

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

Loke, Justin; Akiki, Susanna; Borrow, Julian; Ewing, Joanne; Bokhari, Syed; Chandra, Deepak; Arrazi, Julie; Hazlewood, Peter; Arthur, Katherine; Walsh, Julie; Membwange, Yvonne; Wandroo, Farooq; Watts, Angela; Borg, Anton; Brock, Kristian; Ferguson, Paul; Craddock, Charles; Griffiths, Michael; Raghavan, Manoj

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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
16 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
19 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
22 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).

26 Two hundred and eighty-nine patients diagnosed with *FLT3*-ITD mutated AML are described
27 (Supplementary Table 1). The median age at diagnosis was 61 years. Of 280 patients tested, 45%
28 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
31 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
33 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
35 figure 2a). However, AR is an imperfect surrogate for loss of the wildtype allele as 2 patients with
36 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
55 management of these patients we stratified time-event analysis based on whether patients received
56 SCT or chemotherapy only.

57 In patients treated with chemotherapy alone, an $AR \geq 0.5$ 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
59 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
61 of AID13 to a high AR. High AR patients were therefore stratified into those with and without AID13.
62 The poor prognostic impact of $AR \geq 0.5$ was no longer significant when AID13 patients were not
63 included, measured by both EFS ($AR \geq 0.5$ without AID13 vs $AR < 0.5$ $p=0.064$) and OS ($AR \geq 0.5$ 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
69 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
72 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.

75 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,
77 consistent with data that it is a secondary driver mutation.(11) Consistent with AID13 being a driver
78 of relapse, in 5 out of 6 patients with AID13 with available relapse data there was no loss of AID13 at
79 relapse.

80 In summary, we show that AID13 at diagnosis is associated with impaired overall survival in patients
81 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
89 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
103 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

113 Authors:

114 Justin Ching Ting Loke 1 , Susanna Akiki 2 , Julian Borrow 2, Joanne Ewing 3 , Syed W Bokhari 4 ,
115 Deepak Chandra 5 , Julie Arrazi 1 , Peter Hazlewood 1 , Katherine Arthur 6 , Julie Walsh 7 , Yvonne
116 Membwange 8 , Farooq Ahmad Wandroo 8 , Angela Watts 9 , Anton Borg 10, Kristian Brock 11, Paul
117 Ferguson 1, Charles Craddock 1 , Mike Griffiths 2 and Manoj Raghavan 1

118 1 Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, United Kingdom; 2 West
119 Midlands Regional Genetics Laboratory, Birmingham, United Kingdom; 3 Department of

120 Haematology, Heartlands Hospital, Birmingham, United Kingdom; 4 University Hospitals Coventry
121 and Warwickshire NHS trust; 5 University Hospital of North Staffordshire; 6 Worcestershire Royal
122 Hospital, United Kingdom; 7 New Cross Hospital, Wolverhampton, United Kingdom; 8 Sandwell and
123 West Birmingham Hospitals NHS Trust, West Bromwich, United Kingdom; 9 Russells Hall Hospital,
124 Dudley, United Kingdom; 10 South Warwickshire NHS Foundation Trust; 11 Cancer Research UK
125 Clinical Trials Unit (CRCTU), University of Birmingham, Edgbaston, Birmingham, UK

126 **Corresponding author:**

127 Dr Manoj Raghavan

128 Email: m.raghavan@bham.ac.uk

129 Mailing Address:

130 Centre for Clinical Haematology

131 Queen Elizabeth Hospital

132 Birmingham, B15 2TH, UK

133 Phone number: +44 (0) 121 371 4476

134 Fax: +44 (0) 121 414 9913

135 Supplementary information is available at Leukemia's website

136 **Figure Legends**

137 1) Figure 1 Event free survival (EFS) and overall survival (OS) based on allelic ratio (AR) and
138 acquired isodisomy at chromosome 13 (AID13) stratification for patients treated with
139 intensive chemotherapy alone

140 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

143 **References**

144 1. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of
145 a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important
146 prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy:
147 analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials.
148 Blood. 2001;98(6):1752-9.

149 2. Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M, et al. Differential impact
150 of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation.
151 Blood. 2014;124(23):3441-9. Epub 2014/10/02.

152 3. Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T, Levis M. FLT3-mutant allelic burden and
153 clinical status are predictive of response to FLT3 inhibitors in AML. Blood. 2009;115(7):1425-32.

154 4. Pratcorona M, Brunet S, Nomdedéu J, Ribera JM, Tormo M, Duarte R, et al. Favorable
155 outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation
156 and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 2013;121(14):2734-8.

157 5. Linch DC. Impact of FLT3ITD mutant allele level on relapse risk in intermediate-risk acute
158 myeloid leukemia: implications for consolidation therapy. *Blood*. 2014;124(2):273-6.

159 6. Raghavan M, Smith L-L, Lillington DM, Chaplin T, Kakkas I, Molloy G, et al. Segmental
160 uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia.
161 *Blood*. 2008;112(3):814-21.

162 7. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and
163 treatment outcome in cytogenetically normal acute myeloid leukemia. *The New England journal of*
164 *medicine*. 2008;358(18):1909-18. Epub 2008/05/03.

165 8. Griffiths M, Mason J, Rindl M, Akiki S, McMullan D, Stinton V, et al. Acquired Isodisomy for
166 chromosome 13 is common in AML, and associated with FLT3-itd mutations. *Leukemia*.
167 2005;19(12):2355-8.

168 9. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised
169 recommendations of the International Working Group for Diagnosis, Standardization of Response
170 Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid
171 Leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*.
172 2003;21(24):4642-9. Epub 2003/12/16.

173 10. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, et al. The predictive
174 value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML):
175 analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial.
176 *Blood*. 2001;98(5):1312-20. Epub 2001/08/25.

177 11. Kottaridis PD, Gale RE, Langabeer SE, Frew ME, Bowen DT, Linch DC. Studies of FLT3
178 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia:
179 implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection,
180 and possible therapy with FLT3 inhibitors. *Blood*. 2002;100(7):2393-8.

181 12. Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, et al. Absence of the Wild-
182 Type Allele Predicts Poor Prognosis in Adult de Novo Acute Myeloid Leukemia with Normal
183 Cytogenetics and the Internal Tandem Duplication of FLT3. *Cancer Research*. 2001;61(19):7233-9.

184 13. Green C, Linch DC, Gale RE. Most acute myeloid leukaemia patients with intermediate
185 mutant FLT3/ITD levels do not have detectable bi-allelic disease, indicating that heterozygous
186 disease alone is associated with an adverse outcome. *British Journal of Haematology*.
187 2008;142(3):423-6.

188 14. Lee BH, Tothova Z, Levine RL, Anderson K, Buza-Vidas N, Cullen Dana E, et al. FLT3 Mutations
189 Confer Enhanced Proliferation and Survival Properties to Multipotent Progenitors in a Murine Model
190 of Chronic Myelomonocytic Leukemia. *Cancer Cell*. 2007;12(4):367-80.

191 15. Kharazi S, Mead AJ, Mansour A, Hultquist A, Boiers C, Luc S, et al. Impact of gene dosage,
192 loss of wild-type allele, and FLT3 ligand on Flt3-ITD-induced myeloproliferation. *Blood*.
193 2011;118(13):3613-21. Epub 2011/08/05.

194

195

Figure 1

