| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
| --- | --- | --- | --- | --- | --- |
| Ogawa Y, Tobinai K, Ogura M, Ando K, Tsuchiya T, Kobayashi Y, Watanabe T, Maruyama D, Morishima Y, Kagami Y, Taji H, Minami H, Itoh K, Nakata M, | Phase I and II pharmacokinetic and pharmacodynamic study of the proteasome inhibitor bortezomib in Japanese patients with relapsed or refractory multiple myeloma. | Cancer Sci | 99 | 140-4 | 2008 |
| Maeshima AM, Taniguchi H Nomoto J, Maruyama D, Kim SW, Watanabe T, Kobayashi Y, Tobinai K, Matsuno Y. | Histological and immunophenotypic changes in 59 cases of B-cell non-Hodgkin's lymphoma after rituximab therapy. | Cancer Sci | | (in press) | |
| Chinen Y, Taki T, Nishida K Shimizu D, Okuda T, Yoshida N, Kobayashi C, Koike K, Tsuchida M, Hayashi Y, Taniwaki M | Identification of the novel AML1 fusion partner gene LAF4, a fusion partner of MLL, in childhood T-cell acute lymphoblastic leukemia with t(2;21)(q11;q22) by bubble PCR method for cDNA | Oncogene | 27 | 2249-2256 | 2008 |
| Tanaka R, Kuroda J, Stevenson W, Ashihara E, Ishikawa T, Taki T, Kobayashi Y, Kamitsuji Y, Kawata E, Takeuchi M, Murotani Y, Yokota A, Hirai M, Majima S, Taniwaki M, Maekawa T, Kimura | Fully automated and super-rapid system for the detection of JAK2V617F mutation | Leuk Res | 32 | 1462-1467 | 2008 |
| Hidaka T, Nakahata S, Hatakeyama K, Hamasaki M, Yamashita K, Kohno T, Arai Y, Taki T, Nishida K, Okayama A, Asada Y, Yamaguchi R, Tsubouchi H, Yokota J, Taniwaki | Down-regulation of TCF8 is involved in the leukemogenesis of adult-T cell leukemia/lymphoma | Blood | 112 | 383-393 | 2008 |
| Hiwatari M, Ono R, Taki T, Hishiya A, Ishii E, Kitamura T, Hayashi Y, Nosaka T | Novel gain-of-function mutation in the extracellula domain of the PDGFRA gene in infant acute lymphoblastic leukemia with t(4;11)(q21;q23 | Leukemia | 22 | 2279-2280 | 2008 |
| Taketani T, Taki T, Sako M, Ishii T, Hayashi Y | HLXB9-ETV6 fusion gene in an acute megakaryoblastic leukemia patient and expression of the HLXB9 gene in leukemia and normal B cell line | Cancer Genet Cytogenet | 186 | 115-119 | 2008 |

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Sawada T, Nishiyama C, Kishi T Sasazuki T, Komazawa-Sakon S, Xue X, Piao JH, Ogata H, Nakayama J, Taki T, Hayashi Y, Watanabe M, Yagita H, Okumura | Fusion of OTT to BSAC results in aberrant up-regulation of transcriptional activity | J Biol Chem | 283 | 26820-26828 | 2008 |
| Ohnishi H, Taki T, Yoshino H. Takita J, Ida K, Ishii M, Nishida K, Hayashi Y, Taniwaki M. | Complex t(1;22;11)(q44;q13;q23) translocation causing MLL-p300 fusion gene in therapy-related acute myeloid leukemia | Eur J Haematol | 81 | 475-480 | 2008 |
| Kitoh T, Taki T, Hayashi Y, Nakamura K, Irino T, Osaka M | Transient abnormal myelopoiesis in a newborn wit Down syndrome followed by an acute myeloid leukemia: identification of the same structural chromosomal abnormality at both stage of TAM and leukemi | Cancer Genet Cytogenet | 188 | 99-102 | 2009 |
| Yanada M, Sugiura I, Takeuchi J, Akiyama H, Maruta A, Ueda Y, Usui N, Yagasaki F, Yujiri T, Takeuchi M, Nishii K, Kimura Y, Miyawaki S, Narimatsu H, Miyazaki Y, Ohtake S, Jinnai I | Prospective monitoring of BCR-ABL1 transcript levels in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia undergoing imatinib-combined chemotherapy. | Br J Haematol. | 143 | 503-10 | 2008 |
| 矢ヶ崎　史治、丹羽　敏博、阿部亜紀、石川　真穂、加藤　千明、小倉 健二、佐々木　宏、許　泰一、陣内　逸郎、別所　正美、宮 | Major bcr-abl mRNA定量におけるTMA法(Amp-CML)とRealtime quantitative PCRの相関 | 臨床血液 | | | in press |
| 竹下明裕 | 【末梢血検査異常　何を考え,どう対応するか】分化誘導療法 | Medicina | 45 | 2236-2238 | 2008 |
| 竹下明裕, 直江知樹 | 抗体を用いたがん治療の現状と問題点　白血病(Gemtuzumab, Alemtuzumab | 腫瘍内科 | 3 | 62-71 | 2009 |

**Allogeneic bone marrow transplantation after L-asparaginase-induced pancreatitis in a patient with acute lymphoblastic leukemia**

## 1. Introduction

L-Asparaginase is an important component of anti-leukemic therapy for acute lymphoblastic leukemia (ALL) [1]. Approximately 0.7–18% of patients treated with L-asparaginase develop pancreatitis [2]. Although pancreatitis associated with L-asparaginase in most patients is self-limiting, hemorrhagic pancreatitis or necrosis may occur in <0.5% of patients treated with L-asparaginase [2]. Thus, L-asparaginase-induced pancreatitis is a potentially lethal complication in the treatment of ALL.

In adults, ALL still has a poor prognosis and often requires allogeneic hematopoietic stem cell transplantation (allo-SCT) [1]. To date, it is not established whether allo-SCT can be underwent in a patient with ALL after severe pancreatitis. We present an ALL patient with L-asparaginase-induced severe pancreatitis that was successfully treated with intra-arterial injection of nafamostat mesilate and imipenem/cilastatin sodium. In addition, the patient received allo-SCT 2 months after L-asparaginase-induced pancreatitis.

## 2. Case report

In December 2005, a 23-year-old Japanese man was diagnosed as having precursor B-cell ALL with normal karyotype. A hematological complete remission (CR) was not achieved after five courses of remission induction therapy. In July 2006, a sixth course of remission induction therapy consisting of vincristine, pirarubicin hydrochloride, cyclophosphamide (CY), L-asparaginase and prednisolone (PSL) was initiated [3]. The L-asparaginase was scheduled to give at 6000 U/m$^2$ as six intravenous doses administered within 2 weeks. After five L-asparaginase infusions, the patient complained of upper abdominal pain. Within 12 h, his condition deteriorated and he showed signs of shock on day 13. Laboratory findings showed an amylase level of 511 U/l (normal <116 U/l), pancreas-amylase 455 U/l (<53 U/l), C-reactive protein (CRP) 0.66 mg/dl (<0.3 mg/dl), and leukocyte count $0.5 \times 10^9$/l L-Asparaginase-induced acute necrotizing pancreatitis was suspected and antileukemic therapy was discontinued. Ciprofloxacin, vancomycin and fluconazole were initiated, and anti-proteolytic therapy using urinastatin was administered. Since hypotension and hypoxia persisted on day 14, the patient received mechanical ventilation and continuous hemodiafiltration (CHDF). Abdominal computed tomography (CT) with intravenous contrast agent revealed necrotic change in most of the pancreas, expect for the head, and massive fluid in the peripancreas and the pleural cavity, indicating severe necrotizing pancreatitis (Fig. 1A and B). Although leukocyte counts were $0.1 \times 10^9$/l without neutrophils, intra-arterial catheters were inserted into both the celiac trunk and superior mesenteric artery on day 15. To control proteolysis and secondary bacterial infection, intra-arterial injection of nafamostat mesilate and imipenem/cilastatin sodium was started [4]. CRP increased up to 31.68 mg/dl on day 15 (Fig. 2). Abdominal CT scan

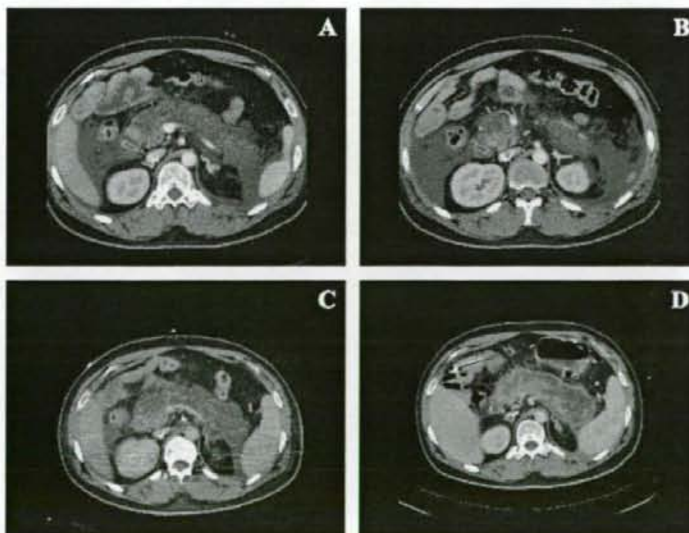


Fig. 1. Computed tomography-scan images of pancreatitis. Upper panels (A and B) indicate enhancement only in the pancreas head, and the presence of peripancreatic fluid on day 14. Decreased peripancreatic fluid seen on day 19 (C). The boundaries of pancreas are enhanced and a naso-jejunic tube is seen on day 27 (D).

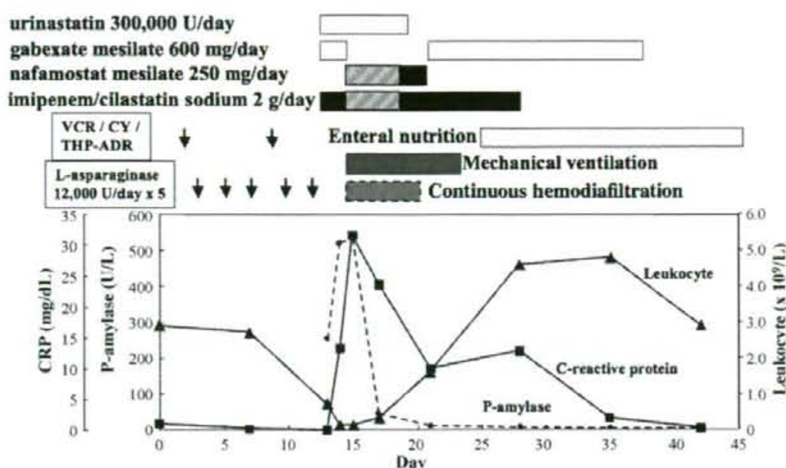


Fig. 2. Clinical course during treatment of pancreatitis. Pancreatitis developed on day 13. Intra-arterial injection (slashed box), continuous hemodiafiltration and mechanical ventilation were carried out in the intensive care unit. VCR: vincristine; THP-ADR: pirarubicin hydrochloride; CY: cyclophosphamide; CRP: C-reactive protein.

showed a decrease in peripancreatic fluid, while most of the pancreas did not show contrast enhancement, expect for the head, on day 19 (Fig. 1C). Since the serum amylase normalized and secondary bacterial infection was not indicated, the intra-arterial catheters were removed on day 19 and then nafamostat mesilate and imipenem/cilastatin sodium were administered intravenously (Fig. 2). CHDF and mechanical ventilation were discontinued on days 20 and 22, respectively. Enteral nutrition was started through a naso-jejunic tube in order to inhibit production of exocrine pancreatic enzymes on day 26. Abdominal CT showed loss of peripancreatic fluid and the boundaries of the pancreas were enhanced after administration of intravenous contrast agent without any sign of pseudocysts on day 28 (Fig. 1D). The patient achieved hematological CR with 2.8% blasts in the bone marrow on day 29.

The patient consented to receive allo-SCT because he did not achieve CR after repeated induction chemotherapy. Two months after development of severe pancreatitis, he received a conditioning regimen consisting of CY (120 mg/kg) and fractionated total body irradiation (12 Gy), followed by bone marrow transplantation from an HLA-matched unrelated donor. Graft-versus-host (GVHD) prophylaxis consisted of short-term methotrexate and tacrolimus. In order to avoid recurrence of pancreatitis, oral intake other than a little water was prohibited. After confirmation of engraftment, he started oral intake on day 14. On day 49, grade III acute GVHD developed in the skin and he received intravenous methylprednisolone (30 mg daily) followed by reduced-dose oral PSL. The patient is still in CR 18 months later, although he developed diabetes mellitus and is receiving insulin therapy.

## 3. Discussion

L-Asparaginase-induced pancreatitis is an uncommon but potentially lethal complication in the treatment of ALL. The mechanism behind L-asparaginase-induced pancreatitis remains unknown. Hypertriglyceridemia due to L-asparaginase has been suggested as a possible mechanism of pancreatitis [5]. Although our patient showed an increase in triglyceride level from 117 mg/dl on day 5 to 531 mg/dl on day 12, it did not reach 1000 mg/dl, which might increase the risk of pancreatitis. Other mechanisms, such as hypersensitivity reactions and inhibition of normal protein synthesis, were mostly not excluded. Pancreatitis has been associated with a myriad of other drugs [6]. Although PSL was given to our patient, pancreatitis occurred on day 12, after tapering PSL dose from 40 mg/m$^2$ to 7.5 mg/m$^2$. Therefore, pancreatitis appears to be attributed to L-asparaginase, which was administered on days 2, 4, 6, 9 and 11. Onset of pancreatitis ranges from 2 days after the beginning therapy to 10 weeks after discontinuing therapy [2]. In our patient, abdominal symptoms started at 12 days after starting L-asparaginase therapy. In addition, the patient received a total of 156,000 U/m$^2$ of L-asparaginase during four courses of chemotherapy.

Early death in patients with acute pancreatitis is mainly due to multi-organ failure, which is probably caused by release of mediators and cytokines from the inflamed and necrotic pancreas [7]. The majority of deaths, however, occur at a later stage and are due to local and systemic septic complications [8]. Mortality rates from L-asparaginase-induced pancreatitis in ALL patients remain unknown, while the mortality rate was 21.4% in patients with the same grade of acute pancreatitis caused by other than L-asparaginase in

Japan [9]. In addition, the mortality rate was 15% in patients with the same grade of acute pancreatitis scored with the Ranson criteria [10]. It is important to inhibit pancreatic exocrine function to prevent autodigestion of the pancreas, along with damage to the surrounding tissues. In the present patient, a high concentration of nafamostat mesilate and imipenem/cilastatin sodium through intra-arterial injection might have prevented the proteolysis and secondary bacterial infection of the pancreas [4], despite the presence of severe necrotizing pancreatitis.

The present patient successfully underwent allo-SCT 2 months after development of severe pancreatitis. To our knowledge, this is the first report of allo-SCT after L-asparaginase-induced severe pancreatitis in a patient with ALL. Allo-SCT also has some risk for pancreatitis. Signs of pancreatitis were found in 28% of recipients in an autopsy series of allo-SCT patients [11]. Many factors including drugs (cyclosporine, tacrolimus and PSL) and viral infections (cytomegalovirus, varicella zoster virus and adenovirus) can be risk for acute pancreatitis in allo-SCT [12]. Allo-SCT is still one of choice of treatment for the ALL patient even with L-asparaginase-induced pancreatitis. This case therefore shows that allo-SCT appears to be safe and effective even in the patients who have developed L-asparaginase-induced severe pancreatitis. It is of importance that pancreatitis can subside without evidence of inflammation at the time of allo-SCT.

### Conflict of interest

None of the authors have any conflict of interest to be documented.

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### References

[1] Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med 2006;354:166–78.

[2] Greenstein R, Nogeire C, Ohnuma T, Greenstein A. Management of asparaginase induced hemorrhagic pancreatitis complicated by pseudocyst. Cancer 1979;43:718–22.

[3] Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. J Clin Oncol 2006;24:460–6.

[4] Takeda K, Matsuno S, Ogawa M, et al. Continuous regional arterial infusion (CRAI) therapy reduces the mortality rate of acute necrotizing pancreatitis: results of a cooperative survey in Japan. J Hepatobiliary Pancreat Surg 2001;8:216–20.

[5] Parsons SK, Skapek SX, Neufeld EJ, et al. Asparaginase-associated lipid abnormalities in children with acute lymphoblastic leukemia. Blood 1997;89:1886–95.

[6] Trivedi CD, Pitchumoni CS. Drug-induced pancreatitis: an update. J Clin Gastroenterol 2005;39:709–16.

[7] Brady M, Christmas S, Sutton R, et al. Cytokines and acute pancreatitis. Baillieres Best Pract Res Clin Gastroenterol 1999;13:265–89.

[8] Beger HG, Rau B, Mayer J, Pralle U. Natural course of acute pancreatitis. World J Surg 1997;21:130–5.

[9] Dervenis C, Johnson CD, Bassi C, et al. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini consensus conference. Int J Pancreatol 1999;25:195–210.

[10] Eachempati SR, Hydo LJ, Barie PS. Severity scoring for prognostication in patients with severe acute pancreatitis: comparative analysis of the Ranson score and the APACHE III score. Arch Surg 2002;137:730–6.

[11] Ko CW, Gooley T, Schoch HG, et al. Acute pancreatitis in marrow transplant patients: prevalence at autopsy and risk factor analysis. Bone Marrow Transplant 1997;20:1081–6.

[12] Nieto Y, Russ P, Everson G, et al. Acute pancreatitis during immunosuppression with tacrolimus following an allogeneic umbilical cord blood transplantation. Bone Marrow Transplant 2000;26:109–11.

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IgA level. The patient was reevaluated after three cycles of VAD. Interestingly, normal haematological values were fond including serum IgA monoclonal component returning to initial levels despite persistence of low IgG and IgM levels and increased $\beta_2$-microglobulin serum concentration. Similarly, BM PC numbers remained stable but with reappearance of normal polyclonal PC. Unexpectedly, no evidence of abnormal blasts or myelodysplasia was found. Multiparameter flow cytometry immunophenotypic analysis of the BM revealed a profile compatible with normal/reactive myeloid and precursor cell components. The patient remained in remission during 14 months with no treatment or transfusions; after this period she relapsed of her MDS with identical clinical and biological characteristics (Table 1). The clonal nature of both CD34 precursors and neutrophils was confirmed by the HUMARA test.

## 4. Discussion

Most frequently, occurrence of cytopenias in MM patients is mainly due to BM failure because of disease severity. Here we show that, at least in some cases, occurrence of cytopenias in MM should forewarn the presence of another haematological malignancy, such as MDS, especially in older patients [1,2]. Notably, diagnosis of MDS was attained due to the presence of a high number of blasts cells and clear dysplastic features detected in a BM smear. In cases showing low blast cell numbers and less pronounced dysplastic features, clonality studies (e.g., HUMARA test for assessing inactivation of chromosome X in females) could help to confirm the clonal nature of purified myeloid cells and supporting the diagnosis of MDS, as found in our patient; otherwise, such cases may remain misdiagnosed [2].

Even though the VAD protocol is not a standard treatment for MDS, it was used here in an effort to control both diseases. To our knowledge, this is the first report about a VAD-induced remission of AREB-II, pointing out the potential utility of this type of therapy in high-risk MDS patients.

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### References

[1] Mauritzson N, Albin M, Rylander L, Billström R, Ahlgren T, Mikoczy Z, et al. Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 1974–2001. Leukemia 2002;16:2366–78.

[2] Ishii Y, Hsiao HH, Sashida G, Ito Y, Miyazawa K, Kodama A, et al. Derivative (1;7)(q10;p10) in multiple myeloma. A sign of therapy-related hidden myelodysplastic syndrome. Cancer Genet Cytogenet June 2006;167(2):131–7.

[3] Tsiara S, Economou G, Panteli A, Isaakidis P, Kapsali E, Bourantas KL. Coexistence of myelodysplastic syndrome and multiple myeloma. J Exp Clin Cancer Res December 1999;18(4):565–6.

[4] Shibata K, Shimamoto Y, Nakazato S, Matsuzaki M, Tadano J. Refractory anaemia with ringed sideroblasts concurrent with multiple myeloma—a brief review of the recent literature. Haematologia (Budapest) 1997;28(4):199–205.

[5] Mittelman M, Floru S, Pick AI. Is granulocyte-macrophage colony-stimulating factor (GM-CSF) safe in myelodysplastic syndromes? Haematologia (Budapest) 1994;26(2):111–5.

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## Treatment of therapy-related acute myeloid leukemia occurring in elderly non-Hodgkin lymphoma patients with low-dose cytarabine, aclarubicin and granulocyte colony-stimulating factor

*To the Editor,*

The addition of rituximab to chemotherapy increases the complete remission (CR) rate and prolongs the overall survival (OS) rate in elderly patients with diffuse large B-cell lymphoma (DLBCL) [1]. However, the benefits of rituximab for patients with B-cell lymphoma may also bring about a high incidence of therapy-related leukemia (TRL). The effects of intensive chemotherapy in elderly patients with acute myeloid leukemia (AML) are not comparable with those in younger patients due to the biologic characteristics of AML cells and a poor tolerance to chemotherapy. In addition, the chemotherapeutic response is generally poorer in patients with TRL than in patients with *de novo* AML. Combination CAG therapy comprising cytarabine (Ara-C), aclarubicin and granulocyte colony-stimulating factor (G-CSF) has shown promising effects in the treatment of elderly patients with AML [2,3]. In the present study, we describe 3 of 142 B-cell

lymphoma patients treated with standard chemotherapy and rituximab between 2002 and 2006 in our hospital who developed therapy-related AML (tAML). Of these three patients who received CAG therapy, two patients achieved CR.

*Patient 1*: A 70-year-old woman had a diagnosis of stage III DLBCL with an immunoblastic variant in July 2003 (Table 1). She received chemotherapy plus rituximab and achieved a durable CR. In July 2005, the patient was diagnosed as having tAML (M4 in the FAB classification). She received two courses of CAG (Ara-C 10 mg/m$^2$, q12h, on days 1–14; aclarubicin 14 mg/m$^2$ on days 1–4; G-CSF 200 µg/m$^2$ on days 1–14) plus vincristine 1.0 mg/m$^2$ and prednisolone 40 mg/m$^2$ (VP) therapy, and obtained a CR (Table 2). Thereafter, she received eight courses of postremission chemotherapy consisting of VP-CAG.

*Patient 2*: A 65-year-old man had a diagnosis of stage IV follicular lymphoma in May 1998. He received multiple chemotherapeutic regimens including autologous peripheral blood stem cell transplantation. In July 2006, approximately 8 years after the onset of lymphoma and during the 4th relapse of lymphoma, he was diagnosed as having tAML (M2) (Table 1). He received three courses of CAG, resulting in no response and no obvious adverse effects.

*Patient 3*: A 74-year-old man was diagnosed as having stage IIE DLBCL in June 2003. In February 2007, at 4 years and 2 months after the onset of lymphoma and during the 2nd CR of lymphoma, he had tAML (M4). He received two courses of VP-CAG therapy and obtained a hematological and cytogenetic CR.

Up to 10% of patients treated with conventional or high-dose therapy for non-Hodgkin lymphoma (NHL) may develop TRL within 10 years [4]. Further long-term follow-up is required to estimate the precise incidence of TRL in our series of patients. Patients 1 and 2 had chromosome 7q abnormalities characteristically seen in TRL associated with alkylating agents, whereas patient 3 had a chromosome 11q23 translocation characteristically observed in tAML associated with topoisomerase II inhibitors (Table 2). All three patients belonged to an unfavorable risk group of AML [5]. The incidence of unfavorable karyotypes in tAML patients at the University of Chicago was about 75% and their median OS was 8 months [6]. In a German AMLCG study, the median OS for an unfavorable cytogenetic group was 6 months and the OS of patients with tAML was significantly inferior to that of patients with *de novo* AML [7].

The CAG priming regimen was initially reported to increase the CR rate in 69 patients with primary resistant AML and secondary AML [2]. Elderly patients with tAML cannot receive the standard remission induction therapy due to poor tolerance of chemotherapy and increased accumulation of anthracyclines. Although aclarubicin is an anthracycline, its cardiotoxicity is estimated to be 10-fold lower than that of doxorubicin [8]. In addition, the combination of G-CSF with Ara-C may not only have a promising effect but also prevent infection by maintaining neutrophils during the chemotherapy. Although infection or febrile neu-

Table 1
Clinical profiles of the patients with NHL who developed tAML

| Patient no. | Age and sex | Histology of NHL | Therapy for NHL | THP-ADR (mg/m$^2$) | ADR (mg/m$^2$) | CY (mg/m$^2$) | ETP (mg/m$^2$) | Years to TRL |
|---|---|---|---|---|---|---|---|---|
| 1 | 69F | DLBCL | THPCOP × 2, R-THPCOP × 6 | 284 | – | 4219 | – | 2.0 |
| 2 | 65M | FL | [a] | – | 633 | 7765 | 4259 | 8.2 |
| 3 | 72M | DLBCL | R-THPCOP × 6, R-EPOCH ×4, radiation | 367 | – | 3571 | 667 | 4.2 |

DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; THPCOP: pirarubicin (THP-ADR), cyclophosphamide (CY), vincristine and prednisolone; R: rituximab; EPOCH: etoposide (ETP), pirarubicin, cyclophosphamide, vincristine and prednisolone; ADR: doxorubicin; TRL: therapy-related leukemia.
[a] Multiple chemotherapeutic regimens including autologous peripheral blood stem cell transplantation.

Table 2
Clinical profiles and outcomes of tAML patients after treatment of NHL

| Patient no. | Age and sex | Hb (g/dl) | Platelets ($\times 10^9$/L) | Leukocytes ($\times 10^9$/L) | %Blasts (PB) | %Blasts (BM) | Karyotype | Response | RFS (months) | OS (months) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 72F | 9.1 | 6 | 10.1 | 32 | 61.6 | 46,XX,del(7)(q22) [18]/46,idem,add (11)(p11) [2] | CR | 24+ | 28+ |
| 2 | 73M | 9.1 | 5.2 | 4.1 | 10 | 43.6 | 46,XY,add(7)(q11),add(20)(q1?) [20] | NR | 0 | 13 |
| 3 | 75M | 8.9 | 5.9 | 16.9 | 3 | 24 | 46,XY,t(11;16) (q23;p13.3) [20] | CR | 4+ | 9+ |

CR: complete remission; NR: no response; RFS: relapse-free survival; OS: overall survival from the initial CAG therapy for tAML.

tropenia was seen during induction chemotherapy in the present patients, all three patients showed amelioration of these infections. In addition, no adverse effects that interrupted repeated CAG chemotherapy courses as postremission therapies were observed. Therefore, CAG seems to be a safe and effective regimen for elderly patients with tAML after treatment of NHL.

## Conflict of interest statement

None.

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## References

[1] Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235–42.
[2] Saito K, Nakamura Y, Aoyagi M, et al. Low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) for previously treated patients with relapsed or primary resistant acute myelogenous leukemia (AML) and previously untreated elderly patients with AML, secondary AML, and refractory anemia with excess blasts in transformation. Int J Hematol 2000;71:238–44.
[3] Qian SX, Li JY, Tian T, et al. Effect of low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor priming (CAG regimen) on the outcome of elderly patients with acute myeloid leukemia. Leuk Res 2007;31:1383–8.
[4] Armitage JO, Carbone PP, Connors JM, et al. Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. J Clin Oncol 2003;21:897–906.
[5] Haferlach T, Schoch C, Loffler H, et al. Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: results of a multiparameter analysis from the German AML Cooperative Group studies. J Clin Oncol 2003;21:256–65.
[6] Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. Blood 2003;102:43–52.
[7] Schoch C, Kern W, Schnittger S, et al. Karyotype is an independent prognostic parameter in therapy-related acute myeloid leukemia (t-AML): an analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. Leukemia 2004;18:120–5.
[8] Hori S, Shirai M, Hirano S, et al. Antitumor activity of new anthracycline antibiotics, aclacinomycin-A and its analogs, and their toxicity. Gann 1977;68:685–90.

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**Occurrence of colon adenocarcinoma in chronic myeloid leukemia patients treated with imatinib: Report of two cases and review of the literature**

The 60-month follow-up of IRIS trial with imatinib in CML demonstrated that grade 3–4 adverse events diminished over time, and there was no clinically significant change in the profile of late effects. So far no peculiar long-term side effects related to imatinib were reported [1].

In 2005, Roy et al. [2] referred on the occurrence of second malignancies in 7/189 CML patients treated with imatinib in late chronic phase (CP) after interferon failure and expressed uncertainty whether the solid tumours were related to the drug. We report here our experience on the occurrence of a second neoplasia in 2 out of 150 CML late CP patients treated with imatinib.

A 69-year-old male, diagnosed as having Ph+ CML in July 1999 was treated with Interferon from 1999 to 2004, when the drug was suspended for intolerance; complete cytogenetic remission (CCR) was reached with persistence of molecular disease. In October 2004 imatinib was started at 400 mg/day and a complete molecular response (CMR) was reached at the 13th month. After 33 months, the patient experienced alternate episodes of stypsis and diarrhoea and a colonoscopy showed a transverse colon stenosis. Surgical treatment was necessary and a diagnosis of mucinous adenocarcinoma (T3, N2, M2; Dukes's stage C) was made. Stadiation revealed metastasis in lymphnodes and peritoneum (pt2, pn1, pmx, m1, g2).

The patient received palliative surgery with anterior sigma resection since perivisceral fat. A PET scan also revealed localization in the first cervical metamer. Presently, the patient is being treated with cycles of bevacizumab associated with 5-FU (FLUFI schedule) and imatinib during the free intervals of chemotherapy.

The second patient was a 76-year-old male, diagnosed as having CML in 2002. IFN was started in October 2002, but the drug was definitively suspended after 3 months because of severe intolerance and imatinib was started at 400 mg/day. After 3 months on this treatment the patient reached a complete cytogenetic remission (CCR), which was confirmed at 6 and 12 months.

In May 2005, the patient complained anorexia and progressive loss of weight, diarrhoea with persistent fever of unknown origin. Tenesmus of the rectum became evident after 20 days and a CT scan revealed a mass in the sigma infiltrating omolateral urethere and vessels. The patients was then surgically treated, with palliative right colostomy for a colon adenocarcinoma (T3, N1, M2; Dukes's stage C), and died after 15 days for bleeding complications.

Similarly to the patients described by Roy et al. [2], also our 2 cases were in late CP, received hydroxyurea and IFN prior to imatinib and developed a second neoplasia after 25 and 33 months, respectively, of imatinib therapy. A surveillance analysis of clinical trials conducted until 2005, recorded 110 malignant neoplasias among imatinib treated CML patients [3], similarly to that expected in the general population with standardized incidence ratio. In our series of 150 patients we did observe only two cases of colon adenocarcinoma. This type of neoplasia represents the fourth most frequent tumour in Europe; the incidence in Italy varies from region to region with new cases being 75.1/100.000 individuals/year for men and 59.0 for women [4].

Imatinib acts through inhibition of the hybrid BCR/ABL but is also effective, albeit at a lesser extent, on the normal cellular ABL, a downstream effectors of the Eph-receptor, which is responsible for mediation of repulsive cell–cell interaction, angiogenesis and epithelial homeostasis, with tumour suppression effect. Crk, an adaptor protein, mediates this action through phosphorylation requiring ABL tyrosine kinase [5]. The ephrin-Eph pathway has tumour suppressor function and this interaction activates bidirectional signalling to regulate different target proteins [5]; in mouse intestine this pathway can regulate cell migration and compartmentalization through crypt-villus axis and can constrain the progression of epithelial tumours [6]. In human carcinoma cells, the ephrin-Eph tumour suppressor function is compromised and imatinib inhibition is directed against the ephrin B2 ligand by blocking the tumour suppressive effects of Eph receptor. The Eph receptor is large and it is not known whether all Eph receptors have tumour suppressive effects [6]. The receptor tyrosine kinase EphB4, is a direct transcriptional target of the TCF/βcatenin complex; the expression of this receptor is frequently lost in colorectal cancer, suggesting that the inhibition of EphB4 signalling may contribute to tumour progression [6].

In our cases a relationship between imatinib therapy, or previous exposure to IFN or cumulative effect of either exposures and development of colon adenocarcinoma cannot be

**bjh** research paper

# Age-associated difference in gene expression of paediatric acute myelomonocytic lineage leukaemia (FAB M4 and M5 subtypes) and its correlation with prognosis

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## Summary

Acute myeloid leukaemia, French-American-British M4 and M5 subtypes (AML-M4/M5) is frequently associated with *MLL* gene rearrangement and its incidence is relatively high among infants. Clinically, paediatric AML-M4/M5 has been considered as an intermediate or undefined prognostic group. In this study, we analysed gene expression of 40 paediatric AML-M4/M5 patients excluding inv(16) and t(8;21) patients, and found striking differences among the patients in an age-associated manner. In particular, most of the infants displayed very distinct gene expression. On the basis of this difference, we divided paediatric patients into three subgroups (A, B and C) with the average age of 0·3, 3·1 and 6·6 years old respectively. All subgroups included patients with *MLL* gene rearrangement as well as normal and other karyotypes. Surprisingly, gene expression signatures of *MLL* gene rearrangement differed substantially among these subgroups. In addition, subgroup C presented extremely poor outcome (3-year event-free survival 28%) whilst eight patients with *MLL* gene rearrangement in subgroup C had all relapsed within 18 months. These results suggest that age is an important factor contributing to the biology of AML-M4/M5 and the sub-grouping procedures developed in this study could be a powerful tool to identify unfavourable risk patients within paediatric AML-M4/M5.

**Keywords:** acute myeloid leukaemia, gene expression profiling, microarray, MLL, prognostic factors.

Acute myeloid leukaemia (AML) is a heterogeneous disease with different morphological features usually classified according to the French-American-British (FAB) classification system into subtypes M0–M7. In addition, a variety of genetic alterations have been ascertained including specific chromosomal translocations, such as t(8;21), t(15;17), inv(16) and 11q23 rearrangement. These major chromosomal translocations are usually associated with specific FAB subtypes. With the introduction of high throughput gene expression analysis using DNA microarrays, many studies have reported unique gene expression signatures for major chromosomal translocations, suggesting that the underlying molecular biology of these leukaemias can be defined by the genetic alterations (Golub *et al*, 1999; Armstrong *et al*, 2002; Schoch *et al*, 2002; Yagi *et al*, 2003; Ross *et al*, 2004). AML classified as FAB M4 or M5 subtypes (AML-M4/M5) have myelomonoblastic or monoblastic morphology. In this subtype, 11q23 rearrangements resulting in the fusion of the *MLL* with other partner genes (*MLL* gene rearrangement) are often observed. Unlike other chromosomal translocations, *MLL* gene rearrangement is also observed in acute lymphoblastic leukaemia (ALL), especially in infant ALL. Different studies have identified common gene expression signatures for acute leukaemia that contain *MLL* gene rearrangement irrespective of their lineage (Armstrong *et al*, 2002; Ross *et al*, 2004; Kohlmann *et al*, 2005).

The biological and clinical features of acute leukaemia differ between children and adults (Downing & Shannon, 2002) and considerable variations exist among different age groups in children. It is well known that infant ALL possess molecular abnormalities of *MLL* gene rearrangement that result in

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extremely poor outcome (Taki *et al*, 1996; Pui *et al*, 2002). For these differences, infant ALL is clinically and statistically handled separately from all the other paediatric ALL. In AML, distribution of FAB subtype differs among the age group. Infant AML generally show myelomonoblastic or monoblasic features (FAB M4 or M5) and often involves *MLL* gene rearrangement (Pui *et al*, 1995; Horibe *et al*, 2001). However, the presence of *MLL* gene rearrangement failed to correlate with treatment response in infant AML and the clinical outcomes were very similar to those of childhood AML (Satake *et al*, 1999; Pui *et al*, 2000; Kawasaki *et al*, 2001). For these reasons, not many studies distinguish infant from older paediatric AML clinically and statistically, and the biological differences between infant AML and childhood AML remain obscure.

In this study, we examined gene expression of paediatric AML-M4/M5, excluding inv(16) and t(8;21) patients, using microarray technology and found that age-associated differences exist among paediatric AML-M4/M5 patients. With the use of expression patterns, we have subdivided the patients into three subgroups and identified differences in the clinical outcome among these subgroups. Also, we have reviewed the gene expression profiles specific to patients with *MLL* gene rearrangement and showed that different signatures exist within different subgroups.

## Materials and methods

### *Patients and samples*

Forty paediatric AML patients (≤15 years old) diagnosed as FAB M4 or M5 subtype, between 1995 and 2001 in Japan, were enrolled in this study. Patients with inv(16) or t(8;21) were not included in this study. Characteristics of the 40 patients are shown in Table SI. These patients were treated with different but similar protocols, ANLL91 (Nagao, 1995; Satake *et al*, 1999) or AML99 (Tsukimoto *et al*, 2005; Shimada *et al*, 2006), which combine cytarabine, etoposide and anthracyclines. All leukaemic samples used in this study were obtained at the time of diagnosis. Morphological diagnosis according to FAB classification, immunophenotype and cytogenetic analysis were performed locally. *MLL* gene rearrangement was determined by cytogenetic analysis and multiplex reverse transcription polymerase chain reaction (RT-PCR) analysis (Pallisgaard *et al*, 1998; Salto-Tellez *et al*, 2003) using the HemaVision kit (DNA Technology, Aarhus, Denmark). Southern blot or fluorescence *in situ* hybridization analysis was also performed in some patients. In addition to the paediatric patients, 14 adult patients (20–79 years old), diagnosed as AML FAB M4 or M5 subtype [excluding inv(16) and t(8;21) patients], were also enrolled to compare paediatric AML to adult AML (Table SII).

This study was approved by the ethics committee of the National Cancer Centre. Patient identities were masked, and study numbers were assigned to all collected samples. To maintain anonymity, only age, sex, FAB subtype, chromosomal abnormality, treatment protocol and clinical outcome of the patients were linked to RNA samples. Informed consent was provided and obtained from the patients and/or the parents as appropriate.

### *Microarray analysis*

For microarray gene expression analysis, mononuclear cells were isolated from the bone marrow cells at the time of diagnosis (median percentage of leukaemic blast cells: 82·3%, see Table SI), and then total RNA was prepared using an RNeasy Mini kit (Qiagen, Hilden, Germany). The integrity of the purified RNA was confirmed using a 2100 Bioanalyzer and an RNA 6000 Nano LabChip kit (Agilent Technologies, Santa Clara, CA, USA). The DNA microarray used was a Human Genome U133 plus 2.0 array (Affymetrix, Santa Clara, CA, USA). Target cRNA was prepared from 20 ng of the purified RNA with a two-cycle cDNA synthesis kit and 3′-amplification reagents for IVT labeling (Affymetrix). Hybridization to the microarrays, washing and staining with the antibody amplification procedure and scanning, were performed according to the manufacturer's instructions. Using the GeneChip Operating Software version 1.4 (Affymetrix), the scanned image data were processed and the expression value (Signal) and detection call (Present, Marginal or Absent) of each probe set were calculated. The Signal values were normalized so that the mean in each experiment was set at 100 to adjust for minor differences between the experiments.

### *Statistical analysis*

For statistical analysis, the Signal values were log-transformed after the addition of 10 to reduce adverse effect caused by the noises at low expression levels. Most of the statistical analysis including principal component analysis (PCA), hierarchical clustering analysis, and Student's *t*-test were performed on the GeneSpring GX software version 7.31 (Silicon Genetics, Redwood City, CA, USA) after the log-transformed expression values were normalized to the median of all samples enrolled in each of the analyses. For the hierarchical clustering analysis, Pearson's correlation was used. To select karyotype discriminating genes, Significance Analysis of Microarray (SAM) was also used (Tusher *et al*, 2001). The Kaplan–Meier method and log-rank test were used for comparison of event-free survival (EFS), which was defined as the time from diagnosis to event (relapse or death of any cause).

### *Quantitative RT-PCR*

Quantitative RT-PCR was carried out using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with FastStart TaqMan Probe Master (Rox) and Universal ProbeLibrary (Roche Applied Science, Mannheim, Germany). cDNA was prepared from 50 ng of total RNA using SuperScript III (Invitrogen), and 1/100 of the cDNA was used as a

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template for each PCR reaction. Probes and primers were designed using the Probe Finder software (Roche Applied Science). Sequences of the primers are listed in Table SIII. Transcript levels were normalized to that of the *GAPDH* transcript. Quantitative RT-PCR was performed on 15 of the 40 paediatric patients.

## Results

### *Expression profile of paediatric AML-M4/M5*

Gene expression of 40 paediatric AML patients diagnosed as FAB M4 or M5 subtype was analysed with an oligonucleotide microarray composed of 54675 probe sets. Patients with inv(16) or t(8;21) were excluded from this study because they are known to display distinct expression profiles and clinical behavior (Yagi *et al*, 2003; Ross *et al*, 2004; Valk *et al*, 2004). Among the 40 paediatric patients, 14 patients (35%) were infants (<1 year old) and 10 out of 14 infants (71%) possessed *MLL* gene rearrangement (Table I and SI).

To obtain the overview of the variations in gene expression among the 40 patients, PCA was performed. Patients were clearly separated into two clusters by the first principal component, a tight cluster and a relatively loose cluster (Fig 1A). The tight cluster was composed of 10 patients that were all infants. Although the remaining four infants were included in the loose cluster, the gathering of infants into one cluster was statistically significant ($P = 6{\cdot}5^{-07}$ in chi-square test). On the other hand, the distribution of *MLL* gene rearrangement (presence or absence) and FAB subtype (M4 or M5) had no significant correlations between the two clusters ($P = 0{\cdot}097$ and $P = 0{\cdot}58$ respectively). Clinical outcomes were also not significantly different between the two clusters ($P = 0{\cdot}23$ in log-rank test), although the patients in the loose cluster seemed to have poorer outcome.

**Table I.** AML-M4/M5 patients enrolled in this study.

| Variables | Patients (*n*) | Ratio (%) |
|---|---|---|
| Total | 40 | |
| Gender | | |
| Male | 22 | 55·0 |
| Female | 18 | 45·0 |
| French-American-British subtype | | |
| M4 | 17 | 42·5 |
| M5 | 23 | 57·5 |
| Age at diagnosis (years) | | |
| <1 | 14 | 35·0 |
| 1–5 | 16 | 40·0 |
| ≥6 | 10 | 25·0 |
| Karyotype/gene rearrangement | | |
| *MLL* gene rearrangement | 23 | 57·5 |
| Normal | 13 | 32·5 |
| Others | 4 | 10·0 |

To assess the differences in gene expression among the 40 patients, we then performed unsupervised two-dimensional hierarchical clustering analysis. In this analysis, the same 10 infants who assembled a strong cluster in PCA again formed a distinct cluster (Fig 1B). This analysis also showed that this infant cluster was characterized by high expression of a very large set of genes (Fig 1B). These results suggest that most of infant AML-M4/M5 possess biological features different from older children with AML-M4/M5. This typical infant cluster comprised both *MLL* gene rearrangement-positive [*MLL*gr(+)] and *MLL* gene rearrangement-negative [*MLL*gr(−)] patients, indicating that a typical infant profile was not restricted by *MLL* gene rearrangement status. In addition, we could not detect any prominent cluster formation of *MLL*gr(+) patients or gene expression signature specific to *MLL* gene rearrangement in this analysis.

### *Comparison with adult AML-M4/M5*

To compare the gene expression of paediatric AML-M4/M5 with that of adult AML-M4/M5, gene expression of 14 adult patients were examined and added to the PCA and hierarchical clustering analysis. PCA with 40 paediatric and 14 adult patients revealed three clusters correlating with patient age. Ten infants and the remaining paediatric patients once again formed separate clusters while 11 out of 14 adult patients also formed a distinct cluster (Fig 1C). These results suggest that older paediatric AML-M4/M5 as well as infant AML-M4/M5 have distinct characteristics that were different from adult AML-M4/M5. In the unsupervised two-dimensional hierarchical clustering analysis, 12 out of 14 adult patients formed a distinct cluster (Fig S1). A large set of genes was commonly overexpressed between adults and infants, and a relatively small set of genes was commonly overexpressed between adults and older children (Fig S1). These results imply that expression patterns are not defined by the linear increment of patients' age. The genes shared by the infant and adult clusters included those encoding transcription factors and regulatory proteins involved in haematopoietic cell differentiation and proliferation (*AFF1, MLLT4, MLLT10, ARHGAP26, ELF2, ETV6, FLI1, FOXP1, FYN, LRRFIP1/2, LYN, MED13, MED13L, RUNX1*).

### *Sub-grouping of paediatric AML-M4/M5*

When we looked into the expression patterns of paediatric patients, the solid clustering of 10 infants was inevitable but we also realized that the remaining patients seemed to be heterogeneous. For those genes that were specifically over-expressed in 10 infants, some of the other patients showed quite a different expression while the others showed somewhat closer expression patterns to 10 infants (Fig 1B). Thus, we proceeded with our analysis to separate the 40 paediatric patients into three subgroups based solely on gene expression without any previous knowledge of the patients' clinical

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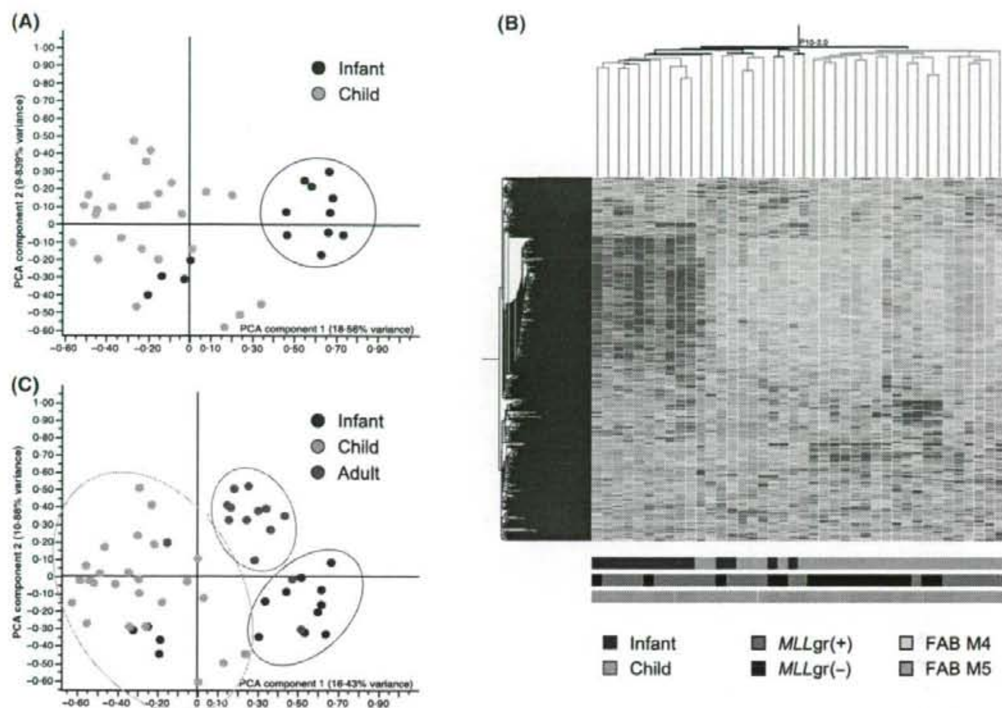


Fig 1. Overview of AML-M4/M5 gene expression. (A) PCA analysis of 40 paediatric AML-M4/M5 patients. 25754 probe sets with Present calls in at least 10 of the 40 patients were used in this analysis. Infant (<1 year old) and child (≥1 year old) patients are indicated by the colours. (B) Two-dimensional hierarchical clustering analysis of 40 paediatric AML-M4/M5 patients. 1865 probe sets with high variation in expression (≥2-fold changes to the median value in at least 10 of the 40 patients) were selected from the 25754 probe sets and used in the analysis. Columns and rows in the matrix represent patients and probe sets, respectively. Red and green indicates high and low expression, respectively. Indicated below the matrix are the age group (infant or child), karyotype [*MLL*gr(+) or *MLL*gr(−)] and FAB subtype (M4 or M5) for each patient by colour codes. (C) PCA analysis of 40 paediatric and 14 adult AML-M4/M5 patients. 25605 probe sets with Present calls in at least 15 of the 54 patients were used in the analysis. Infant, child and adult patients are indicated.

information. First, we isolated 2421 probe sets overexpressed in 10 distinct infants by selecting probe sets that exhibited *P*-values of <0·01 in Students *t*-test and twofold higher expression when the 10 infants were compared with the other remaining patients (Fig 2A and genes listed in Table SIV). With the average normalized expression value of these genes, we then divided the 40 patients into subgroups, designated as A, B and C. Subgroup A had an average normalized value greater than 2·0, whereas subgroup C had an average normalized value less than 1·0 (Fig 2A and B, see also Table SI). All of the 10 distinct infants were selected as subgroup A, and 12 and 18 of the remaining 30 patients were divided into subgroup B and C, respectively.

As shown in Fig 2C and D, age distribution and karyotypes were different among the three subgroups. The average age of subgroup A was 0·3 years old with eight out of 10 patients' possessing *MLL* gene rearrangement. The average age of subgroup B was 3·1 years old, and seven of the 12 patients possessed *MLL* gene rearrangement with t(9;11) as being the dominant karyotype. Subgroup C included many of the older children with the average age of 6·6 years old, and eight of the 18 patients possessed *MLL* gene rearrangement. Interestingly, all the t(6;11) patients (average age 10·0 years old) were placed in subgroup C. FAB subtypes were equally distributed among each subgroup (Fig 2E).

To compare the clinical outcomes among the subgroups, we performed Kaplan–Meier analysis and log-rank test. Subgroup C had the worst outcome (3-year EFS 28%), which was significantly lower than those of subgroup A (3-year EFS 70%) and subgroup B (3-year EFS 75%) ($P = 0{\cdot}023$) (Fig 2F). The outcome of subgroup C was definitely lower than that of the intermediate-risk patients in AML 99 study (3-year EFS 60%) (Tsukimoto *et al*, 2005).

To investigate the underlying biology in paediatric AML-M4/M5, we selected the genes that differentially expressed in subgroups A and C (Table II and SV). Three thousand and thirty-three probe sets were identified by selecting genes with *P*-values of <0·0001 and fold change values of >2·0 or <0·5. Among these genes, 2487 probe sets (82%) were higher in subgroup A and 546 probe sets (18%) were higher in subgroup C. Almost all of

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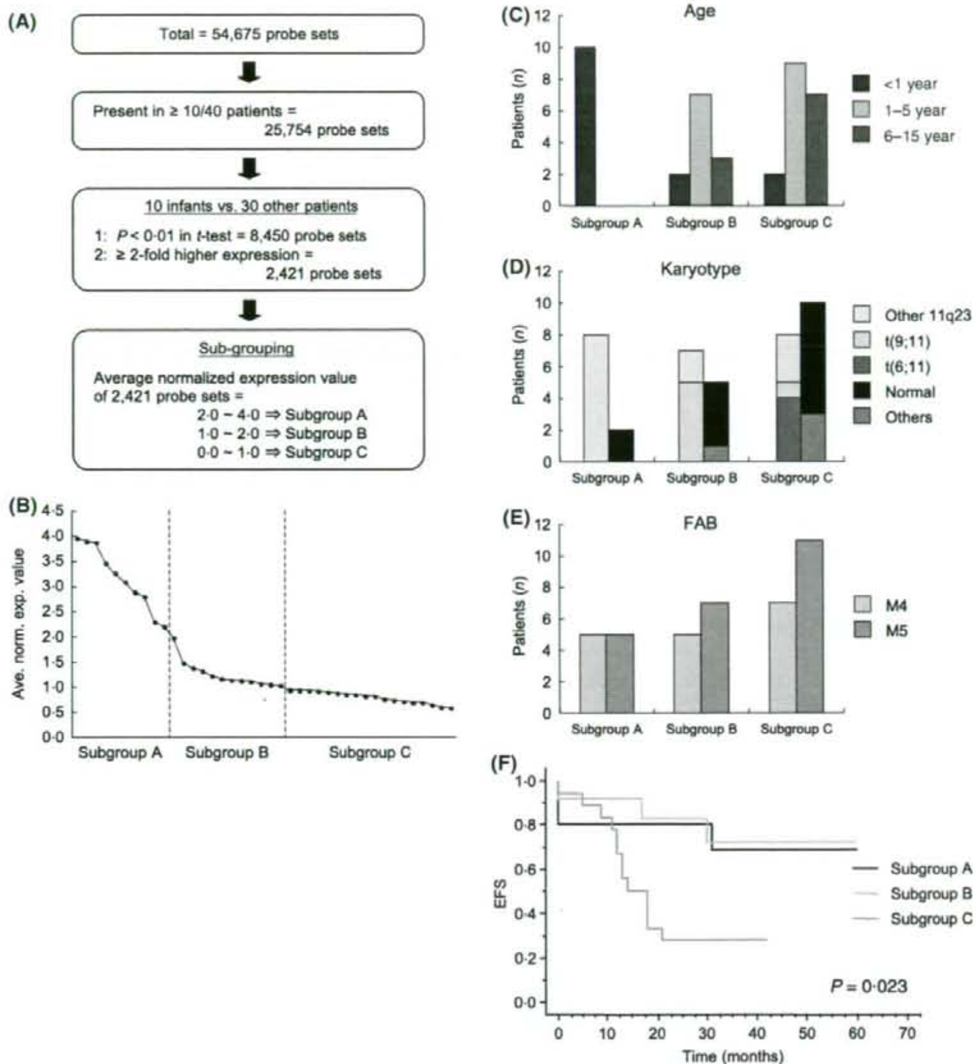


Fig 2. Sub-grouping of paediatric AML-M4/M5. (A) Sub-grouping scheme. First, subgroup-discriminating probe sets were selected as follows: (i) probe sets without Present calls in at least 10 of 40 paediatric patients were removed (25754 probe sets); (ii) probe sets with *P*-value of <0·01 in Student's *t*-test comparing 10 distinct infants and the remaining 30 patients were selected (8450 probe sets) and (iii) probe sets with ≥2-fold higher expression in the 10 distinct infants were chosen (2421 probe sets). Then, the average of normalized expression values of the 2421 subgroup-discriminating probe sets was calculated for each patient and used for sub-grouping. 10 patients with the average values between 2·0 and 4·0 were assigned as subgroup A, 12 patients with the average values between 1·0 and 2·0 as subgroup B, 18 patients with the average values lower than 1·0 were assigned as subgroup C. (B) Distribution of the average normalized expression values of 40 patients. (C) Age distribution of each subgroup. The average age was 0·3, 3·1 and 6·6 years old for subgroup A, B and C, respectively. (D, E) Karyotype and FAB subtype distribution of each subgroup. (F) Kaplan–Meier analysis of event-free survival (EFS). *P*-value was obtained by log-rank test.

the selected genes (2995 probe sets, 99%) showed medium expression level in subgroup B whether their expression were higher in subgroup A or in subgroup C, confirming that subgroups A, B and C have a linear correlation in gene expression. Among the genes overexpressed in subgroup A, the top-ranked gene (25·3-fold) was a DNA-binding zinc finger homeobox protein gene *ZEB2* (Table II). The expression of this gene was higher in adult patients than in subgroup C patients (Fig 3A). Interestingly, *MLL* translocation partner genes such as *ARHGAP26* and *FOXO3* were also overexpressed

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**Table II.** Genes expressed differentially between subgroups A and C.

| Fold rank | Probe set ID | Gene symbol | Fold change |
|---|---|---|---|
| Highly expressed in subgroup A | | | |
| 1 | 239296_at | *ZEB2* | 25·3 |
| 2 | 242008_at | *AGTPBP1* | 21·3 |
| 3 | 240652_at | Unknown | 21·0 |
| 4 | 1556590_s_at | Unknown | 18·1 |
| 5 | 1562194_at | *ZEB2* | 17·3 |
| 6 | 244548_at | *ARHGAP26* | 17·0 |
| 7 | 1557797_a_at | *ZEB2* | 16·6 |
| 8 | 239555_at | *LYN* | 16·5 |
| 9 | 1569477_at | *FOXO3* | 16·4 |
| 10 | 232307_at | *MED13L* | 16·4 |
| Highly expressed in subgroup C | | | |
| 1 | 206310_at | *SPINK2* | 23·8 |
| 2 | 203373_at | *SOCS2* | 13·5 |
| 3 | 201427_s_at | *SEPP1* | 12·8 |
| 4 | 205051_s_at | *KIT* | 9·6 |
| 5 | 209160_at | *AKR1C3* | 8·9 |
| 6 | 206067_s_at | *WT1* | 7·1 |
| 7 | 206772_at | *PTH2R* | 6·8 |
| 8 | 210140_at | *CST7* | 6·7 |
| 9 | 200923_at | *LGALS3BP* | 6·5 |
| 10 | 236738_at | *LOC401097* | 6·2 |

Probe sets were selected with Student's *t*-test and fold changes. Top 10 probe sets were listed in the order of their fold change values.

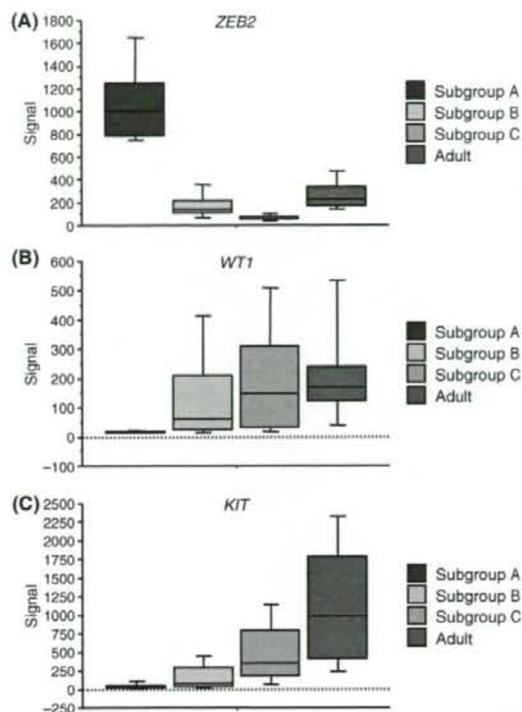


**Fig 3.** Differentially expressed genes between subgroup A and subgroup C. Box and whiskers plots are shown for *ZEB2* (A), *WT1* (B) and *KIT* (C). Signal values were compared among subgroup A, B and C paediatric patients and adult patients.

in subgroup A regardless of the karyotype. In subgroup C, poor prognostic factors such as *WT1* and *KIT* were overexpressed, coinciding with its poor clinical outcome (Table II and Fig 3B and C). *WT1* and *KIT* exhibited linear increment with the adult patients expressing highest of all.

### *Gene expression signatures of MLL gene rearrangements*

More than a half of the patients enrolled in this study possessed *MLL* gene rearrangement (Table I), but with unsupervised hierarchical clustering analysis, no prominent cluster was observed for *MLL*gr(+) patients (Fig 1B). To evaluate the differences between *MLL*gr(+) AML-M4/M5 and *MLL*gr(−) AML-M4/M5, we used SAM and selected differentially expressed probe sets, at 5% false discovery rate (FDR), between *MLL*gr(+) and *MLL*gr(−) patients among all patients and also within each subgroup (Table III and SVI). When all the patients were compared, 112 probe sets were selected. Comparison within subgroup A did not exhibit any significant difference between *MLL*gr(+) and *MLL*gr(−) patients and this was probably due to the scarcity of *MLL*gr(−) patients. Only 11 and 55 probe sets were selected at 5% FDR when compared within subgroup B and subgroup C, respectively (Table III). Surprisingly, commonly selected probe sets were very small (Fig 4A), indicating that the gene expression signatures of *MLL* gene rearrangement differed considerably among the different subgroups identified. Expression patterns of selected genes are shown in Fig 4B. Among the 112 probe sets selected in comparison of all patients, only 17 probe sets (15%) were upregulated by *MLL* gene rearrangement. On the other hand, among the 55 probe sets selected within subgroup C, 53 probe sets (96%) were upregulated by *MLL* gene rearrangement, and some of these probe sets were also upregulated in some *MLL*gr(+) patients of subgroup B but not in subgroup A patients (Fig 4B), indicating a clear difference of *MLL* gene rearrangement signatures among the subgroups.

**Table III.** The numbers of probe sets showing differential expression between *MLL*gr(+) and *MLL*gr(−).

| | Patient no. *MLL*gr(+) vs. *MLL*gr(−) | 5% FDR |
|---|---|---|
| All patients | (23 vs. 17) | 112 |
| Subgroup A | (8 vs. 2) | 0 |
| Subgroup B | (7 vs. 5) | 11 |
| Subgroup C | (8 vs. 10) | 55 |

Probe sets were selected using SAM with 5% false discovery rate (FDR).

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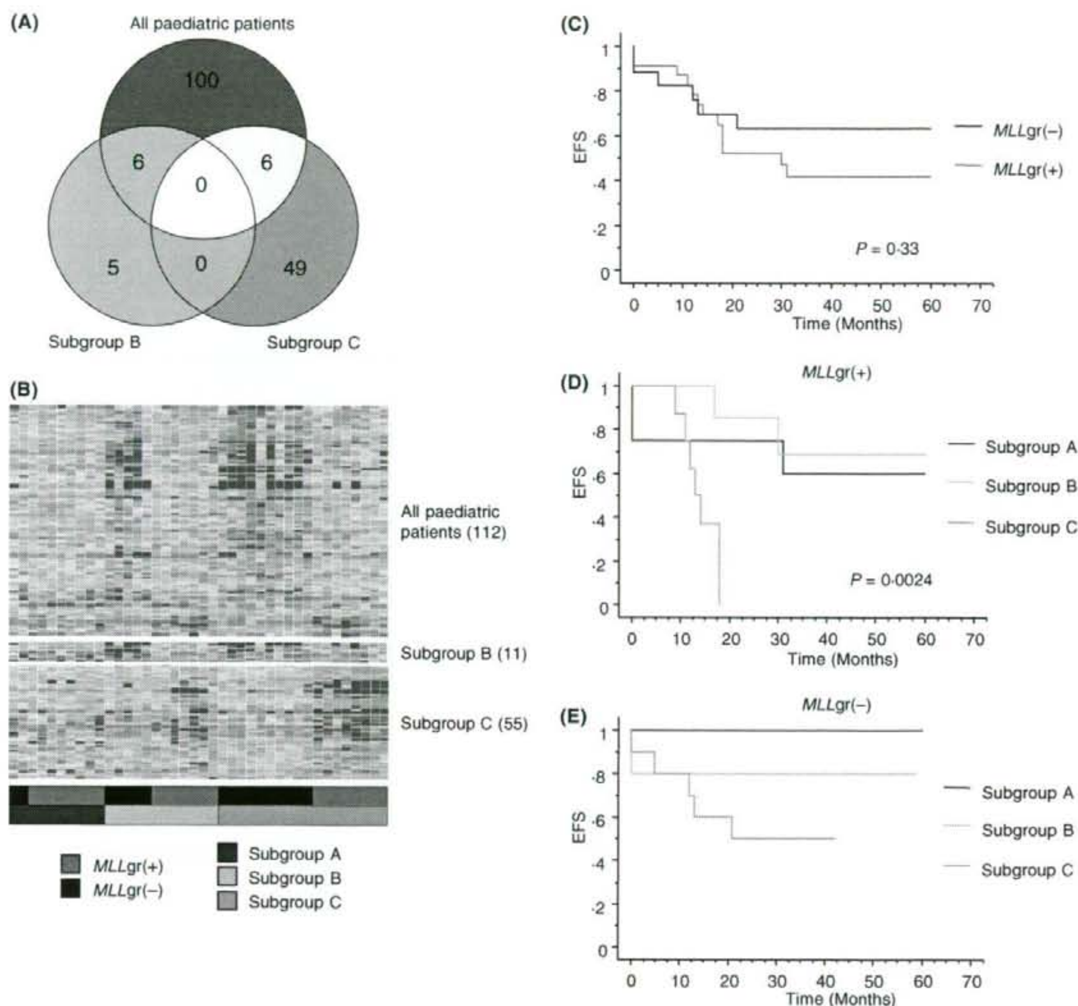


Fig 4. The significance of *MLL* gene rearrangements in paediatric AML-M4/M5. (A) Venn diagram comparing the differentially expressed genes between *MLL*gr(+) and *MLL*gr(−) patients. The probe sets selected by SAM with 5% FDR were compared among subgroup B, subgroup C and all paediatric patients. (B) Expression pattern of the SAM-selected probe sets. Columns and rows in the matrix represent patients and probe sets, respectively. The normalized expression level for each probe set is represented by colour, with red representing deviation above the median and green representing deviation below the median. The *MLL* gene rearrangement [*MLL*gr(+) or *MLL*gr(−)] and paediatric AML-M4/M5 subgroup (A, B or C) of each patient are indicated by colour codes below the matrix. (C–E) Kaplan–Meier analysis of event-free survival (EFS). *MLL*gr(+) and *MLL*gr(−) patients within paediatric patients (C), subgroups A, B and C within *MLL*gr(+) patients (D) and subgroups A, B and C within *MLL*gr(−) patients (E) were compared. *P*-value was obtained by log-rank test.

Genes specifically overexpressed throughout all of the *MLL*gr(+) patients included a zinc finger protein gene *ZNF521* (Table IV and Fig 5A), which has been reported previously (Kohlmann *et al*, 2005). However, frequently reported genes such as *HOXA* family members and *MEIS1* (Armstrong *et al*, 2002; Yeoh *et al*, 2002; Tsutsumi *et al*, 2003; Bullinger *et al*, 2004; Ross *et al*, 2004; Valk *et al*, 2004; Andersson *et al*, 2005) were not selected with SAM and *HOXA9* had no correlation with *MLL* gene rearrangement (Fig 5D). Rather, the *HOXB2* and *HOXB3* genes were selected as underexpressed genes among *MLL*gr(+) patients (Table IV and Fig 5B). A transcription factor gene *EVI1*, whose overexpression is known to associate with poor prognosis, was upregulated in subgroup C *MLL*gr(+) patients (Table IV and Fig 5C) (Doorn-Khosrovani *et al*, 2003; Valk *et al*, 2004). These data were also confirmed by quantitative RT-PCR (Fig 6).

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**Table IV.** Genes expressed differentially between *MLL*gr(+) and *MLL*gr(−) patients.

| Fold rank | Probe set ID | Gene symbol | Fold change |
|---|---|---|---|
| Highly expressed in *MLL*gr(+) patients among all patients | | | |
| 1 | 226676_at | *ZNF521* | 19·0 |
| 2 | 226677_at | *ZNF521* | 15·8 |
| 3 | 1552665_at | *MGC14425* | 7·9 |
| 4 | 227929_at | Unknown | 4·9 |
| 5 | 217520_x_at | *LOC646278* | 4·9 |
| 6 | 206440_at | *LIN7A* | 3·1 |
| 7 | 205472_s_at | *DACH1* | 3·0 |
| 8 | 1557450_s_at | *WHDC1L1* | 2·8 |
| 9 | 225785_at | *REEP3* | 2·7 |
| 10 | 205471_s_at | *DACH1* | 2·5 |
| Highly expressed in *MLL*gr(−) patients among all patients | | | |
| 1 | 228904_at | *HOXB3* | 13·1 |
| 2 | 205453_at | *HOXB2* | 10·8 |
| 3 | 205033_s_at | *DEFA1* | 10·2 |
| 4 | 236892_s_at | Unknown | 8·5 |
| 5 | 223828_s_at | *LGALS12* | 5·1 |
| 6 | 202833_s_at | *SERPINA1* | 5·0 |
| 7 | 200923_at | *LGALS3BP* | 4·8 |
| 8 | 211429_s_at | *SERPINA1* | 4·5 |
| 9 | 206761_at | *CD96* | 4·4 |
| 10 | 239791_at | *LOC404266* | 4·3 |
| Highly expressed in *MLL*gr(+) patients among subgroup C patients | | | |
| 1 | 235700_at | *CT45-4* | 28·2 |
| 2 | 1567912_s_at | *CT45-4* | 25·4 |
| 3 | 226676_at | *ZNF521* | 22·3 |
| 4 | 236858_s_at | Unknown | 17·9 |
| 5 | 226677_at | *ZNF521* | 16·9 |
| 6 | 226420_at | *EVI1* | 12·1 |
| 7 | 1552665_at | *MGC14425* | 11·2 |
| 8 | 1559266_s_at | *LOC730417* | 10·5 |
| 9 | 236859_at | *RUNX2* | 10·0 |
| 10 | 1557261_at | *WHDC1L1* | 10·0 |
| Highly expressed in *MLL*gr(−) patients among subgroup C patients | | | |
| 1 | 209298_s_at | *ITSN1* | 2·7 |
| 2 | 209357_at | *CITED2* | 2·6 |

Probe sets were selected with SAM at 90th percentile with 5% false discovery rate (FDR). Top 10 or all selected probe sets were listed in the order of their fold change values.

When compared between all patients, EFS did not differ statistically ($P = 0{\cdot}33$) between *MLL*gr(+) and *MLL*gr(−) patients whilst *MLL*gr(+) patients seemed to have a poorer prognosis (Fig 4C). The EFS of each subgroup was compared within each *MLL* gene arrangement status and subgroup C presented the poorest outcome for both *MLL*gr(+) and *MLL*gr(−) karyotypes (Fig 4D and E). It was astounding to see that all subgroup C *MLL*gr(+) patients (eight patients) had relapsed within 18 months (3-year EFS 0%). Subgroup C represented an extremely poor prognostic subgroup with early relapse especially with *MLL* gene rearrangement.

## Discussion

Microarray-based gene expression profiling has been applied to the diagnosis of many different types of leukaemia and used to explore their underlying molecular pathology (Golub *et al*, 1999; Armstrong *et al*, 2002; Schoch *et al*, 2002; Yeoh *et al*, 2002; Yagi *et al*, 2003; Ross *et al*, 2004). However, no previous study has been reported that focussed solely on the gene expression profile of AML-M4/M5 or on the difference between infants and older children with AML. The present study analysed the gene expression of 40 paediatric AML-M4/M5 patients and found that typical infants displayed very unique and distinct expression patterns that are clearly different from the older patients. These distinctly expressed genes were used to separate 40 patients into three subgroups without any previous knowledge of the patients' clinical information, and showed that these subgroups differed in age distribution and clinical outcome. In addition, the gene expression signature of *MLL* gene rearrangement and outcomes of *MLL*gr(+) patients were different among the different subgroups. These results indicate that paediatric AML-M4/M5 is heterogeneous in an age-associated manner and this heterogeneity is correlated with their prognosis.

The three subgroups that were separated by gene expression were different in age distribution and in clinical outcome, but not in FAB subtype (Fig 2). The difference between subgroup A and subgroup C was extremely prominent. A large set of differentially expressed genes was isolated and these genes were medianly expressed in subgroup B. Among the genes over-expressed in subgroup A, many transcription factor genes involved in haematopoietic cell differentiation and proliferation were included such as *FOXO3*, *MLL3* and *ELF2* (Table II and S5). These observations suggest that underlying biology is transitionally different among the three subgroups. The top-ranked gene overexpressed in subgroup A is a DNA-binding zing finger homeobox protein gene *ZEB2*. *ZEB2* mutations are responsible for Mowat–Wilson syndrome and Hirschsprung disease (Wakamatsu *et al*, 2001; Mowat *et al*, 2003). It functions in the transforming growth factor β signaling pathway and may promote tumor invasion in hepatic, renal and breast cancer (Miyoshi *et al*, 2004; Elloul *et al*, 2005; Krishnamachary *et al*, 2006), but the association between *ZEB2* and leukaemia is still unknown. Genes that were overexpressed in subgroup C included *WT1* and *KIT*; overexpression of these genes is known to be associated with poor prognosis (Ashman *et al*, 1991; Inoue *et al*, 1994; Weisser *et al*, 2005; Paschka *et al*, 2006; Shimada *et al*, 2006) and this overexpression may be responsible for the dismal outcome of subgroup C patients.

When adult AML-M4/M5 patients were included in the analysis, it was very interesting to find that adult patients not only shared common expression pattern with older children (subgroup C) but many of the genes upregulated in infants (subgroup A) were similarly highly expressed among the adult patients (Fig S1). It is well known that certain types of leukaemia are common only in the paediatric population and

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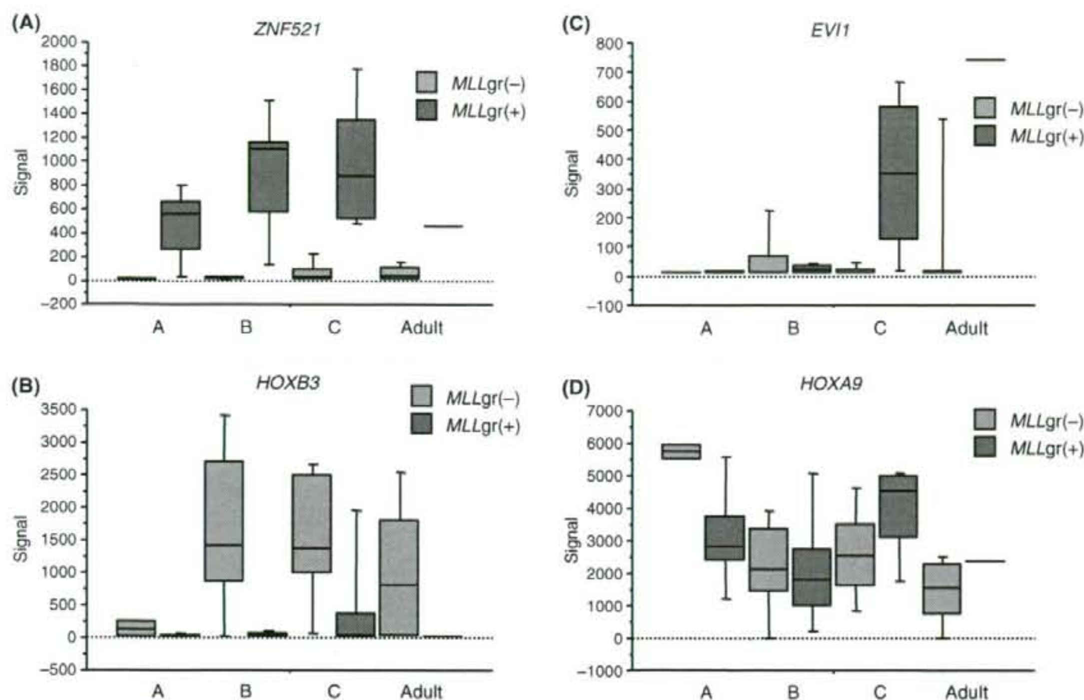


Fig 5. Differentially expressed genes between *MLL*gr(+) and *MLL*gr(−) patients. Box and whiskers plot are shown for *ZNF521* (A), *HOXB3* (B), *EVI1* (C) and *HOXA9* (D). Signal values were compared between *MLL*gr(+) and *MLL*gr(−) patients within subgroup A, B and C paediatric patients and adult patients.

vice versa. For example, *MLL* gene rearrangements are much more frequent among younger populations while *NPM1* mutations and *FLT3*-internal tandem duplication (ITD) are typically prevalent in the adult population (Iwai *et al*, 1999; Meshinchi *et al*, 2001; Cazzaniga *et al*, 2005; Falini *et al*, 2005; Brown *et al*, 2007). With these differences in genetic alterations, we had expected that gene expression would be transitional in an age-increasing manner. In previous studies, the gene expression signatures of genetic alterations were compared between adult and paediatric leukaemia. Kohlmann *et al* (2004) have reported that gene expression signatures identified in paediatric ALL were applicable to adult ALL patients. Ross *et al* (2004) have also shown that the expression signatures generated from paediatric AML were able to accurately classify adult AML with the same genetic lesions. The similarities or differences between infant AML and adult AML has never been thoroughly discussed before, and thus we have identified for the first time that infant AML-M4/M5 and adult AML-M4/M5 share expression patterns. Among the commonly shared genes between infant AML-M4/M5 (subgroup A) and adult AML-M4/M5, transcription factor genes involved in haematopoietic cell differentiation and proliferation such as *ETV6* and *RUNX1* were included, and this may suggest that these two groups might share common underlying biology.

Many studies have already reported that AML with *MLL* gene rearrangement present distinct gene expression profiles (Armstrong *et al*, 2002; Yeoh *et al*, 2002; Tsutsumi *et al*, 2003; Bullinger *et al*, 2004; Ross *et al*, 2004; Valk *et al*, 2004; Andersson *et al*, 2005; Kohlmann *et al*, 2005), but with our unsupervised analysis, no prominent cluster of *MLL*gr(+) patients was observed. This inconsistency may be attributable to the fact that we focused solely on AML-M4/M5, while previous studies investigated the whole AML population to identify the signature of *MLL* gene rearrangements. We speculated that *MLL*gr(+) AML-M4/M5 and *MLL*gr(−) AML-M4/M5 might share similar gene expression especially in paediatric patients. When we examined the differences between *MLL*gr(+) and *MLL*gr(−) patients in AML-M4/M5, relatively small differences (112 probe sets at 5% FDR) were found and almost all of these genes were under-expressed in *MLL*gr(+) patients. In addition, *HOXA* family genes and *MEIS1*, which have been repeatedly identified as overexpressed genes in *MLL*gr(+) leukaemia (Armstrong *et al*, 2002; Yeoh *et al*, 2002; Tsutsumi *et al*, 2003; Bullinger *et al*, 2004; Ross *et al*, 2004; Valk *et al*, 2004; Andersson *et al*, 2005; Kohlmann *et al*, 2005), were not included in the selected gene lists. Rather, under-expression of *HOXB2* and *HOXB3* in *MLL*gr(−) patients was observed (Table IV). Among the

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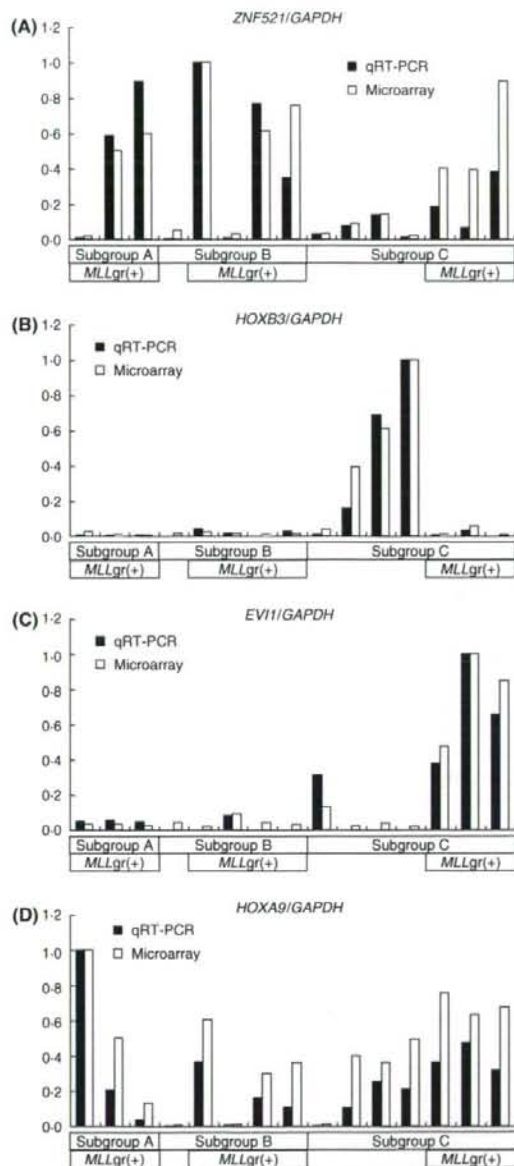


**Fig 6.** Quantitative RT-PCR confirmation of differential expression between *MLL*gr(+) and *MLL*gr(−) patients. Quantitative RT-PCR was performed for *ZNF521* (A), *HOXB3* (B), *EVI1* (C) and *HOXA9* (D) on RNA samples from 15 patients. Included were 3 subgroup A [3 *MLL*gr(+)], 5 subgroup B [4 *MLL*gr(+) and 1 *MLL*gr(−)] and 7 subgroup C [3 *MLL*gr(+) and 4 *MLL*gr(−)] patients. Transcript levels of those 4 genes were normalized to that of *GAPDH*, and presented as ratios to the highest patient. Their expression levels determined by microarray analysis were also shown as the same ratios after normalization to the *GAPDH* expression level.

previously reported *MLL* gene rearrangement-associated genes, *ZNF521* was specifically overexpressed among all of the *MLL*gr(+) patients in our study. *ZNF521* is an early haematopoietic zinc finger protein gene that is highly expressed in primitive human haematopoietic cells (Bond *et al*, 2004), and *PAX5-ZNF521* gene fusion has been recently reported among B-progenitor ALL (Mullighan *et al*, 2007). This gene, which is likely to be involved in normal haematopoiesis and ALL development, may also be active in the leukaemogenesis of *MLL*gr(+) AML-M4/M5. On the other hand, we also found that some *MLL* gene rearrangement-associated genes were subgroup-specific. When compared within subgroup C, 55 probe sets were selected as differentiating genes for *MLL*gr(+) and *MLL*gr(−) patients at 5% FDR, but the expression patterns of these probe sets were not shared by the other subgroups, especially subgroup A. This is noteworthy because EFS of *MLL*gr(+) patients were very different among different subgroups and subgroup C *MLL*gr(+) patients had the poorest outcome (Fig 4D). These findings suggest that the target genes of *MLL* fusion proteins might be different among each subgroup.

Our gene expression profiling analysis revealed that paediatric AML-M4/M5 consists of different subgroups with distinctively different outcomes. Subgroup C, which comprised older children, presented 3-year EFS of 28%, which was extremely lower than the previously reported EFS of intermediate risk patients (60%) (Tsukimoto *et al*, 2005). Our sub-grouping procedure enabled us to extract these poor prognostic patients from the other intermediate risk patients. It is known that some mutations, such as *FLT3*-ITD, *KIT* mutation and *NPM1* mutation, influence the prognosis of AML and their incidences increase in an age-dependent manner. In our analysis, however, these mutations were rare not only in subgroup B but also in subgroup C (Table SI), indicating that these factors are not the main reason for the dismal outcome of subgroup C. Subgroup C contained both *MLL*gr(+) and *MLL*gr(−) patients but subgroup C *MLL*gr(+) patients especially had a disappointing outcome, as all eight patients had relapsed within 18 months. Subgroup C included different types of *MLL* gene rearrangement, but t(6;11) was present only in subgroup C. Previous reports have also suggested that the prognosis of t(6;11) is dismal (Welbron *et al*, 1993; Martineau *et al*, 1998). In our study, the clinical outcome of t(6;11) was also very disappointing as all four patients had relapsed within 18 months (Fig S2A). In addition, we were able to isolate the genes discriminating t(6;11) and the other *MLL* gene rearrangements with SAM (Table SVII and SVIII). These results provide another possibility that patients with t(6;11) exhibit different expression profiles and dismal outcome. However, some of the upregulated genes in t(6;11) patients were also upregulated in *MLL*gr(−) patients of subgroup C (Fig S2B). So some of the selected genes for t(6;11) represent not only t(6;11) patients but rather represent subgroup C patients. Moreover, the outcome of other subgroup C *MLL*gr(+) patients is also dismal, although they

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did not show high expression of those genes. These data suggest that t(6;11) karyotype is not an independent prognostic factor but rather a surrogate marker for subgroup C *MLL*gr(+) patients.

In summary, paediatric AML-M4/M5 was identified as a group of heterogeneous diseases comprised different outcomes. Adverse prognostic subgroup could be distinguished with the use of gene expression profiling. Our results have shown that age is an important factor contributing to the biology of AML-M4/M5. These findings also suggest that gene expression profiling may be very useful for risk estimation and our sub-grouping procedures could be a powerful risk stratification tool to identify unfavourable risk patients within the heterogeneous paediatric AML-M4/M5. Further studies are needed to explain the biology underlying different gene expression between infants and older children and to verify the usefulness of our sub-grouping procedures for risk-stratified therapeutics.

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## References

Andersson, A., Olofsson, T., Lindgren, D., Nilsson, B., Ritz, C., Eden, P., Lassen, C., Rade, J., Fontes, M., Morse, H., Heldrup, J., Behrendtz, M., Mitelman, F., Hoglund, M., Johansson, B. & Fioretos, T. (2005) Molecular signatures in childhood acute leukemia and their correlations to expression patterns in normal hematopoietic subpopulations. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 19069–19074.

Armstrong, S.A., Staunton, J.E., Silverman, L.B., Pieters, R., den Boer, M.L., Minden, M.D., Sallan, S.E., Lander, E.S., Golub, T.R. & Korsmeyer, S.J. (2002) MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nature Genetics*, **30**, 41–47.

Ashman, L.K., Cambareri, A.C., To, L.B., Levinsky, R.J. & Juttner, C.A. (1991) Expression of the YB5.B8 antigen (c-kit proto-oncogene product) in normal human bone marrow. *Blood*, **78**, 30–37.

Bond, H.M., Mesuraca, M., Carbone, E., Bonelli, P., Agosti, V., Amodio, N., De Rosa, G., Di Nicola, M., Gianni, A.M., Moore, M.S., Hata, A., Grieco, M., Morrone, G. & Venuta, S. (2004) Early hematopoietic zinc finger protein (EHZF), the human homolog to mouse Evi3, is highly expressed in primitive human hematopoietic cells. *Blood*, **103**, 2062–2070.

Brown, P., McIntyre, E., Rau, R., Meshinchi, S., Lacayo, N., Dahl, G., Alonzo, T.A., Chang, M., Arceci, R.J. & Small, D. (2007) The incidence and clinical significance of nucleophosmin mutations in childhood AML. *Blood*, **110**, 979–985.

Bullinger, L., Dohner, K., Bair, E., Frohling, S., Schlenk, R.F., Tibshirani, R., Dohner, H. & Pollack, J.R. (2004) Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *The New England Journal of Medicine*, **350**, 1605–1616.

Cazzaniga, G., Dell'Oro, M.G., Mecucci, C., Giarin, E., Masetti, R., Rossi, V., Locatelli, F., Martelli, M.F., Basso, G., Pession, A., Biondi, A. & Falini, B. (2005) Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. *Blood*, **106**, 1419–1422.

Doorn-Khosrovani, S.B., Erpelinck, C., van Putten, W.L., Valk, P.J., Luytgaarde, S., Hack, R., Slater, R., Smit, E.M., Beverloo, B., Verhoef, G., Verdonck, L.F., Ossenkoppele, G.J., Sonneveld, P., de Greef, G.E., Lowenberg, B. & Delwel, R. (2003) High EVI1 expression predicts poor survival in acute myeloid leukemia: a study of 319 de novo AML patients. *Blood*, **101**, 837–845.

Downing, J.R. & Shannon, K.M. (2002) Acute leukemia: a pediatric perspective. *Cancer Cell*, **2**, 437–445.

Elloul, S., Elstrand, M.K., Nesland, J.M., Trope, C.G., Kvalheim, G., Goldberg, I., Reich, R. & Davidson, B. (2005) Snail, Slug, and Smad-interacting protein 1 as novel parameters of disease aggressiveness in metastatic ovarian and breast carcinoma. *Cancer*, **103**, 1631–1643.

Falini, B., Mecucci, C., Tiacci, E., Alcalay, M., Rosati, R., Pasqualucci, L., La Starza, R., Diverio, D., Colombo, E., Santucci, A., Bigerna, B., Pacini, R., Pucciarini, A., Liso, A., Vignetti, M., Fazi, P., Meani, N., Pettirossi, V., Saglio, G., Mandelli, F., Lo-Coco, F., Pelicci, P.G. & Martelli, M.F. (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *The New England Journal of Medicine*, **352**, 254–266.

Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D. & Lander, E.S. (1999) Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*, **286**, 531–537.

Horibe, K., Tsukimoto, I. & Ohno, R. (2001) Clinicopathologic characteristics of leukemia in Japanese children and young adults. *Leukemia*, **15**, 1256–1261.

Inoue, K., Sugiyama, H., Ogawa, H., Nakagawa, M., Yamagami, T., Miwa, H., Kita, K., Hiraoka, A., Masaoka, T., Nasu, K., Kyo, T., Dohy, H., Nakauchi, H., Ishidate, T., Akiyama, T. & Kishimoto, T. (1994) WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *Blood*, **84**, 3071–3079.

Iwai, T., Yokota, S., Nakao, M., Okamoto, T., Taniwaki, M., Onodera, N., Watanabe, A., Kikuta, A., Tanaka, A., Asami, K., Sekine, I., Mugishima, H., Nishimura, Y., Koizumi, S., Horikoshi, Y., Mimaya, J., Ohta, S., Nishikawa, K., Iwai, A., Shimokawa, T., Nakayama, M., Kawakami, K., Gushiken, T., Hyakuna, N., Katano, N., Tsurusawa, M. & Fujimoto, T. (1999) Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. *Leukemia*, **13**, 38–43.

Kawasaki, H., Isoyama, K., Eguchi, M., Hibi, S., Kinukawa, N., Kosaka, Y., Oda, T., Oda, M., Nishimura, S., Imaizumi, M., Okamura, T., Hongo, T., Okawa, H., Mizutani, S., Hayashi, Y., Tsukimoto, I., Kamada, N. & Ishii, E. (2001) Superior outcome of infant acute myeloid leukemia with intensive chemotherapy: results of the Japan Infant Leukemia Study Group. *Blood*, **98**, 3589–3594.

Kohlmann, A., Schoch, C., Schnittger, S., Dugas, M., Hiddemann, W., Kern, W. & Haferlach, T. (2004) Pediatric acute lymphoblastic

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