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

DOI:
10.1038/leu.2015.148

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

Internal tandem duplication (ITD) mutations in the FLT3 gene on chromosome 13 occur in 25% of patients with acute myeloid leukaemia (AML) and result in impaired overall survival.(1) Patients with a high allelic ratio (AR) of ITD mutant to wildtype *FLT3* in genomic DNA have an even poorer prognosis.(2) AR may be a predictor of response to FLT3 inhibitors(3) and may also interact with other mutations in influencing disease risk.(4, 5)

AR may be dependent on a number of factors including loss of the wildtype allele. Acquired isodisomy (AID) results in the loss of the wildtype allele, through duplication of the mutant allele with segmental loss of the wildtype allele. Although studies(6) have shown the importance of AID at chromosome 13 (AID13) at relapse, the impact of AID13 at diagnosis is unclear. This study aimed to identify the relationship between AID13 and *FLT3*-ITD AR and investigated the outcomes of patients with AID13.

All patients diagnosed with AML underwent *FLT3* mutation analysis in the West Midlands Regional 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 using fluorescent based fragment analysis (Applied Biosystems, US). Allelic ratio (AR) (mutation: wild 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) (supplementary figure 1).

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 compared using the log rank test. Variables were compared using Wilcoxon or chi-squared test as appropriate. Statistical analyses were performed with R 3.0.3, and the R-packages 'survival' and 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 patients were of intermediate risk.

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 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. 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. AID13 itself was associated with an increased white cell count, which was not statistically significant (Wilcoxon test: $p=0.096$) (supplementary figure 2b). AID13 was significantly associated with an intermediate cytogenetic risk profile and the presence of a NPM1 mutation (chi-squared: $p<0.05$) (supplementary figure 2c).

AR is also thought to be associated with the size of ITD, and the presence of contaminating normal cells. Therefore, we also investigated the relationship between these factors and AR. There was no association between AR and ITD size. The presence of contaminating cells is inversely correlated with the percentage of blasts in the sample. Although the association between AR and blast percentage was statistically significant, the correlation was very weak (Pearson's correlation coefficient +0.154 (p=0.02)) (Supplementary figure 3 a) and b)). Having accounted for these other factors, this suggests AID13 is the key factor affecting AR.

Outcomes were analysed for 179 patients treated with curative intent who did not have APML. The majority of these patients were treated on the concurrent national AML cooperative trials, with a standard combination of anthracyclines and cytarabine (supplementary table 1). Patients obtained CR independent of AID13 or AR level (supplementary figure 4a and b). An AR level of 0.5 to split the patient population was based on its use in previous studies (2, 4, 5). Post-remission outcome is strongly influenced by choice of consolidation treatment, in particular allogeneic stem cell transplant (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.

In patients treated with chemotherapy alone, an AR ≥0.5 was compared to an AR <0.5. A high AR 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) (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. The poor prognostic impact of AR≥0.5 was no longer significant when AID13 patients were not included, measured by both EFS (AR≥0.5 without AID13 vs AR<0.5 p=0.064) and OS (AR≥0.5 without AID13 vs AR<0.5 p=0.119).

Of the 179 non-APML patients treated with curative intent, 70 received a SCT (supplementary table 3). High AR (≥0.5) did not have a poor prognostic impact in the SCT treated patients (EFS: p=0.477; OS: p=0.669), supplementary figure 5a). Similarly, AID13 did not confer a poor prognostic impact in 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 without SCT (EFS: p=0.281; OS: p=0.823, supplementary figure 5c).

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 intensive chemotherapy alone died with active disease. This suggests that SCT may ameliorate the poor risk of AID13.

AID has been a common finding at relapse.(6) Of 45 patients who had sequential relapsed bone 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.

A smaller study (12) also used microsatellite markers to investigate the impact of AID13, and suggested that AID13 resulted in a decreased OS. The frequency of AID13 in our study of consecutive patients (12.8%) was lower than that described by this smaller study (8/23 patients, 34%). The frequency of AID13 at an intermediate mutant level (0.25-0.5) was low in our study (1/60). Another study (13) identified only 2 of 34 patients with AID13 using single nucleotide polymorphism arrays, confirming this result.

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. Our data is consistent with a prospective study from the German Austrian AML Study group who showed an SCT improved OS compared to intensive chemotherapy alone in patients with *FLT3*-ITD with a high AR, but no benefit was seen in those with a low AR.(2)

Several studies have implicated the AR of *FLT3*-ITD as an important factor in determining the outcomes of patients with this mutation.(1, 3-5) This study suggests mitotic recombination leading to AID13 is a major mechanism of increasing the allelic ratio of *FLT3*-ITD. Importantly, it is detectable by an accessible laboratory assay with a binary outcome. In contrast to allelic ratio, which is a continuous variable. Our data demonstrates differences in the outcomes of patients with AID13 suggesting patients with heterozygous and homozygous *FLT3*-ITD mutations are distinct cohorts. This is consistent with murine models where homozygous *FLT3*-ITD mutation results in a more severe myeloproliferative phenotype than those with either a heterozygous (14) or hemizygous mutation.(15) The loss of the wildtype allele is also important, as seen in *FLT3*-ITD 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*-ITD allele and the loss of the wildtype allele.

Acknowledgements

We acknowledge Christina Yap (University of Birmingham CRCTU), the scientists in the West Midlands Regional Genetics Laboratory, and the haematologists of the West Midlands.

Authorship contributions

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

Conflicts of Interest

None

Authors:

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

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

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

Corresponding author:

Dr Manoj Raghavan

Email: m.raghavan@bham.ac.uk

Mailing Address:

Centre for Clinical Haematology

Queen Elizabeth Hospital

Birmingham, B15 2TH, UK

Phone number: +44 (0) 121 371 4476

Fax: +44 (0) 121 414 9913

Supplementary information is available at Leukemia's website

Figure Legends

1) 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
    a) EFS and OS for AR ≥0.5 vs AR<0.5
    b) EFS and OS for presence or absence of AID13
    c) EFS and OS for AR ≥0.5 without AID13 vs AR<0.5

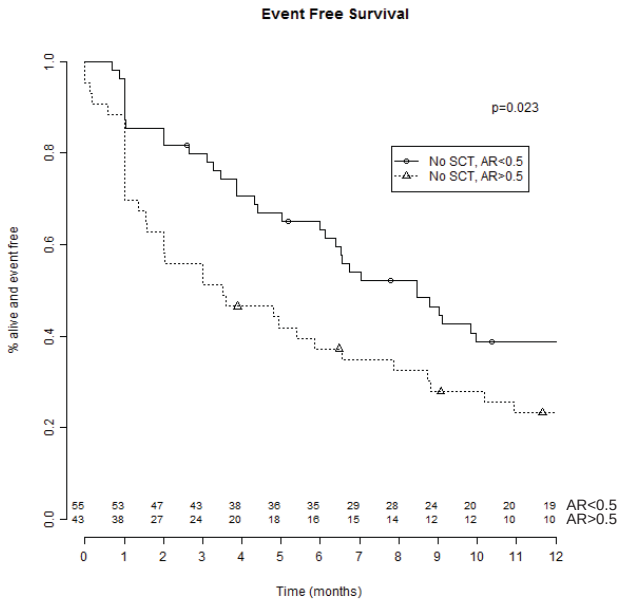
References

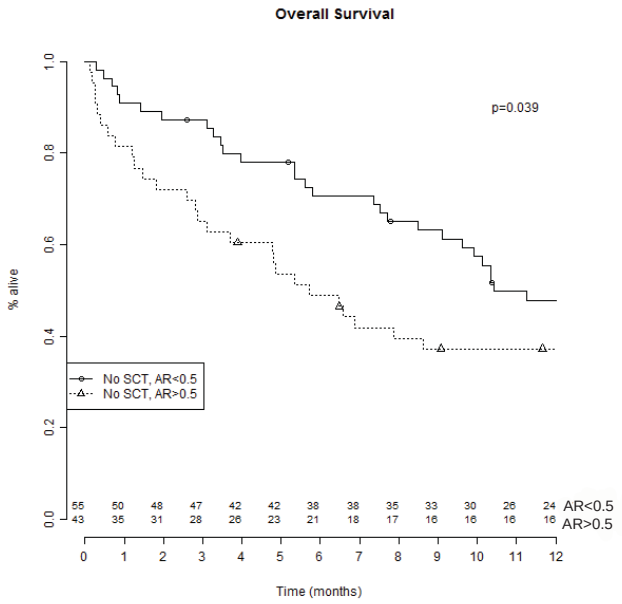
1. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001;98(6):1752-9.
2. Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M, et al. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. Blood. 2014;124(23):3441-9. Epub 2014/10/02.
3. Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T, Levis M. FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. Blood. 2009;115(7):1425-32.
4. Pratcorona M, Brunet S, Nomdedéu J, Ribera JM, Tormo M, Duarte R, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 2013;121(14):2734-8.

5. Linch DC. Impact of FLT3ITD mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia: implications for consolidation therapy. Blood. 2014;124(2):273-6.
6. Raghavan M, Smith L-L, Lillington DM, Chaplin T, Kakkas I, Molloy G, et al. Segmental uniparental disomy is a commonly acquired genetic event in relapsed acute myeloid leukemia. Blood. 2008;112(3):814-21.
7. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. The New England journal of medicine. 2008;358(18):1909-18. Epub 2008/05/03.
8. Griffiths M, Mason J, Rindl M, Akiki S, McMullan D, Stinton V, et al. Acquired Isodisomy for chromosome 13 is common in AML, and associated with FLT3-itd mutations. Leukemia. 2005;19(12):2355-8.
9. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21(24):4642-9. Epub 2003/12/16.
10. Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood. 2001;98(5):1312-20. Epub 2001/08/25.
11. Kottaridis PD, Gale RE, Langabeer SE, Frew ME, Bowen DT, Linch DC. Studies of FLT3 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia: implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection, and possible therapy with FLT3 inhibitors. Blood. 2002;100(7):2393-8.
12. Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, et al. Absence of the Wild-Type Allele Predicts Poor Prognosis in Adult de Novo Acute Myeloid Leukemia with Normal Cytogenetics and the Internal Tandem Duplication of FLT3. Cancer Research. 2001;61(19):7233-9.
13. Green C, Linch DC, Gale RE. Most acute myeloid leukaemia patients with intermediate mutant FLT3/ITD levels do not have detectable bi-allelic disease, indicating that heterozygous disease alone is associated with an adverse outcome. British Journal of Haematology. 2008;142(3):423-6.
14. Lee BH, Tothova Z, Levine RL, Anderson K, Buza-Vidas N, Cullen Dana E, et al. FLT3 Mutations Confer Enhanced Proliferation and Survival Properties to Multipotent Progenitors in a Murine Model of Chronic Myelomonocytic Leukemia. Cancer Cell. 2007;12(4):367-80.
15. Kharazi S, Mead AJ, Mansour A, Hultquist A, Boiers C, Luc S, et al. Impact of gene dosage, loss of wild-type allele, and FLT3 ligand on Flt3-ITD-induced myeloproliferation. Blood. 2011;118(13):3613-21. Epub 2011/08/05.

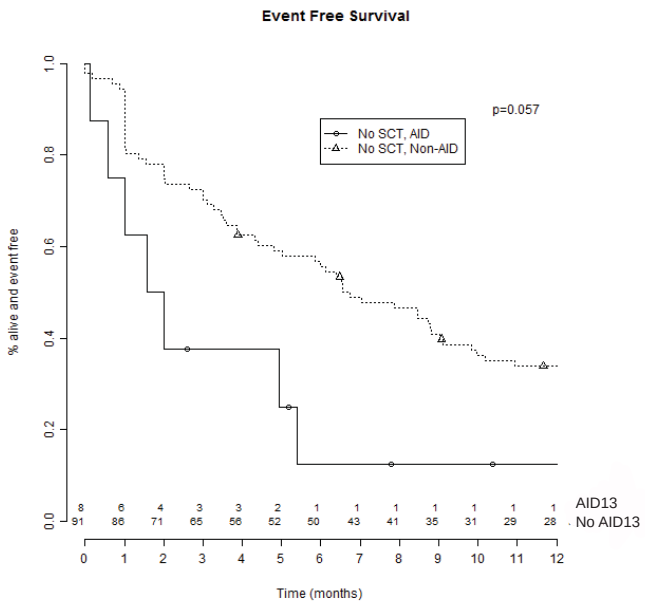
Figure 1

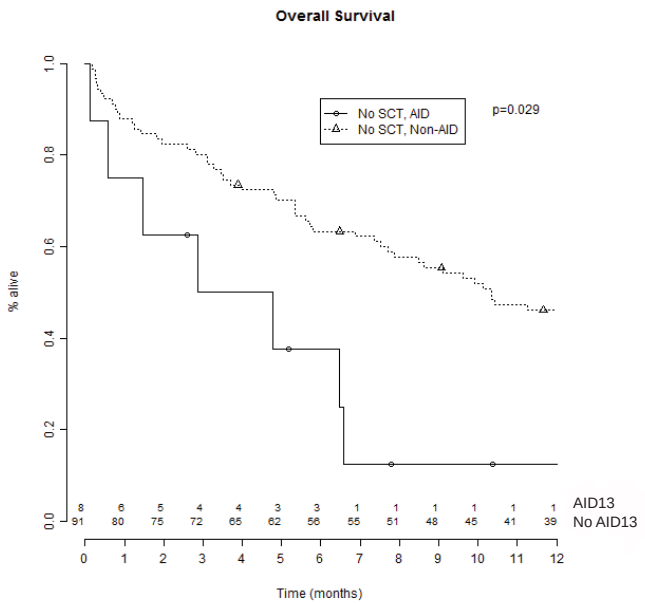
a)


Event Free Survival
p=0.023
No SCT, AR<0.5
No SCT, AR>0.5
% alive and event free
AR<0.5
AR>0.5
Time (months)

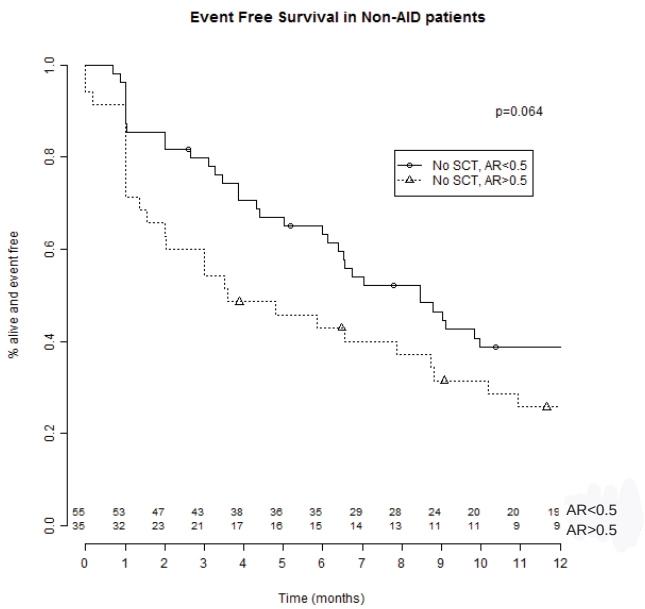

Overall Survival
p=0.039
No SCT, AR<0.5
No SCT, AR>0.5
% alive
AR<0.5
AR>0.5
Time (months)

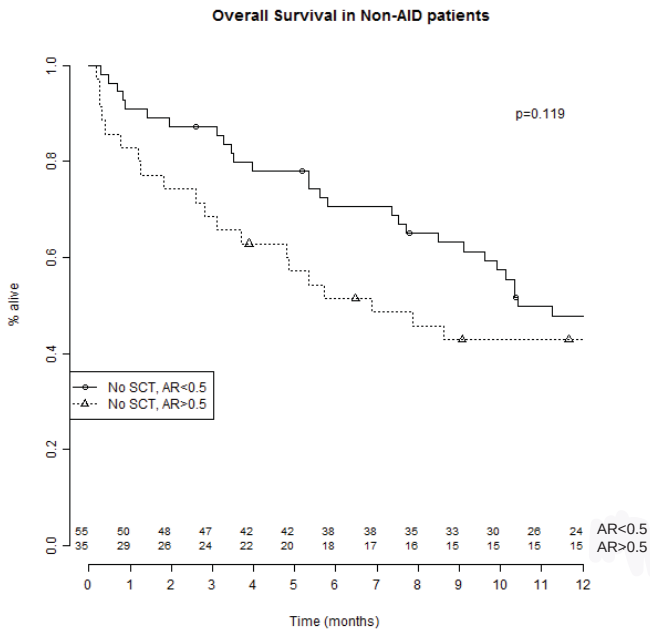
b)


Event Free Survival
p=0.057
No SCT, AID
No SCT, Non-AID
% alive and event free
AID13
No AID13
Time (months)


Overall Survival
p=0.029
No SCT, AID
No SCT, Non-AID
% alive
AID13
No AID13
Time (months)

c)


Event Free Survival in Non-AID patients
p=0.064
No SCT, AR<0.5
No SCT, AR>0.5
% alive and event free
AR<0.5
AR>0.5
Time (months)


Overall Survival in Non-AID patients
p=0.119
No SCT, AR<0.5
No SCT, AR>0.5
% alive
AR<0.5
AR>0.5
Time (months)