deaths) were from cardiac causes. Other causes of death were: infection 9 (14%), accident 6 (9%), other 6 (9%), not known 5 (8%), malignancy 4 (6%), liver disease 3 (5%). Within the Birth Cohort, 18 (6%) patients died, 7 from cardiac causes.

Three-hundred and four (55.9%) patients switched to CCT and 269 (49.4%) continued for at least six months. Indications for switching were: high serum ferritin (66.6%), deteriorating left ventricular function on echocardiography or clinically (25.2%), low myocardial T2* (2.8%), and intolerable side effects of frequent DFO infusions (5.4%).

Results of multivariable analysis are shown in Table 2. For CCT, the hazard ratio of 0.14 equates to 7.4-fold improved survival for each year on therapy. In the birth cohort, CCT was the only independent factor associated with survival. For cardiac deaths, the effect of CTT could not be calculated since there were no events in those initiated on therapy. Making an arbitrary assumption that the patient with the median duration of CCT had in fact died of a cardiac cause, CCT is associated with a 4-fold increased chance of survival from cardiac death.

Seventy-five patients (24.7%) stopped CCT. This high incidence of discontinuations was consistent with most other long-term studies in CCT.[6-9] Reasons for stopping were: agranulocytosis (5%), recurrent neutropenia (2.9%), gastrointestinal disturbance (5.6%), non-compliance with DFO (3.3%), pregnancy (2.6%), arthralgia (1.6%), allergy (0.7%), weight gain (0.7%), increased liver enzymes (0.3%), other (2%). For those treated for at least six months, the median (standard deviation) therapy duration was 3.59 (1.96) years. There was one death in a patient during treatment with CCT. This was due to *E. coli* sepsis and was not associated with neutropenia or agranulocytosis.

This study provides good evidence that CCT is effective in controlling body iron stores in moderately to heavily iron loaded patients and prevents iron overload related deaths. It is not clear whether equal benefits would be obtained with DFP monotherapy, as shown in another study,[10] or with the new oral iron chelating drug deferasirox, where the efficacy in terms of survival has not yet been established. We observed a high rate of discontinuations and of severe but manageable adverse events with CCT, highlighting the need for supervision from a specialist center. Management of the risk of agranulocytosis requires weekly full blood counts, and periodically reminding patients about this potential risk.

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What is the optimal treatment for biphenotypic acute leukemia?

We read with great interest the paper written by Xu *et al.*[1] about a comprehensive investigation in clinical and biological characteristics of adult biphenotypic acute leukemia (BAL) in China. We agree that the prognosis of BAL patients is poor when compared with *de novo* acute myeloid leukemia (AML) or acute lymphoblastic

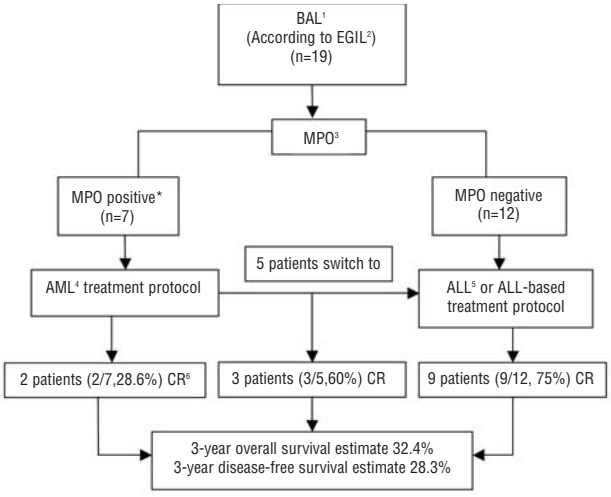

Figure 1. Treatment schedule on the BAL patients. *The MPO was considered to be positive when expressed in >20% of blasts. [1]BAL: biphenotypic acute leukemia; [2]EGIL: European Group for the Immunological Characterization of Leukemias; [3]MPO: myeloperoxidase; [4]AML: acute myeloid leukemia; [5]ALL: acute lymphoblastic leukemia; [6]CR: complete remission AML treatment protocol: (1) Induction therapy: DA (daunorubicin 45 mg/m^2 d1-3, cytarabine 150 mg/m^2 d1-7); (2) Consolidation therapy: DA (daunomycin 45 mg/m^2 d1-3, cytarabine 150 mg/m^2 d1-7), IA (idarubicin 8 mg/m^2 d1-3, cytarabine 150 mg/m^2 d1-7), MA (mitoxantrone 8 mg/m^2 d1-3, cytarabine 1 g/m^2 d1-3), HA (homoharringtonine 2 mg/m^2 d 1-7, cytarabine 150 mg/m^2 d1-7), ME (mitoxantrone 8 mg/m^2 d1-3, etoposide 100 mg/m^2 d4-8). ALL treatment protocol (1) Induction therapy (phase I): DVLD (vincristine 2 mg d1, 8, 15 and 22; daunorubicin 45mg/m^2 d1, 2 and 15, 16; L-Asparaginase 6000 U/m^2 d19-26; dexamethasone 6 mg/m^2 d1-28); Induction therapy (phase II): CAM (cyclophosphamide 600 mg/m^2 d1,15; cytarabine 75 mg/m^2 d3-6, 10-13; 6-mercaptopurine 50 mg/m^2 d1-14); (2) Intensification (3 cycles): MA (mitoxantrone 8 mg/m^2 d1-3, cytarabine 1 g/m^2 d1-3), HD-MTX+ L-Asparaginase (methotrexate 2 g/m^2 d1, L-Asparaginase 10,000U/m^2 d2) ; (3) Central nervous system (CNS) prophylaxis: include at least 12 IT injections of methotrexate (10 mg/m^2) and dexamethasone (5 mg), and 24 Gy cranial irradiation; (4) Consolidation therapy: EAOD (cytarabine 75 mg/m^2 d1-5; etoposide 100 mg/m^2 d1-5; VCR 2 mg d1, 8, 15 and 22; dexamethasone 6 mg/m^2 d1-15), EA (cytarabine 75 mg/m^2 d1-5; etoposide 100 mg/m^2 d1-5), DCAT (DNR 25 mg/m^2 d1, 8, 15 and 22; cyclophosphamide 600 mg/m^2 d 29; cytarabine 75 mg/m^2 d 31-34, 38-41; 6-thioguanine 50 mg/m^2 d 29-42), EA (cytarabine 75 mg/m^2 d1-5; etoposide 100 mg/m^2 d1-5); (5) Maintenance therapy (a total of two years): VP (vincristine 2 mg d1, prednisone 60 mg/m^2 d1- 5) (every three months), MM (6-mercaptopurine 50 mg/m^2 orally each day, methotrexate 12 mg/m^2 orally once a week). ALL-based treatment protocol is based on the treatment schedule of the ALL treatment protocol, in which the first induction therapy is combined with continuous infusion of cytarabine (100 mg/m^2/d) for seven days; other treatment is the same as the ALL treatment protocol.

leukemia (ALL), and BAL patients showed a much higher incidence of CD34 antigen expression, complex abnormal karyotype, and extramedullary infiltration. Nevertheless, we would like to focus on the particular clinical situation of BAL about the chemotherapeutic considerations. We continue to ask ourselves, what is the optimal treatment for BAL?

Carefully reading through the article, we found that 16 of 21 BAL patients were treated by ALL induction protocol (DVP, CDVP, CDVLP) or ALL-based induction protocol (VPDA, VPHA), of which 14 obtained complete remission (CR) (87.5%, 14/16). Also with the above treatment approaches, 3 patients still survived with persistent CR. Otherwise, 5 patients received induction chemotherapy with AML protocols (DA, HA, HAE), only one patient achieved CR (20%, 1/5). From the article, we found that 18 patients died of leukemia relapse or treatment complications, at least 14 patients died of chemotherapeutic complications including fatal infections (57.1%, 8/14) and hemorrhagic complications (42.9%, 6/14). Eleven patients relapsed and were resistant to original induction therapy, but infection and hemorrhage related death were the main reasons for poor overall results. These interesting data told us that induction treatment in BAL patients with ALL or ALL-based protocol may lead to higher CR rate than treatment with AML protocol, and intensive supportive care could improve the outcome of BAL patients.

From 326 adult acute leukemia patients, including 105 ALL and 221 AML (FAB classification), presenting to our center between January 2002 and June 2007, 19 patients (5.8%) were diagnosed as BAL according to the criteria based on the previously described scoring system adopted by the European Group of Immunological Classification of Leukemia (EGIL).[2] Immunophenotyping was performed by two or four-color immunofluorescence using flow cytometry, focusing on the blast cell population, and employed a panel of monoclonal antibodies to B-cell (CD10, CD19, CD20, CD22, CD79a, smIg), T-cell (CD1, CD2, CD3, CD4, CD5, CD7, CD8), myeloid (CD13, CD14, CD15, CD33, CD65, CD117, myeloperoxidase), and precursor cell (terminal deoxynucleotidyl transferase, CD34, HLADR) associated antigens. There were 11 female and 8 male patients with a median age of 36 years (range, 16-65). The median count for WBC was 35.6×10^9/L. Thirteen cases had a myeloid and B-lymphoid phenotype, and 6 cases had a myeloid and T-lymphoid phenotype.

Myeloperoxidase (MPO) activity, recognized as a very important hallmark of myeloblasts,[3,4] has also been shown to have a good prognostic value in AML patients:[5] a high percentage of MPO-positive blasts correlate to favorable prognosis. We designed a clinical trial to investigate the relationship between the expression of MPO in BAL blasts and the response to chemotherapeutic regimens. The study was approved by the Ethics Committee (Anhui Provincial Hospital) and all patients gave signed informed consent. The treatment schedule is shown in Figure 1. Out of 12 BAL patients at first diagnosis treated with ALL or ALL-based induction regimens such as DVLD, DVLD+Cytarabine, 75% (9/ 12) achieved CR. Out of 7 patients treated with AML regimens, such as DA, only 2 achieved CR (28.6%). Of note, 3 of the 5 patients (60%, 3/5) who failed to respond or had only a PR to AML therapy achieved a CR after switching to ALL-based induction therapy. With intensive supportive care, fatal infectious and hemorrhagic complications occurred only in 5 patients (26.3%, 5/19). With a median follow-up of 40 months, the median disease-free survival and overall survival were 12 and 16 months. The 3-year disease-free survival and overall survival estimates were 28.3% and 32.4%, respectively. In a recent publication, Rubnitz *et al.*[6] reviewed the pathological and clinical features, including response to therapy, of 35 pediatric patients with mixed lineage leukemia (same as BAL) at St Jude hospital. In the subgroup of 23 patients initially given AML therapy, 12 (52%) achieved complete remission (CR) and 2 attained partial remission (PR). By contrast, of the 12 patients who first received ALL therapy, 10 (83%) achieved CR. Interestingly, 8 of the 10 patients who failed AML induction achieved CR after treatment with ALL regimens (vincristine, prednisone, and L-

asparaginase). But the overall outcomes (5-year survival 36~54%) were clearly inferior to those seen for a contemporaneous sample of patients treated for standard ALL (5-year survival 84.6% in children).

With a similar clinical poor prognosis to Ph+ALL,[1,7] patients with BAL present with high WBC counts and expression of CD10, high extramedullary infiltration, and low rate of long-term survival. This leads us to think that BAL and Ph+ALL perhaps have the same clinical entity, and that this could be why BAL patients adapt to the treatment of ALL or ALL-based approach. We agree with the authors that multi-center cooperative studies should be carried out in both clinical and basic research to further characterize the features of BAL.

We conclude that MPO perhaps does not confer a good prognostic value in BAL patients as in AML patients. Intention-to-treat analysis showed that treatment with ALL or ALL-based approach, not AML protocol, resulted in high induction CR rates in BAL patients, but the long-term survival was still dismal.

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What is the optimal treatment to biphenotypic acute leukemia? Authors' reply

First of all we would like to thank Dr. Zheng *et al.*[1] for their interest in our paper recently published in Haematologica and for sharing their experience with a cohort of 19 BAL patients. They raised the question "what is the optimal treatment to biphenotypic acute leukemia?" which we are also interested in. Based on our data and the observation of Dr. Zheng and colleagues, they proposed that remission induction for BAL patients with ALL or ALL-like regimens may lead to higher CR rate than with AML regimens. Here we would like to share some experience and thoughts with our colleagues.

Based on our experience and the pathology of BAL, we believe that a combined regimen for both AML and ALL might be the best choice for the induction therapy for BAL, a unique entity with biological and clinical features of both myeloid and lymphoid leukemia. The ALL or ALL-based induction regimens Dr Zheng *et al.* mentioned, such as DVLD, DVLD+cytarabine in their center or DVP, CDVP, CDVLP, VPDA, VPHA in our department, all included anthracyclines and/or Ara-C. It has already been widely accepted that anthracycline is a main component in the treatment of both AML and ALL. In other words, the protocol described by Zheng *et al.* should be effective for BAL with both ALL and AML features. On the other hand, AML-type induction regimen did not include the effective component such as steroids for ALL, so the outcome was poor.

During our data analysis, our first conclusions were that it was better to adopt ALL-type regimens such as VPDA than AML-type regimen such as DA. After an extensive exchange of ideas with our colleagues and reviewers during revision of our manuscript, we finally gave up this conclusion since case numbers in our report are too small for statistical analysis and we just left a few comments in the text.

With the above facts in mind, we think that it is too early to draw a conclusion that *induction treatment in BAL patients with ALL or ALL-based protocol may lead to higher CR rate than treatment with AML protocol* until we can obtain more evidence from a well conducted prospective multi-center clinical trial to elucidate this conclusion.

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