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Acquired Isodisomy on Chromosome 13 at diagnosis results in impaired overall survival in Patients with FLT3-ITD mutant Acute Myeloid Leukaemia

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1 Acquired Isodisomy on Chromosome 13 at diagnosis results in impaired overall survival in Patients

- 2 with FLT3-ITD mutant Acute Myeloid Leukaemia
- 3 Internal tandem duplication (ITD) mutations in the FLT3 gene on chromosome 13 occur in 25% of

4 patients with acute myeloid leukaemia (AML) and result in impaired overall survival.(1) Patients with

5 a high allelic ratio (AR) of ITD mutant to wildtype *FLT3* in genomic DNA have an even poorer

6 prognosis.(2) AR may be a predictor of response to FLT3 inhibitors(3) and may also interact with

7 other mutations in influencing disease risk.(4, 5)

8 AR may be dependent on a number of factors including loss of the wildtype allele. Acquired

9 isodisomy (AID) results in the loss of the wildtype allele, through duplication of the mutant allele

10 with segmental loss of the wildtype allele. Although studies(6) have shown the importance of AID at

- 11 chromosome 13 (AID13) at relapse, the impact of AID13 at diagnosis is unclear. This study aimed to
- 12 identify the relationship between AID13 and *FLT3*-ITD AR and investigated the outcomes of patients
- 13 with AID13.
- 14 All patients diagnosed with AML underwent *FLT3* mutation analysis in the West Midlands Regional

15 genetics laboratory between 2002 and 2015 and are included in this study. *FLT3* and *NPM1* mutation

analysis by PCR of genomic DNA was undertaken as described.(7) PCR products were identified

17 using fluorescent based fragment analysis (Applied Biosystems, US). Allelic ratio (AR) (mutation: wild

18 type ratio in genomic DNA) was determined from the relative peak heights. AID was determined by

analysis of microsatellite markers along chromosome 13 in patients with AR above 0.25 (8)

20 (supplementary figure 1).

21 Complete remission (CR), event-free survival (EFS) and overall survival (OS) were defined as

described.(9) OS and EFS were estimated by the Kaplan-Meier method. Survival curves were

23 compared using the log rank test. Variables were compared using Wilcoxon or chi-squared test as

24 appropriate. Statistical analyses were performed with R 3.0.3, and the R-packages 'survival' and

25 SPSS (version 19).

Two hundred and eighty-nine patients diagnosed with *FLT3*-ITD mutated AML are described
(Supplementary Table 1). The median age at diagnosis was 61 years. Of 280 patients tested, 45%
had the *NPM1* exon 12 mutation. Cytogenetic classification by MRC criteria (10) showed 77% of 267

- 29 patients were of intermediate risk.
- 30 We investigated which factors influenced allelic ratio (AR). The first was the loss of the wildtype

allele. Loss of the wildtype allele can occur through the loss of all or part of chromosome 13 but was

32 seen in only 3 patients. The most frequent mechanism for the loss of the wildtype allele is the

presence of AID13; this was seen in 12.8% (n=34/266) of patients with *FLT3*-ITD AML at diagnosis.

34 AID13 was associated with a significant increase in AR (Wilcoxon test p<0.0001, supplementary

- figure 2a). However, AR is an imperfect surrogate for loss of the wildtype allele as 2 patients with
- AID13 had an AR less than 0.5, and conversely, 106 patients with an AR over 0.5 did not have AID13.

37 AID13 itself was associated with an increased white cell count, which was not statistically significant

38 (Wilcoxon test: p=0.096) (supplementary figure 2b). AID13 was significantly associated with an

39 intermediate cytogenetic risk profile and the presence of a NPM1 mutation (chi-squared: p<0.05)

40 (supplementary figure 2c).

41 AR is also thought to be associated with the size of ITD, and the presence of contaminating normal

- 42 cells. Therefore, we also investigated the relationship between these factors and AR. There was no
- 43 association between AR and ITD size. The presence of contaminating cells is inversely correlated
- 44 with the percentage of blasts in the sample. Although the association between AR and blast
- 45 percentage was statistically significant, the correlation was very weak (Pearson's correlation
- 46 coefficient +0.154 (p=0.02)) (Supplementary figure 3 a) and b)). Having accounted for these other
- 47 factors, this suggests AID13 is the key factor affecting AR.
- 48 Outcomes were analysed for 179 patients treated with curative intent who did not have APML. The
- 49 majority of these patients were treated on the concurrent national AML cooperative trials, with a
- 50 standard combination of anthracyclines and cytarabine (supplementary table 1). Patients obtained
- 51 CR independent of AID13 or AR level (supplementary figure 4a and b). An AR level of 0.5 to split the
- 52 patient population was based on its use in previous studies (2, 4, 5). Post-remission outcome is
- 53 strongly influenced by choice of consolidation treatment, in particular allogeneic stem cell transplant
- 54 (SCT). To understand the influence of AR and AID13 on outcomes and the role for SCT in the
- management of these patients we stratified time-event analysis based on whether patients received
 SCT or chemotherapy only.
- 57 In patients treated with chemotherapy alone, an AR ≥0.5 was compared to an AR <0.5. A high AR
- 58 conferred a worse prognosis in both EFS (p=0.023) and OS (p=0.039) (figure 1a). In the same
- patients, the presence of AID13 also conferred a worse prognosis in EFS (p=0.057) and OS (p=0.029)
- 60 (figure 1b). Because AID13 increases the AR, it is important to understand the relative contribution
- of AID13 to a high AR. High AR patients were therefore stratified into those with and without AID13.
- 62 The poor prognostic impact of AR≥0.5 was no longer significant when AID13 patients were not
- 63 included, measured by both EFS (AR \ge 0.5 without AID13 vs AR<0.5 p=0.064) and OS (AR \ge 0.5 without
- 64 AID13 vs AR<0.5 p=0.119).
- 65 Of the 179 non-APML patients treated with curative intent, 70 received a SCT (supplementary table
- 66 3). High AR (≥ 0.5) did not have a poor prognostic impact in the SCT treated patients (EFS: p=0.477;
- 67 OS: p=0.669), supplementary figure 5a). Similarly, AID13 did not confer a poor prognostic impact in
- 68 patients treated with SCT (EFS: p=0.663; OS: p=0.536), supplementary figure 5b). There was also no
- impact from a high AR (≥ 0.5) in patients without AID13 treated with SCT, as seen with those treated
- 70 without SCT (EFS: p=0.281; OS: p=0.823, supplementary figure 5c).
- 71 Of the 70 SCT treated patients, 28 (40%) remain alive. 17 of the 70 had AID13, and, of these, 6
- remain alive. 11 died, 6 from relapse and 5 from NRM. 7 out of 8 patients with AID treated with
- 73 intensive chemotherapy alone died with active disease. This suggests that SCT may ameliorate the
- 74 poor risk of AID13.
- AID has been a common finding at relapse.(6) Of 45 patients who had sequential relapsed bone
- 76 marrow samples, 5 developed AID13 as a new finding. 6 relapsed with a *FLT3*-ITD negative clone,
- consistent with data that it is a secondary driver mutation.(11) Consistent with AID13 being a driver
- of relapse, in 5 out of 6 patients with AID13 with available relapse data there was no loss of AID13 at
 relapse.
- In summary, we show that AID13 at diagnosis is associated with impaired overall survival in patients
 who are treated with chemotherapy alone, and that this is the major part of the effect of a high AR.

- 82 A smaller study (12) also used microsatellite markers to investigate the impact of AID13, and
- 83 suggested that AID13 resulted in a decreased OS. The frequency of AID13 in our study of
- 84 consecutive patients (12.8%) was lower than that described by this smaller study (8/23 patients,
- 85 34%). The frequency of AID13 at an intermediate mutant level (0.25-0.5) was low in our study (1/60).
- 86 Another study (13) identified only 2 of 34 patients with AID13 using single nucleotide polymorphism
- 87 arrays, confirming this result.
- 88 This study has demonstrated the poor prognosis of patients with AID13 or a high AR in patients
- treated with chemotherapy alone. The poor outcomes were ameliorated in those who received SCT.
- 90 Our data is consistent with a prospective study from the German Austrian AML Study group who
- 91 showed an SCT improved OS compared to intensive chemotherapy alone in patients with *FLT3*-ITD
- 92 with a high AR, but no benefit was seen in those with a low AR.(2)
- 93 Several studies have implicated the AR of *FLT3*-ITD as an important factor in determining the
- 94 outcomes of patients with this mutation.(1, 3-5) This study suggests mitotic recombination leading
- 95 to AID13 is a major mechanism of increasing the allelic ratio of *FLT3*-ITD. Importantly, it is
- 96 detectable by an accessible laboratory assay with a binary outcome. In contrast to allelic ratio,
- 97 which is a continuous variable. Our data demonstrates differences in the outcomes of patients with
- 98 AID13 suggesting patients with heterozygous and homozygous *FLT3*-ITD mutations are distinct
- 99 cohorts. This is consistent with murine models where homozygous FLT3-ITD mutation results in a
- 100 more severe myeloproliferative phenotype than those with either a heterozygous (14) or
- 101 hemizygous mutation.(15) The loss of the wildtype allele is also important, as seen in FLT3-ITD
- 102 hemizygous cells which show a more aggressive phenotype than the heterozygous cells, which retain
- the wildtype copy of *FLT3*.(15) AID13 is a single event which results in both a gain of a second *FLT3*-
- 104 ITD allele and the loss of the wildtype allele.

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108 Authorship contributions

- 109 JL, SA, JB collected and analysed data. JE, SWB, DC, JA, PH, JW, KA, YM, FAW, AW, AB, PF, CC, MG
- 110 provided data. KB, MG, MR analysed data. JL, SA, JB, MR wrote the manuscript.

111 Conflicts of Interest

- 112 None
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- 135 Supplementary information is available at Leukemia's website
- 136 Figure Legends
- Figure 1 Event free survival (EFS) and overall survival (OS) based on allelic ratio (AR) and
 acquired isodisomy at chromosome 13 (AID13) stratification for patients treated with
 intensive chemotherapy alone
- 139
- a) EFS and OS for AR ≥0.5 vs AR<0.5
- 141 b) EFS and OS for presence or absence of AID13
- 142 c) EFS and OS for AR ≥0.5 without AID13 vs AR<0.5

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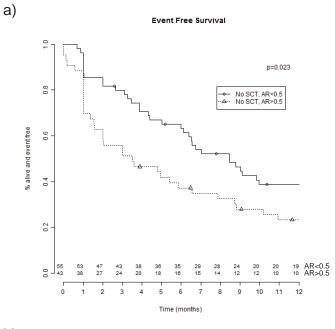
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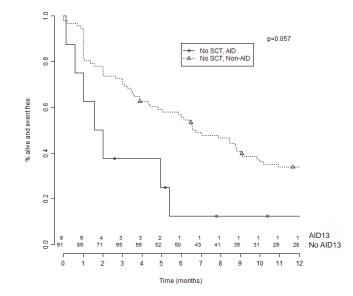
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Figure 1



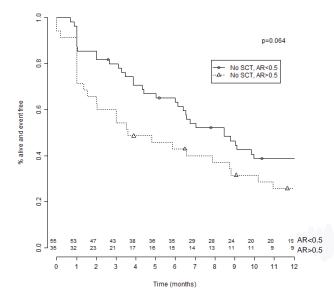


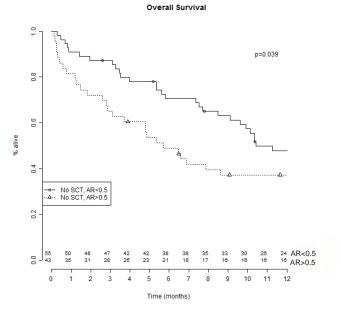




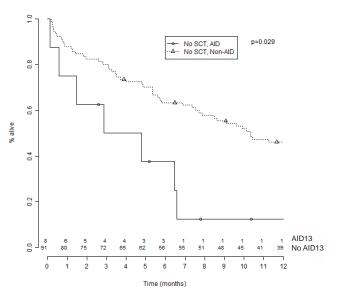


Event Free Survival in Non-AID patients





Overall Survival



Overall Survival in Non-AID patients

