SH2017-0124

A CASE OF PERSISTANT NEUTROPHILIA: *BCR-ABL* NEGATIVE

John R Goodlad[1], Pedro Martin-Cabrera[2], Catherine Cargo[2]

1. Department of Pathology, NHS Greater Glasgow & Clyde, QEUH, Glasgow
2. Haematological Malignancy Diagnostic Services, St James's University Hospital, Leeds

CLINICAL HISTORY

- **An 80 year old male presented with anaemia and persistently raised white cell count (70.2 x 10^9/l)**

- **Blood film**
  - **Differential:**
    - **Neutrophils 81% (57 x 10^9/l)**
    - Lymphocytes 3.6%
    - Monocytes 2%
    - Eosinophils 2%
    - **Promyelocytes 1.6%**
    - **Myelocytes 4.3%**
    - **Metamyelocytes 5%**
    - **Blasts 0.5%**
    - No basophils

    11.4%
  - **Neutrophil morphology:**
    - Some very well granulated/toxic granulation
    - Some dysplastic
      - Hypogranular cytoplasm
      - Hypolobated nuclei
      - Pseudo-Pelger Huet forms

- **Molecular testing:**
  - No evidence of *BCR-ABL* fusion
  - *CALR* normal
  - *JAK2 V617F* normal

BONE MARROW ASPIRATE AND TREPHINE

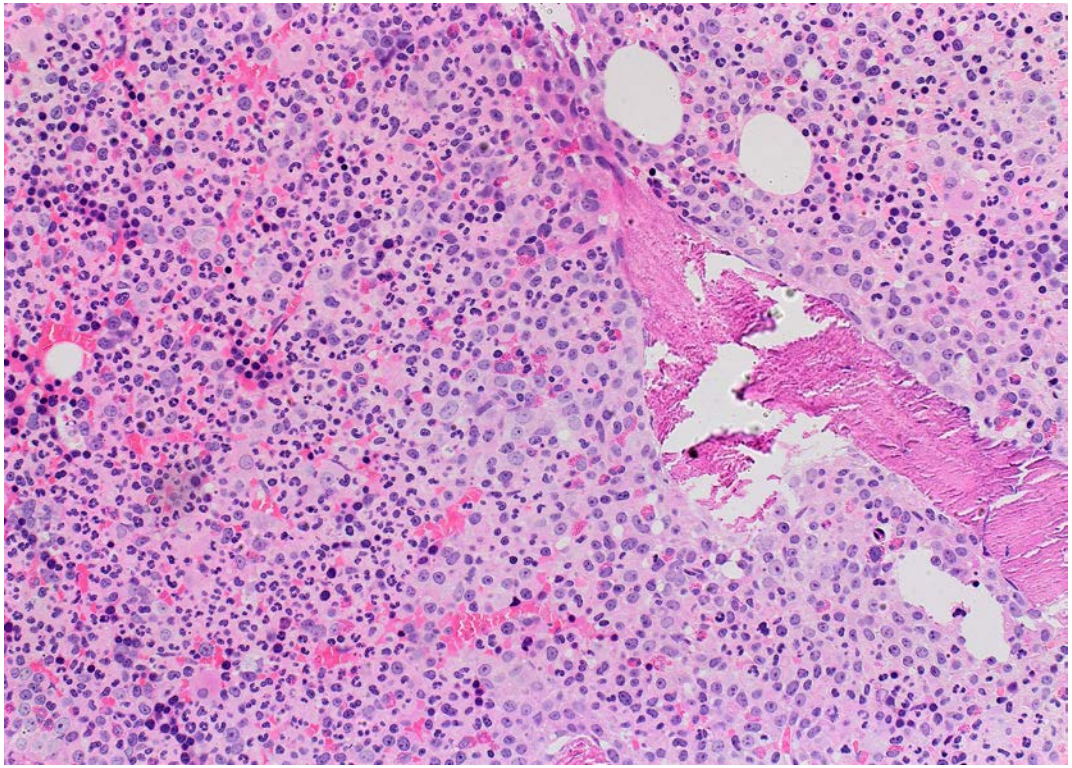
Flow Cytometry:

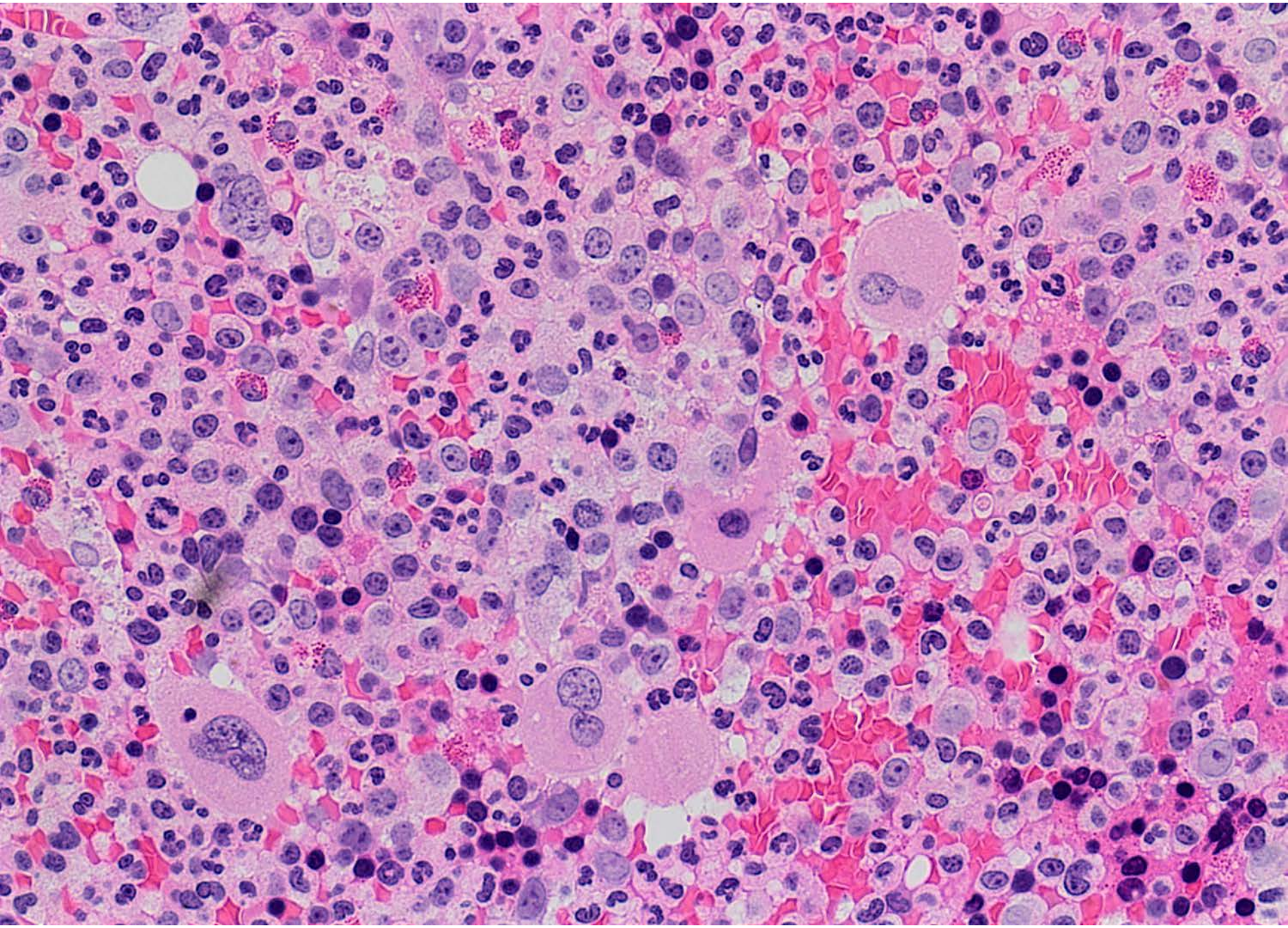
Neoplastic myeloid progenitors = 1.0% of total cells, composite phenotype:

- CD45+CD34+CD117+ CD15-CD13+HLADR+ CD33+/-CD7+/- CD64-CD56-
- Monocytic cells (CD64+CD14(78%)+CD56-) = 3.44% of total cells.

Trephine:

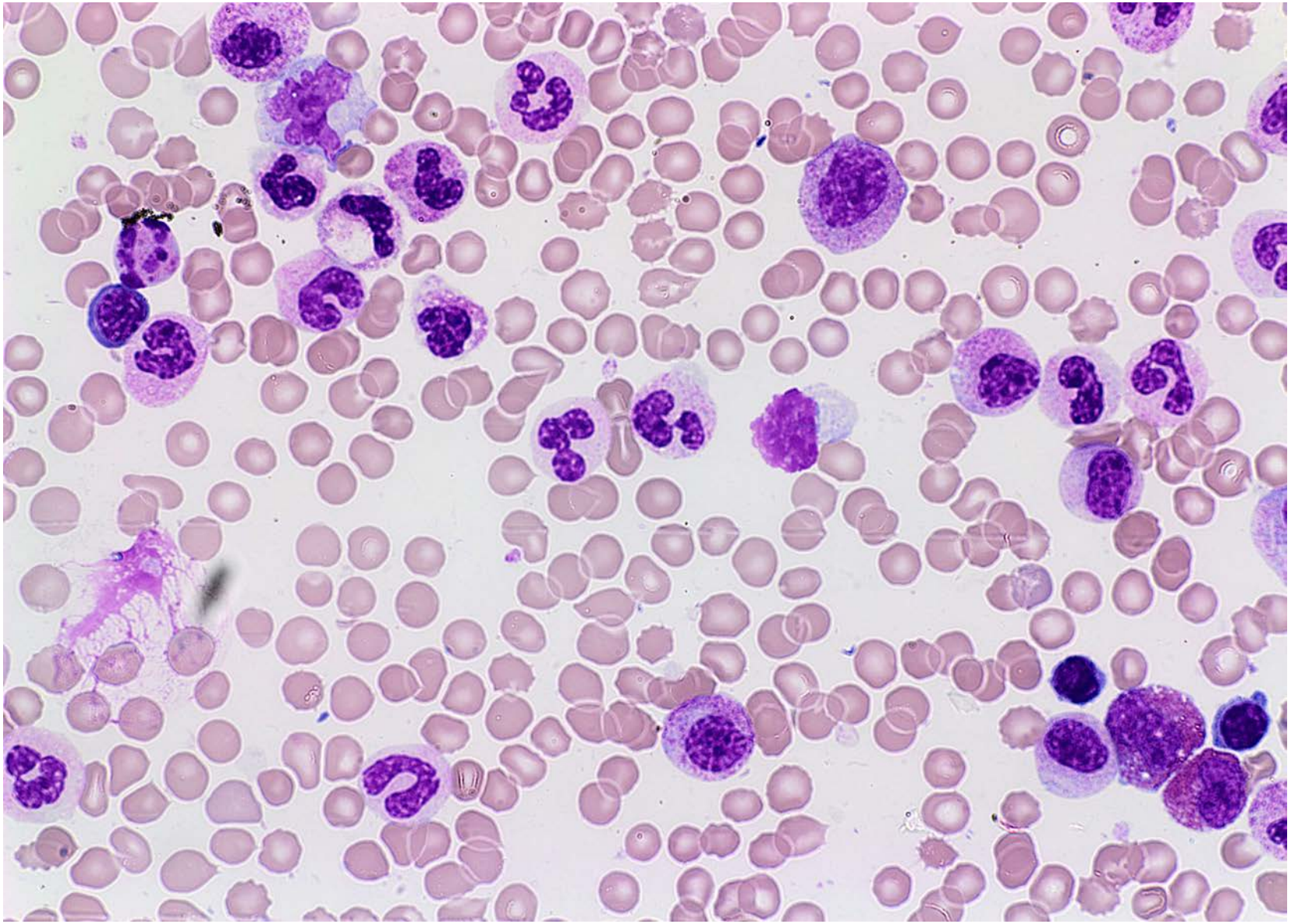
Hypercellular marrow with expanded myeloid series and increased and atypical megakaryocytes

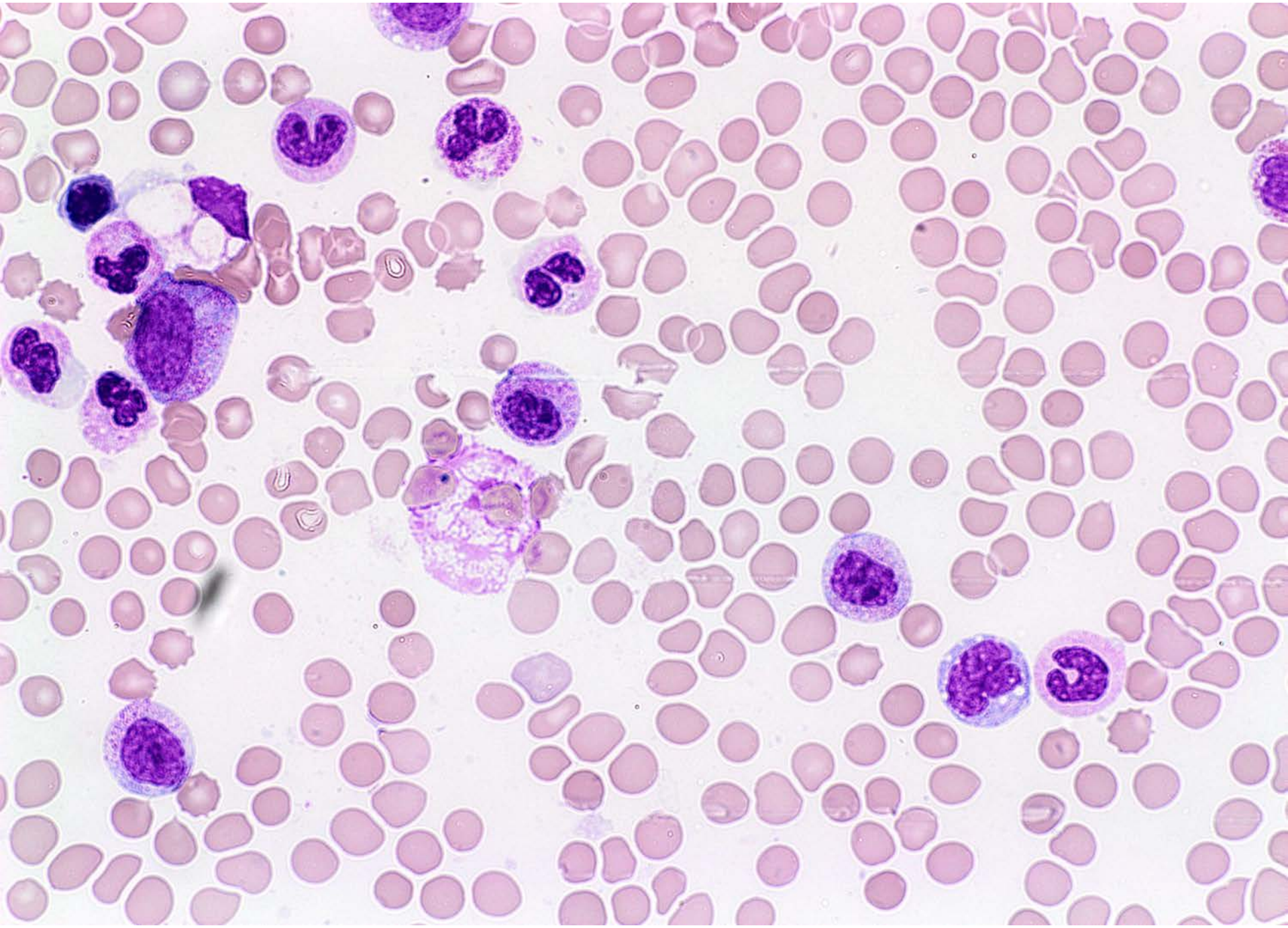




ASPIRATE

Dysplastic changes in myeloid series (no ring sideroblasts)





ADDITIONAL RESULTS

Cytogenetic/FISH:

- Normal male karyotype

Targeted High throughput sequencing:

- *CSF3R* mutated (p.Thr618Ile, c1853C>T)
- *SETBP1* mutated (p.Asp868Asn, c2602G>A)

Sanger sequencing:

- *SRSF2* mutated (p.P95L, c.284C>T)
- *MPL* exon 10 normal

High Throughput Sequencing Report

Sample: H21399/16

Gene Panel (370 amplicons covering 27 genes)

Functional Pathway	Gene
DNA Methylation	TET2, DNMT3A, IDH1, IDH2
Chromatin Modification	ASXL1, EZH2
Splicing	SF3B1, SRSF2, U2AF1, ZRSR2
Transcription Factors	NPM1, RUNX1, BCOR, WTI, TP53
Signalling	FLT3, NRAS, KRAS, CBL, cKIT, JAK2, MPL, CSF3R, STAT3
Cohesin complex	STAG2
Other	SETBP1, CALR

Test Specification

Library preparation:	Fluidigm 48x48 Access Array
Sequencing:	Illumina MiSeq 300v2
Analysis:	In-house pipeline, aligned to GRCh37p13, annotated using Ensembl VEP (v76)
Reporting thresholds:	Minimum coverage X100 Minimum variant allele fraction (VAF) 5%
Annotation Database:	Catalogue of Somatic Mutations in Cancer (COSMIC) http://grch37-cancer.sanger.ac.uk/cosmic

Results Summary

(please refer to Page 2 for evidence summary)

1. Variants with indicative pathogenicity

Sample	Variant	VAF	Read Depth	Consequence	CDS	Protein	Annotation
H21399/16	CSF3R_1_36933434_G/A	0.166	2016	missense	c.1853C>T	p.Thr618Ile	COSM1737962
H21399/16	SETBP1_18_42531907_G/A	0.427	2515	missense	c.2602G>A	p.Asp868Asn	COSM1318400

PROPOSED DIAGNOSIS

ATYPICAL CHRONIC MYELOID LEUKAEMIA

No follow-up available

Diagnosis of atypical CML generally associated with poor prognosis

- Overall median survival approx. 25 months
- 40% transform to acute leukaemia

(Breccia M et al, Haematologica 2006)

Diagnostic criteria for atypical CML (2016)

Peripheral blood leukocytosis ≥ 13 x 10^9

- Due to increased neutrophils and precursors ✓
  - Dysgranulopoiesis, may include abnormal chromatin clumping ✓
  - Immature granuloyctes (promyelocytes, myelocytes, metamyelocytes) account for ≥ 10% of white cells ✓
  - Myeloblasts <20% white cells ✓

No or minimal absolute basophilia; <2% leukocytes ✓

No or minimal monocytosis; monocytes <10% of leukocytes ✓

Hypercellular bone marrow

- Granulocytic proliferation and dysplasia +/- dysplasia in erythroid and megakaryocyte lineages ✓
- Myeloblasts <20% nucleated cells ✓

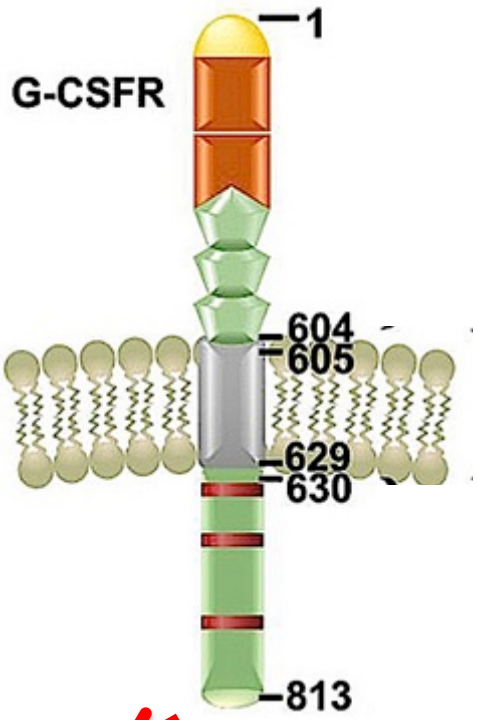
No evidence of *PDGFRA, PDGFRB* or *FGFR1* rearrangement, or *PCM1-JAK2*

Not meeting criteria for *BCR-ABL1+* CML, PMF, or ET ✓

CSF3R MUTATIONS (Dwivedi P and Greis KD, Exp Hematol 2017;46:9-20)

CSF3R is the receptor for Granulocyte Colony Stimulating Factor

- Transmembrane protein of 813 amino acids
- Binding by ligand induces conformational change and activation of downstream pathways



JAK/STAT

PI3K/AXT

MAPK/ERK

Neutropenia
(severe congenital neutropenia & chronic idiopathic neutropenia)

e.g. T618I

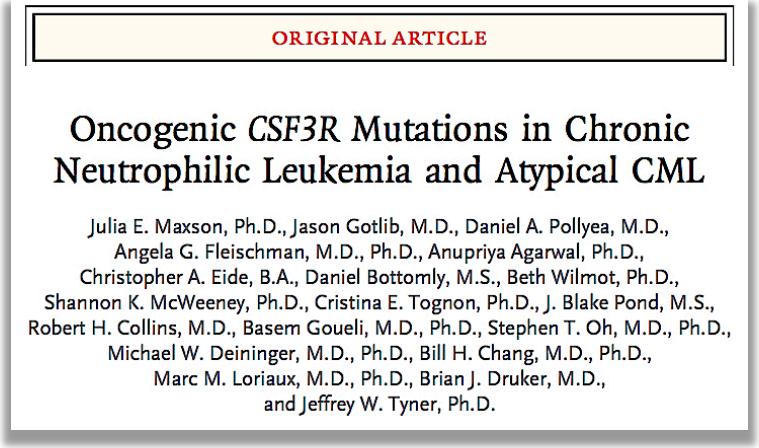
Myeloid neoplasms
(constitutive activation of JAK/STAT)

SCN➔AML/MDS
(SRC Kinase activity↑)

GRANULOCYTE PROLIFERATION

GRANULOCYTE DIFFERENTIATION

CSF3R MUTATIONS IN ATYPICAL CML



ORIGINAL ARTICLE

Oncogenic *CSF3R* Mutations in Chronic Neutrophilic Leukemia and Atypical CML

Julia E. Maxson, Ph.D., Jason Gotlib, M.D., Daniel A. Pollyea, M.D., Angela G. Fleischman, M.D., Ph.D., Anupriya Agarwal, Ph.D., Christopher A. Eide, B.A., Daniel Bottomly, M.S., Beth Wilmot, Ph.D., Shannon K. McWeeney, Ph.D., Cristina E. Tognon, Ph.D., J. Blake Pond, M.S., Robert H. Collins, M.D., Basem Goueli, M.D., Ph.D., Stephen T. Oh, M.D., Ph.D., Michael W. Deininger, M.D., Ph.D., Bill H. Chang, M.D., Ph.D., Marc M. Loriaux, M.D., Ph.D., Brian J. Druker, M.D., and Jeffrey W. Tyner, Ph.D.

2013: 1st descriptions of *CSF3R* mutations in myeloid neoplasms

- 8/17 (47%) cases of aCML (n=4) or suspected aCML (n=3)
- 8/9 (89%) cases of CNL

N Eng J Med 2013.368.1781.

Later studies suggest real incidence of *CSF3R* mutations in aCML likely to be much lower

Author	Number of cases harbouring mutated CSF3R
Pardanani A et al, Leukemia 2013	0/9 (0%)
Wang SA et al, Blood 2014	0/27 (0%)
Meggendorfer M et al, Haematologica 2014	2/58 (3%)
Patnaik MM et al, Am J Hematol 2017	2/25 (8%)
Gambacorti-Passerini CD et al, Blood 2015	0/15 (0%)
TOTAL	**4/134 (3.0%)** (7.9% incl Maxon cases)

CSF3R MUTATIONS IN CNL AND OTHER MYELOID NEOLASMS

***CSF3R* mutations in myeloid neoplasms** (combined results)

	Number	%	Refs
CNL	32/49	**65%**	1-3,7
aCML	12/151	**7.9%**	1-3
CMML/JMML	8/470	**1.7%**	1,3,5,6
MDS	0/88	**0%**	5,6
PMF	0/76	**0%**	1
ET	0/21	**0%**	5
De novo AML	20/2364	**0.8%**	2,4-6

1. Pardanani A, Leukemia 2013
2. Maxson JE, NEJM 2013
3. Meggendorfer M, Haematologica 2014
4. Tefferi A, Haematologica 2013
5. Sano H, Br J Haematol 2015
6. Hwang SY, Ann Hematol 2015
7. Cui Y, J Hematol Oncol
8. Kosmider O, Leukemia 2013
9. Wang SA, Blood 2014
10. Patnaik MM, Hematology 2017
11. Gambacorti-Passerini CB, Blood 2015

***CSF3R* mutations are relatively specific for CNL amongst myeloid neoplasms**

SETBP1 MUTATIONS IN ATYPICAL CHRONIC MYELOID LEUKAEMIA

Initial studies indicated relatively high frequency in aCML, e.g.

Piazza R et al, Nat Genet 2013:	17/70 (**24.3%**)
Gambacorti-Passerini CB et al, Blood 2015:	4/15 (26.7%)
Meggendorfer M et al, Leukemia 2013:	19/60 (**31.7%**)

May also present in CNL, e.g.

Piazza R et al, Nat Genet 2013:	1/4 (**25%**)
Pardanani A et al, Leukemia 2013:	4/12 (**33%**)

Low incidence/not present in other myeloid neoplasms, e.g./

SETBP1 MUTATIONS IN MYELOID NEOPLASIA

Piazza R et al, Nat Genet 2013.45.18-24

Table 1 Frequency of *SETBP1* mutations in 644 patient samples and 344 cancer cell lines

Tumor type	Number of samples	Number of mutated samples	Percent mutated samples
AML	106	0	0
ALL	62	0	0
CLL	32	0	0
MDS	100	0	0
MPN			
CML	42	0	0
PMF	33	0	0
PV	42	0	0
ET	36	0	0
CNL	4	1	25
MDS/MPN			
aCML	70	17	24
Unclassified MDS/MPN	30	3	10
CMML	82	3	4
JMML	5	0	0

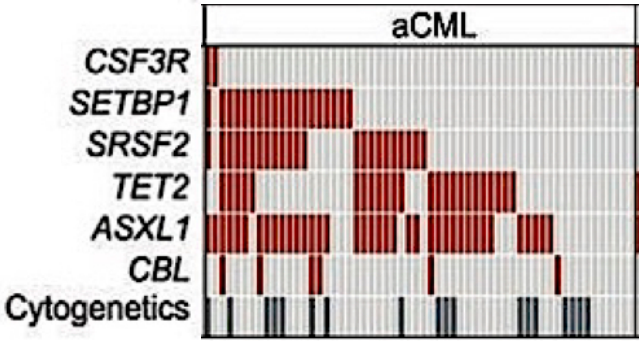
CSF3R AND *SETBP1* MUTATIONS MAY CO-EXIST IN aCML & CNL

Cases of CNL with *CSF3R* mutation often also harbour mutation of *SETBP1*

- Pardanani A et al 2013: 40% (4/10 cases)
- Cui Y et al 2014: 60% (6/8 cases)

Cases of aCML with *SETBP1* mutation may also have *CSF3R* mutation, e.g.

- Meggendorfer M et al, 2014



Hypothesized that presence of both mutations infers bad prognosis/resistance to JAK inhibitor (Ruxolitonib) in CNL and aCML:

Chronic neutrophilic leukemia with concurrent *CSF3R* and *SETBP1* mutations: single colony clonality studies, *in vitro* sensitivity to JAK inhibitors and lack of treatment response to ruxolitinib

Leukemia (2014) **28**, 1363–1365; doi:10.1038/leu.2014.39

Lasho TL et al, Leukemia 2014

Ann Hematol (2015) 94:879–880
DOI 10.1007/s00277-014-2272-0

LETTER TO THE EDITOR

Atypical chronic myeloid leukemia with concomitant CSF3R T618I and SETBP1 mutations unresponsive to the JAK inhibitor ruxolitinib

Emanuele Ammatuna · Matthias Eefting · Kirsten van Lom · François F. Kavelaars · Peter J. M. Valk · Ivo P. Touw

Ammatuna E et al, Ann Hematol 2015

SUMMARY

Initial study of this case raised a differential diagnosis (aCML vs CNL)

Targeted molecular testing, performed to help refine the diagnosis, revealed mutations of *SETBP1* and *CSF3R*

A diagnosis of aCML made in light of:

- **Appropriate morphological findings**
  - **Dysplastic features present**
  - **Left shift in peripheral blood**

  } **Help exclude CNL**
- **Compatible/suggestive mutational profile, i.e.**
  - ***SETBP1* mutations associated with aCML**
  - ***CSF3R* mutations more specific for CNL**
  - **But both mutations may co-exist in aCML**

Highlights the importance of interpreting molecular abnormalities in the context of other findings

ACKNOWLEDGEMENTS

HMDS, St James's University Hospital, Leeds
Jan Taylor
Paul Evans
Sharon Barrans
Matthew Cullen

FINAL PANEL DIAGNOSIS

Atypical chronic myeloid leukemia, *BCR-ABL1*-negative