**Background and Objectives.** Recent investigations in acute myeloid leukemia (AML) have clearly demonstrated that specific karyotypic abnormalities result in distinct biological and clinical entities. We focus on recent advances on biology and treatment of AML with t(8;21).

**Data sources and Methods.** The information presented here derives from literature data and experience of the authors. The most relevant studies are critically analyzed and discussed.

**State of Art.** Peculiar molecular, morphologic, immunophenotypic and epidemiologic findings of AML with t(8;21) as well as current methods for the evaluation of minimal residual disease are presented. Results from current therapeutic options including consolidation chemotherapy or transplantation procedures are critically reviewed.

**Perspectives.** Innovative therapeutic approaches based on risk-adapted, patient-oriented approaches would be possible in this AML subtype, provided that answers to many unresolved questions are given.

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Key words: acute myeloid leukemia, t(8,21), AML1/ETO, chemotherapy, stem cell transplantation

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Acutely myeloid leukemia (AML) is a relatively uncommon malignancy, accounting for about 1–1.5% of all cancers in Western countries. Nonetheless, at a cytogenetic level AML is one of the most extensively investigated human neoplastic disorders. Since the introduction of chromosome banding techniques in 1970s, clonal chromosome aberrations have been routinely studied in AML and cytogenetic findings have pivotaly contributed to our understanding of the morphologic, immunophenotypic and clinical heterogeneity of the disease. More recently, advances in molecular biology have demonstrated that genomic rearrangements deriving from chromosomal translocations result in the generation of chimeric genes and fusion proteins, providing convincing evidence for their primary role in leukemogenesis.

On a clinical ground, chromosome findings are currently considered as one of the most relevant prognostic factors for achievement of complete remission (CR), duration of first CR, and survival in AML. Translocation (8;21) (q22;q22) and inversion of chromosome 16 [inv(16)(p13q22)] are two of the most common AML cytogenetic abnormalities, occurring in 7–8% and 4–5% of the adult cases, respectively. Higher frequencies of t(8;21) AML (11.7%) have been reported in children. Both t(8;21) and inv(16) are characterized at molecular level by the disruption and transcriptional deregulation of genes encoding subunits of the core binding factor (CBF), an αβ-heterodimeric transcriptional factor involved in the regulation of normal hematopoiesis, and are therefore encompassed as CBF-AML. However, AML with t(8;21) and AML with inv(16) are substantially different with regards to several morphologic, immunophenotypic and clinical features. Here we will focus on the biomolecular, morphologic and immunophenotypic peculiar characteristics of AML with t(8;21)(q22;q22) as well as on recent advances in the treatment of this subtype of AML.
Molecular biology

The molecular biology of CBF-AML has been extensively reviewed in previously reported articles. As a consequence, such recruitment to form a complex with histone deacetylase that ETO can aberrantly recruit nuclear corepressors to form a complex with histone deacetylase that ETO can aberrantly recruit nuclear corepressors to form a complex with histone deacetylase that ETO can aberrantly recruit nuclear co-repressors, leading to the inhibition of gene expression.

Recent studies have shown that ETO and AML1 phosphoproteins are expressed in CD34+ hematopoietic progenitors, suggesting that both genes are expressed at an early stage of hematopoiesis and that their expression is downregulated during differentiation. Notwithstanding, there is no conclusive evidence as yet that the AML1/ETO chimeric gene is sufficient per se to induce leukemia. As matter of fact, transgenic animals carrying the AML1/ETO fusion do not develop leukemia and it is conceivable that, as demonstrated for the CBFβ/MYH11 lesion associated with inv(16) or t(16;16), a further hit event is required in addition to AML1/ETO to develop a full blown leukemia phenotype.

Morphologic features

According to FAB classification, patients with t(8;21) AML typically present with M2 morphology, with a minority of cases presenting M1 or M4. Occasionally, the translocation can occur in patients with refractory anemia with excess blasts (RAEB) or RAEB in transformation (RAEB-t). The leukemic cells from AML patients carrying t(8;21) often exhibit several characteristic morphologic features, raising suspicion of the translocation and/or the molecular transcript at morphologic observation of marrow specimen. Seven parameters, including bone marrow eosinophilia (more than 5%), presence of Auer rods, abnormal granulopoiesis defined as the presence of myelocytes and metamyelocytes with homogeneous salmon-colored granules and a rim of basophilia in the cytoplasm, cells with abnormal cytoplasmic vacuoles, myeloperoxidase (MPO) positivity and FAB M2 subtype were considered by Nuñó et al. in order to correlate morphologic and molecular findings. By retrospectively reviewing cytogenetically negative and morphologically suspected cases, the authors demonstrated that 2 cases out of 2 carrying at least 5 out of the above morphologic characteristics were positive for the AML1/ETO chimeric transcript. Contrariwise 2 cases, one with 4 features and one with 3, were negative. A group of 13 patients with t(8;21) detectable by conventional cytogenetics were adopted as controls and all cases showed a minimum of 6 features. More recently, Andrieu et al. developed a well-defined scoring system, called the Weighted Score (WS), based on the attribution of different weights to definite morphologic parameters, including FAB classification, Auer rods, pseudo-Chediak, marrow eosinophilia, large blasts with prominent Golgi and abnormal cytoplasmic granules. By applying the WS, positivity for AML1/ETO was found by molecular investigation in 9 cases out of 9 (100%) lacking the translocation and suspected at morphologic level; however, there was a false positive rate of 7% (4/55). In our own experience, additional morphologic characteristics, including hypogranularity, acquired Pelger-Huet abnormality and Auer rods in maturing granulocytic cells can also be found (Figure 1). Obviously, any morphologic score needs to be...
tested in a prospective manner by different observers working independently. However, since the practical value of correlating morphology with AML1/ETO is potentially to limit patients undergoing molecular screening, predictive score systems should be taken in account when molecular evaluation of AML cases is not routinely feasible. Nonetheless, the above data demonstrate that AML with t(8;21) is characterized by peculiar morphologic features (detailed in Table 1), which are predictive of the cytogenetic translocation and/or of the chimeric transcript in more than 90% of cases.

Immunophenotypic findings

Immunophenotypic analysis has demonstrated that blast cells from AML with t(8;21) show high levels of CD34 and DR expression as well as more frequent positivity for CD19 and CD56 surface markers when compared to AMLs with normal or other aberrant karyotypes. Conversely, CD7 and CD2 are rarely expressed and CD33 expression is characteristically weak. In an attempt to develop an immunophenotypic model possibly predictive of t(8;21), we adopted an approach based on the exploration of outcome-driven statistical methods for the definition of antigen cut-off points significantly related to biological phenomena. Since this methodology implies that even a very low percentage of positive cells can be discriminating, critical immunophenotypic data need to be confirmed by dual-staining experiments, at least. In addition, flow cytometry CD45 gating is needed in order to discriminate the leukemic blast cells correctly from other mononuclear cells as well as from myeloid maturing cells. According to such a technique, we investigated a group of 93 AML cases with a minimum of 20 fully evaluable metaphases. When the t(8;21) AML group was compared to the control group including patients with either normal or other abnormal karyotypes, statistically significant differences in the median percentages of positive cells were found for different antigens. In particular, the t(8;21) AML group showed significantly higher expression of CD19, CD34, CD56 and CD54. In contrast, CD45R0, CD33, CD36, CD11b and CD14 were significantly less expressed in AML with t(8;21) than in controls. The Classification and Regression Tree model (CART) was then imposed on each of these antigens to determine the cut-off point which would achieve the most homogeneous subsets with regard to cytogenetic pattern, i.e. t(8;21) or not. Once cut-off points had been established, multivariate analysis was performed using a logistic regression model. Taking into account antigens

Table 1. Morphologic features associated with AML with t(8;21).

<table>
<thead>
<tr>
<th>Features included in the Nuclora’s predictive score</th>
<th>Features included in the Weighted Score*</th>
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<tbody>
<tr>
<td>Bone marrow eosinophilia (more than 5 %)</td>
<td>Bone marrow eosinophilia (more than 5 %)</td>
</tr>
<tr>
<td>Auer rods (also found in maturing cells)</td>
<td>Auer rods</td>
</tr>
<tr>
<td>Abnormal granulopoiesis including hypogranularity and acquired Pelger-Huet abnormality</td>
<td>Abnormal cytoplasmic granules</td>
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<tr>
<td>Cells with abnormal cytoplasmic globules containing pink, waxy inclusions</td>
<td>Myeloperoxidase positivity</td>
</tr>
<tr>
<td>Cytoplasmic vacuoles</td>
<td>FAB M2 subtype</td>
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<tr>
<td>Myeloperoxidase positivity</td>
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<td>FAB M2 subtype</td>
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*Based on the attribution of different scores to single parameters.
found to be differently expressed above the best discriminating cut-off points, all the possible combinations of two or more antigens were tested. The simple combination of CD19 and CD34 expression at more than 10% and 35%, respectively, correctly classified 92 out of 93 cases (99%, \( p = 0.00001 \)).

It is worthy of note that the addition of any other antigen combination did not improve the reliability of the predictive model as indicated in Table 2.

The 99% reliability of the predictive model based on the combination of CD19 and CD34 expression was definitely confirmed by updating our data in a larger series of 144 patients, 18 of whom (12%) had t(8;21). In conclusion, the above findings demonstrate that AML with t(8;21) is associated with distinct immunophenotypic features, highly predictive of the cytogenetic pattern. Cases of AML with simultaneous expression of CD19 and CD34 above 10% and 35%, respectively, in which the t(8;21) is not detectable at cytogenetic level as well as those with insufficient number of metaphases, must be investigated at molecular level for the detection of the AML1/ETO hybrid gene. Laboratories interested in converting the CART model system to a simplified alternative approach could identify putative t(8;21) cases as those characterized by highest expression of CD34 (3rd-4th logarithmic decade on a cytofluorometric histogram) along with expression of CD19 (often accompanied by CD56) on myeloid blast cells. Figure 2 shows a classical cytometric pattern of t(8;21) AML, with typical coexistence of immature blast cells (green) and maturing myeloid cells (yellow), along with the brightest expression of CD34 and the simultaneous presence of CD19 and CD56 on blasts.

Molecular diagnosis

Following cytogenetic analysis, t(8;21) is found in about 7-8% of AML cases; however, several studies have demonstrated that a reverse transcriptase polymerase chain reaction (RT-PCR) assay using specific primers may detect the translocation in cases with an apparently normal karyotype.\(^{29,31,42,43}\)

For example, Andrieu et al. reported the use of both cytogenetic and RT-PCR for t(8;21) detection in a series of 64 patients and found that this abnormality was identified by the techniques in, respectively, 8% and 16% of cases. Similarly, a recent survey from the Cancer and Leukemia Group B (CALGB) reported detection of AML1/ETO by RT-PCR in 6 cases in which karyotyping had not clearly shown t(8;21).\(^{43}\)

Of interest, AML1/ETO fusion transcripts were detected in a patient with t(8;10) (q22;q26) as well as in a patient with t(1;10;8) (p22;p13;q22), thus indicating that these abnormalities may represent variants of t(8;21). Of interest, in this study a cryptic insertion of the translocation was also identified by the fluorescence in situ hybridization (FISH) method. Furthermore, in two AML patients, one with t(8;21)(p21;q22) and one with t(8;20)(q22;p13), dual-color FISH, using appropriate ETO and AML1 probes, revealed an insertion of AML1 into 8q22 on the derivative chromosome 8 in patient 1 and of ETO into 21q22 on one chromosome 21 in patient 2, leading to AML1/ETO fusion signals.\(^{44}\)

In both cases, expression of AM1/ETO transcripts was demonstrated by RT-PCR and cDNA sequencing. Complex mechanisms involving translocation and insertion of chromosomal fragments probably account for the creation of AML1/ETO hybrid genes in these variant t(8;21).

In conclusion, it has been demonstrated that molecular screening for this aberration is feasible in large prospective multicenter clinical trials, resulting in the detection of most true CBF AMLs.\(^{43,45}\) In our opinion, RT-PCR is strictly required when cytogenetic analysis fails, in cases with suspected variants of t(8;21) and in those with morphologic and/or immunophenotypic features reminiscent of t(8;21) AML. However, for at least two reasons RT-PCR should not replace conventional cytogenetics and should be not be used as the only diagnostic test for the detection of CBF AMLs. In fact, karyotyping is useful to confirm results obtained by a technique, RT-PCR, prone to artifacts and contaminations resulting in the possibility of obtaining false positive and false negative results. Moreover, karyotype studies may provide relevant information on additional lesions that in certain cases accompany t(8;21) and whose significance remains a subject of investigation at both biological and clinical levels.

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Table 2. Immunophenotypic predictive model for AML with t(8;21).

<table>
<thead>
<tr>
<th>Model</th>
<th>Percentage of cases correctly classified</th>
</tr>
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<tr>
<td>Any without CD19</td>
<td>86%</td>
</tr>
<tr>
<td>CD19&gt;10%/CD34&gt;35%</td>
<td>99%</td>
</tr>
<tr>
<td>Any additional model including CD19/CD34</td>
<td>99%</td>
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Epidemiology

AML with t(8;21) is much more frequently found in patients aged less than 60 years than in older individuals and in the majority of cases occurs in primary de novo AML cases. However, cases with radiation-associated AML involving AML1 gene translocations have been recently reported. There is not a clear sex preponderance; a male prevalence has been described in some series, but not substantiated by others studies. Of interest, a geographically heterogeneous distribution has been reported, suggesting the design of specific screening strategies adapted to the incidence in each country. Conversely, in the largest series of elderly AML patients investigated at cytogenetic level, the frequency of AML with t(8;21) was lower than 2% of cases. In a recent study from the UK Medical Research Council (MRC) dealing with 1,065 patients with a median age of 66 years, t(8;21) was found in only 23 patients (2.1%) and in 17 out of these 23 it was associated with additional chromosomal abnormalities, mostly numerical. Furthermore, three cases out of 164 (1.8%) were reported by Leith et al. and more recently, an ECOG study reported only one case of t(8;21) among 103 elderly patients with adequate cytogenetic analysis who were randomized to post-remission therapy. Such a low incidence makes it difficult to evaluate the prognostic impact of t(8;21) in elderly individuals with AML, so that in most cases...
studies dealing with older patients, t(8;21) is combined with normal karyotype that is considered intermediate in prognosis. However, in the MRC series, in spite of a CR rate of 87%, a relapse risk of 84% at 5 years was observed, suggesting that t(8;21) plays a different prognostic role in elderly AML patients. Obviously, more attenuated induction and post-remission chemotherapy as well as the unfeasibility in most cases of undergoing stem cell transplantation (SCT) account for poorer therapeutic results. Notwithstanding this, the above data confirm that AML in the elderly is characterized by distinct biological features, including more frequent adverse cytogenetics and a strikingly low percentage of cases with t(8;21). Of note, t(8;21) in older AML patients is infrequent in both secondary and de novo forms; in addition, it is often associated with additional karyotypic aberrations, such as deletion of chromosome 9 [del(9q)], loss of chromosomes Y (-Y) or X (-X) and other numerical abnormalities.

Prognostic factors

With the sequential advances made in AML induction and post-remission treatment, patients with t(8;21) have been consistently reported to have a higher CR rate and more prolonged survival than those with either normal or other aberrant karyotypes. Nevertheless, a significant proportion of patients experience relapse that may result in a relatively low rate of second remission. Therefore, the identification of pre-treatment parameters potentially predictive of clinical outcome could direct therapy toward a patient-oriented, risk-adapted approach. Apart from age, different characteristics at presentation including extramedullary leukemia (EML), immunophenotypic findings, additional chromosomal abnormalities, white blood cell (WBC) count and differentiation potential of the leukemic clone have been investigated (Table 3).

The presence of granulocytic sarcomas is preferentially associated with AML with t(8;21). EM L was found at diagnosis in 8 patients out of 84 (9.5%) in Byrd’s series, and mainly involved the spinal cord. In this study, the CR rate was 50% in t(8;21) patients with EML, as compared to 94% in those without EML. In addition, patients with EML had a significantly shorter survival (p = 0.002, median 5.4 months versus 59.5 months). The poor outcome was related to residual recurrent EML following induction therapy as well as to permanent neurologic deficits. Of interest, the only patient remaining alive in CR was consolidated with high dose ARA-C and most patients did not receive adequate site-directed therapy. Therefore, the prognostic relevance of EML in t(8;21) AML remains unclear and needs to be confirmed in patient cohorts receiving more aggressive local and systemic intensification.

WBC count has also been reported as associated with poor prognosis in some studies; however, in a recent large series of 158 patients with t(8;21) AML, WBC did not retain statistical significance in the multivariate analysis. Interestingly, in this study the so-called WBC index (calculated as the product of WBC count by the percentage of bone marrow blasts, to take into account the differentiation potential of the leukemic clone) was a more potent predictive factor than WBC count itself and remained independently predictive of relapse-free survival, after adjustment for age and trials in the multivariate analysis.

As previously outlined, AML with t(8;21) has a distinctive immunophenotype, characterized by high frequency of expression of different membrane antigens including CD56, a neural cell adhesion molecule expressed on neoplastic cells of several hematologic malignancies. Recently, the expression of CD56 has been proven to be associated with adverse clinical outcome in a series of 100 patients with acute promyelocytic leukemia (APL) uniformly treated with all-trans retinoic acid (ATRA) plus chemotherapy as well as in other AML FAB subtypes. As far as specifically concerns AML with t(8;21), positivity for CD56 was demonstrated in 55% of cases in a small series of 29 patients and was significantly related to inferior disease-free survival. Of interest, there was no difference in CR rate between the two groups of CD56+ and CD56- AMLs (88% versus 92%: p = 1); conversely, in spite of comparable post-remission therapy, CR duration was significantly shorter for cases expressing CD56 at presentation. In this study,

| Table 3. Prognostic factors in AML with t(8;21).* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age             | Additional chromosome abnormalities | RT-PCR positivity in apparently normal karyotype | Extramedullary leukemia | CD56 expression |
| Extra chromosomal chromosome abnormalities | WBC index (product of WBC count by percentage of bone marrow blasts) | Post-remission therapy |

*Apart from age, the prognostic relevance of all parameters indicated needs to be confirmed by multivariate analysis in large series of homogeneously treated patients.
granulocytic sarcomas were present exclusively in cases with CD56 expression and this may have played a role in the inferior outcome of these CD56+ cases. A possible correlation between CD56 and P-glycoprotein expression was hypothesized, but no data are currently available to demonstrate such a relationship. Of note, 24 of 26 patients received at least one cycle of HDARA-C as consolidation and the number of courses were similar in cases with or without CD56 expression; on this basis, the authors concluded that positivity for CD56 may represent a useful parameter for stratifying therapy for patients with t(8;21) AML. In our opinion, this finding needs further confirmation by multivariate analysis in larger series of uniformly treated patients, managed with repetitive courses of HDARA-C or transplantation procedures.

Additional chromosomal abnormalities can be found in AML with t(8;21). While loss of chromosome Y or X is not an apparent predictor of outcome, the prognostic impact of del (9q) on patients’ outcome was found to be variable, depending on the study.66,67 The current MRC criteria consider t(8;21) as favorable irrespectively of the presence of additional aberrations, including del(9q) or complex karyotypes.10 In contrast, the Southwest Oncology Group coding requires absence of both del 9q and complex karyotype.12 Of note, only 17 patients with del(9q) were observed in the SWOG study, while results from the MRC, pooling data from 3 MRC trials, report a survival at 5 years of 80% for patients with concomitant t(8;21) and del (9q) as opposed to 36% and 31% for those with del(9q) alone or del(9q) with other abnormalities. These results demonstrate that t(8;21) can markedly soften the potential adverse prognostic significance of concomitant chromosomal abnormalities, including del(9q). Finally, a recent study by Sarriera et al. attempted to compare the outcome in AML patients with t(8;21) between those in whom the translocation was found by standard cytogenetic analysis and those with AML1/ETO fusion transcript found only by PCR testing.68 Ninety percent of the patients with t(8;21) shown at cytogenetic level and 40% of the patients with t(8;21) shown by RT-PCR alone achieved CR (p = 0.03), suggesting a significant difference in the clinical outcome between the two groups. Once again, this finding needs to be confirmed by multivariate analysis including other potential predictive factors. In conclusion, it remains to be established whether patients with solely molecular evidence of the gene rearrangement really fare worse than those in whom it is confirmed by conventional cytogenetics.

Minimal residual disease

An important goal in the treatment of acute leukemia is to determine whether there is a level of minimal residual disease (MRD) below which relapse is unlikely. Molecular evaluation has definitively demonstrated that MRD detection by RT-PCR is highly predictive of relapse in APL, leading to the concept of molecular relapse.69 Of note, molecular relapse is currently treated at most institutions to prevent hematologic recurrence of APL.70 An identical approach has been investigated in AML with t(8;21). Surprisingly, detection of the AML1/ETO fusion transcript by nested RT-PCR has been reported in patients in long-term CR treated with either consolidation chemotherapy or autologous stem cell transplantation (ASCT).71,72 Similarly, persistence of the hybrid gene has been detected in bone marrow or peripheral blood samples from patients undergoing allogeneic SCT (allo-SCT), despite a variety of conditioning regimens and occurrence of acute and/or chronic graft-versus-host disease.73 These findings suggest that the complete eradication of AML1/ETO and RT-PCR negativity are not invariably needed to achieve cure from AML with t(8;21). Apparently discordant results were reported in a multicenter study of 51 patients with t(8;21) in first or second CR, in which all samples were tested by two different RT-PCR techniques (a nested technique and a one step technique with a sensitivity of 10⁻⁶ and 10⁻⁵, respectively).74 Samples from 14 potentially cured patients (median follow-up 112 months) were taken at least twice and all were PCR negative by both techniques. In addition, samples from 37 patients were prospectively taken after CR1 and/or CR2 achievement, before consolidation treatment, and every 3 to 6 months after completion of therapy. Of interest, better prognosis was observed for patients who converted to PCR negativity after CR achievement as well as for those who became PCR-negative with the one step technique before intensive consolidation treatment. The one-order lower sensitivity of one-step PCR may be the explanation for its better clinical usefulness. RT-quantitative PCR, based on more sophisticated and reproducible technology in prospective studies of homogeneously treated patients, will certainly help to clarify the clinical relevance of molecular detection of AML1/ETO transcripts in patients with t(8;21) while in hematologic CR.

Reagents for FISH that identify both derivative 8 and 21 chromosomes with a high analytical sensitivity have been developed.75,76 Studies based on the combination of May-Grünwald-Giemsa stain-
ing and FISH had previously suggested that t(8;21) is restricted to the granulocytic lineage, being exclusively found in myeloblasts and maturing neutrophilic cells. More recently, the expression of AML1/ETO fusion transcripts has been clearly detected in residual normal progenitors as well as in the CD34 positive leukemic lineage-negative cell fraction.

As far as concerns MRD detection, in remission patients 1-4/2000 positive cells (0.05-0.19%) have been found in more than 60% of cases. Although higher frequencies were found in two patients in early relapse and in one patient in early remission, a negative test did not exclude relapse. Since false positives are negligible and most AMLs with t(8;21) express CD34, FISH in combination with CD34 selection by cell sorting would be more sensitive for MRD detection.

Cytometric detection of minimal residual disease in t(8;21) AML should start from the concept that CD34 expression on t(8;21) AML is higher than on the vast majority of normal CD34+ progenitor cells, often reaching the 4th decade on a cytometric logarithmic histogram. Thus, by gating CD34+ cells it is possible to enrich blast cells and search for the expression of CD19 or the co-expression of CD19 and CD56 on the selected population. Figure 3 shows the detection of 15 cells (out of one million events analyzed), characterized by high expression of CD34 as well as the co-expression of the two antigens. We are now testing the sensitivity of such an approach; preliminary results suggest that cytometric detection of MRD in t(8;21) AML is able to reach the level of 10^-5.

Treatment of AML with t(8;21)

All large studies conducted in young/adults primary AML have shown that the best results in terms of CR rate (85%-90%) are achieved in AML with t(8;21). Of interest, highest CR rates are reported following either a standard 3+7 combination or more aggressive regimens based on 3-drug combinations and 10 days of continuous infusion of ARA-C, such as ADE. Whatever the induction therapy, favorable cytogenetics will ultimately result in a large proportion of cured patients, provided that aggressive post-remission therapy is administered. Following laboratory studies demonstrating a steep in vitro dose-response curve in experimental models, high dose cytarabine (HDARA-C) has represented a major focus of interest in AML clinical research over the past years either as induction or as post-remission treatment. In particular, a large randomized study conducted by the CALGB demonstrated that HDARA-C offers substantial advantage in terms of
CR duration as compared to intermediate or conventional dose treatment.

However, subsequent examination of outcome emphasized that, following HDARA-C, the frequency of long-term survival was strictly related to cytogenetic subtype, best results being obtained in t(8;21) AMEL. In addition, a more recent survey from CALGB showed that patients with t(8;21) AML treated with repetitive cycles of HDARA-C had a significant advantage in terms of disease-free and overall survival (DFS and OS) as compared to those who had not received sequential HDARA-C therapy. At our institution, we administered consolidation with 3 cycles of high dose ARA-C (3 g/m² q12h on days 1, 3, 5) to 14 consecutive patients affected by AML with t(8;21) who had achieved CR after ICE (idarubicin, ARA-C and etoposide). Before giving HDARA-C, the NOVIA regimen (mitoxantrone + intermediate dose ARA-C) was administered with the aim of collecting peripheral blood stem cells (PBSC). Collection of more than 2 × 10⁶/L CD34⁺ cells was successful in 80% of cases, but no patient received ASCT as post-remission therapy. These CD34⁺ cells are being stored and will be used for ASCT in CR2 in case of relapse. Of note, all 14 patients received the three programmed courses and survival at 5 years was 84%. One relapse occurred and CR2 was easily achieved by the FLAG regimen.

The patient underwent allo-SCT and died from severe GVHD while in CR. On the basis of these results, incorporation of repetitive courses of HDARA-C into consolidation therapy for AML with t(8;21) seems strictly necessary for the achievement of long-term survival and cure.

On the other hand, MRC data from the AML10 trial demonstrated a favorable impact of either ASCT or allo-SCT for patients with t(8;21). In this study, also including children, the CR rate for t(8;21) AML was 98% and the addition of ASCT resulted in a significant reduction of the risk of relapse compared to that in a group of patients who received no further therapy. Nonetheless, treatment-related mortality was significantly higher in the ASCT group than in the group managed with chemotherapy. Accordingly, the conclusion of the authors was that in patients with favorable cytogenetics as well as in children it is reasonable to delay autografting until second remission, given the good chance of salvage therapy. Nowadays, the extensive use of PBSCs have substantially reduced morbidity and mortality from ASCT in most hematologic malignancies including AML. The toxicity of this procedure should, therefore, be reconsidered. Moreover, in the MRC study consolidation therapy was not based on HDARA-C and this renders a direct comparison between CALGB and MRC results unreliable. Notwithstanding, therapeutic results from either HDARA-C or ASCT are comparable to those reported after allo-SCT, with substantially lower morbidity and mortality. We feel that a randomized study based on the comparison of consolidation with 3 courses of HDARA-C versus ASCT performed whenever possible with PBSC collected after consolidation with NOVIA or similar regimens would define the optimal post-remission approach to AML with t(8;21). Apart from survival and disease-free survival, such a trial would provide important results on treatment-related morbidity and mortality, duration of hospitalization and costs. Allo-SCT should be considered in CR2, when a compatible donor is available.

Although rarely, AML with t(8;21) may occur in elderly or very elderly patients. In this age category therapeutic results are significantly poorer than those observed in the young/adults. In addition, a substantial number of patients are unsuitable for induction and post-remission aggressive therapy. Experimental observations have demonstrated that certain non-random chromosomal translocations in AML may provide the biological basis to explain the differentiating effect of hematopoietic growth factors. In particular, blast cells from AML with t(8;21) have been shown to undergo neutrophilic differentiation following in vitro exposure to granulocyte colony-stimulating factor (G-CSF). Moreover, in vitro treatment with G-CSF of the t(8;21) positive cell line Kasumi-1 has been reported to enable activation of the STAT pathway as well as expression of the myeloid differentiation antigens CD11b, CD13, CD15 and CDw85. On this basis, we treated a 75-year old patient with t(8;21) AML, considered unable to receive aggressive therapy because of concomitant metastatic prostate cancer, with only G-CSF at 450 µg/m² as induction therapy. During the treatment a progressive increase of WBC count was observed along with a progressive increase in maturing myeloid cells. After 2 weeks, CR was demonstrated by normal blood count and differential and bone marrow myeloblasts < 5%. Following relapse the patient was restarted on G-CSF and achieved a second CR. Notably, in vitro studies performed to compare the effects of G-CSF with other differentiating agents definitively demonstrated that exposure to G-CSF induced striking neutrophilic differentiation, while ATRA, GM-CSF and As₂O₃ exerted negligible activity. While occasional reports had previously described CR in AML patients in whom G-
CSF had been given with the aim of increasing neutrophil count to control infection. This case is unique in that the growth factor was successfully used as targeted induction therapy aiming at CR achievement. If confirmed in additional cases, this finding could stimulate clinical trials based on the administration of G-CSF before or concomitantly with chemotherapy in AML with t(8;21), parallel ing what is routinely done with ATRA for APL.

A tentative approach to the diagnosis and treatment of AML with t(8;21), ideally in the context of a clinical trial, is illustrated in Figure 4.

**Future directions**

A number of observations have clearly demonstrated that AML with t(8;21) is a distinct clinicobiological entity within the field of a heterogeneous disease such as AML. Extensive molecular investigations have been conducted to characterize the molecular counterpart of the translocation as well as its potential role in leukemogenesis. The lack of an effect of the AML1/ETO chimeric gene sufficient per se to induce leukemia is particularly intriguing, so that further research is needed to identify additional hit events. On the other hand, clinicians wait for the answer to many unresolved questions to develop more effective and less toxic therapeutic approaches. The investigation of large series of patients with solely molecular detection of gene rearrangement will clarify whether these represent a subset with different biological and clinical characteristics. More sophisticated quantitative RT-PCR techniques and/or immunologic methods will allow correct monitoring of MRD enabling individual, risk-adapted post-remission strategies. Innovative clinical trials, based on biological response modifiers such as G-CSF and phenyl-butyrate, aiming at induction of differentiation and/or apoptosis, either alone or in combination with chemotherapy, could clarify whether the successful current clinical approach to APL can be translated to AML with t(8;21).

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