Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by a specific genetic alteration, affecting the retinoic acid receptor-α (RARα), and leading to a blockage in the differentiation of the granulocytic cells. The accumulation of the promyelocytic blasts in the bone marrow produces intense peripheral blood cytopenias or, less commonly, hyperleucytosis, both of which are frequently associated with a life-threatening consumptive coagulopathy. The body of available biological information on APL establishes this leukemia as a unique entity that has to be promptly recognized and clearly distinguished from all other acute leukemias, especially in light of its striking response to treatment with anthracyclines and differentiating agents such as all-trans retinoic acid (ATRA) or arsenic trioxide (ATO). Current state-of-the-art treatments, which include simultaneous administration of ATRA and anthracycline-based chemotherapy for induction and consolidation, as well as ATRA-based maintenance, have dramatically transformed APL into the most curable acute leukemia in adults, with approximately 80% of long-term survivors. Risk-adapted strategies to modulate treatment intensity may be an effective approach to minimizing therapy-related morbidity and mortality while maintaining the potential of cure. Nonetheless, a sizeable proportion of patients will relapse after the ATRA-based upfront therapy. Given the high anti-leukemic efficacy observed with ATO in patients who relapse, this agent is currently regarded as the best treatment option in this setting. In this article, we will review the current treatment strategies in the management of newly diagnosed and relapsed APL. We also highlight other aspects that can be crucial for the outcome of individual patients, including supportive care, recognition and treatment of life-threatening complications, management of ATRA- and ATO-associated adverse events, and the role of minimal residual disease (MRD) monitoring.

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Acute promyelocytic leukemia (APL) is a particular subset of acute myeloid leukemia (AML) with characteristic clinical, morphologic, and genetic features. These include the association at diagnosis of a severe bleeding diathesis, a high sensitivity of leukemic blasts to chemotherapy with anthracyclines, and the response in vitro and in vivo to the differentiation agent all-trans retinoic acid (ATRA). More recently, it was shown that APL patients are also exquisitely sensitive to arsenic trioxide (ATO), which selectively induces partial differentiation and apoptosis of APL blasts.14

The annual incidence of APL in the United States is estimated to be approximately 600 to 800 cases. The disease is very uncommon in children under 10 years of age. Its incidence gradually increases, reaching a plateau during early adulthood, then remains constant until it diminishes after 60 years age.5 This is in marked contrast to other subtypes of AML, in which the incidence increases with advanced age. Earlier reports suggesting an increased prevalence in Hispanics were not confirmed in a recent study on APL incidence as a function of race and ethnicity.6

During the past two decades, dramatic progress in the biology and treatment of APL has contributed to the transformation of this once rapidly fatal disease into the most curable acute leukemia. In addition, APL is nowadays regarded as a paradigmatic model for translational research in medicine. In this article, we will review the diagnostic and therapeutic progress recently made in the management of APL with special emphasis on front-line treatment.

**BIOLOGICAL FEATURES**

The French-American-British (FAB) classification recognizes two main morphologic APL subtypes, including a more frequent hypergranular form (M3) featured by abnormal dysplastic promyelocytes with abundant cy-
toplasmic granules and Auer bodies (the latter are sometimes piled together to form the so-called “faggots”), and a less frequent microgranular form (also referred to as variant or M3v) characterized by leukemic blasts with bi-lobed nuclei with dusty and minute cytoplasmic granules. Unlike the hypergranular type, the M3v type is frequently associated with an elevated white blood cell (WBC) count. With respect to cytochemical characteristics, either the typical or the variant APL subtypes show a strongly positive staining for myeloperoxidase with Sudan Black.1-3

As regards immunophenotypic features, APL blasts show a characteristic surface antigen profile, including strong positivity for CD33, expression of CD9, CD13, and CD117, infrequent expression of human leukocyte antigen (HLA)-DR and CD34, low expression of proteins associated with multidrug resistance (MDR) such as PGP, and lack of CD7, CD11a, CD11b, CD14, and CD18.4 Aberrant expression of the T-cell-associated antigen CD2 has been reported in a proportion of cases associated with M3v and increased leukocyte counts at presentation.8

At the genetic level, APL is characterized by a unique reciprocal chromosome translocation t(15;17)(q22; q11–12), leading to a fusion between the promyelocytic (PML) gene on chromosome 15 and the retinoic acid receptor-α (RARA) gene on chromosome 17. Based on the PML breakpoint location, the PML/RARA transcript subtypes bcr1, bcr2 (also referred to together as long transcript type), and bcr3 (short type) may be formed. The PML/RARA fusion is detectable in more than 95% of morphologically characterized APLs, while in the remaining cases several variant rearrangements have been described, including at low frequency (<3%) the promyelocytic leukemia zinc finger (PLZF)/RARA fusion, which is not sensitive to ATRA and ATO.2 A number of in vitro and in vivo studies have shown that PML/RARA acts as a constitutive and potent transcriptional repressor of RARα-target genes and has a crucial role in disease development. Importantly, the PML/RARα protein is targeted by both ATRA and ATO treatment and undergoes degradation in leukemic blasts upon exposure to these agents. As a consequence, diagnostic identification of PML/RARA is considered of paramount clinical relevance in light of its specific association with the disease and because it predicts response to tailored treatments containing ATRA and/or ATO.2

FLT3 receptor mutations, including internal tandem duplications (ITD) in the juxtamembrane domain and point mutations in the tyrosine kinase (TK) II domain, have been detected at high frequency in APL (up to 45% of cases). These mutations have been associated with higher WBC count at presentation and with the short-type PML/RARA fusion.9 FLT3 receptor is currently regarded as an interesting target for tailored treatment in non-M3 AMLs, which disclose ITD and TK mutations in 20% to 30% of cases. FLT3 inhibitors are being evaluated in clinical trials for AML patients, while they have not been tested so far in the treatment of patients with APL owing to the availability of several highly effective agents already in use in this disease.

CLINICAL PRESENTATION, DIAGNOSTIC WORK-UP, AND SUPPORTIVE MEASURES

APL usually has an abrupt onset. Due to the risk of early death (10%-20%) and the high potential for cure (>80%), immediate recognition and prompt initiation of specific treatment are extremely important. Patients frequently present with normal or decreased hemoglobin and WBC count, low platelet levels, and mucocutaneous hemorrhagic signs. Lymph node and hypochondric organ enlargements are extremely uncommon. Laboratory analyses typically show increased coagulation time and marked signs of hyperfibrinolysis.10 Both morphologic and genetic tests to confirm the diagnosis have to be preferentially performed in the bone marrow, especially in cases with a low WBC count. However, although genetic confirmation of the diagnosis is mandatory for the reasons detailed below, treatment should not be delayed and should be started even before the results of genetic tests are available.

When APL is suspected based on clinical and morphologic criteria, rapid patient hospitalization in a specialised onco-hematologic center is recommended and the following actions must be undertaken simultaneously11,12:

(1) Institution of supportive measures to correct the coagulopathy and decrease the risk of severe bleeding. Treatment of the coagulopathy should consist of fibrinogen, fresh frozen plasma, and platelet transfusions to maintain fibrinogen and platelets above 150 mg/dL and 30 to 50 × 10^9/L, respectively, and until disappearance of all clinical and laboratory signs of coagulopathy. The use of heparin for the control of the coagulopathy and that of tranexamic acid to counteract hyperfibrinolysis is not recommended as their benefits are uncertain.

(2) Start targeted treatment with ATRA without waiting for genetic confirmation of the diagnosis. Indeed, ATRA has been shown to rapidly ameliorate the signs of the coagulopathy.13 Tailored chemotherapy should be added as soon as possible. Regimens with ATRA and chemotherapy combinations that are currently considered as the gold standard approach in front line therapy are discussed below.

(3) Confirm the diagnosis at the genetic level, by demonstrating the presence of the t(15;17), or its underlying gene fusion PML/RARA, in leuke-
mia cells. The genetic hallmark of the disease is detectable in virtually 100% of APL patients with appropriate laboratory methods. Compared to karyotyping, fluorescence in situ hybridization (FISH) and reverse transcriptase—polymerase chain reaction (RT-PCR) offer the ability to analyze nondividing cells as well as samples with few or poor-quality metaphases. Furthermore, cases in which the PML/RARA fusion is formed as a result of cryptic or complex rearrangements in the absence of the classic t(15;17) also are identified with FISH or RT-PCR. Because RT-PCR is notoriously prone to contamination and artefacts, it is recommended that diagnostic and monitoring samples be sent to experienced reference laboratories.2

As an alternative, anti-PML monoclonal antibodies may be conveniently used for rapid and low cost identification of a characteristic “microspeckled” distribution of the PML protein that results from the translocation in APL blasts.14 However, this method is not intended to be a substitute of RT-PCR. The latter technique, in fact, represents the only one able to define the type of PML/RARA isoform to be used as a target for minimal residual disease (MRD) evaluation in the individual patient.2

Advantages and pitfalls of diagnostic methods in APL are detailed in Figure 1.

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**Table 1. Methods for genetic diagnosis of APL.**

<table>
<thead>
<tr>
<th>Target aberration</th>
<th>Method</th>
<th>Time required hrs</th>
<th>Visualization</th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
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<tbody>
<tr>
<td>CHROMOSOME</td>
<td></td>
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<tr>
<td>t (15;17)</td>
<td>Karyotyping</td>
<td>16-48</td>
<td>Highly specific</td>
<td>No need of dividing cells</td>
<td>No information on the type of PML/RARA fusion</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>6-24</td>
<td>No need of dividing cells</td>
<td>No information on the type of PML/RARA fusion</td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>PML/RARA fusion</td>
<td>4-6</td>
<td>Highly sensitive; rapid; defines target for MRD</td>
<td>Poor RNA yield at diagnosis; contamination and artifacts (false positives)</td>
<td></td>
</tr>
<tr>
<td>NUCLEUS</td>
<td>Microspeckled nuclear distribution of the PML protein</td>
<td>2-3</td>
<td>Rapid; simple; low cost</td>
<td>Artifacts due to cellular degradation; no information on the type of PML/RARA fusion</td>
<td></td>
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</tbody>
</table>

**Figure 1.** Methods for genetic diagnosis of APL. FISH, fluorescence in situ hybridization; RT-PCR, reverse transcriptase—polymerase chain reaction; MRD, minimal residual disease.
dation, translated into a lower relapse rate due to the favorable impact on patients with WBC counts greater than $10 \times 10^9/L$. As to the type of anthracycline, no prospective studies have so far compared idarubicin versus daunorubicin.

Using the simultaneous ATRA plus anthracycline-containing chemotherapy approach, several large multicenter studies have reported CR rates of 90% to 99%. Remarkably, no resistant cases have been reported in series where the diagnosis of APL was confirmed at the genetic level.1,3,11,12

Consolidation
The use of at least two further cycles of anthracycline-based consolidation is commonly adopted in most trials as it has been shown that this strategy results in the achievement of molecular remission (defined below) rates of 90% to 99%. The Spanish PETHEMA (Programa Español Tratamientos en Hematología) and the Italian GIMEMA (Gruppo Italiano Malattie Ematologiche dell’ Aduloto) cooperative groups reported improved outcomes using risk-adapted strategies in which treatment intensity during consolidation was modulated according to predefined groups at risk for relapse.1,12,18,19 In addition, both cooperative groups reported a statistically significant improvement in outcomes using a standard ATRA dose in conjunction with chemotherapy during consolidation. However, such benefit was suggested by a comparison with historical controls and to date no randomized trials have addressed the role of ATRA in consolidation.

As to the role of cytarabine, the aforementioned randomized study of the European APL group15 reported an increased risk of relapse for patients not receiving this agent for induction and consolidation therapy. However, a recent joint analysis of the PETHEMA and the European APL groups16 showed a significantly lower cumulative incidence of relapse for patients in the low- and intermediate-risk groups (i.e., those with $<10 \times 10^9/L$ WBCs at presentation) treated with anthracycline monotherapy in the PETHEMA LPA99 trial as compared to those in the best arm of the European APL 2000 trial including cytarabine. In this study, a trend in favor of cytarabine use was observed only for high-risk patients. The latter observation is in agreement with a recent study of the GIMEMA group suggesting a benefit in using cytarabine for consolidation in the group of patients at high risk.19

Given the high cure rate obtained using upfront ATRA and chemotherapy, hematopoietic stem cell transplantation (HSCT) is not indicated for patients achieving molecular remission at the end of consolidation.

Maintenance
The advantage of using ATRA for maintenance therapy in APL has been demonstrated in two randomized studies conducted in Europe and the United States in which this agent was given on an intermittent or continuous schedule, respectively.1,3 The APL93 study of the European APL group showed a lower relapse rate by combining intermittent ATRA, methotrexate, and 6-mercaptopurine, which was particularly effective for patients with high WBC counts at presentation. In contrast, two more recent reports including a Japanese20 and an Italian study,21 failed to demonstrate a benefit of maintenance therapy in APL. These discrepancies are most likely attributable to the intensity of prior induction and consolidation therapy in the two series. In other words, maintenance therapy would be less useful when induction and consolidation therapy have been more effective.

MANAGEMENT OF ATRA AND ATO-ASSOCIATED ADVERSE EVENTS
Besides the control of the coagulopathy, physicians caring for patients with APL should be aware of a severe and potentially life-threatening complication frequently occurring during treatment with either ATRA or ATO and referred to as differentiation syndrome. This condition should be suspected clinically in the presence of one of the following symptoms or signs: dyspnea, unexplained fever, weight gain, peripheral edema, unexplained hypotension, acute renal failure or congestive heart failure, and, in particular, a chest radiograph demonstrating pulmonary infiltrates or pleuroperticardial effusion.1,12 Because the overt syndrome is associated with high mortality rates, early recognition and therapeutic intervention are crucial. Specific treatment consists of dexamethasone at a dose of 10 mg twice daily by intravenous injection for at least 4 days or until disappearance of symptoms. Temporary discontinuation of ATRA or ATO is indicated only in the most severe cases. At present there is no evidence that prophylaxis with corticosteroids is advantageous in reducing rates of morbidity and mortality associated with the differentiation syndrome.1,12

Severe headache and pseudo-tumor cerebri are frequently observed in children during ATRA treatment. Treatment consists of temporary discontinuation or dose reduction of ATRA and administration of dexamethasone, osmotic diuretics, and analgesics.5

Treatment with ATO also is associated with prolongation of the QT interval, and careful monitoring of the electrocardiogram results and serum electrolytes is required in this setting. In particular, maintenance of the serum K and Mg levels above the lower normal limits is recommended.1,11 The most serious toxicities related to ATO and ATRA treatment of APL and recommendations for management are summarized in Table 1.
<table>
<thead>
<tr>
<th>Agent and/or ATO</th>
<th>Side Effects</th>
<th>Symptoms</th>
<th>Incidence</th>
<th>Management</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA and/or ATO</td>
<td>Differentiation syndrome (DS)</td>
<td>Dyspnea, unexplained fever, weight gain, peripheral edema, unexplained hypotension, acute renal failure or congestive heart failure</td>
<td>5-25%</td>
<td>Dexamethasone 10 mg twice daily until disappearance of symptoms and signs</td>
<td>DS (formerly retinoic acid syndrome). Variable incidence of DS is reported depending on different treatment schedules (ATRA, ATO, ATRA ± ATO ± chemotherapy). Temporary discontinuation is required only in severe DS</td>
</tr>
<tr>
<td>ATRA</td>
<td>Pseudo-tumor cerebri</td>
<td>Papilledema, headache, nausea, vomiting, and visual disturbances</td>
<td>5-15% in children; rare in adults</td>
<td>Temporary discontinuation; dexamethasone, osmotic diuretics, and analgesics</td>
<td></td>
</tr>
<tr>
<td>ATO</td>
<td>Prolongation of the QTc interval</td>
<td>Symptomatic palpitations or syncope</td>
<td>50%</td>
<td>Maintenance of the serum potassium &gt;4.0 mmol/L (4.0 mEq/L) and magnesium &gt;0.82 mmol/L (2.0 mg/dL)</td>
<td>Possible evolution to torsade de pointes and sudden death has been occasionally reported. Severe QTc prolongation requiring withdrawal of ATO treatment has been reported in rare cases.</td>
</tr>
</tbody>
</table>
RESPONSE ASSESSMENT AND MRD MONITORING

Morphological assessment of the bone marrow after induction therapy has been well studied for its ability to provide useful prognostic information in non-M3 AML subtypes. In patients with APL receiving ATRA-containing regimens, early bone marrow evaluation after induction therapy may reveal a relatively hypercellular pattern that reflects residual differentiation of leukemic cells. Moreover, persistence of atypical promyelocytes is occasionally detectable several weeks after the start of induction therapy with ATRA (up to 40–50 days). Such features may be misleading and erroneously considered to indicate leukemia resistance. In such cases, treatment should be continued until terminal differentiation of blasts and achievement of hematological CR that invariably occurs in all APL patients who survive ATRA-based induction chemotherapy.11,12 Results of RT-PCR, karyotyping, and FISH analyses performed early after induction also may be confounding. In fact, several large prospective studies have failed to demonstrate any correlation between the post-induction PCR status and subsequent patient outcome.11,12 Therefore, clinicians should refrain from making therapeutic decisions based on morphologic or laboratory observations at this time point and should wait until CR is obtained.

Unlike assessment of response performed after induction, molecular evaluation of bone marrow samples after completion of consolidation is of paramount importance to determine the risk of relapse in the individual patient.17,22 The predictive value of post-consolidation PCR with respect to successive outcome has been demonstrated in several prospective studies using techniques with relatively low sensitivity (ie, with detection threshold levels between 10−3 and 10−4 cells). Patients who test PCR-positive for PML/RARA at this time point have persistent MRD in their marrow and are operationally defined as molecularly resistant. Given their overall poor prognosis, these patients are candidates for further intensification and aggressive treatment approaches, including allogeneic stem cell transplantation.23 To avoid false-positive results, the detection of residual PML/RARA transcripts at the end of consolidation should be confirmed by sending a new marrow sample to a reference laboratory with very experienced personnel using a low-sensitivity assay.22

By contrast, patients who test PCR-negative after consolidation (ie, those achieving molecular remission) are at very low risk of relapse and usually undergo maintenance therapy or observation. In light of its association with prolonged survival, molecular remission is nowadays recommended as the therapeutic objective in APL and represents a clinically relevant surrogate marker for survival.21 In fact, a number of studies have demonstrated that patients with repeatedly negative PCR after consolidation experience long-term remission, whereas conversion to PCR positivity is associated with hematologic relapse.22

The increasing anti-leukemic efficacy reported with current standard treatments, which results in the achievement of molecular remission in greater than 95% of patients, has questioned the benefit of systematic molecular monitoring following consolidation therapy.22 At present, it is reasonable to adapt the frequency of MRD sampling according to the initial risk of relapse as defined by clinical criteria. For example, patients with hyperleukocytosis at presentation may be worth monitoring by a more stringent timetable (ie, every 2 months in the first year post-consolidation and thereafter every 3 months for 2 additional years). By contrast, molecular monitoring of patients at low risk is less cost-effective and should be performed on a less stringent schedule.

PCR monitoring of PML/RARA has been implemented in recent years through the advent of quantitative real-time techniques (RQ-PCR). This method allows more precise assessment of the kinetics of response and relapse by quantitation of leukemia-associated transcripts. While being marginally more sensitive than conventional RT-PCR for PML/RARA detection, RQ-PCR provides more precise and reproducible evaluations and proves useful in particular for the identification of poor-quality samples. Whatever the assay employed for MRD molecular assessments, as indicated above for molecular diagnosis, the use of reference laboratories with extensive specific experience is highly recommended.22,25,26

ROLE OF ATO

ATO has a dual dose-dependent effect in APL cells, including the induction of differentiation at low concentrations and of apoptosis at higher concentrations. Cell differentiation is observed to a lesser extent as compared to that obtained with ATRA. At higher concentrations, ATO inhibits APL cell growth through apoptosis that follows the induction of the proenzymes of caspase 2 and caspase 3, and activation of both caspase 1 and caspase 3.27

Initial studies using ATO for recurrent APL were again reported from China in the late 1990s. These studies, subsequently confirmed in Western populations, reported remission rates of more than 80% and high rates of molecular remission in patients relapsing after previous ATRA.28,29 Based on these findings, several trials were conducted worldwide to investigate the role of ATO in front-line therapy. Shen et al30 reported a randomized study in which three groups of patients received ATRA, ATO, or both. While CR rates were high in the three arms (≥90%), patients in the combination arm achieved CR in a shorter time and with more rapid kinetics of PML/
**RARA** clearance. Estey et al also reported high remission rates with the ATO and ATRA combination for patients with low WBC counts, while high-risk patients were also given an anti-CD33 monoclonal antibody conjugated to calicheamicin (gemtuzumab ozogamicin [GO]). Other studies in newly diagnosed patients conducted in India and Iran used ATO as a single agent and reported high remission rates (86%) with better outcomes for patients presenting with low WBC counts. Overall, these studies demonstrate high anti-leukemic activity of ATO, which is likely the most active single agent in APL.

Because these promising results have been obtained in small series lacking prolonged follow-up, the current recommendation for induction therapy in newly diagnosed APL remains the standard approach with ATRA plus chemotherapy. While appropriate comparisons between the standard treatment and ATO-based regimens are being assessed in randomized clinical trials, front-line induction with ATO should be recommended only for patients unfit to receive chemotherapy.

As to the role of ATO in front-line consolidation of APL, this has been explored in a large US Intergroup trial in which after ATRA and chemotherapy induction, patients in CR were randomized to receive or not two courses of 25 days of ATO prior to consolidation with ATRA and daunorubicin. In this study, recently presented in abstract form, patients receiving ATO had significantly better event-free and overall survivals compared to those receiving ATRA and chemotherapy only. However, the survival rate in the control arm in this trial was relatively low compared to rates reported by several other groups using standard ATRA and anthracycline chemotherapy-based schedules. Thus, it appears that further studies are needed to investigate whether inclusion of ATO may allow de-intensification of chemotherapy in APL without compromising the cure rates currently achieved with ATRA and anthracycline-based protocols.

Finally, in developing countries with healthcare systems that are unable to afford the expenses of the standard therapeutic agents, or where anthracyclines and retinoic acid are not available, ATO produced locally under good quality control at low cost might provide a reasonable and practical alternative in the treatment of APL.

**SALVAGE THERAPY**

Prior to the advent of ATO, salvage therapy of APL mostly consisted of the re-administration of ATRA and chemotherapy for induction and of further consolidation with chemotherapy usually followed by HSCT. Given the high anti-leukemic efficacy of ATO in relapsed patients, this agent is presently regarded as the best treatment option in this setting. Current evidence suggests using at least two ATO cycles, as this approach results in the achievement of second molecular CR in nearly 80% of cases. Several patient- and disease-related variables, including age, performance status, donor availability, duration of first CR, and particularly PCR status after consolidation, should be considered for the choice of transplant modality. In principle, to further consolidate CR after ATO, autologous HSCT may be a valid option for patients without detectable MRD and prolonged (>1 year) first CR, while allogeneic HSCT would be recommended for patients failing to achieve a second molecular remission and for those with a short first CR duration.

For patients unfit to proceed to SCT, several options are still available, including repeated cycles of ATO, and combination of ATO and/or ATRA with standard chemotherapy or GO. The latter agent is highly effective in APL and has been shown to induce molecular responses even when used as a single agent in advanced disease. Therefore, this drug should be further explored in the treatment strategy for relapsed patients.

Finally, several studies have independently shown that the outcome of patients with relapsed APL may be improved by pre-emptive administration of salvage therapy. In these studies, a survival advantage was shown by administering ATRA plus chemotherapy or ATO salvage therapy at the time of molecular relapse as compared to treating patients for overt hematologic disease recurrence.

**CONCLUSIONS**

Genotypic and phenotypic characteristics of APL have provided targets for more rational and tailored treatment; today, cure of this leukemia is a reality for the vast majority of patients. The clinical impact of laboratory evaluation on patient management and the rarity of the disease indicate the need for cooperation between basic and clinical researchers at both national and international levels. Challenges for the future include the extension of these clinical results to less privileged countries and the improvement of risk-adapted strategies to minimize treatment-related toxicities.

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