The role of *BCR/ABL* isoforms in the presentation and outcome of patients with Philadelphia-positive acute lymphoblastic leukemia: a seven-year update of the GIMEMA 0496 trial

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To verify the potential clinical and prognostic value of *BCR/ABL* isoforms, we analyzed 101 consecutive adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia enrolled in the GIMEMA 0496 trial between October 1996 and December 1999. A p190 or p210 with or without p190 *BCR/ABL* transcript was documented in 59 (58.5%) and 42 cases (41.5%), respectively. At diagnosis, a white cell count <16x10^9/L and a higher level of CD34 and CD33 expression were associated with the p190 BCR/ABL transcript (p<0.05, p=0.009 and p=0.03, respectively). A complete remission was achieved in 62/92 (67.4%) patients, while 16/92 (17.4%) were resistant and 14/92 (15.2%) died of therapy-related complications. Fifty-two patients underwent intensive re-induction treatment, which was followed by stem cell transplant consolidation in the 36 in persistent complete remission (allogeneic = 20 patients; autologous = 16 patients). Response rates to induction therapies were similar in the two *BCR/ABL* isoform groups. By contrast, the p190 emerged as the only independent prognostic factor favorably affecting the 5-year overall survival and disease-free survival rates (p=0.008 and p=0.02, respectively).

Key words: BCR/ABL isoforms, ALL.

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The Philadelphia chromosome (Ph) marks the largest genetically defined group among adult patients with acute lymphoblastic leukemia (ALL).[1-6] At the molecular level, the t(9;22) translocation gives origin to the *BCR/ABL* chimeric gene[1-2,6] that encodes for a 210 kd protein (p210) or for a 190 kd protein (p190). Experimental data indicate that these two proteins have different biological properties,[1] although this heterogeneity does not, apparently, translate into substantially different clinico-biological subtypes of *BCR/ABL*+ ALL. Based on these considerations, the aim of the present study was to evaluate the therapeutic and clinical outcome of 101 cases of *BCR/ABL*+ ALL and to assess whether, after a prolonged follow-up of 7 years, the type of *BCR/ABL* isoform could result in different clinical outcomes.

Design and Methods

Between October 1996 and December 1999, the presence of a t(9;22) or of a *BCR/ABL* fusion, was detected in 101 patients out of the 402 adult patients enrolled in the GIMEMA 0496 trial. Cytogenetic and molecular studies on all patients were performed at diagnosis in the laboratories of the Department of Cellular Biotechnology and Hematology of the University "La Sapienza" of Rome, Department of Biomedical Sciences and Human Oncology of the University of Turin, Department of Hematology of the University of Perugia, Department of Biomedical Sciences, Hematology Unit, of the University of Ferrara and Unit of Molecular Onco-Hematology of the University "Federico II" of Naples. The diagnosis of ALL was based on standard morphologic and immunophenotypic criteria.[7] Cytogenetic and molecular studies were performed at diagnosis following the methods described elsewhere.[8-10]

Treatment protocol

All patients were treated according to the GIMEMA 0496 protocol. The induction phase was administered over a 6-week period and consisted of four drugs: prednisone, vincristine, high-dose daunorubicin (total dose 270 mg/m^2) and L-asparaginase. After the induction treatment, all *BCR/ABL*+ patients received further consolidation therapy that consisted of high dose Ara-C and mitoxantrone followed by an autologous or allogeneic stem cell transplantation (SCT) in first complete remission, based on the availability of a HLA identical sibling and according to the EIGLE study.[11] Patients were considered to be in complete remission if, after the induction treatment, they had normal peripheral blood counts and <5% blast cells in bone marrow with a normal cellularity.

Statistical analysis

The primary study end-points were: achievement of complete remission, duration of disease-free survival and duration of

overall survival. The probabilities of overall and disease-free survival were estimated using the Kaplan-Meier method and compared by the log-rank test. Multivariate analysis was performed by logistic regression and Cox proportional hazard regression models.

Results and Discussion

Ninety-two out of the 101 *BCR/ABL*+ ALL patients were evaluable for therapeutic response. After the induction phase, the high-dose Ara-C and mitoxantrone were administered to 52 patients. Afterwards, among the 51 patients who remained in complete remission, 36 patients were transplanted with allogeneic (20 patients) or autologous stem cells (16 patients). At the time of the analysis, 6/20 and 4/16 patients of the allogeneic and autologous transplant recipients, respectively, remain in continuous complete remission. None of the remaining 16 patients who did not undergo either an allogeneic or an autologous SCT is still in remission at the time of writing this report. Overall, 59 patients (59%) carried the p190 transcript, 23 (22%) had the p210 fusion and 19 patients (19%) expressed both the p190 and p210 gene fusions. These last 19 patients were classified as p210 positive for further comparisons according to the well-described findings demonstrating that in these cases DNA breakpoints fell within the minor break point cluster region (m-bcr), and that the two types of mature mRNA resulted from alternative splicing mechanisms. In addition, our own observations showed: (i) a high prevalence of p210 copies with respect to copies of the p190 transcript in p190/p210$^+$(*unpublished data*), and (ii) similar disease-free and overall survivals in the groups of p190/p210+ and p210+ ALL patients as observed in the present study (*data not shown*). Table 1 illustrates the clinico-biological characteristics of the Ph$^+$ ALL patients according to the type of *BCR/ABL* isoforms, whereas the therapeutic response to induction treatment, observed in all 92 evaluable Ph$^+$ ALL patients and in the two distinct genetic subgroups, is reported in Table 2. The percentage of patients with a white cell count >16×10^9/L was significantly higher among patients with p210$^+$ than among p190$^+$ patients (p=0.05). Concerning the immunophenotype, p190+ blasts more frequently expressed the CD33 (44% vs 23%; p=0.03) and CD34 antigens (100% vs 87%; p=0.009) than did the p210$^+$ blasts. The overall response to induction therapy did not differ significantly between the two groups of p190$^+$ and p210$^+$ patients. By contrast, the probabilities of disease-free survival and overall survival were significantly affected by the type of *BCR/ABL* transcript (Figure 1). Among the 22 p190$^+$ and the 14 p210$^+$ patients whose complete remission persisted after the consolidation chemotherapy, 12 (54%) and 8 (57%) respectively, underwent allogeneic SCT from related donors, whereas an autologous SCT was performed in 10 (46%) and 6 (43%) patients, respectively. The type of transplants did not affect the clinical outcome of patients. In fact, none of 14 p210$^+$ patients who underwent SCT (8 allogeneic and 6 autologous) survived in complete remission whereas among p190$^+$ patients, 6/12 and 2/10 p190$^+$ patients who received an allogeneic or autologous transplant survived in complete remission. Multivariate analysis performed in *BCR/ABL*$^+$ patients showed that among the variables analyzed (ie. age, white cell count, expression of CD34, CD10, CD13 and CD33, type of *BCR/ABL* transcript), the presence of the p190 fusion transcript was the only powerful independent prognostic factor that favorably influenced disease-free survival (HR=0.52; CI = 0.28 – 0.94; p=0.031). This study describes the clinico-biological characteristics of 101 *BCR/ABL*$^+$ ALL patients, prospectively enrolled in the Italian multicenter GIMEMA 0496 study. For number of patients and length of follow-up, the present study is one of the three largest multicenter studies reported to date. Concerning the distribution of the different *BCR/ABL* fusion products within our pop-

Table 1. Clinico-biological characteristics of the Ph$^+$ ALL patients entered the GIMEMA 0496 study according to the type of BCR/ABL isoforms.

Features	*All patients (n=101)*	*Patients with p190 n (%) 59 (59%)*	*Patients with p210±p190 n (%) 42 (41%)*	*p*
Age				
≤ 30 years	22	14 (24)	8 (23.5)	0 .6
> 30 years	79	45 (76)	34 (76.5)	
White cell count				
≤ 16×10^9/L	38	27 (46)	11 (27)	0.05
> 16×10^9/L	62	32 (54)	30 (73)	
CD33				
negative	61	31 (56)	30 (77)	0.03
positive	33	24 (44)	9 (23)	
CD34				
negative	5	0	5 (3)	0.009
positive	91	57 (100)	34 (87)	

Platelet count, CD10 expression, splenomegaly (> 4 cm) and percentage of blasts in the peripheral blood samples did not differ significantly between the two groups.

Table 2. Response rate of the 92 Ph$^+$ ALL evaluable patients according type of the BCR/ABL transcript.

	All patients n (%) 92 (100%)	*p190-positive n (%) 53 (57.6%)*	*p210+positive n (%) 39 (42.4%)*	*p*
Complete remission	62 (67.4)	37 (69.8)	25 (64.1)	n.s.
Resistant	16 (17.4%)	7 (13.2)	9 (23.0)	
Death during induction	14 (15.2)	9 (17.0)	5 (12.9)	
Alive in continuous CR/total CR patients	10/62 (16.1)	10/37 (27.0)	0/25 (0.0)	–
Relapsed/total CR patients	42/62 (67.8)	23/37 (62.2)	19/25 (76.0)	–
Dead in CR/total CR patients	10/62 (16.1)	4/37 (10.8)	6/25 (24.0)	–

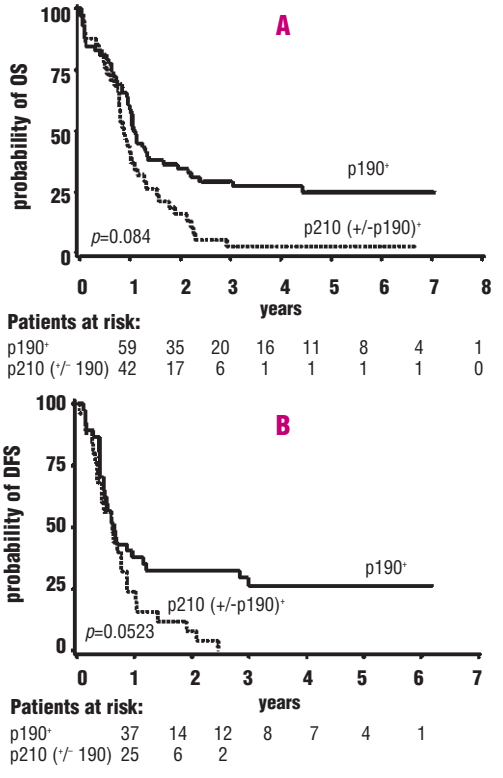

Figure 1. Actuarial probability of overall survival (OS) (A) and of disease-free survival (DFS) (B) according to the type of *BCR/ABL* gene fusion.

ulation of patients, the p190 product was detected in the majority of our patients (59%), while the remaining cases (41%) were p210+. It should be noted that 45% of p210+ cases (19 patients) co-expressed both the p190 and the p210 *BCR/ABL* fusions. This distribution is different from that recently published by French and German groups,[4,5] who reported higher prevalences of p190+ cases (77% and 68%, respectively). Conversely, we noted a higher incidence of *BCR/ABL*+ patients co-expressing both the p190 and p210 *BCR/ABL* fusions, who accounted for 19% of the entire series. This latter discrepancy, detected uniformly by the three reference laboratories who performed the molecular analyses, may be related to the relatively higher sensitivity of our assays that, in our hands, can amplify one p190+ cell among 10^6 normal cells. In this respect, it is worth noting that using very sensitive methods Saglio *et al.*[12] detected low amounts of the p190 fusion in p210+ leukemic cells from patients with chronic myeloid leukemia or ALL. Looking at the potential influence of the different *BCR-ABL* subtypes, we observed that patients with p190+ and p210+ ALL differed significantly with respect to both clinical and biological characteristics at diagnosis and outcome. In fact, hyperleukocytosis was more frequent in p210+ patients, whereas p190+ leukemic cells more frequently expressed the CD33 and CD34 molecules. Moreover, in our series the p190 fusion was the only independent prognostic factor conferring a significantly better probability of disease-free and overall survival. The demonstration of a discrete oncogenic potential of the different *BCR/ABL* transcripts for a long time led to the speculation that the breakpoint regions may have an impact on the biology of the leukemic clone and on the clinical manifestations and outcome of patients. However, none of the studies with a large series of patients, has provided evidence of hematologic differences between the two types of breakpoints. The heterogeneous clinical outcome of *BCR/ABL*+ ALL patients was first suggested by Secker-Walker and Craig in 1993,[13,14] who discussed on the controversy surrounding a Ph+ ALL and an ALL blast crisis following a Ph+ chronic myeloid leukemia. These authors found that Ph+ ALL cases classified as *stem-cell*, because of the presence of the Ph alteration in both myeloid and lymphoid cells, differed from the Ph+ ALL *lymphoid restricted* cases by having a paradoxical but significantly better event-free survival (median: 35 vs 4 months; $p<0.01$). However, data from this study were unable to correlate the observed differences with the two types of *BCR/ABL* junctions. Thereafter, a putative association of the minor break point cluster region with *stem cell* features, was suggested by Haferlach *et al.*,[15] who combined May-Grünwald-Giemsa staining with fluorescence *in situ* hybridization at the single cell level in four cases of Ph+ ALL with the minor breakpoint cluster region. Moreover, in the same study they showed that co-expression of myeloid antigens on blast cells was indicative of the *stem cell* origin of the leukemic clone, because of a clear demonstration of the Ph+ chromosome through the myeloid lineage cells. To this end, it is worth noting the significantly higher level of expression of the CD34 and CD33 molecules detected on leukemic cells of our p190+ patients, which might suggest the putative *stem cell* origin of such cases and, therefore, justify, according to the above cited data of Secker-Walker and Craig,[13,14] the higher chemo-sensitivity shown in the present study by this category of patients. A favorable prognostic impact of the p190 gene fusion on the clinical outcome of *BCR/ABL*+ ALL patients was not statistically proven in the GMALL 04/89 and 05/93 studies, in which only a trend towards a better overall survival for the p190+ patients was noted.[5] However, at least three differences between the two studies may explain the discrepancy of the results. (i) the different age distribution between the two groups in the German study; (ii) the higher proportion of p190+ cases in the German report, compared to a more homogeneous distribution of p190+ and p210+ ALL subgroups in our study; and, finally, (iii) the high doses of anthracyclines scheduled in the induction phase of the GIMEMA 0496 trial. Finally, the prognostic significance of BCR/ABL transcripts seemed not to be affected by the type of transplant procedure, as suggested not only by the results of the multivariate analysis, but also by the absence of long survivors in the p210 group, irrespective of the type of transplant. Due to the relatively small number of patients we cannot confirm the statistically significant superiority of allogeneic SCT with respect to autologous SCT recently demonstrated by the updated results of the LALA-94 study.[16]

In conclusion, the present study demonstrates that

among adult Ph+ ALL patients the type of *BCR/ABL* fusion transcript characterizes subtypes of leukemia with distinct clinico-biological features and clinical outcome after intensive conventional chemotherapies. Therefore, it will be of great relevance to verify whether the biological, clinical and prognostic heterogeneities reported in the present study affect the response and clinical outcome of BCR/ABL+ ALL patients treated with the new BCR/ABL tyrosine kinase inhibitors, which seem to be very effective also in this subtype of leukemia.[17,18,19]

GC, FP, RF and FM conceived the study. The study was performed in the GIMEMA group under the direction of FM, who also performed all the pilot experiments and most of the analysis. LE, MM, performed the benchwork and contributed to the writing of the manuscript with the contribution of LA, GM, AT, FDR, GS, GF, PL who also provided most of the patients enrolled in the study. PF performed the statististical analysis. AC, CM, GS, supervised the cytogenetic and molecular analyses and critically reviewed the manuscript. GC, EF, and FP wrote the manuscript with contributions from other authors. The authors declare that they have no potential conflicts of interest.

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