Complete remission after the first cycle of induction chemotherapy determines the clinical efficacy of relapse-preventive immunotherapy in acute myeloid leukaemia

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Expected ATL cases in Brazil and its regions estimated accounting for differences in HTLV-1 prevalence between Brazil's regions, age and gender.

References

da Barbosa, S.F.C. (2012) *Soroprevalência da infecção pelo vírus linfotrópico de células T humanas em portadores de doenças linfoproliferativas atendidos no Hospital Ophir Loyola*. Belém, Pará. Available at: http://repositorio.ufpa.br/jspui/bitstream/2011/9140/1/Dissertacao_SoroprevalenciaInfeccaoVirus.pdf.

Carneiro-Proietti, A.B.F., Ribas, J.G.R., Catalan-Soares, B.C., Martins, M.L., Brito-Melo, G.E.A., Martins-Filho, O.A., Pinheiro, S.R., de Araújo, A.Q.-C., Galvão-Castro, B., de Oliveira, M.S.P., Guedes, A.C. & Proietti, F.A. (2002) Infecção e doença pelos vírus linfotrópicos humanos de células T (HTLV-I/II) no Brasil. *Revista da Sociedade Brasileira de Medicina Tropical*, **35**, 499–508. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pxml:id=S0037-86822002000500013&lng=pt&tlng=pt.

Gessain, A. & Cassar, O. (2012) Epidemiological aspects and world distribution of HTLV-1 Infection. *Frontiers in microbiology*, **3**, 388. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artxml:id=3498738&tool=pmcentrez&rendertype=abstract.

Iwanaga, M., Watanabe, T. & Yamaguchi, K. (2012) Adult T-cell leukemia: a review of epidemiological evidence. *Frontiers in microbiology*, **3**, 322. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22973265.

Ministério da Saúde do Brasil (2012) Informação dos Registros Hospitalares de Câncer como estratégia de transformação Perfil do Instituto Nacional de Câncer José Alencar Gomes da Silva em 25 anos. Available at: http://bvsms.saude.gov.br/bvs/publicacoes/inca/Informacao_dos_registros_hospitalares.pdf.

Nunes, D., Boa-Sorte, N., Grassi, M.F.R., Taylor, G.P., Teixeira, M.G., Barreto, M.L., Dourado, I. & Galvão-Castro, B. (2017) HTLV-1 is predominantly sexually transmitted in Salvador, the city with the highest HTLV-1 prevalence in Brazil. *PLoS ONE*, **12**, e0171303. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28158226.

Oliveira, P.D., de Carvalho, R.F. & Bittencourt, A.L. (2017) Adult T-cell leukemia/lymphoma in south and central America and the Caribbean: systematic search and review. *International Journal of STD & AIDS*, **28**, 217–228. Available at: http://journals.sagepub.com/doi/10.1177/0956462416684461.

Oliveira, P.D., Kachimarek, A.C. & Bittencourt, A.L. (2018) Early onset of HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATL): systematic search and review. *Journal of Tropical Pediatrics*, **64**, 151–161. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28582585.

Pombo De Oliveira, M.S., Loureiro, P., Bittencourt, A., Chiattone, C., Borducchi, D., De Carvalho, S.M., Barbosa, H.S., Rios, M., Sill, A., Cleghorn, F. & Blattner, W. (1999) Geographic diversity of adult t-cell leukemia/lymphoma in Brazil. The Brazilian ATLL Study Group. *International journal of cancer*, **83**, 291–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10495418.

Rosadas, C., Malik, B., Taylor, G.P. & Puccioni-Sohler, M. (2018) Estimation of HTLV-1 vertical transmission cases in Brazil per annum. *PLOS Neglected Tropical Diseases*, **12**, e0006913.

Zihlmann, K.F., de Alvarenga, A.T. & Casseb, J. (2012) Living Invisible: HTLV-1-infected persons and the lack of care in public health. *PLoS Neglected Tropical Diseases*, **6**, e1705. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3373594/pdf/pntd.0001705.pdf.

Complete remission after the first cycle of induction chemotherapy determines the clinical efficacy of relapse-preventive immunotherapy in acute myeloid leukaemia

Relapse after the completion of induction and consolidation chemotherapy remains a significant cause of mortality in the post-chemotherapy phase of acute myeloid leukaemia (AML). Several studies have questioned whether AML patients who require two or more courses of induction chemotherapy to attain complete remission (CR) are at increased risk of relapse or death. While studies performed in the 1980's and 1990's yielded inconclusive results (Rowe et al., 2010), contemporary studies have identified that needing more than one cycle of induction chemotherapy to achieve CR is a risk factor for relapse and death in younger adult patients (Othus et al., 2019).

Aspects of immunity are relevant to the relapse risk in AML and several immunotherapies aimed at preventing relapse have been developed (Martner et al., 2013; Weinstock et al., 2017; Beyar-Katz & Gill, 2018; Liu et al., 2019). Immunotherapy with histamine dihydrochloride in conjunction with low-dose interleukin-2 (HDC/IL-2) is approved for relapse prevention in AML patients within the European Union (EU). For this study, we analysed the potential impact of previous induction chemotherapy on the clinical efficacy of HDC/IL-2.

Three hundred and twenty patients with AML (18–84 years, median 55), who were not eligible for allogeneic stem cell transplantation, were randomly assigned to receive relapse-preventive immunotherapy with HDC/IL-2 or no treatment (control group) in a phase III trial. HDC/IL-2 was initiated in CR after consolidation chemotherapy (Brune et al., 2006). Patients in the treatment arm were scheduled to receive 10 consecutive three-week cycles of HDC/IL2 with three- (cycles 1–3) or six-week (cycles 4–10) rest periods. In each cycle, these patients received HDC (Noventia Pharma,

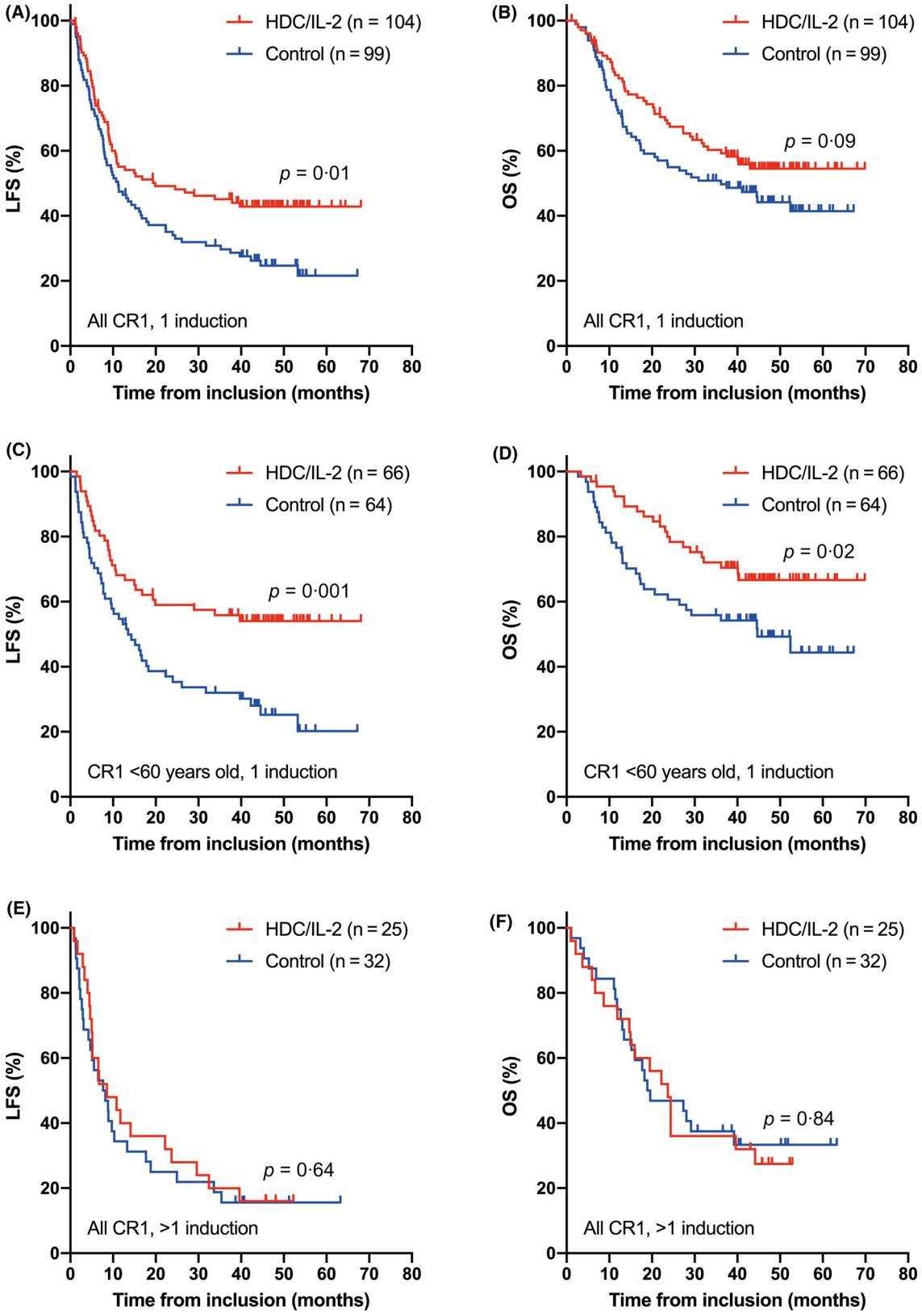

Fig 1. Panels A and B show Kaplan-Meier plots of LFS and OS in HDC/IL-2 treated patients or control patients who attained CR1 after the first cycle of induction chemotherapy. Panels C and D show corresponding results in patients <60 years old. Panels E and F show LFS and OS in patients (all ages) who required >1 induction cycle to attain CR1. Statistical analysis was performed by the logrank test.

Milan, Italy) at 0·5 mg and human recombinant low-dose IL-2 (aldesleukin; 16 400 IU/kg; Chiron Corporation, Emeryville, CA) subcutaneously twice a day. After 18 months of treatment, patients were monitored for at least 18 additional months. The median follow-up time was 48 months.

In the CR1 population ($n = 261$), the number of induction cycles was known in 260 patients, and 203 (78%) of these attained CR after one induction cycle. Among the 57 remaining patients, 47 attained CR after two cycles, while three ($n = 7$) or four ($n = 3$) cycles were required to induce CR in 10 patients. Among control patients, there was a non-significant trend towards inferior clinical outcome in terms of leukemia-free survival (LFS, defined as the time from inclusion to relapse or death) in those who required more than one induction to attain CR, with a similar trend in younger patients (<60 years old; Figure S1A-B). In the HDC/IL-2 arm, outcome was significantly superior in patients who attained CR1 after one induction for all patients as well as for younger patients (Figure S1C-D). In multivariable analyses, it was found that the number of induction cycles required to attain CR independently predicted outcome for patients in the treatment arm (Table S1).

We compared clinical outcomes for HDC/IL-2-treated and control patients who achieved CR after one cycle of induction. In this group, the HDC/IL-2 arm was significantly superior to the control arm for LFS, with a similar trend for overall survival (OS; Figure 1A-B). In patients less than 60 years old, treatment with HDC/IL-2 entailed significantly improved LFS and OS (Figure 1C-D). The survival advantage for patients in the treatment arm over the control arm remained significant in multivariable analyses (Table I), correcting for predefined potential confounders (Brune et al., 2006). Treatment with HDC/IL-2 did not affect LFS or OS in patients who required more than one cycle of induction to attain CR1 (Figure 1E-F) and was not significantly beneficial in older patients (>60 years old; data not shown). A test of interaction on the effect of HDC/IL-2 *versus* controls for LFS supported the finding that it was more pronounced in younger patients who attained CR after one (HR = 0·48) *versus* more than one (HR = 1·33) cycle of induction ($P = 0·04$).

The HDC component of the HDC/IL-2 regimen aims at countering immunosuppression triggered by reactive oxygen species, formed by the NOX2 enzyme expressed by normal and malignant myeloid cells (Martner et al., 2013; Martner et al., 2019). In AML, functional NOX2 is co-expressed with histamine H_2 receptors (H_2R) by leukaemic cells of the M4 and M5 FAB classes (Aurelius et al., 2012a; Aurelius et al., 2012b). We analysed the impact of induction chemotherapy on treatment efficacy in younger patients with FAB M4/M5 AML and observed pronounced efficacy of HDC/IL-2 *versus* controls in terms of LFS and OS in patients who attained CR1 after one induction in univariable and multivariable analysis (Figure S2, Table I).

We conclude that the clinical efficacy of relapse-preventive immunotherapy with HDC/IL-2 hinges on the effectiveness of induction chemotherapy. In this regard, our results confirm and extend previous findings in a single-arm phase IV trial in which 84 AML patients in CR1 received HDC/IL-2 (Aurelius et al., 2019). In addition, similar results of favourable outcome in patients achieving CR after one *versus* several cycles of induction were obtained in AML patients undergoing allogeneic transplantation (Walter et al., 2013). Collectively, these results suggest that efficient elimination of leukemic cells during induction treatment favours immune-mediated clearance of residual leukaemia, thus preventing relapse and death in the post-chemotherapy phase.

Within the group of patients attaining CR after one induction cycle, our data also point to subgroups of patients, including FAB-M4/M5 AML, in whom the clinical efficacy of HDC/IL-2 for relapse prevention is pronounced. These results thus provide means to identify patients who are likely to benefit from immunotherapy and, conversely, patients in

Table I. Cox regression analysis of effects of HDC/IL-2 treatment *versus* control on LFS or OS in patients in CR1 who attained CR after one induction cycle. Covariates that tended to affect LFS or OS in univariable analyses (*P*-values below 0·1) were included in multivariable analyses.

		Univariable analysis			Multivariable analysis†		
Patient groups	Outcome	HR†	95% CI‡	*P*-value	HR	95% CI	*P*-value
All patients	LFS	0·65	0·46–0·92	0·01	0·64	0·45–0·91	0·01
	OS	0·71	0·48–1·05	0·09	0·76	0·51–1·14	0·18
<60 years	LFS	0·48	0·30–0·76	0·002	0·46	0·29–0·72	0·0008
	OS	0·53	0·31–0·92	0·02	0·49	0·28–0·85	0·01
<60 years M4/M5§	LFS	0·28	0·13–0·62	0·002	0·28	0·13–0·62	0·002
	OS	0·28	0·10–0·74	0·01	0·33	0·12–0·91	0·03

*Covariates considered: age; gender (female vs. male); karyotype (favourable vs. intermediate vs. unfavourable); French-American-British (FAB) class M0/M1/M5/M6 (yes vs. no); FAB class M2/M3/M4 (yes vs. no); >3 days cytarabine treatment (yes vs. no); secondary AML (yes vs. no); and months from CR1 to random assignment (>6 mo vs. ≤6 mo).

†HR = hazard ratio between HDC/IL-2 and control.

‡CI = confidence interval.

§M4/M5 = French-American-British class M4 and M5.

whom the therapy is inefficacious. The exploratory nature of these results should be emphasised.

In summary, our results imply that the efficiency of induction chemotherapy independently determines the clinical benefit of relapse-preventive immunotherapy with HDC/IL-2 in adult AML patients who are not candidates for allogeneic transplantation. This finding, along with similar results achieved in patients receiving allo-SCT, suggests that future immunotherapy trials should take the efficiency of induction therapy into account.

Author contributions

Authors MN and AH performed the research. Authors MN, AH, AM, FT and SN analysed the results. Authors MB and KH designed the clinical study. Authors KH, FT and AM wrote the manuscript. All authors approved the manuscript prior to submission.

Conflict of interest

Authors FT, AM and KH hold issued or pending patents protecting the use of NOX2 inhibitors in cancer. Authors MN, AH, MB and SN declare no potential conflicts of interest.

Malin S. Nilsson[1]
Alexander Hallner[1]
Mats Brune[2]
Staffan Nilsson[3,4]
Fredrik B. Thorén[1]
Anna Martner[1]
Kristoffer Hellstrand[1]

[1]TIMM Laboratory, Sahlgrenska Cancer Center, Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, [2]Department of Haematology, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, [3]Department of Laboratory Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg and [4]Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden.
E-mail: kristoffer.hellstrand@microbio.gu.se

Keywords: aml, chemotherapy, immunotherapy, histamine dihydrochloride, IL-2

First published online 18 December 2019
doi: 10.1111/bjh.16320

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Cox regression analysis of the effect of 1 *versus* >1 induction cycle of chemotherapy on LFS or OS in patients in CR1. Covariates with P-values below 0.1 in univariable analyses were included in multivariable analyses.

Figure S1. Kaplan-Meier plots of LFS in all control arm patients in CR1 (A) and in younger control patients (<60 years old, B) who attained CR after 1 *versus* >1 course of induction chemotherapy. Panels C and D show corresponding results in the HDC/IL-2 arm. Statistics by the logrank test.

Figure S2. Kaplan-Meier plots of LFS (A) and OS (B) in younger patients (<60 years old) with AML of FAB M4/M5 classes receiving HDC/IL-2 or control who attained CR1 after the first course of induction chemotherapy. Statistics by the logrank test.

References

Aurelius, J., Martner, A., Brune, M., Palmqvist, L., Hansson, M., Hellstrand, K. & Thoren, F.B. (2012a) Remission maintenance in acute myeloid leukemia: impact of functional histamine H2 receptors expressed by leukemic cells. *Haematologica*, **97**, 1904–1908.

Aurelius, J., Thoren, F.B., Akhiani, A.A., Brune, M., Palmqvist, L., Hansson, M., Hellstrand, K. & Martner, A. (2012b) Monocytic AML cells inactivate antileukemic lymphocytes: role of NADPH oxidase/gp91(phox) expression and the PARP-1/PAR pathway of apoptosis. *Blood*, **119**, 5832–5837.

Aurelius, J., Mollgard, L., Kiffin, R., Ewald Sander, F., Nilsson, S., Bergh Thoren, F., Hellstrand, K. & Martner, A. (2019) Anthracycline-based consolidation may determine outcome of post-consolidation immunotherapy in AML. *Leukemia & Lymphoma*, **60**, 2771–2778.

Beyar-Katz, O. & Gill, S. (2018) Novel approaches to acute myeloid leukemia immunotherapy. *Clinical Cancer Research*, **24**, 5502–5515.

Brune, M., Castaigne, S., Catalano, J., Gehlsen, K., Ho, A.D., Hofmann, W.K., Hogge, D.E., Nilsson, B., Or, R., Romero, A.I., Rowe, J.M., Simonsson, B., Spearing, R., Stadtmauer, E.A., Szer, J., Wallhult, E. & Hellstrand, K. (2006) Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. *Blood*, **108**, 88–96.

Liu, Y., Bewersdorf, J.P., Stahl, M. & Zeidan, A.M. (2019) Immunotherapy in acute myeloid leukemia and myelodysplastic syndromes: The dawn of a new era? *Blood Reviews*, **34**, 67–83.

Martner, A., Thoren, F.B., Aurelius, J. & Hellstrand, K. (2013) Immunotherapeutic strategies for relapse control in acute myeloid leukemia. *Blood Reviews*, **27**, 209–216.

Martner, A., Aydin, E. & Hellstrand, K. (2019) NOX2 in autoimmunity, tumor growth and metastasis. *The Journal of Pathology*, **247**, 151–154.

Othus, M., Estey, E.H., Garcia-Manero, G., Wood, B.L., Stirewalt, D.L., Godwin, J.E., Weick, J.K., Anderson, J.E., Appelbaum, F.R., Erba, H.P. & Walter, R.B. (2019) Second cycle remission achievement with 7+3 and survival in adults with newly diagnosed acute myeloid leukemia: analysis of recent SWOG trials. *Leukemia*, **33**, 554–558.

Rowe, J.M., Kim, H.T., Cassileth, P.A., Lazarus, H.M., Litzow, M.R., Wiernik, P.H. & Tallman, M.S. (2010) Adult patients with acute myeloid leukemia who achieve complete remission after 1 or 2 cycles of induction have a similar prognosis: a report on 1980 patients registered to 6 studies conducted by the Eastern Cooperative Oncology Group. *Cancer*, **116**, 5012–5021.

Walter, R.B., Buckley, S.A., Pagel, J.M., Wood, B.L., Storer, B.E., Sandmaier, B.M., Fang, M., Gyurkocza, B., Delaney, C., Radich, J.P., Estey, E.H. & Appelbaum, F.R. (2013) Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood*, **122**, 1813–1821.

Weinstock, M., Rosenblatt, J. & Avigan, D. (2017) Dendritic cell therapies for hematologic malignancies. *Molecular Therapy - Methods & Clinical Development*, **5**, 66–75.

In *trans* early mosaic mutational escape and novel phenotypic features of germline SAMD9 mutation

Mutations in *SAMD9* or *SAMD9L* are found in patients with familial predisposition to myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) associated with both MIRAGE syndrome (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes, and Enteropathy) and ATXPC (ataxia-pancytopenia syndrome) (Li *et al*., 1981; Narumi *et al*., 2016; Chen *et al*., 2016), respectively. The wild-type (WT) function of *SAMD9* and *SAMD9L* in haematopoietic cell lines is antiproliferative. Germline mutations are thought to be gain-of-function (GOF) mutations, exacerbating the antiproliferative effects of the WT gene (Tesi *et al*., 2017; Buonocore *et al*., 2017; Davidsson *et al*., 2018). Functional somatic reversion through either loss of heterozygosity (−7 or 7q-) or uniparental disomy (UPD), or through acquisition of loss of function (LOF) mutations, has been described in affected individuals. Thus far, LOF mutations have been described in *cis* for *SAMD9*, but rarely in *trans* for *SAMD9L* (Nagata *et al*., 2018; Wong *et al*., 2018). Here we present a four-generation pedigree affected by AML and MDS, with novel disease features and a reversion mutation in *trans,* consistent with germline mosaicism.

The proband (III-1) presented in 1986 aged 10 with a history of excessive bruising. She was one of four siblings, two of which were stillborn. She was also noted to have an extensive family history of MDS and AML; specifically, her mother (II-1) had MDS, an aunt (II-4) died of AML aged 11 following MDS, a cousin (III-5) died of AML aged eight and her mother's cousin (II-8) died of leukaemia (Fig 1A). Her haematological parameters at presentation were normal, except for abnormal platelet function tests. In 1998 (aged 22) she developed tri-lineage cytopenias. Bone marrow aspirate (BMA) at this time showed MDS with 7q- in 5/30 metaphases (46,XX,del(7)(q22q33)[5]/46,XX[25]). Additional multisystem abnormalities were identified over her 20-year follow-up; imaging undertaken for urinary tract infections, adenomyosis and an iliofemoral DVT in 2002 (aged 26) revealed duplex left kidney and ureters, intermediate situs, including right-sided stomach and spleen, multiple small splenenculi, reversed pancreas, midline liver, mispositioned transverse colon and abnormal anatomy of venous drainage.

Follow-up BMA in 2004 (aged 28) showed MDS with normal karyotype (NK) with similar findings in 2015 (aged 39). Illumina Trusight myeloid panel showed an MDS-associated *ZRSR2* variant (pGly438_Ser439insSerArg).

Her daughter, IV-1, was born in 1996 at 28 weeks due to severe IUGR. Blood count at birth showed thrombocytopenia ($90x10^9$/l), presumed to be due to prematurity. However, aged two, she was noted to have anaemia (Hb 9.1g/dl) as well as thrombocytopenia ($65x10^9$/l). A BMA showed MDS with NK. She underwent an unrelated donor transplant at the age of four. Pre-transplant assessment noted that she had global developmental delay. She died two months post-transplant due to Epstein-Barr virus-related disease.

The proband had five additional pregnancies. IV-3 was complicated by severe polyhydramnios and the pregnancy was terminated because of concerns regarding congenital anomalies. IV-5 is alive and well, aged 10.

Informed consent was obtained from the UCL/UCLH Biobank for Studying Health and Disease. Whole exome sequencing was undertaken on stored bone marrow DNA of the proband from 2015, which identified two non-synonymous base changes in *SAMD9* c.2958C> G – resulting in S986C – and c.1447C> G – resulting in p.L483V (Fig 1B). *In silico* analyses of these variants suggested both mutations were likely to be pathogenic (Fig 1C).

To determine if the mutations found in the proband were present in other family members, we undertook targeted deep sequencing of the regions surrounding the *SAMD9* variants in IV-1 and the proband's mother (II-1) along with additional available samples from IV-5 and III-1 (Fig 1D). III-1, IV-1 and II-1 carried L483V at allele frequencies consistent with germline origin. The S986C variant was present only in samples from III-1. Here it was present at an allele frequency of 21% in the peripheral blood (2001) and 30·4% in the BMA from 2015. Matched buccal swab (2015) also showed this variant but at a lower allele frequency (17%). To determine whether this was due to blood contamination, we obtained nail clippings from III-1. This revealed the presence of the S986C mutation at a low frequency of 3·6%. This suggests that the proband