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Targeted NGS in AML and MDS: the first 1,000 samples from WMRGL

David Hill
10/06/19
ACGS summer meeting

Introduction

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- In AML, patients are stratified in to adverse, intermediate and favourable risk categories dependent on cytogenetics as defined by the MRC AML trials
  - If stratified in to Adverse risk group, patients receive intensified therapy
- As of 2017 new European LeukaemiaNet (ELN) guidelines incorporated additional molecular risk markers

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

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Clinical utility of NGS in AML testing strategy

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ELN genetic Risk Category	Genetic Abnormality
Favourable	t(8;21)(q22;q22.1); RUNX1-RUNX1T1 inv(16)(p13.1q22) or t(16;16) (p13.1q22); 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* with *FLT3*-ITD t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favourable 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*

Testing strategy requires:

- G-banding
- FISH for cryptics
- Molecular techniques
  - Multiple assays or 1 NGS approach

Dohner et al., Blood (2017); 129(4);424-447

TruSight Myeloid Panel (TSMP) design

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- In conjunction with Illumina, re-designed TruSight Myeloid Panel (TSMP) from amplicon to Nextera rapid capture chemistry (hybridisation based)
- Bespoke BI pipeline which allows us to detect:
  - FLT3-ITDs
  - KMT2A structural variants
  - More accurate quantification of variants
  - CN-LOH of chromosome 13

The current service

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In silico panel	Genes on Panel
24 gene for all AML (diagnosis/relapse) High risk MDS Low risk MDS on specific clinical request	*ASXL1, BCOR, CALR, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KMT2A, MPL, NPM1, PHF6, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*
29 gene for CMML referrals	*ASXL1, BCOR, CALR, CBL, CEBPA, CSF3R, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KMT2A, KRAS, MPL, NPM1, NRAS, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*
32 gene for JMML referrals	*ASXL1, BCOR, CALR, CBL, CEBPA, CSF3R, DNMT3A, GATA2, EZH2, FLT3, IDH1, IDH2, JAK2, JAK3, KIT, KMT2A, KRAS, MPL, NPM1, NRAS, PHF6, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*

Classification and reporting

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- We have developed in house guidelines for the reporting of variants based on the AMP guidelines for somatic variant interpretation (Li et al., 2017 The Journal of Molecular Diagnostics:19(1);4-23).
- Once variants are tiered they are collated in an integrated report with the cytogenetic findings
- Example report:

Cytogenetic analysis has already been reported to show an abnormal karyotype with rearrangements of 11p and 14q (A19.20849). An abnormal karyotype with rearrangements of 11p and 14q is classified in the intermediate cytogenetic risk group in AML (MRC trial data).

BCOR, IDH2, RUNX1 and FLT3 TKD variants are recurrent in myeloid neoplasias, including AML. AML with mutated RUNX1 is a provisional entity in the WHO classification (Arber et al., Blood 2016, 127;20:2391-2405). Using the 2017 ELN guidelines RUNX1 variants are classified within the adverse genetic risk group (Dohner et al. Blood 2017; 129(4):424-447).

IDH2 and FLT3 variants are therapeutic targets for IDH and FLT3 inhibitors (Saygin and Carraway. J Hematol. & Oncol. 2017;10:93).

Testing by referral reason

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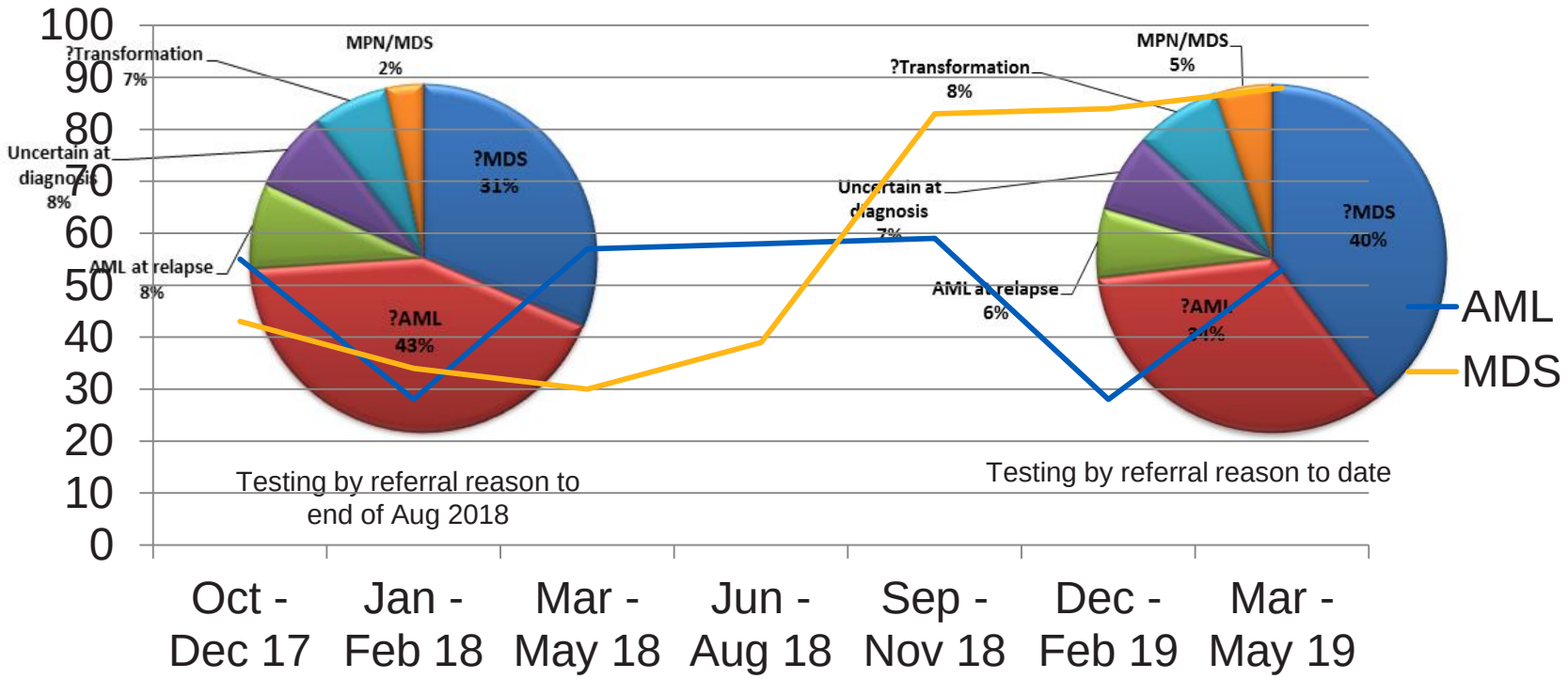

- Increased from ~35 MDS to ~80 MDS requests per quarter. Why?
- Increased awareness amongst clinical users
- molecular info increasingly used to guide patient management
- Increase in 'softer' MDS referrals

Abnormality rates

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	Total number of samples activated for NGS (reported)	% positive reports (1 or more clinically significant variant)
AML/?AML at diagnosis	338 (320)	82%
AML at relapse	65 (61)	77%
MDS/?MDS at diagnosis	401 (375)	59%
Uncertain at diagnosis	73 (69)	71%
Other	53 (50)	70%
?Transformation	80 (74)	74%
Total	1010 (949)	71%

Abnormality rates by NGS

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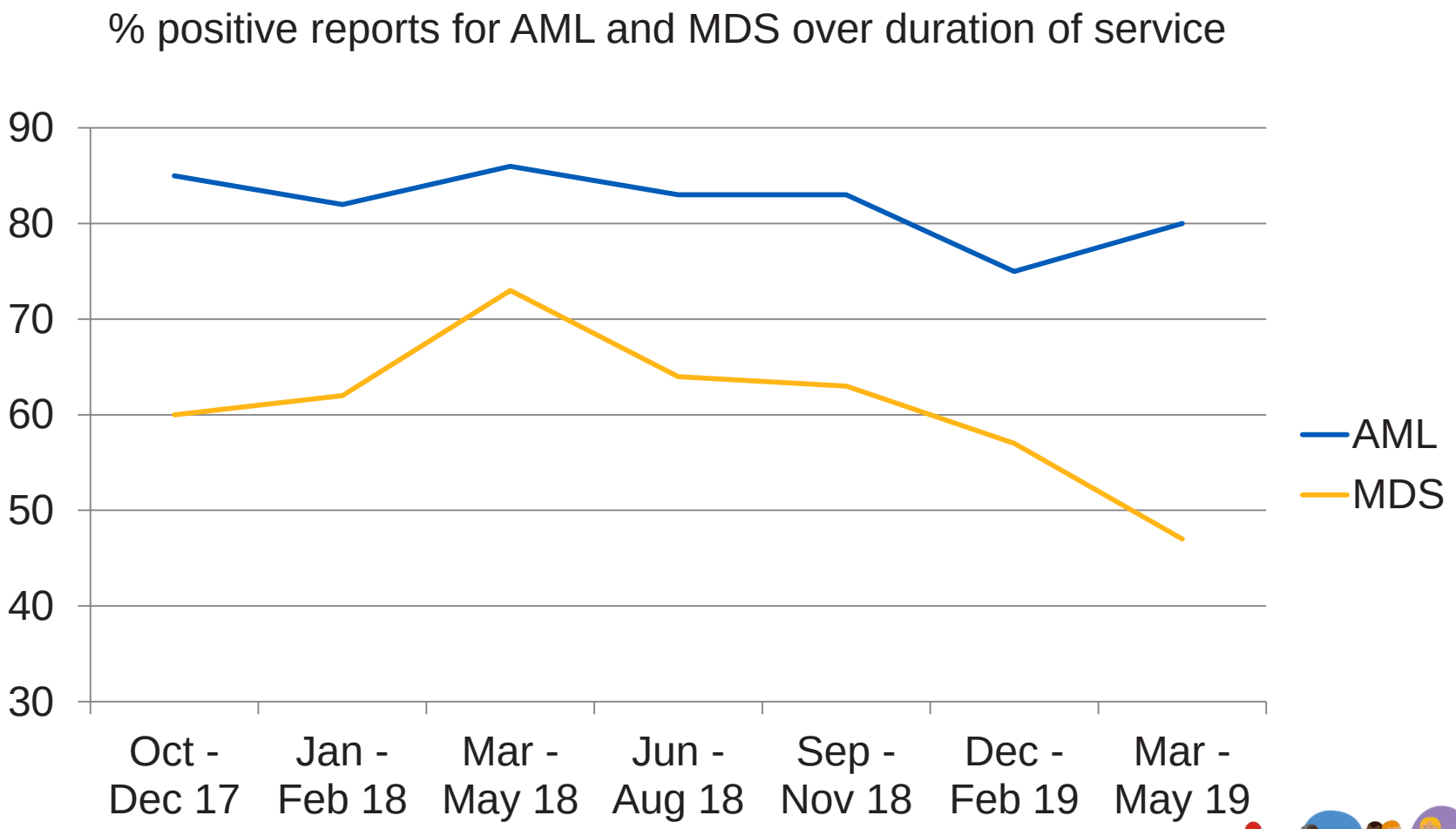

Variant burden

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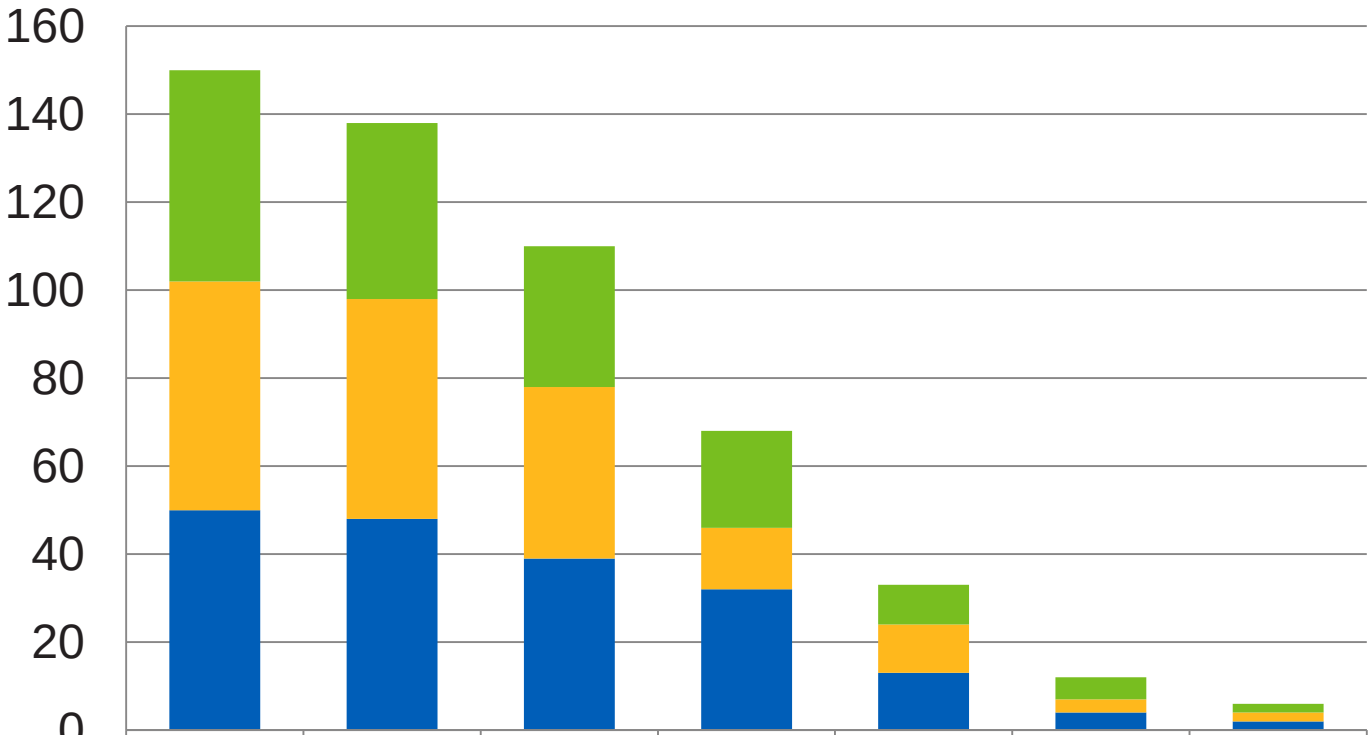
Number of clinically significant variants per patient per disease



	1 variant	2 variants	3 variants	4 variants	5 variants	6 variants	7 variants
Other	48	40	32	22	9	5	2
MDS	52	50	39	14	11	3	2
AML	50	48	39	32	13	4	2

AML versus MDS

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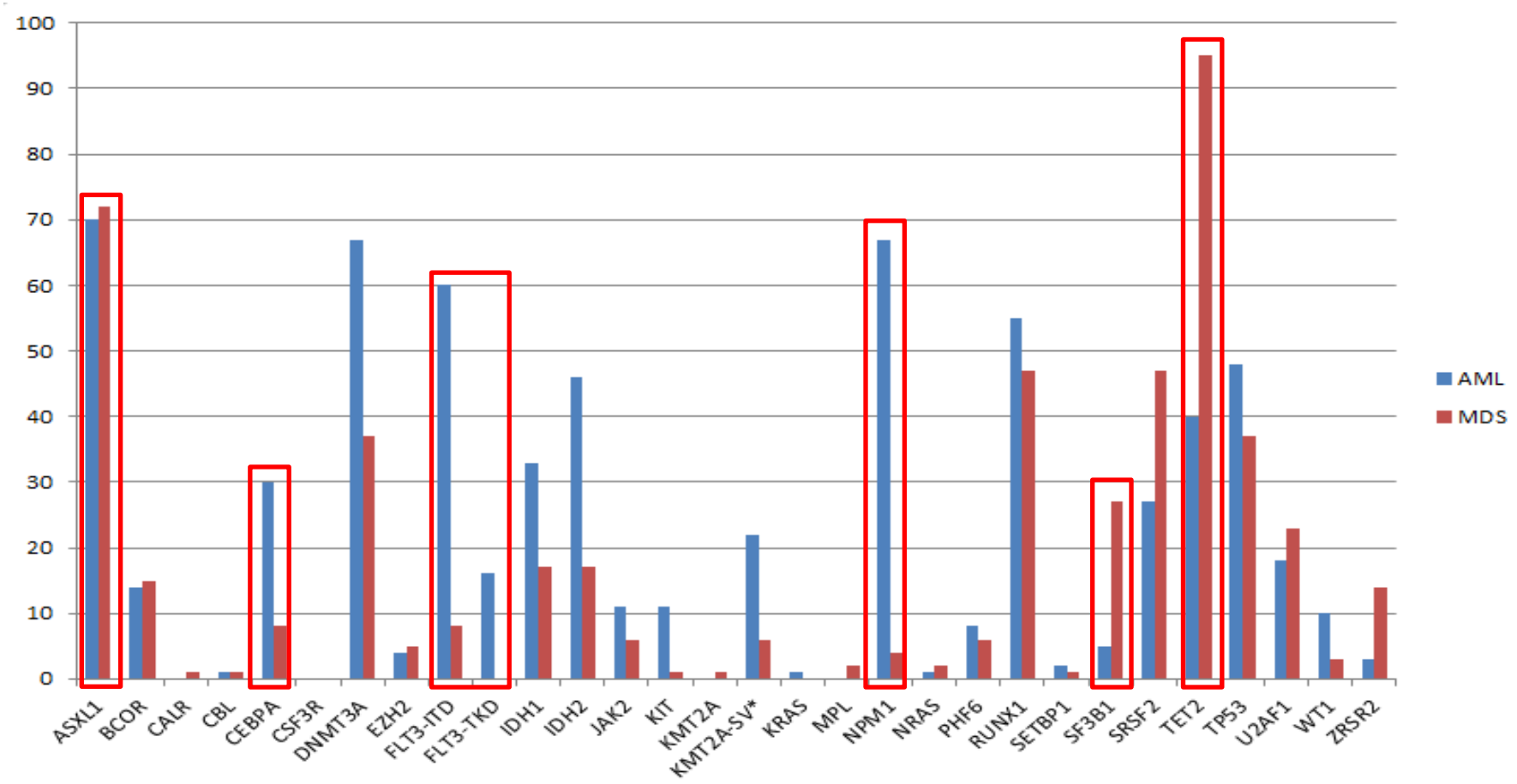

Total number of clinically significant variants in AML and MDS

Variant profile of AML referrals

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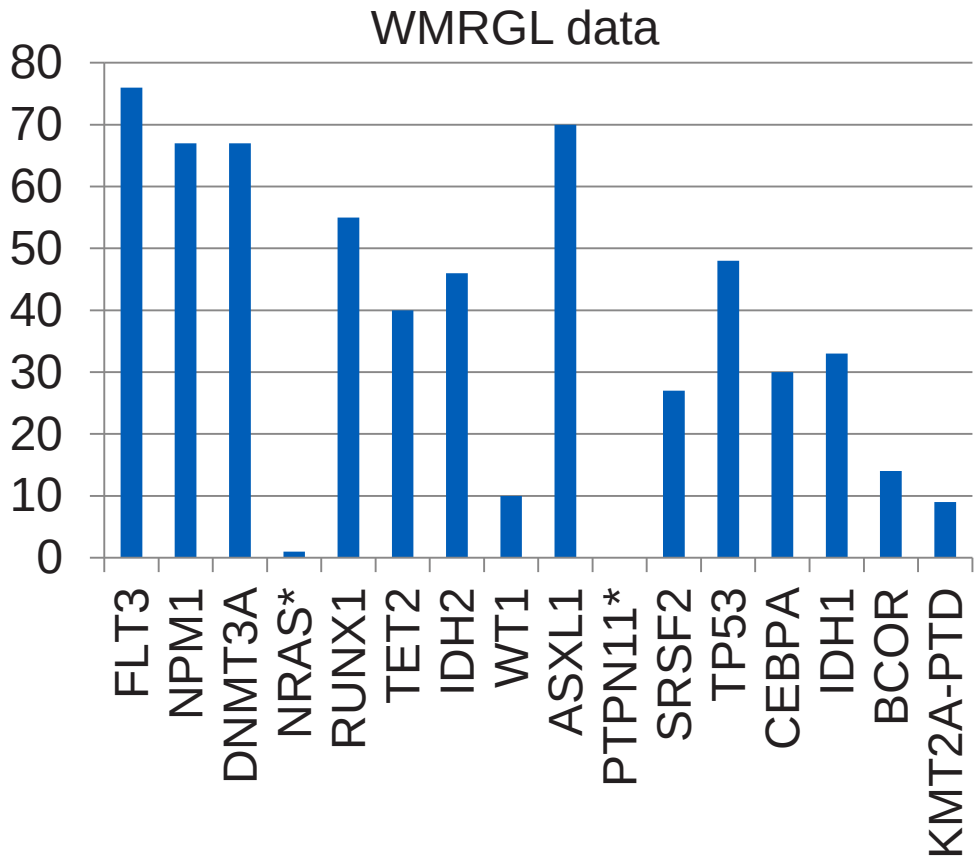

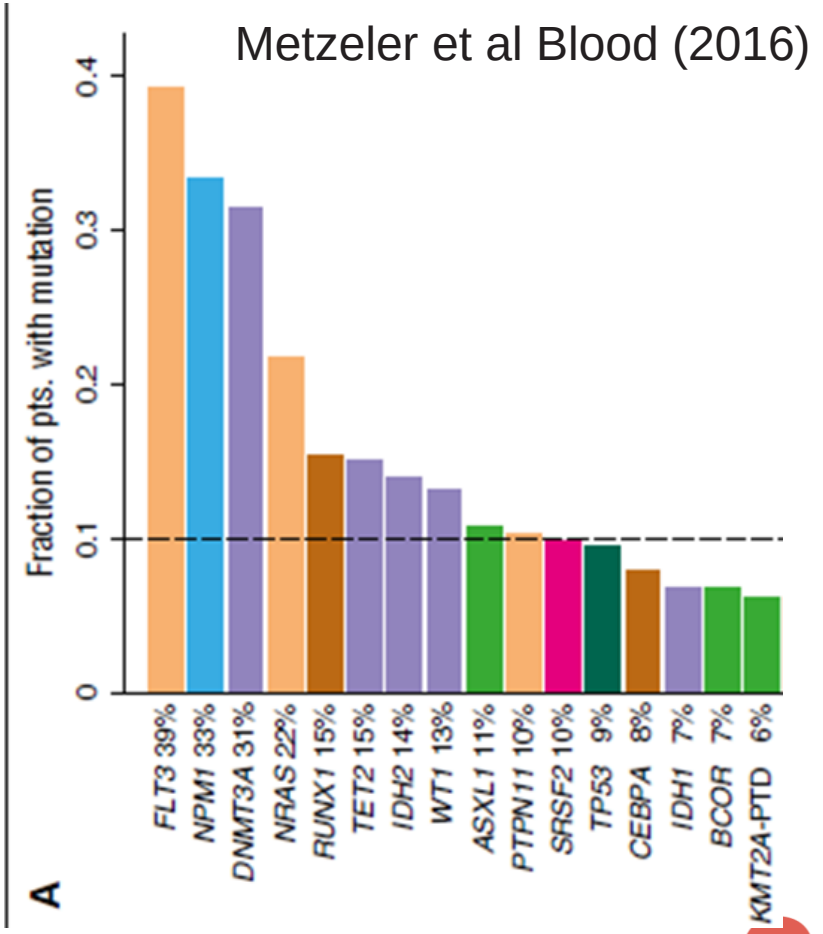

Metzeler et al. – 664 patients aged 18-84 with a diagnosis of AML from the German AML cooperative group

* Not included as part of AML in silico panel

Variant profile of MDS referrals

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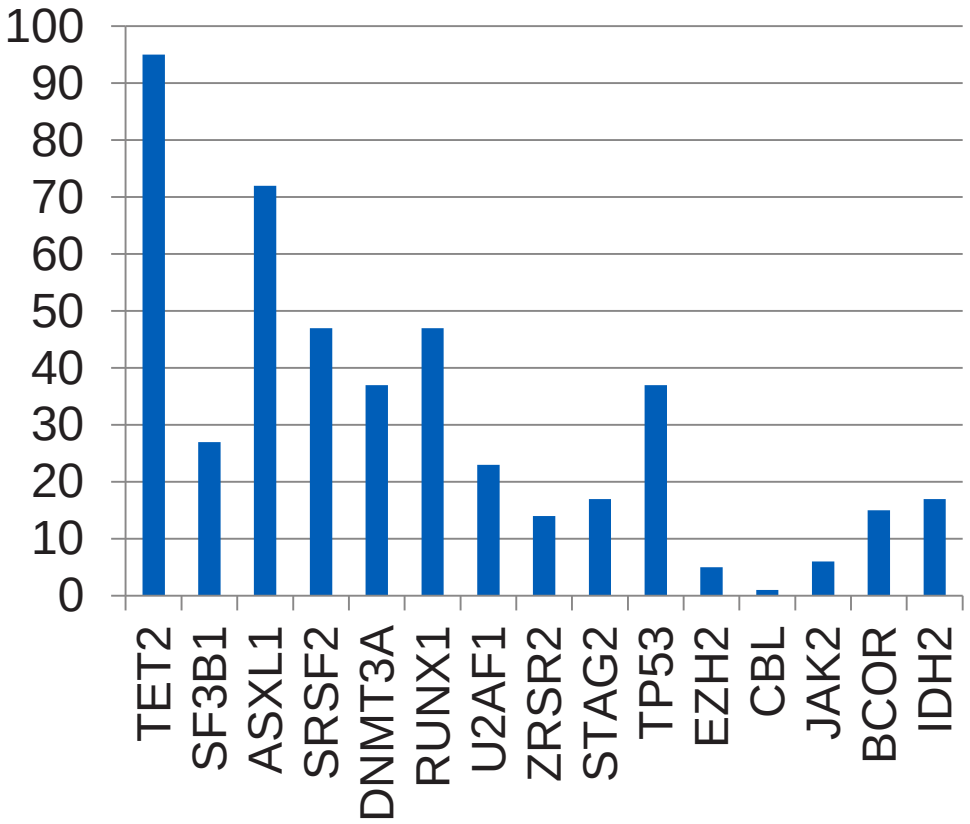
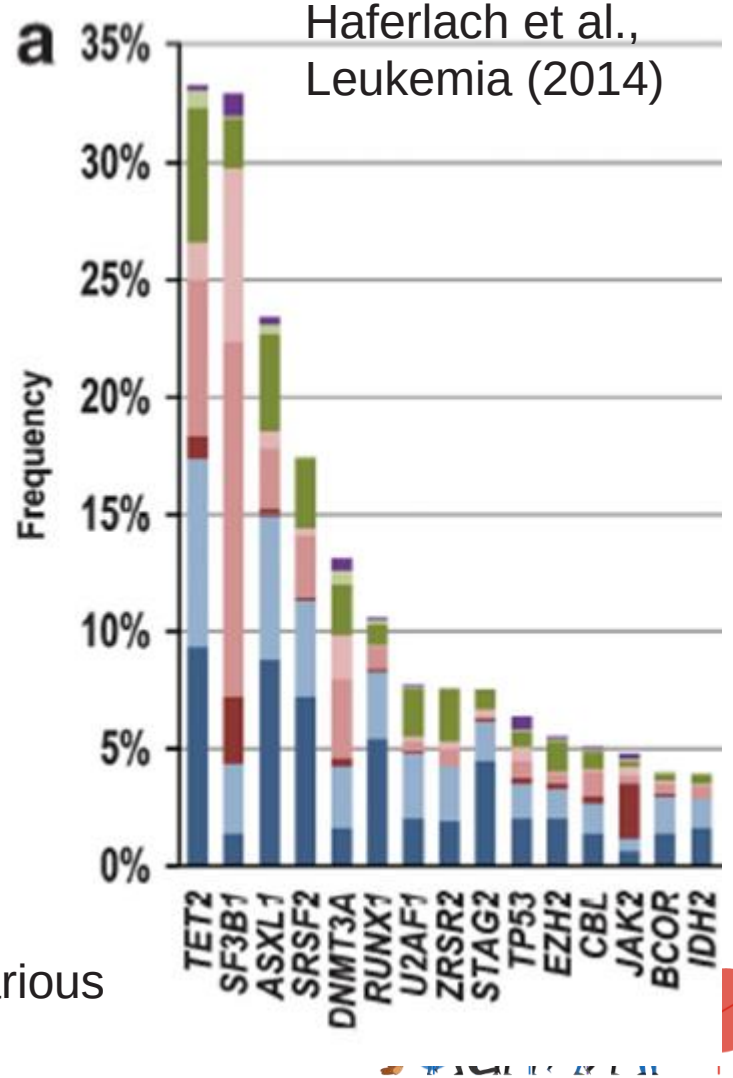
WMRGL data



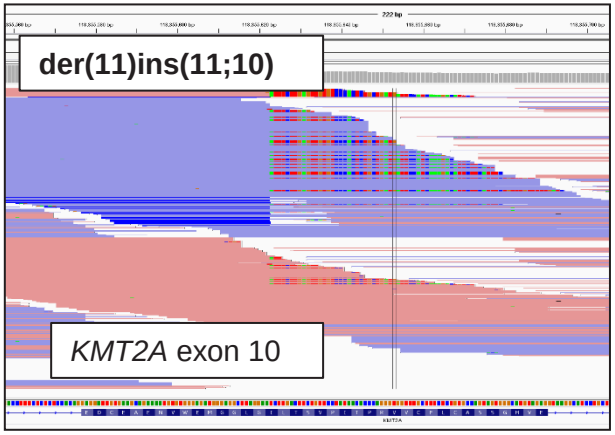
Haferlach et al., Leukemia (2014)

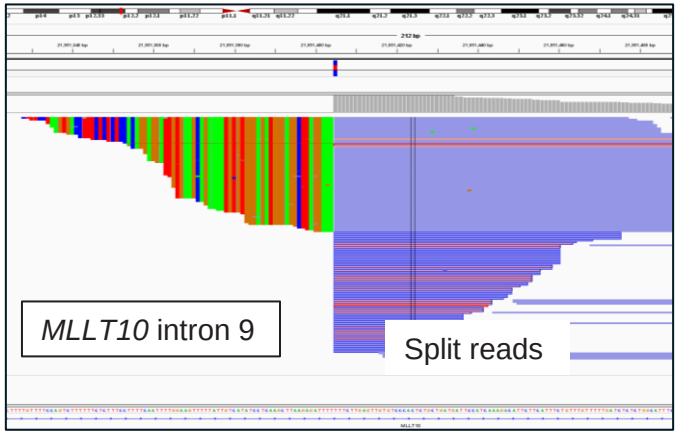


Haferlach et al cohort – 944 patients across various WHO, 2008 MDS subtypes.

Structural variants

- Structural variants have been identified in 135 patients (through a combination of pindel and Manta VC):
  - 83 with *FLT3*-itd mutations
  - 35 with *KMT2A* PTDs
  - 14 with *KMT2A* translocations
  - 1 translocation involving *NPM1*
  - 1 translocation involving *TP53*
  - 1 470kb *RUNX1* deletion





Clinical utility

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- 94% (267/285) of AML/?AML patients identified a relevant genetic aberration using a combination of cytogenetics and NGS.
- 6% (18/285) had no variant and ANK
  - 4/18 patients failed G-band analysis and so may have an undetected cytogenetic abnormality
  - 2/18 have confirmed AML, ANK and no variant detected
  - 12/18 may not have AML, or have AML with as yet unidentified genomic abnormalities
- Detection rate is lower in MDS/?MDS at 59% (as expected).
- Adverse risk variants according to published literature/guidelines are reported in: 40% of AML patients and 38% of MDS patients
  - Such patients would be considered for treatment intensification/SCT
- MRC AML19 trial amendment (2019): Patients with adverse risk genomics (now to include TP53, RUNX1, ASXL1) to enter high-risk randomisation arm (previously adverse risk **cytogenetics only**)
- Targeted therapies

Recent developments

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- Re-designed TSMP for compliance with Cancer test directory (mandated genes for AML, MDS, MPN, JMML*, ALL)
- Also contains genes in familial AML and familial ALL test directory panels https://panelapp.genomicsengland.co.uk/panels/

Inherited predisposition to acute myeloid leukaemia (AML) Version 0.45	14 of 14 100%	5 reviewers

- New panel launched this week

Acknowledgements

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WMRGL

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Questions?

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