using the HbA_2 value of 3.4% as cut off.[8]

Using the value of 30 μg/L for serum ferritin as cut off, 861 samples showed iron deficiency (ID) (group A) and 271 were without ID (group B) (Figure 1). The mean HbA_2 value was 2.8%±0.79 in group A and 3.50%±1.23 in group B, with a significant difference (P=0.00001) among between the two groups.

The distribution of the 253 samples with molecular analysis between groups A and B showed that 170 samples were part of group A and 83 of group B (Figure 1).

From 170 samples of group A, 21 resulted positive for a β-globin gene mutation with a mean of HbA_2 value of 4.90%±1.29. From 83 samples of group B, 29 resulted positive for a β-mutation (mean of HbA_2 value: 5.37%±0.87).

The comparison between the HbA_2 mean value of β-thalassemia carriers with (group A) or without (group B) ID, using the value of 30 μg/L as serum ferritin cut off, does not show a significant difference (P=0.060) and in both groups HbA_2 levels are more than 3.4%. Reduction of HbA_2 has been reported to be linked to the severity of anemia[9] so that it is possible that the value of 30 μg/L for serum ferritin defines an ID not sufficiently severe or not sufficiently prolonged to significantly reduce the level of HbA_2.

The ROC analysis, performed with samples of group A, at the 3.4% HbA_2 cut-off value, showed sensitivity and specificity of 74.19% and 95.8%, respectively. The false negative samples were 8 of 30 (26,6%): 3 presented Hb β variants, co-eluting with HbA, (Hb Valletta, Hb Ernz and Hb City of Hope) that do not require prenatal diagnosis, one showed an undefined single nucleotide polymorphism (SNP) in the β-globin gene of ambiguous diagnostic significance,[8] 2 samples presented, respectively, the α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$), and the β-globin gene promoter mutation, -101 (HBB c.-151C→T), and finally, 2 samples showed co-heredity of β^+ and δ^+ mutation.

The samples with $\alpha\alpha\alpha^{anti3.7}$ and the -101 beta mutation showed, respectively, HbA_2 values of 3.0% and 3.2% with a lower value of Hb (<12 g/dL) and MCV (<75 fL). The contemporary analysis of hematologic and hemoglobin data enable us to identify these subjects as samples that must be submitted to molecular analysis if their partners are carriers of β thalassemia.[6] While, in other cases, it is recommended to remeasure HbA_2 after ID treatment.

In the 2 samples showing co-heredity of β^+ and δ^+ mutation, the large reduction is principally associated with the presence of delta mutation rather than serum ferritin value.

Our results show that the presence of iron deficiency did not preclude the detection of classical β carrier in our population. There could be some problems in the presence of silent β mutation or α gene triplication with ID, because HbA_2 shows almost normal levels. However, the reduction in total Hb and MCV, and possible persistently low MCV and MCH after iron suppletion, should suggest greater attention is needed and molecular analysis exploring both the α and the β genes should be carried out, especially if the subject is a partner of a classical β thalassemia carrier.

Cristina Passarello, Antonino Giambona, Monica Cannata, Margherita Vinciguerra, Disma Renda, and Aurelio Maggio

U.O. C. Ematologia e Malattie Rare del Sangue e Organi Ematopoietici, Azienda Ospedaliera Ospedali Riuniti "Villa Sofia-Cervello", Palermo, Italy

Correspondence: Antonino Giambona, Lab. Diagnosi Prenatale e Molecolare-talassemia, Azienda Ospedaliera Ospedali Riuniti "Villa Sofia-Cervello", via Trabucco 180, 90146 Palermo, Italy.
E-mail: giambic@libero.it

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Efficacy of combining dasatinib and FLAG-IDA for patients with chronic myeloid leukemia in blastic transformation

The prognosis of patients with chronic myeloid leukemia (CML) has improved considerably over the last ten years with the introduction of ABL tyrosine kinase inhibitors (TKI) into clinical practice. TKIs induce complete cytogenetic remissions (CCyR) in 10-45% of patients who are treated with these drugs in advanced phase with minimal toxicity; unfortunately, these remissions are typically short lasting.[1,2] Dasatinib alone induces CCyR in 20-40% of patients.[2,3] However, the majority relapse within one year and the median survival is eight months.[2] Conventional chemotherapy regimens, such as FLAG-IDA, can induce CCyR in 30-40% of patients who have progressed to blastic phase, but again most patients relapse within six months and survival is poor.[4] A logical approach might be to combine both strategies in order to improve the outcome. The proposed schedule of combination TKI and chemotherapy is supported by two clinical

Table 1. Patients' characteristics and responses.

Patients' characteristics						Response to the #1dasatinib-FLAG-IDA			Response to #2dasatinib-FLAG-IDA		
Patient ID	Age/ sex	% blasts (BM)	Cytogenetics	BCR-ABL/ABL (%)	Phenotype	% blasts (BM)	Cytogenetics	BCR-ABL/ABL (%)	% blasts (BM)	Cytogenetics ABL/ABL(%)	BCR-
1	65/ F	45	46,XX,(9;22)(q34;q11)[1] /46,XY[9]	95.6	lymphoid	2	46,XX[20]	0.037	0	46 XX[30]	0.08
2*	44/ F	67	G-banding failed	139.7	biphenotypic	1	46,XX[30]	0.015	2	46 XX[30]	0.70
3#	41/ M	33	G-banding failed	24.8	myeloid	3	46,XY[30]	0.019	1	46 XY[30]	0.09
4	55/ M	73	46,XY,(9;22)(q34;q11)[28] /46,XY[2]	115.7	myeloid	5	46,XY,(9;22) (q34;q11)[1] /46,XY[29]	1.2	1	46 XY[30]	0.09

**Patient 2 harbored the ABL kinase domain mutation E459K at the time of starting chemotherapy. Dasatinib was permanently discontinued on Day +16 of the first course of chemotherapy due to HRCT chest abnormalities later attributed to a fungal infection. # Dasatinib was interrupted during the second cycle of chemotherapy due to the development of pleural effusions but was uneventfully reintroduced at a reduced dose of 70mg after three weeks.*

observations: i) a number of chemotherapeutic agents commonly used in the management of blastic phase CML have been shown to have synergism with a TKI (e.g. cytarabine);[5-8] and ii) TKIs have been used successfully in combination with conventional chemotherapy for the treatment of Ph^+ ALL.[9] We report our experience of dasatinib/FLAG-IDA chemotherapy in 4 CML patients who progressed to blastic phase while on imatinib.

The 4 patients received first-line imatinib for 11, 7, 26 and 10 months, respectively, before progressing to blastic phase. Table 1 shows patients' characteristics. Patients received two courses of FLAG-IDA (G-CSF 300 μg/day sc Days 0 to 6, fludarabine 30mg/m^2 iv Days 1 to 5, cytarabine 2g/m^2 iv Days 1 to 5, and idarubicin 12 mg/m^2 iv Days 1 to 3) together with dasatinib 100 mg daily. Dasatinib was administered continuously from Day 0 of the first course of chemotherapy. The combination was fairly well tolerated (Table 1) and all patients recovered a neutrophil count over 1×10^9/L within 30 days of the start of combination therapy.

After the first course of chemotherapy, all patients achieved morphological remission (95CI 40-100%); one achieved major cytogenetic response and the remaining 3 achieved both CCyR and major molecular response (MMR). The patients who failed to achieve CCyR after the first course of chemotherapy achieved CCyR and MMR after the second cycle. Currently all 4 patients are alive; Patients 1, 2 and 3 have undergone allogeneic stem cell transplantation (continuing in remission 3, 12 and 13 months post transplant) and patient 4 is scheduled to do so shortly. Dasatinib was reintroduced on Day +30 after transplant in all 3 patients with good tolerance. We have shown that dasatinib can be safely combined with conventional chemotherapy, and although this approach should be tested in a larger number of patients, the combination seems to induce deep remissions in patients with CML in blastic phase, allowing for further therapeutic strategies to enable a continuing response.

Dragana Milojkovic, Amr Ibrahim, Alistair Reid, Letizia Foroni, Jane Apperley and David Marin

Department of Haematology, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 OHS, UK

Correspondence: David Marin, Department of Haematology, Imperial College London, Du Cane Road, London W12 0NN, UK. E-mail: d.marin@imperial.ac.uk

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