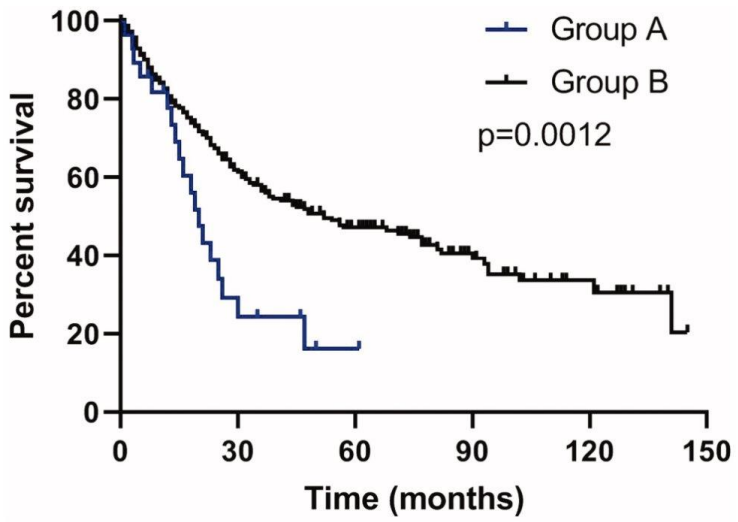
Sorted FISH BCR::ABL1 Fusion Patterns



Fusion in blasts only (lymphoid only disease)

Fusion in sorted segmented granulocytes and blasts (multilineage disease) similar to CML blast phase



Chen et al. *Leuk Lymphoma* 61:12, 2831

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CML Blast Phase vs Ph+ B-ALL with multilineage involvement

- The multi-lineage pattern resembles CML in blast phase.
- However, these cases are distinct because:
  - They present de novo, not from progression of chronic-phase CML.
  - They occur much more frequently in children.
- Cannot be distinguished simply based on p190 and p210.

BCR::ABL1 Transcript Variants

- Most common BCR::ABL1 transcripts in CML are:e13a2 (b2a2) and e14a2 (b3a2) found in >95% of CML (p210)
- Most common transcript in Ph+ B-ALL: e1a2 (p190)
- Rare transcripts lacking ABL1 exon 2, such as in our case: e13a3 (b2a3) and e14a3 (b3a3) found in <1% of CML (p203)
  - More commonly seen in CML in adults, not in de novo childhood ALL
  - Despite the unusual transcript, the immunophenotype is clearly B-ALL in our case

Summary

- Diagnostic challenge: Overlap between Ph-positive B-ALL with multilineage involvement and CML in lymphoid blast phase.
- Evaluation of BCR::ABL1 fusion on flow sorted myeloid cells is helpful to determine multilineage involvement.
- Ph-positive B-ALL carries a high risk of multilineage involvement, making it critical to distinguish lymphoid-only from multilineage cases, as this distinction may carry significant clinical implications.
- Evaluating BCR::ABL1 fusion in myeloid cells is essential for accurate diagnosis and treatment planning in Ph+ B-ALL

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Thank You

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