Progresos en el tratamiento de la leucemia linfoblástica aguda

Progress in the treatment of acute lymphoblastic leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder that derives from hemopoietic precursors of B- or T-cell origin. The acquisition of a series of genetic aberrations are likely to lead to an impaired maturation, with an arrest in the differentiation process and an abnormal proliferation. As a consequence, the accumulation of leukemic cells occurs both in the bone marrow, where it suppresses the physiologic hemopoiesis, and in extra-medullary sites[1]. ALL is the most common neoplasm in childhood, while it is relatively rare in adults. Among the potential causes of ALL initiation, exposure to ionising radiations has been identified as a predisposing cause and an inherited predisposition has been proposed, at least in children. For the latter, genome wide association studies have identified some variants -i.e. *IKZF1*, *ARID5B*, *CEBPE*, *CDKN2A* and *PAX5*, *BMI1-PIP4K2A* and *GATA3*- that are associated with leukemia occurrence[2]. In addition to the different incidence of the disease and possibly the initiation causes, the clinical course and outcome vary profoundly between children and adults; in fact, nowadays about 85% of children are potentially curable, while the prognosis for adult cases is much more unfavorable, with only 40-50% of patients being long-term survivors. Nonetheless, over

the years an improvement in the survival likelihood has been observed also in adult patients. This has been contributed by: i) the therapeutic intensification of the current protocols; ii) the improvement of the overall clinical conditions of adult patients with ALL; iii) a better knowledge of the molecular background; and iv) the introduction of novel therapeutic drugs, represented mostly by immunotheraputic compounds. Significant advances have been observed in specific subsets, such as Philadelphia (Ph/BCR-ABL1)+ ALL, Burkitt leukemia, T-lineage ALL and, more in general, in adolescents and young adults (AYA), which benefit from pediatric-based approaches. These entities will be discussed separately. For an optimal management of patients with ALL, some steps are of pivotal importance: they include a precise and rapid diagnostic work-up, a prognostic stratification, a correct therapeutic strategy -particularly in specific subsets- and an accurate monitoring of minimal residual disease (MRD) for a more precise risk stratification and decision making in case of MRD persistence or reappearance.

Diagnosis

For a precise diagnosis, a morphologic bone marrow assessment still represents the first step in the diagnostic work-up and for a differential diagnosis with acute myeloid leukemia (AML). By definition, ALL blasts are negative for myeloperoxidase and other myeloid cytochemical reactions. The classification of leukemic blasts has been recapitulated by the WHO classification[3], recently updated[4]. Immunophenotyping, by means of multi-channel flow cytometry, is pivotal for the diagnosis and subclassification of ALL, and is also useful for MRD monitoring. The European Group for the Immunological Characterization of Leukaemias (EGIL) has revised the general guidelines[5]. Among the novel subgroups that can be recognized by immunophenotyping, early-T precursor (ETP) ALL must be underlined[6]. Cytogenetics/molecular analyses[7,8] are necessary for a precise work-up and aim at identifying major translocations/rearrangements, namely t(1;19)(q23;p13)/E2A/PBX1, t(12;21)(p13;q22)/ETV6/RUNX1, MLL rearrangements, translocations involving chromosome 8 (c-myc gene) -i.e. t(8;14) (~90% of cases), t(8;22) (~10% of cases) and t(2;8) (very rare)- present in virtually 100% of cases of mature B-ALL with L3/Burkitt morphology, and t(9;22)(q34;q11)/BCR-ABL1. The analysis of BCR-ABL1 rearrangement should be performed in the shortest possible time in patients, e.g. during the steroid pre-phase used in many protocols, to optimize the management of these cases who benefit by the upfront administration of a tyrosine kinase inhibitor (TKI) (see below). Thus, BCR/ABL1 investigation should be included in the minimal diagnostic work-up requirement for all ALL patients, including the elderly. The introduction of high-throughput techniques such as gene expression profiling (GEP), SNP array analysis and, more recently, next generation sequencing (NGS) and whole exome sequencing (WES) has allowed to identify novel subgroups, such as the so-called BCR-ABL1-like cases. Furthermore, a set of lesions involving the JAK, RAS and PI3K pathways have been identified; these analyses, though still investigational, represent a further step towards the concept of personalized medicine.

Principles of therapy

The therapeutic backbone of ALL treatment is based on an induction phase based on 4-5 drugs (steroids, vincristine, antracyclines, asparaginase and cyclophosphamide) followed by early intensification/consolidation with high-dose methotrexate and cytarabine, and maintenance, with or without reinduction cycles, that can be prolonged to 24-36 months. Allogeneic stem cell transplantation (allo-SCT) in first complete remission (CR) should be reserved to patients with high risk factors, e.g. MLL rearrangements, in some t(9;22)(q34;q11)/BCR/ABL1+ cases and in patients with MRD persistence. Some subgroups have specific genetic features that translate into different therapeutic strategies. These will be discussed below.

Specific subgroups

Ph+ ALL

Ph+ ALL is the most frequent aberration in adult ALL: its incidence increases progressively with age (more than 50% from the 6th decade of life), while it is extremely rare in children (less than 5% in patients younger than 10 years)[9]. Historically, Ph+ ALL was the ALL subgroup with the worse outcome, since the rate of complete hematologic remissions (CHR) with chemotherapy regimens was lower than that observed in other subsets and the 5-year event-free survival (EFS) was below

20%[8]. The management and outcome of Ph+ ALL have dramatically changed since the introduction of TKIs. The inclusion of a TKI in induction represents today the gold standard treatment of Ph+ ALL patients, because it leads to much higher CHR rates and improved long-term outcome, also in elderly patients[10-17]. The use of imatinib as TKI, together with or following conventional induction treatment, is associated with remission rates >90% and improvements in disease-free survival (DFS) and overall survival (OS). Similar results have been obtained with the 2nd generation TKI dasatinib, a more potent oral inhibitor of the BCR/ABL1, c-KIT and SRC kinase families, although the use of dasatinib might lead to a greater and more rapid acquisition of resistant mutations (personal observation). Limited data are instead available with nilotinib. In spite of the clinical success achieved with these integrated approaches, it must be underlined that toxic deaths have been recorded during induction in about 5% of cases treated with a TKI associated with conventional chemotherapy. The GIMEMA cooperative group[18-21] has, over the years, adopted an induction strategy based on the administration of a TKI -either imatinib or dasatinib or imatinib in rotation with nilotinib, or, more recently, ponatinib- plus steroids in induction, together with intrathecal central nervous system (CNS) prophylaxis, but without systemic chemotherapy. These regimens, utilized in adult and elderly populations (roughly 200 cases have been treated so far), have led to CHR rates in 96-100% of patients without toxic deaths in induction, thus indicating that this strategy is effective, feasible and safe (also in the elderly), and partly doable at home. As a proof of principle, the use of imatinib (plus steroids) upfront, without the addition of systemic chemotherapy in induction, followed by chemotherapy (and transplant, when applicable) (GIMEMA LAL0904 third amendement) has allowed to obtain the best long-term OS and DFS rates -48.8% and 45.8% at 60 months- so far reported[21].

Along the same line of thought, the PETHEMA[22] and GRAAL[23] groups have both shown that a de-intensified chemotherapy treatment in induction is capable of inducing the same or better long-term outcomes as intensive treatments with less toxicity. Impressive results have been recently reported with a combination based on the pan-TKI ponatinib and the Hyper-CVAD regimen[24]. A CHR was achieved in all cases, a major molecular response in 75% of patients, with a 2-year event-free survival of 81%; however, it must be underlined that 6/31 patients enrolled have died in CHR, 3 due to cardiac-related toxicities. It remains to be established if ponatinib alone (plus steroids and CNS prophylaxis) in induction, without systematic chemotherapy (or with mild chemotherapy) might provide similar results, sparing toxicity.

Upon CHR achievement, consolidation/intensification treatment -which varies according to the different study groups but generally includes high-dose chemotherapy (particularly for younger patients)- is administered to further reduce and possibly eradicate MRD. Consolidation/intensification should be performed in all cases with persistent MRD positivity, and even more importantly in individuals who are not suitable to receive an allo-SCT. At present, allo-SCT still remains the only curative option for Ph+ ALL, mostly for younger adult patients. The EBMT[25] group has provided a comprehensive update on the role of allo-SCT in the TKI era in a cohort of 390 individuals. The main conclusions are as follows: 1) TKI administration prior to transplant is associated to a significantly better OS both in univariate (47% vs 38% in the pre-TKI era) and multivariate analysis, and also correlates with a lower relapse rate in univariate (33% vs 50%) and multivariate analysis; 2) MRD at the time of transplant is not associated with differences in OS, leukemia-free survival (LFS), relapse rate and non-relapse mortality. It must, however, be underlined that MRD levels were not uniformly evaluated and this may represent a confounding factor; 3) the prophylactic administration of a TKI following allo-SCT improves LFS, OS and relapse rates. Given the toxic effects of this procedure, efforts are ongoing to identify patients who may possibly be spared post-transplant TKI. Autologous transplantation (auto-SCT) has been revisited in Ph+ ALL, mostly because TKI treatment allowed the achievement of sustained molecular responses and therefore better survival rates. Giebel et al[27] compared the outcome of patients stratified into three categories, according to the period in which the procedure was performed: 1996-2001 (no TKI treatment), 2002-2006 (TKI administered in sporadic cases) and 2007 onwards (TKI administered in all patients). OS and LFS significantly increased among the three categories, being 16%

and 11%, 48% and 39%, and 57% and 52%, respectively. Similar results have been recently published by Chalandon et al who showed comparable results between allo- and auto-SCT[23]. An emerging issue is represented by the presence of mutations, with a predominance of P-loop and T315I mutations[28], the latter being insensitive to most TKIs with the exception of ponatinib. Another clinical challenge is represented by the occurrence of the so-called compound mutations[29], defined as the presence of two or more mutations in the same molecule. For such cases, the use of alternative approaches is urgently required, and novel compounds are under development[30,31].

Finally, the management of Ph+ ALL is likely to undergo additional changes, and hopefully improve further, with the introduction of novel immunotherapeutic compounds (see below) -particularly blinatumomab, whose utility has been reported in relapsed/refractory cases[26]- that are currently being introduced in the front-line setting. These strategies should lead to increased rates of MRD-negative patients.

Burkitt leukemia

Burkitt leukemia, characterized by a peculiar morphology and immunophenotype, as well by rearrangements involving the c-MYC gene, can be considered as a therapeutic success in hematology. In fact, the prognosis of patients with a diagnosis of Burkitt leukemia (L3 in the old FAB classification) has largely improved with the use of short-term, dose-intensive treatment programs. CR rates now exceed 80%, with 2-year DFS rates of 60% to 80%. Relapses, when present, are usually observed within the first year of remission. Intensive early prophylactic intrathecal therapy, together with high dose cytarabine and methotrexate, reduce the rates of CNS relapse. The addition of rituximab to chemotherapy has further improved the cure rates of Burkitt leukemia[32,33].

BCR/ABL1-like ALL

The BCR/ABL1-like subgroup, identified by means of GEP, represents a subgroup of ALL that can be found in both pediatric and adult cohorts[34-36]: this entity is particularly frequent in the AYA group, where it where it accounts for 25-30% of cases, as opposed to ~10% of children. Several genetic lesions have been unraveled: in fact, these patients often harbor deletions of the transcription factor *IKZF1*, deregulation of CRLF2 -sustained either by IGH-CRLF2 rearrangements or by an interstitial deletion of the pseudoautosomal region of sexual chromosomes (P2RY8-CRLF2)- and a large set of lesions that involve several tyrosine kinases. Among the most frequent, it is worth mentioning NUP214-ABL1, in-frame fusions of EBF1-PDGFRB, BCR-JAK2, STRN3-JAK2 and the cryptic IGH-EPOR rearrangements, *ABL1*, *ABL2* and *CSF1R* rearrangements, and *JAK1/2* mutations[34,37]. While much is known about the genetic lesions associated with this subset, it must be underlined that, so far, the recognition of these cases relies mostly on GEP analysis, not routinely performed in most centers, and that there is not a recurrent common lesion underlying the signature identified. Efforts are ongoing to identify rapidly these cases. Clinically, the recognition of this subgroup is of great importance for two main reasons: 1) The prognosis is usually poor, although it has been shown that in childhood cases MRD-based risk-directed therapy[38], including transplant procedures, might overcome the unfavorable outcome. It is not clear if MRD-directed therapy is capable of overcoming this poor prognosis molecular signature also in adults. 2) Given that the majority of alterations detected in such patients involve tyrosine kinases and their downstream targets, it is plausible that the use of TKIs and/or mTOR inhibitors may be effective in these patients. While few reports are available on the upfront management of BCR/ABL1-like ALL, refractory patients treated with TKIs may achieve rapid and sustained responses[34]. Similar findings have been reported in 2 cases harboring a EBF1-PDGFRB or ATF7IP/PDGFRB rearrangement treated with imatinib or dasatinib[39,40]. Thus, the integration of a TKI in the therapeutic program for these patients could be a suitable approach, if identified early. The optimal timing for the inclusion of a TKI needs to be investigated, possibly in prospective clinical trials.

T-lineage ALL

The outcome of patients with T-ALL is nowadays similar, if not better, to that of patients with B-lineage ALL, thanks to the use of more intensive treatments. Furthermore, while in the past the spectrum of genetic lesions was limited to rearrangements involving the T-cell receptor (TCR) genes, knowl-

edge on the genetic landscape of T-ALL has greatly increased in recent years[41]. A large set of mutations has been identified in T-ALL by sequencing, re-sequencing and NGS, and include *NOTCH1*, *FBW7*, *BCL11B*, *JAK1*, *PTPN2*, *IL7R* and *PHF6*. Some of these lesions are of prognostic significance: *NOTCH1* and/or *FBW7* mutations, which occur in >60% and ~20% of cases, respectively, are usually associated with a more favorable outcome. In light of this, a prognostic model has been proposed by the GRAAL group[42] that defined as low-risk patients those harboring *NOTCH1* and *FBW7* mutations, and as high risk those without these mutations or harboring lesions involving RAS/PTEN. In addition, mutations affecting the JAK/STAT pathway are of prognostic significance[43-47]. Taken together, these results suggest that a correct prognostic stratification should include the analysis of RAS and JAK/STAT mutations also considering that inhibitors for both mutations are available. A specific subgroup identified within T-ALL is represented by ETP-ALL[6]. As mentioned, this subset can be easily recognized by flow cytometry since it is characterized by distinct cell surface features: absence of CD1a, weak CD5 expression and expression of one or more myeloid or stem cell-associated markers. Several genomic lesions have been identified, including mutations in *DNMT3A*, *FLT3*, *IDH1*, *IDH2* and *ETV6*. Interestingly, *FLT3* mutations can be detected in up to 35% of cases, thus implying the possibility of novel therapeutic strategies[48]. Furthermore, mutations occurring in genes regulating cytokine receptors and RAS signaling (67%), inactivating lesions disrupting hematopoietic development (58%) and histone-modifying genes (48%) have been reported, suggesting that ETP-ALL share a similar genomic background with AML. Clinically, this subgroup was initially defined as a poor prognosis subset; however, the prompt recognition of ETP cases is improving their outcome. Allo-SCT in first CR should be considered the optimal choice for these patients. Finally, the presence of AML-related features prompts to investigate the use of myeloid-directed therapies.

Adolescents and young adults (AYA)

Although survival rates are in the order of 85% for childhood ALL, older AYAs have a less favorable prognosis. The differences in outcome are sustained by the heterogeneity in disease biology, physiologic and psychosocial factors, the therapeutic approach and the referral center, being either a pediatric or an adult care hospital. The definition of this age group is somehow confusing, although the National Cancer Institute has defined the AYA cancer population as being between 15 and 39 years[49]. Among the biologic factors observed, a T-lineage phenotype, as well as an ETP phenotype, is more common in AYA. Good-risk cytogenetic aberrations, such as *ETV6/RUNX1* rearrangements and hyperdiploidy disappear with age progression, whereas the *BCR/ABL1* rearrangement and the BCR/ABL1-like features tend to increase; as mentioned above, the latter seems to prevail in the AYA group. As for host factors, several features are responsible for increased treatment toxicity, including differences in the metabolism of chemotherapeutic agents, depleted marrow reserve and increased extramedullary toxicity, overall resulting in an increased frequency of life-threatening infections, organ failure, treatment delays and dose reductions in planned chemotherapy compared to childhood ALL. Nonetheless, it has been documented that treatment intensification and the use of pediatric-oriented regimens in AYA improve the outcome of these patients. Indeed, several comparative retrospective studies have shown a clear advantage in treating AYA with these approaches, leading to a 5-6-year event-free survival (EFS) that overcomes 60%[50-54], as opposed to 30-50% with adult-based protocols (with few exceptions[55]). These differences are likely to be due to the use of higher doses of drugs and introduction of asparaginase. Currently, several prospective clinical trials worldwide are using pediatric-inspired[56-58] or unmodified pediatric protocols[59] for AYA with extremely variable age limits. While these trials confirm the overall efficacy of this strategy, it must be noted that in older AYA patients, the percentage of chemotherapy-related deaths in CR may be high, suggesting that these regimens should be possibly administered up to the age of 40.

Minimal residual disease

Monitoring of MRD has become routine clinical practice in the frontline treatment of virtually all childhood ALL and in many adult ALL patients. MRD has proven to be the strongest prognostic factor, allowing for risk group assignment into different treatment arms and has led to significant treatment

reduction or intensification[60]. The MRD techniques need to be sensitive ($\leq 10^{-4}$), broadly applicable, accurate, reliable, fast and affordable. The most common techniques are represented by flow cytometry, polymerase chain reaction (PCR) analysis of rearranged immunoglobulin (Ig) and TCR (allele-specific oligonucleotide [ASO]-PCR) genes and RQ-RT-PCR methods for fusion genes, if present. All methods have some disadvantages: flow cytometry is less sensitive than ASO-PCR and RQ-RT-PCR, mostly when 4- and 6-colors are used; ASO-PCR represents the most reliable approach, but it is time-consuming since it is based on the identification of at least one patient-specific target, not feasible in a proportion of cases (up to 10%) and requires a strong expertise; finally, RQRT-PCR is highly sensitive (10^{-4}-10^{-6}) and relatively easy to perform; however, full standardization of all steps and international QA systems are not yet available. Intensive research is ongoing[61] to validate novel tools to improve MRD monitoring, such as NGS and digital droplet PCR (ddPCR)[62,63]. While the time points for MRD evaluation can slightly differ between childhood and adult ALL, MRD monitoring during the course of treatment is an important prognostic factor and can drive therapeutic intensification. The time points for MRD evaluation adopted in pediatric and adult protocols differ slightly[64]. Nonetheless, MRD is performed during induction (also at early phases in children, i.e. day +15[65]) and at the end of induction for treatment intensification. MRD evaluation during consolidation and follow-up is also crucial, because MRD persistence or reappearance is associated with hematologic relapse and, again, can drive therapeutic decisions. Nowadays, the primary endpoint of treatment should be a state of MRD negativity.

Monoclonal antibodies

Monoclonal antibodies directed towards B cells are worthy of specific attention, because of their impact in the management of ALL patients.

In the front-line setting, rituximab (directed against CD20) has been associated to the hyper-CVAD scheme: this led to a significant (p=0.003) improvement in the 3-year OS rate (75%) as opposed to 47% in the non-rituximab arm and was coupled to a higher percentage of MRD negativity (81% vs 58%, p=0.02) in adolescents and young adults, but not in the elderly population[66]. Along the same line, the GRAALL group has shown -in a randomized trial- that the administration of rituxumab improved the 2-year EFS (65% vs 52%, p=0.038) and OS (74% vs 63%, p=0.018), and increased the rate of patients undergoing transplant procedures[67].

In the relapsed/refractory (R/R) setting, extremely promising results have been reported with blinatumomab, a bispecific antibody that targets CD19 and CD3[68-72], and inotuzumab ozogamicin, that targets CD22. These compounds can induce second CR and represent a potential bridge to allo-SCT. Blinatumomab was first assessed in patients with MRD+ ALL[68]. Adult patients with molecularly refractory disease or with a molecular relapse were eligible. Twenty-one patients were enrolled: MRD negativity was achieved in 16 (80%) cases and at the latest updated follow-up 50.8% of patients are in continuous complete remission at 5 years[69]. Notably, no differences were observed in survival among transplanted vs non-transplanted patients. In the "true" R/R setting[70,72], the results of the phase III multicenter international TOWER study have been reported: 376 patients were enrolled (267 in the blinatumomab arm and 109 in the standard of care -SOC- arm). With a median follow-up of 11 months, the percentage of CR was significantly higher in the blinatumomab arm compared to SOC (43% vs 25%), translating into an advantage also in OS (7.7 vs 4 months, p=0.012), even after censoring for allo-SCT[72]. As mentioned, blinatumomab has been also utilized in R/R Ph+ ALL patients (ALCANTARA study[26]): of the 45 heavily pre-treated patients, 36% achieved a hematologic CR and 88% of them achieved a status of MRD negativity; 55% underwent allo-SCT. The toxicity profile of blinatumomab consists of fever chills and hypogammaglobulinemia, related to a cytokine release syndrome (CRS), that occur few hours after starting infusion. Tremor, headache, other mental status changes (e.g. confusion) and, rarely, seizures have been reported, but are transient.

Inotuzumab ozogamicin, directed towards CD22 and linked to calicheamicin, has been used in single institution experiences[73,74]. Recently, the results of the phase III multicenter international trial (INO-VATE ALL study) on 218 patients have been published[75]. Overall, this study showed that CR achievement was significantly (p<0.001) higher in the inotuzumab arm compared to SOC (80 *vs* 29%), also in terms of MRD negativity, though evaluated

by flow cytometry (78% *vs* 28%, p<0.001). Inotuzumab proved superior in all subgroups, with the exception of patients with MLL rearrangements. CR duration was longer in patients receiving inotuzumab than SOC (4.6 *vs* 3.1 months, p=0.03); again, a higher number of patients underwent a transplant (41% *vs* 11%). Nevertheless, the OS benefit was less evident (7.7 *vs* 6.7 months, p=0.04) and it is likely to be sustained not only by the monoclonal antibody, but also by the possibility of perfoming a transplant. As for toxicity, the major concern derives by the development of veno-occlusive disorders (VOD), often occurring after transplant.

CAR-T

A potentially exciting development is represented by the clinical use of CAR-T cells, autologous-engineered T cells with an antigenic receptor[76]: they can be of first, second, third and forth generation, depending on both the type of manipulation and the number of costimolary molecules. Their role is being investigated mostly in the R/R setting, where high rates of CR, coupled with MRD negativity have been reported in both pediatric and adult cohorts. Currently, multicenter international trials are ongoing, and follow-up is getting longer, to draw some definitve conclusion on their role and impact. Concerns with regard to toxicity, mainly CRS and neurologic side effects, have been reported with a variable degree of severity by all studies.

Concluding remarks

Management of adult ALL has changed considerably in recent years and advancements in outcome are a reality. These derive mainly from a more refined recognition of specific subgroups, for which treatment is different/tailored, from the incorporation of MRD monitoring for a further personalization of treatment and from the use of targeted approaches, when feasible. The inclusion of monoclonal antibodies in front-line strategies and the progressive broadening of targeted treatment strategies are likely to further improve the results obtained so far and lead to scenarios more alike to those observed in pediatric patients.

Declaración de conflictos de interés:
Los autores declaran que no poseen conflictos de interés.

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