



First case of near haploid philadelphia negative B-Cell acute lymphoblastic leukaemia relapsing as acute myeloid leukemia following allogeneic hematopoietic stem cell transplantation

Claire Horgan^a, Charalampos Kartsios^a, Emmanouil Nikolousis^a, Paneesha Shankara^a, Bhuvan Kishore^a, Richard Lovell^a, Vidhya Murthy^a, Zbigniew Rudzki^a, Sara Dyer^b, Pam Holtom^a, Gillian Thompson^a, Maria Kaparou^a, Evgenia Xenou^a, Rebecca Lloyd^a, Indrani Venkatadasari^a, Alexandros Georgios Kanellopoulos^{a,*}

^a University Hospitals Birmingham NHS Foundation Trust, Heartlands Hospital

^b West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's NHS Foundation Trust

ARTICLE INFO

Keywords:

Acute lymphoblastic leukemia
Acute myeloid leukemia
Lineage switch
Allogeneic stem cell transplantation
Inotuzumab Ozogamicin
Near-Haploidy
TP53

ABSTRACT

Herein we present a female patient aged 61 with Philadelphia negative acute lymphoblastic leukaemia demonstrating near haploid karyotype and abnormal TP53 expression at diagnosis, who relapsed with lineage switch as Acute Monocytic Leukemia post allogeneic stem cell transplantation. Molecular analysis established that both neoplasms were derived from the same founder clone. The leukemic lineage switch phenomenon has recently re-attracted interest as mechanism of leukemic evasion post treatment with chimeric antigen receptor T-cells but there is paucity of data on its presence post allograft or following novel antibody treatments such as Inotuzumab Ozogamicin or Blinatumomab. Our proposition for cancer research is that near haploidy in ALL could be linked to leukemic stem cell plasticity evading stem cell transplantation and other immunotherapy approaches.

Acute leukemia consists of heterogeneous group of clonal malignancies classified by their cell lineage as lymphoid (ALL), myeloid (AML, or mixed phenotype (MPAL)). Each subtype has distinct molecular and genetic alterations associated with it that have significant prognostic implications and are used to inform management decisions. Rare cases of lineage switching have been described during the disease course [1–4] and are typically associated with a poor prognosis. The precise mechanism behind this phenomenon remains unclear; however, studies have suggested that lineage commitment of plastic hematopoietic progenitors may be multidirectional and reversible upon specific signals provided by intrinsic and environmental cues [5]. More recently, lineage switching has been described as a mechanism of chimeric antigen receptor (CAR) T-cell therapy resistance. Genomic analysis and gene editing techniques have enabled us to establish that this occurs as a result of global reprogramming of ALL with inherent lineage plasticity. We describe a unique case of relapsed leukaemia with lineage switch from lymphoid to myeloid three months post allogeneic stem cell transplant.

Patient X, a 61 year old female, with a background of hypothyroidism, lichen planus, vitiligo, spinal canal stenosis (operated in May 2018) and hypertension, was diagnosed with Philadelphia negative B-acute lymphoblastic leukaemia in December 2018 (CD34+/19+/22+/79a+/10weakly+/20+/Lysozyme-/P53+). The leukemic blasts were small cells with only minimally polymorphic nuclei and very scant faintly cytoplasm albeit sporadic large blasts with vacuolated cytoplasm were seen (Figure 2 in Supplement). Cytogenetics demonstrated an abnormal karyotype with a doubled-up near haploid clone (54, XX, +X, +X, +6, +6, +11, +11, der(11;21)(p10;q10)x2, +22, +22 [8]/46,XX [2]). She was treated on the UKALL 14 protocol (registration arm) and completed phase 1 on 31st January 2019, achieving flow negative MRD. This was complicated by raised bilirubin attributed to Pegylated Asparaginase induced hepatic toxicity (her bilirubin increased to 180 mmol/l after a single dose). She went on to receive Phase 2 induction which she completed on 5th April 2019. This was complicated by intrathecal methotrexate-induced confusion and weakness. She subsequently received CNS intensification with intravenous

* Corresponding author. Consultant in Bone Marrow Transplantation, University Hospitals Birmingham NHS FT, Heartlands Hospital, Bordesley Green East, Birmingham, West Midlands, United Kingdom, B17 0PN.

E-mail address: Alexandros.Kanellopoulos@heartofengland.nhs.uk (A.G. Kanellopoulos).

<https://doi.org/10.1016/j.lrr.2020.100213>

Received 13 April 2020; Received in revised form 7 June 2020; Accepted 16 June 2020

Available online 18 June 2020

2213-0489/ © 2020 University Hospitals Birmingham. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Methotrexate.

Unfortunately, the bone marrow biopsy post CNS intensification demonstrated early disease relapse with a moderate to heavy disease burden of at least 50% of the cellular material being lymphoid blasts. The phenotype was noted to have changed slightly with loss of expression of CD20, CD79a and CD10 from the previous specimen. She commenced salvage chemotherapy with Inotuzumab Ozogamycin (InO) on 27th June 2019 and achieved MRD negativity post cycle 2. She received further cycle of InO along with 2 doses of prophylactic intrathecal Cytarabine.

She went on to receive a Fludarabine, Melphalan 100 mg/m², Alemtuzumab 30mg- conditioned matched unrelated donor allograft on 20th September 2019 (day 0). To reduce mucositis and hepatic toxicities the melphalan was reduced to 100 mg/m² and the Alemtuzumab was reduced to 30 mg in order to boost the graft versus leukaemia effect. Graft versus host disease (GvHD) prophylaxis also incorporated Cyclosporine and Mycophenolate Mofetil. Early transplant stay was uneventful- a mild increase in liver enzymes resolved after Posaconazole prophylaxis was paused for a few days whilst liver USS was normal. She achieved neutrophil engraftment on day +16 and was discharged home on day +18 clinically well. Day +28 chimerism was 100% whole blood and T-cells and in the absence of GvHD the Mycophenolate Mofetil and Cyclosporin were suspended on day +60 and +75 respectively.

On the 10th December 2019 patient X underwent her day +100 bone marrow assessment and was admitted to hospital with high fevers, thrombocytopenia, vomiting and deranged LFTs. Abdominal ultrasound demonstrated hepatomegaly and the MRCP on 17th December found multiple liver lesions but no biliary duct pathology. She succumbed to rapid liver failure with associated with pancytopenia, multi-organ failure, sepsis and Disseminated Intravascular Coagulation (DIC) on 19th of December 2019. The bone marrow results were exceptional: Morphology demonstrated a 45% infiltrate of abnormal cells with vacuolated cytoplasm. The flow cytometry demonstrated monocytic AML with blast immunophenotype: (CD56+/CD15+/HLADR+/CD64+/cMPO+/CD11b-/CD13-/CD61-/CD2-/CD11c-/CD14-/CD19-/CD10-/CD32-). The biopsy also advocated the heavy infiltrate of acute leukaemia differed markedly from the disease seen prior to transplant in terms of morphology (see Fig. 1) and also immunohistochemistry. Blasts post-transplant were much larger with much more abundant cytoplasm and more irregular nuclei comprising acute myeloid leukaemia with strong P53 expression on immunohistochemistry (CD34-/TdT-/CD15+/Lysozyme strongly+/P53 strongly+/Ki-67 100%).

Cytogenetic analysis corroborated re-emergence of initial abnormal clone with masked near-haploidy and gain of two copies of BCR, similar to that seen at presentation, whilst the molecular measurable residual disease assessment (MRD) confirmed the presence of founder ALL leukemic clone (3.1×10^{-1} to the level seen in diagnosis). In retrospect, re-analysis of the bone marrow at diagnosis in terms of flow cytometry (December 2018) revealed 1.7% CD56+ cells in the blast region with very low level CD13 and were CD34+ whilst myeloid blasts on relapse lacked CD34 expression. Question remains over whether these cells represented the sporadic large vacuolated cells seen in the diagnostic slide, and whether they were linked to the AML diagnosis post-transplant which also expressed CD56 in a proportion of the cells. In conclusion, we deduced leukemic relapse with phenotypic switch rather than de novo, or therapy-related AML.

Lineage conversion of acute leukaemia has been reported rarely in the literature (see Table 1) [6], supporting the existence of stem cell plasticity with a common pathway for B-cell and myeloid progenitors. The fact that lineage switching appears to be more common with specific genetic subtypes of leukaemia, including those with MLL gene rearrangements, which may have a more inherent plasticity, further supports this notion.

The presented case is unique for two reasons: i) It is the first time that the lineage switch phenomenon has been described in Philadelphia

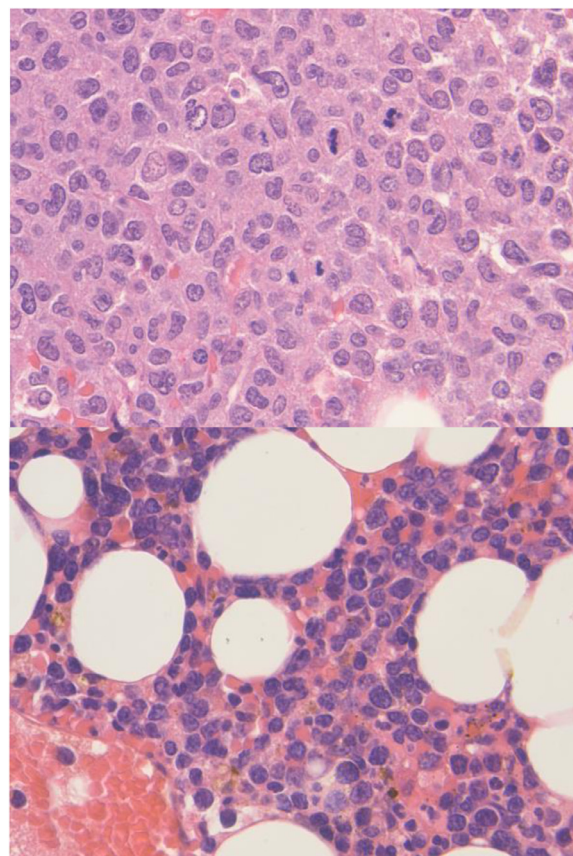


Fig. 1. Upper Panel: Bone marrow biopsy in May 2019 illustrating ALL relapse pre-transplant in June 2019. Lower Panel: Bone marrow biopsy revealing monocytic AML post-transplant in December 2019.

Negative ALL with near haploidy and TP53 mutations [7], and ii) Leukemic Lineage switch has been presumably driven by strong immunological pressure exerted by the graft versus leukemia effect as well salvage with InO. Lineage switch leukemia in patients treated on chemotherapy stems from either clonal selection or genomic editing that reinforces lineage reprogramming. The leukemic switch in this patient might be explained on the basis of second mechanism because the flow cytometry repeat analysis and the bone marrow biopsy did not show convincing evidence of bi-lineage or bi-phenotypic leukemia at diagnosis with consequent clonal selection. This is in line with Jakoby et al. who demonstrated in a series of experiments that relapsing myeloid clones were not detectable prior to CD19 CART administration [4]. Lineage switching in leukemias is more frequent in children than in adults and most cases are ALL converting to AML. Lineage switch mechanisms remain to be elucidated but they might be operating at different levels: i) individual cellular level defined by transcription and epigenetic factors; and ii) at the cell/environmental interaction wherein stem cell reprogramming takes place in response to immune and other extrinsic stress factors [7]. The latter argument is supported by the observation that late relapses to CD19 CART cell treatment can occur as lineage switch AML and this is rather mediated by abrogated expression of the B-cell transcription factors resulting in complete loss of B-cell differentiation pathways. Similar process of plasticity-driven following CD19-directed immunotherapy has been described in CLL with Richter transformation into a plasmablastic lymphoma and in two patients with MLL rearranged ALL transformed to AML and following Blinatumomab treatment [8,9]. Intrinsic factors precipitating the switch to AML in the present case were probably correlated with near haploid chromosomal status. Near haploidy (24–30 chromosomes) has only been once reported in adult ALL and confers very poor prognosis [10]. It is often

Table 1
Reported cases of phenotypic switch in acute leukaemia.

Study	Circumstances of switch
Stass 1984	5 patients with ALL at diagnosis converted to AML and 1 from AML to ALL All patients had received lineage-specific multi-agent chemotherapy and achieved a complete remission prior to lineage switch 3 patients from ALL to FAB M1,, 1 patient to FAB M4 and 1 patient unclassifiable 1 patient converted from AML to T-ALL
Shendi 1995	13 -year old girl with B-ALL relapsed with AML FAB M2 1-year post cessation of treatment
Lounici 2000	46-year old female with AML FAB M4 relapsed with BCP-ALL 6 months post autologous transplant for AML
Rossi 2012	9 cases of lineage switch in childhood acute leukaemia 7 from lymphoid to myeloid, 2 from myeloid to lymphoid 4 patients pro B-ALL to AML M5, 2 patients switched from pre B-ALL (1 to AML M4 and 1 to M5), 1 from common ALL to AML M5 and 2 patients switched from AML M5 to pro B-ALL- in both these cases the B cell precursor lymphoblasts already co-existed at the moment of diagnosis as a minor population (25% and 4% respectively) with the predominant myeloblasts and should therefore be considered as bilineal leukaemias Translocations with involvement of the 11q23 region or mixed lineage leukaemia (MLL) gene were detected in 7 of the 9 of these cases, all of them infants, and the other 2 cases had unspecific abnormalities of chromosome 6 in 1 case and chromosome 19 in the other. All patients reported in this case series died- 2 for sepsis and CNS bleeding during induction, 5 of progressive disease, , 1 patient died in complete remission due to sepsis and another patient died of relapse

associated with aberrant TP53 expression and consistently with our report, hypodiploid ALL is sometimes characterised by blast cells of two different sizes wherein the smaller blasts display near haploidy and the larger are hyperdiploid via endo-reduplication (replication of the chromosomes without subsequent cytoplasm division). Near-haploid ALL bears mutations targeting receptor tyrosine kinase and RAS signaling (mainly NF), histone modifiers (CREBBP), cell cycle genes CDKN2A/B, the6p22 histone gene cluster, and B-cell differentiation factors IKZF3, and PAG1 [11]. Taking all this together, we could speculate that the lineage switch to monocytic AML in this case was driven by stochastic constellation of mutations perturbing the cell cycle, DNA repair and epigenetic differentiation pathways under immune selection exerted by the graft immune cells and InO treatment. Notably, our patient exhibited overwhelming leukemic infiltration of the liver with sudden liver failure and it is to be hypothesised the inciting transforming event to AML might have taken place extramedullary, in the liver, where the graft versus leukemia surveillance is less potent.

To summarize, we reflected on the (first to be reported) case of an adult near haploid ALL patient who relapsed as monocytic AML post allograft. The present study opens questions for research with regards to the nature of leukemic stem cell as well as transdifferentiating and clonal selection pathways in hematological malignancies.

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in

the online version, at [doi:10.1016/j.lrr.2020.100213](https://doi.org/10.1016/j.lrr.2020.100213).

References

- [1] S. Stass, J. Mirro, S. Melvin, C.H. Pui, S.B. Murphy, D Williams, Lineage switch in acute leukemia, *Blood*. 64 (1984) 701–706.
- [2] J. Rossi, A.R. Bernasconi, C.N. Alonso, P.L. Rubio, M.S. Gallego, C.A. Carrara, et al., Lineage switch in childhood acute leukemia: an unusual event with poor outcome, *Am. J. Hematol.* 87 (2012) 890–897.
- [3] E. Dorantes-Acosta, R. Pelayo, Lineage switching in acute leukemias: a consequence of stem cell plasticity? *Bone Marrow Res.* 2012 (2012) 406796.
- [4] E. Jacoby, S. Nguyen, T. Fountaine, K. Welp, B. Gryder, Qin Haiying, et al., CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukaemia lineage switch exposing inherent leukaemic plasticity, *Nat Commun.* 7 (2016) 12320.
- [5] A.C. Shende, A.L. Zaslav, A. Redner, V.R. Bonagura, L. Hatam, C. Paley, et al., A *de novo* lineage switch from B-cell acute lymphoblastic leukemia to acute myelocytic leukemia: a case report, *Am. J. Hematol.* 50 (1995) 75–77.
- [6] A. Lounici, P. Cony-Makhoul, P. Dubus, F. Lacombe, J.P. Merlio, J Reiffers, Lineage switch from acute myeloid leukemia to acute lymphoblastic leukemia: report of an adult case and review of the literature, *Am. J. Hematol.* 65 (2000) 319–321.
- [7] E. Dorantes-Acosta, R. Pelayo, Lineage Switching in Acute Leukemias: a Consequence of Stem Cell Plasticity? *Bone Marrow Res.* (2012) Article ID 406796.
- [8] A.G. Evans, P.G. Rothberg, W.R. Burack, S.F. Huntington, D.L. Porter, J.W. Friedberg, J.L. Liesveld, et al., Evolution to plasmablastic lymphoma evades CD19-directed chimeric antigen receptor T cells, *Br. J. Haematol.* 171 (2015) 205–209.
- [9] R. Gardner, S. Cherian, M. Fang, L.A. Hanafi, O. Finney, H. Smithers, et al., Acquisition of a CD19 negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T cell therapy, *Blood*. 127 (2016) 2406–2410.
- [10] W. Gonzalez-Marques, A.L. Zaslav, D. Balakrishnan, L. Hogan, Y. Ma, D. Tully, et al., A rare case of near-haploid acute lymphoblastic leukemia, *Leuk Res Rep.* 11 (2019) 34–37.
- [11] S. Safavi, K. Paulsson, Near-haploid and low-hypodiploid acute lymphoblastic leukemia: two distinct subtypes with consistently poor prognosis, *Blood*. 129 (2017) 420–423.