Remaining challenges in pediatric and adolescent acute lymphoblastic leukemia

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A B S T R A C T

Acute Lymphoblastic Leukemia (ALL) in children is one of the most curable cancers, associated to an overall survival of 90% in the most privileged countries. Nevertheless this success has been obtained with an overall increasing intensity of highly toxic treatments, essentially chemotherapy in first line and hematopoietic stem cell transplantation in second line. Relapse is still very difficult to predict and to treat successfully. A complex biology integrating massive data is emerging which leads to consider ALL as a phenotype resulting from different somatic mutations occurring on a background of inherited genetic variants. The complexity is even amplified by the emerging knowledge of subclones, selected during the course of the disease by the treatment. Finally genetic variation among individuals contributes to inter-individual differences in efficacy and toxicity of drugs. Some of the current challenges linked to age, constitutional background, pharmacogenomics, disease biology, inadequate early response in a context of emergence of targeted therapies are reviewed hereunder

Learning goals

- To identify remaining challenges in ALL
- To clearly define the difficult to treat subgroups
- To consider innovative biology and therapeutics for the next future.

Introduction

Acute Lymphoblastic Leukemia (ALL) in children is one of the success stories of oncology. Indeed in five decades, a deadly disease has been transformed in one of the most curable cancers associated to an overall survival of 90% in the current protocols applied in the most privileged countries. The major contributors to this result are the biology-driven risk-adapted therapies, the central nervous system directed therapy and the improvement of the supportive care administered by dedicated teams using clinical research-grade protocols, including essentially drugs brought to the market before the mid-seventies. The role of international collaboration is also to be underlined.

Nevertheless 1) this success has been obtained with an overall increasing intensity of chemotherapies and with the use of hematopoietic stem cell transplantation (HSCT) for few patients in first line but for a majority in second line. All this being associated to serious morbidities and mortality 2) despite increasingly refined upfront and secondary (based on early response to treatment) stratifications, the major contributors to relapse in terms of absolute numbers are still the so-called standard and medium risk-groups[1] 3) 80% of the children with ALL live outside the privileged countries, without access to these sophisticated diagnostics and treatments.

Moreover current biology leads us to a profound change of paradigm. ALL can now be seen as a phenotype corresponding to a constellation of different somatic mutations sometimes occurring on a background of inherited genetic variant that influences the risk of leukemia development. The complexity is even amplified by the Darwinian behavior of subclones, selected during the course of the disease by the treatment itself[2].

Finally the increasing knowledge of inherited genetic differences in drug metabolic pathways will impact on the future therapeutic strategies. A simple summary of the challenges could then be: how to know better any leukemia, how to cure more patients at the first attempt and how to cure them better?

Three non-exclusive ways of progresses can be envisaged, raising a lot of issues in particular related to small numbers (any subgroup of ALL is rare disease), costs, access to new technologies and new drugs.

- An implementation of modern biology at diagnosis to better know the patient (host pharmacogenomics, underlying susceptibility to leukemia), his/her leukemia, and to better monitor the response
- A better way to use current drugs by 1) decreasing the use of drugs associated to short term or long term side effects: anthracyclines, dexamethasone in older children (replaced or modified scheme); 2) ameliorating current drugs e.g. pegylation of asparaginase; 3) using up to date biology and measurement of response decrease treatment intensity in very good prognostic subgroups and increase intensity in the bad

prognostic ones; 4) implementing host pharmacogenomics as a useful tool towards individualized therapy
- to introduce new targeted therapies (small molecules, antibodies, engineered cells) first in combination to current treatments, but may be in the long run with the aim of replacing chemotherapy.

Description of selected topics will help us to define some of these challenges and to open some of the newest possibilities.

Challenges linked to age

Infants

Infant ALL is a rare disease (~2% of all ALLs) characterized by an adverse prognosis and the high incidence of the MLL gene rearrangement (MLL-R). The Interfant-99 study has gathered 17 cooperative groups from 22 countries. 482 evaluable infants, aged 0 to 12 months have been included between 1999 and 2005 to investigate the efficacy of a treatment regimen using less anthracyclines and more cytarabine[3]. The study achieved a 4-year event-free survival of 47.0% and survival of 55.3%, outcomes better than those achieved with most previous protocols, but showed no benefit from delayed intensification of therapy with high-dose cytarabine and methotrexate. The current study, Interfant-06 will determine if early intensification with two blocks of AML induction therapy improve outcome, and the role of hematopoietic stem cell transplantation in infants at high-risk of relapse (age <6 months, *MLL* rearrangement and initial leukocyte count ≥300×10^9/L).

The mutational landscape of MLL ALL has been very recently described by combining whole-genome, exome, RNA and targeted DNA sequencing on 65 infants (47 MLL-R and 18 non-MLL-R cases) and 20 older children (MLL-R cases) with leukemia.[4] The data show that infant MLL-R ALL has one of the lowest frequencies of somatic mutations of any sequenced cancer, with the predominant leukemic clone carrying a mean of 1.3 non-silent mutations. In contrast to infant cases, MLL-R leukemia in older children have more somatic mutations (mean of 6.5 mutations/case versus 1.3 mutations/case, P=7.15×10^{-5}) and frequent mutations (45%) in epigenetic regulators, a category of genes rarely mutated in infant MLL-R ALL.[4]

New drugs are urgently awaited in this group of children. One way currently explored is related to the role of DOT1L in MLL leukemias. DOT1L is a methyltransferase responsible for catalyzing the methylation of histone H3 on lysine 79 (H3K79). The ectopic activity of DOT1L, associated with the chromosomal translocation involving MLL, is a required driver of leukemogenesis. DOT1L inhibitors such as EPZ-5676[5] are currently being tested in humans. Stumpel et al demonstrated that treatment with HDAC inhibitors induced cytotoxicity in t(4;11) MLL-r primary infant ALL cells and HDAC inhibitor treatment was shown to neutralize the MLL-AF4 fusion protein[6]. Another possible way to attack pro-B ALL cells of infants as other B lineage ALLs by targeting surface antigens e.g. CD19, with monospecific (unconjugated or conjugated) or bispecific T-cell engaging (BiTE) antibodies (e.g. blinatumomab)[7].

Adolescents and young adults

The underlying biological and clinical features associated to adolescent ALL is strikingly different from childhood ALL. Schematically adolescence begins at around 10 years in ALL (no difference between the 10 to 15 and 15 to 20 year-old) and is characterized by an increase of high risk factors and a decrease of good prognostic factors (Figure 1). A recent report has shown an increase of the prevalence of Ph-like ALL in AYA compared to children: from 10% among children with standard-risk ALL and 13% among those with high-risk ALL to 21% among adolescents with ALL and 27% among young adults with ALL (P<0.001 for the comparisons of children with adolescents and children with young adults)[8]. Kinase-activating alterations were identified in 91% of patients with Ph-like ALL. This finding is of major importance since it opens the possibility to introduce tyrosine-kinase inhibitors and JAK-STAT pathway inhibitors in the frame of the treatment (see the Ph-like ALL paragraph under). The prognosis of adolescents and young adults (AYA) is significantly better as soon as their treatment is undertaken according to pediatric protocols or pediatric-inspired ones[9-11]. Compliance problems in AYA with ALL interfere with the probability of success, considering the importance and length of classical maintenance/continuation treatment in ALL, some authors considering that non-compliance to maintenance could explain up to 59% of the relapses[12].

Obviously AYA are also more susceptible to toxicity of current procedures as they experience more asparaginase-related complications (pancreatitis, thrombo-embolism), more corticosteroid-related complications (diabetes, osteonecrosis), more infections, more toxic deaths, and more complications after HSCT.[13,14]

Thus, here also more targeted and less toxic approaches are eagerly awaited.

Challenges linked to underlying condition

Down syndrome

Children with DS represent 2% of the population of children with ALL. DS children with ALL have a worse prognosis due to a diminished tolerance to chemotherapy (espe-

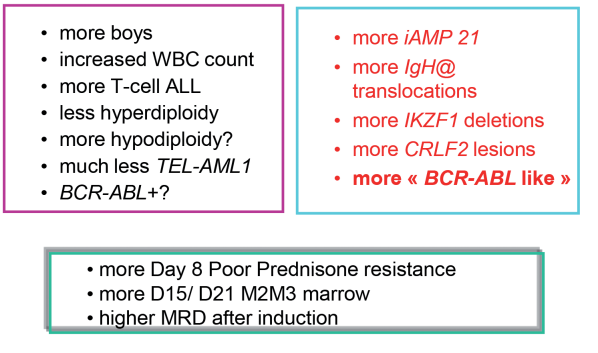

Figure 1. Specific features of ALL in children/adolescents above ten years of age. In the blue square, the more recent findings in adolescent ALL biology.

cially methotrexate) and an increased rate of severe infections. An international retrospective study evaluated the outcome of 653 patients treated from 1995 to 2004[15]. DS-ALL patients had a higher 8-year cumulative incidence of relapse (26% ± 2% versus 15% ± 1%, P<0.001) and 2-year treatment-related mortality (TRM) (7%±1% versus 2.0%± <1%, P<0.0001) than non-DS patients, resulting in lower 8-year event-free survival (EFS) (64%±2% vs 81%±2%, P<0.0001) and overall survival (74%±2% versus 89%± 1%, P<0.0001). Independent favorable prognostic factors include age <6 years, white blood cell count <10 G/L, and ETV6-RUNX1 for EFS. Treatment Related Mortality (TRM) was the major cause of death in DS children with ETV6-RUNX1 and high hyperdiploidy DS-ALLs. Thus, while relapse is the main contributor to poorer survival in DS-ALL, infection-associated TRM was increased in all protocol elements, unrelated to treatment phase or regimen. The very low rates of relapse among Down syndrome patients with *ETV6-RUNX1* fusion or high hyperdiploidy with trisomies 4 and 10 suggest that treatment reduction may be warranted for these subgroups of patients[15]. Since up to 60% of Down syndrome ALL cases are characterized by CRLF2 expression and JAK-STAT activation, they may potentially benefit from future therapy targeting kinase pathways.

Other constitutional predispositions

Noonan syndrome

Noonan syndrome (NS) is a congenital genetic disorder characterized by certain facial features, short stature, and congenital heart disease. The disorder is caused by genetic alterations in the RAS/MAPK signal pathway. NS patients show a predisposition to malignancy; the risk of ALL is increased despite rare descriptions (12 published cases in a 2009 review of the literature)[16]. Of note, somatic PTPN11 mutations may be found in 10% of children with ALL without NS[17]. No clear data on toxicity and efficacy of ALL treatment in NS patients are available. A theoretical possibility in the future could be to use MEK inhibitors since RAS belongs to the Ras-Raf-MEK-ERK pathway.

Ataxia-telangiectasia

AT is a rare autosomal recessive inherited disorder associated with progressive cerebellar ataxia, oculocutaneous telangiectasia, variable degrees of immune deficiency, and cancer susceptibility. AT is caused by biallelic mutations in the ATM gene located on chromosome 11q22.3-23.1. Earlier studies pointed to a high incidence of lymphoid malignancies, most notably T-cell ALL (children) and T-cell prolymphocytic leukemia (T-PLL, adults) in patients with AT. It is known that chemotherapy in children with DNA instability syndromes is hampered by serious therapy-associated side effects, such as severe mucosal inflammation or life-threatening infectious complications. However, detailed information about the administered chemotherapy and its relation to outcome is rather sparse. Two retrospective studies including in total 16 patients with ALL argue in favor of treating those patients with ALL for a potential final outcome in the range of 50% survival.[18,19] One study recommends dose reduction without focus on specific drug(s). The major risks are toxic events and secondary malignancies. Of note, germ-line ATM gene alterations are associated with susceptibility to sporadic T-cell acute lymphoblastic leukemia in children[20]. Some of the possible mutations ATM in T-ALL patients have been associated with a higher white blood cell count at diagnosis and an increased relapse-risk compared to patients with wild-type ATM[21].

Challenges linked to pharmacogenomics

Genetic variation among individuals contributes to inter-individual differences in efficacy and toxicity of drugs. The attempts to understand these variations began with candidate gene studies and have now moved to analysis of variants across the entire genome. Numerous studies have been published related to the increase risk of certain toxicities such as vincristine-induced peripheral neuropathy[22], avascular necrosis, allergy to asparaginase, methotretaxate clearance (see[23] for review). But only genetic testing of ThioPurine MethylTransferase (TPMT) is currently used routinely, since mercaptopurine is the only antileukemic agent for which the evidence is sufficient to warrant clinical use of germline genetic variation at this time. One in 300 individuals lack enzyme activity and 11% are heterozygous for a variant low activity allele and have an intermediate activity. Mercaptopurine and thioguanine, substrates for TPMT, exhibit well documented myelosuppressive effects on haematopoietic cells. The development of severe bone marrow toxicity, in patients taking standard doses of thiopurine drugs, is associated with TPMT deficiency while TPMT heterozygotes are at an increased risk of developing myelosuppression, but are also prone to better early response as measured by MRD after mercaptopurine including consolidation.[24,25]

Other genetic variants will be undoubtedly identified but their clinical utility remains questionable. Indeed current major ALL drugs are not easily dispensable even in the presence of a high risk allele which would increase the relative risk by a factor 2 or 3 but still with a low incidence. Nevertheless the example of the recently described high risk alleles for cardiac toxicity may change the view. Several studies have identified variants associated to an increased risk of late cardiac toxicity of anthracyclines: *SLC28A3*, *ABC*, *NADPH*, *CBR*, *SULT2B1*, *GST*, *UGT1A6* and *CAT*[26,27]. Replication of these associations in independent patient cohorts has been shown for variants in *SLC28A3* and *UGT1A6*[26]. In the high-risk group of the study implicating *SLC28A3*, on the basis of the prediction model combining genetic and clinical factors, 36% of patients developed cardiotoxicity within 1 year of treatment and 57% within 5 years, and this number continued to rise[26]. This impact, if confirmed, should clearly point towards a reduction of cumulative dose of anthracyclines for the patients at risk or the use of a cardioprotectant.

Challenges linked to the biology of leukemia

Hypodiploid ALL

ALL with hypodiploidy <44 chromosomes has a bad prognosis but comprises less than 2% of childhood B-

Lineage ALL. An international retrospective study of 130 hypodiploid patients who were included in clinical trials between 1986 and 1996, has shown a poor outcome with a 8-year event-free survival (EFS) of 38.3%±4.4%[28]. No induction failure was observed. The outcomes of the 46 patients with near-haploidy (24 to 29 chromosomes), the 26 with low-hypodiploidy (33 to 39 chromosomes), and the 13 with high-hypodiploidy (40 to 43 chromosomes) were comparable. Patients with less than 44 chromosomes fared significantly worse than the 54 patients with 44 chromosomes, among whom those with either monosomy 7, a dicentric chromosome or both had a significantly worse EFS[28]. The efficacy of HSCT could not be evaluated since only 9 patients were transplanted. Mehta et al recently described outcomes for 78 children with hypodiploid ALL who underwent HSCT between 1990 and 2010[29]. Thirty nine (50%) patients had ≤43 chromosomes, 12 (15%) had 44 chromosomes and 27 (35%) had 45 chromosomes. Forty three (55%) patients were transplanted in first remission (CR1) while 35 (45%) were transplanted in ≥CR2. All patients received a myeloablative conditioning regimen. The 5-year probabilities of leukemia-free survival, overall survival, relapse, and treatment related mortality (TRM) for the entire cohort were 51%, 56%, 27% and 22% respectively. Multivariate analysis confirmed that mortality was higher for patients transplanted in CR2 (HR 2.16, P=0.05), with chromosome number ≤43 (HR 2.15, P=0.05) and for those transplanted in the first decade of the study period (HR 2.60, P=0.01). Similarly, treatment failure risks were higher with chromosome number ≤43 (HR 2.28, p=0.04) and the earlier transplant period (HR 2.51, P=0.01).[29]

Heterogeneity in biology, early response to treatment (MRD) and outcome of hypodiploid cases is highly probable. In this line recent genomic profiling of 124 hypodiploid ALL cases identified two subtypes that differ in the severity of aneuploidy, transcriptional profiles and submicroscopic genetic alterations[30]. Near-haploid ALL with 24–31 chromosomes harbor alterations targeting receptor tyrosine kinase signaling and Ras signaling (71%) and the lymphoid transcription factor gene IKZF3 (13%). Low-hypodiploid ALL cases with 32–39 chromosomes are characterized by alterations in *TP53* (91.2%), *IKZF2* (53%) and *RB1* (41%). Both near-haploid and low-hypodiploid leukemic cells show activation of Ras-signaling and PI3-kinase-signaling pathways and are sensitive *in vitro* to PI3K inhibitors, indicating that these drugs should be explored as a new therapeutic strategy for this aggressive form of leukemia. One of the main findings of the study was the fact that the *TP53* alterations observed in low-hypodiploid ALL were also present in non-leukemic cells (40% of the cases), suggesting that these mutations are germline. Low-hypodiploid ALL could therefore represent a manifestation of Li-Fraumeni syndrome, at least in some cases[30]. Thus, a diagnosis of low hypodiploid ALL now implicates a TP53 testing in ALL and non ALL cells. If positive in the latter, a genetic family testing should be undertaken.

ALL with iAMP21

Intrachromosomal amplification of chromosome 21 (iAMP21) defines a distinct cytogenetic subgroup representing 2-3% of the childhood B-cell precursor acute lymphoblastic leukemia. Breakage-fusion-bridge cycles followed by chromothripsis and other complex structural rearrangements of chromosome 21 are involved in the mechanism giving rise to iAMP21. Patients with iAMP21 are older (median age: 9 years), with a low white cell count, and relapse at a high rate if treated as standard risk ALL. Recent studies have shown improved outcome on intensive therapy (see[31] for review). No molecular target has been yet identified.

Philadelphia chromosome-positive ALL

Philadelphia chromosome-positive ALL is a rare entity in children, accounting for ~3% of the cases. Before the tyrosine kinase inhibitors (TKI) era, a collaborative international retrospective study of 326 children and adolescents (diagnosed between 1985 and 1996) demonstrated a 5-year event-free survival (±SE) of only 28%±3%. Bad prognostic factors included an older age, a high initial leukocyte count, and a poor response to initial treatment with steroids and intrathecal methotrexate. Geno-identical transplantation improved outcome[32]. A successor study of 610 patients diagnosed between 1995 and 2005 still treated without TKI showed an improvement of EFS (32%±2% at 7 years). Both matched-related and matched-unrelated transplantation improved outcome as compared with chemotherapy alone[33]. The Children's Oncology Group (COG) then showed that continuous post-induction imatinib plus a very intensive chemotherapy improved disease-free survival to 70%±12% at 5 years[34]. In parallel the ESPhALL randomized study has shown a trend in favor of imatinib for good-risk Ph+ patients, most of the patients of the study being transplanted and the exposure to imatinib being less important than in the US study[35]. An international study run in the USA, UK, and Italy is currently testing another TKI, dasatinib, on top of an "ESPhALL type" chemotherapy backbone aiming to show a non-inferior or improved outcome despite reducing the indications of HSCT.

Childhood BCR-ABL1-positive B-cell precursor ALL shows a high frequency of IKAROS (IKZF1) deletions[36]. In a recent study, the prognostic value of IKZF1 deletions was evaluated in 2 cohorts of BCR-ABL1-positive BCP-ALL patients, before tyrosine kinase inhibitors (pre-TKI) and after introduction of imatinib in the EsPhALL protocol[37]. An IKZF1 deletion was detected in 126 out of 191 cases (66%). In the pre-TKI cohort, IKZF1-deleted patients had an unfavorable outcome compared with wild-type patients (4-year disease-free survival of 30.0±6.8% versus 57.5 ± 9.4%; p = .01). In the EsPhALL cohort, the IKZF1 deletions were associated with an unfavorable prognosis in patients stratified in the good-risk arm based on early clinical response (4-year DFS of 51.9 ± 8.8% for IKZF1-deleted vs 78.6 ± 13.9% for IKZF1 wild-type; P = .03), even when treated with imatinib (4-year DFS of 55.5 ± 9.5% for IKZF1-deleted vs 75.0 ± 21.7% for IKZF1 wild-type; P = .05)[37]. The unfavorable outcome for childhood BCR-ABL1-positive BCP-ALL with IKZF1 deletions, underscores the need for alternative therapies. In contrast, good-risk patients with IKZF1 wild-type responded remarkably well to imatinib-containing regimens, providing a rationale to potentially avoid hematopoietic stem-cell transplantation at least in this subset of patients.

Philadelphia chromosome-like (BCR-ABL-like) ALL

Two groups independently identified a novel subtype of

B-lineage ALL showing a gene expression profile similar to Philadelphia chromosome (Ph)-positive ALL with a high frequency of alterations of the *IKZF1* gene but lacking the BCR-ABL1 fusion[38,39]. These cases are characterized by resistance to asparaginase and daunorubicin in vitro, a high level of MRD at the end of induction and an overall poor outcome but not as dismal as the one of BCR-ABL ALL cases in the pre TKI era. These cases essentially belong to the so-called "B-other" group, meaning that no other recurrent abnormality is found (e.g. ETV6-RUNX1, high hyperdiploidy etc)[38,39]. The *BCR-ABL*-like expression profile and *IKZF1* deletions have independent poor prognostic impact in a Dutch-German collaborative study on 1128 children[40].

The genetic basis of Ph-like ALL is still a moving field. Recently more than 30 rearrangements targeting 13 cytokine receptors and tyrosine kinases have been described[8]. Several subgroups have been identified: 1) "ABL-class" rearrangements sensitive to TKI, involving *ABL1, ABL2, CSF1R* and *PDGFRB*; 2) *EPOR* and *JAK2* rearrangements sensitive to JAK inhibitors; 3) *CRLF2* rearrangements associated to *JAK* mutations in ~ 50% of the cases potentially sensitive to JAK inhibition; 4) other *JAK-STAT* activating mutations and deletions, including those involving *IL7R, FLT3, SH2B3* and others; 5) rare targets of rearrangement including *NTRK3* (potentially sensitive to crizotinib) and others[8]. Case reports with EBF1-PDGFRB rearrangements have been published favouring the addition of TKI to chemotherapy[41,42].

Many questions are currently raised. Is the prognostic value of this high risk feature abolished with the current strategies relying on sequential MRD monitoring[43]? Is the identification of these cases possible in real-time? How to introduce targeted agents beside classical TKIs as very few data are available concerning JAK inhibitors in children and none in combination? Could these combinations obviate the need for HSCT currently necessary in some of these cases identified after an induction failure or because of a very high MRD at the end of induction?

Prospective studies are currently underway to answer these questions.

Early T-cell precursor (ETP) ALL

ETP ALL is a recently described subgroup of T-ALLs, identified by a distinct gene expression profile and a peculiar immunophenotype, accounting for 10-15% of T-cell cases[44]. ETP cells derive from immature hematopoietic progenitor cells with maturation arrest at a very early stage. ETP-ALL blasts are CD8 and CD1a negative, CD5weak, and express one or more myeloid or stem cell marker[44]. While the initial report showed that ETP+ patients had a high risk of remission induction failure or hematological relapse, two recent studies, analyzing respectively 35 and 130 ETP+ patients, showed that, if treated with intensive chemotherapy, these patients have finally a good outcome (5-year of 76.7% and 87% respectively)[45,46]. A whole-genome sequencing study of 12 ETP ALL has shown that ETP ALLs are characterized by activating mutations in genes regulating cytokine receptor and RAS signalling (NRAS, KRAS, FLT3, IL7R, JAK3, JAK1, SH2B3, BRAF), inactivating lesions disrupting haematopoietic development (GATA3, ETV6, RUNX1, IKZF1, EP300) and histone-modifying genes (EZH2, EED, SUZ12, SETD2, EP300)[47]. The mutational spectrum and global transcriptional profile of ETP ALL are close to the ones observed in myeloid leukemias. Finally, recent experiments showed an hyperactivation of STAT5 in response to interleukin-7, an effect that was abrogated by the JAK1/2 inhibitor ruxolitinib. Ruxolitinib displayed activity in 6 of 6 patient-derived murine xenograft models of ETP-ALL[48]. These findings establish the preclinical efficacy of ruxolitinib in ETP-ALL and point towards possible clinical trials of ruxolitinib in this subgroup.

Challenges in relation to an inadequate early response

Induction failure

Failure to achieve a complete morphological remission after induction therapy is a rare event associated to an overall bad prognosis. A Ponte Di Legno group initiative collecting data on 44,017 patients, has allowed to study 1041 patients (2.4%) with IF[49]. Patients with IF frequently presented with high-risk features, including older age, high leukocyte count, a T-cell phenotype, the Philadelphia chromosome, and 11q23 rearrangement. With a median follow-up period of 8.3 years, the 10-year survival rate was only 32±1%. An age of 10 years or older, a T-cell leukemia, the presence of an 11q23 rearrangement, and 25% or more blasts in the bone marrow at the end of induction therapy were associated with a particularly poor outcome. High hyperdiploidy (modal chromosome number >50) and an age of 1 to 5 years were associated with a favorable outcome in patients with precursor B-cell leukemia. Allogeneic stem-cell transplantation from geno-identical donors was associated with improved outcomes in T-cell leukemia. Children younger than 6 years of age with precursor B-cell leukemia and no adverse genetic features had a 10-year survival rate of 72±5% when treated with chemotherapy only. The group of children with ALL presenting an induction failure is, thus, highly heterogeneous[49].

For B-Lineage ALL, current use of TKI during and not after induction therapy has nearly abolished induction failures in BCR-ABL positive ALLs. Moreover, case reports of so-called Ph like ALL exhibiting an IF in older children were linked to a 5q35 deletion generating an EBF1-PDGRB fusion. A nice and sustained response to the combination of chemotherapy and TKI was shown.[41,42]

For T-lineage ALL, two studies are to be mentioned. Firstly, a 116-member genomic classifier that could accurately distinguish IF cases has been proposed; network analyses suggest a prominent role for genes mediating cellular quiescence[50]. Secondly ETP ALLs (see above) have been associated to IF[46]. The recent COG report on a very large series of T-cell ALL associates indeed ETP+ ALLs to a higher risk of IF but no final inferior outcome[46].

Minimal Residual Disease

Irrespective of the method (flow cytometry, IG-TCR – based), the exact threshold defined in any protocol (10^{-3}, 10^{-4}) and the exact time point for bone marrow sampling (D15, end of induction, D78), high values of MRD and slowly decreasing MRD are associated to a worse prognosis and to the need of intensification of the treatment through a more intensive chemotherapy and/or HSCT.[1,51-53] This is

logical since these early MRD levels integrate all the leukemic-cell biological features (intrinsic drug sensitivity), host pharmacodynamics and pharmacogenomics, treatment adherence, and efficacy of the treatment regimen[51].

Nevertheless, as mentioned in the introduction, most of the relapses come from the so-called standard and medium risk-groups[1].

Consequently two ways of progress should be pursued: 1) identification of the real good responders with more sensitive techniques giving also access to clonal heterogeneity (Next Generation Sequencing);[54] 2) implementation of stratification tools independent of MRD such as IKZF1 deletions[38,40,55-57].

The challenge of the new targeted therapies

Current studies in pediatric and adolescent ALL focus on the inhibition of critical cell signaling pathways [BCR-ABL, FLT3, mTOR, JAK, RAS/MEK pathway, proteasome inhibition, inhibition of epigenetic regulators of gene expression [DNA methyltransferase (DNMT) inhibitors, histone deacetylase (HDAC) inhibitors, and disruptor of telomeric signaling-1 (DOT1L) inhibitors], monoclonal antibodies and immunoconjugated toxins, bispecific T-cell engaging (BiTE) antibodies, and chimeric antigen receptor-modified (CAR) T cells. Exciting data have indeed been published, particularly regarding the two last ones (see reference 58 for review).

All these exciting areas, belonging to the so-called precision medicine, nevertheless raise very difficult challenges including: how to identify the adequate subgroup? How to test? When to test (advanced disease compared to first line)? How to integrate these new agents in the current strategies giving excellent results in the majority of the children? Will these new agents have no long-term side effects? At what cost?

No simple answers exist to all these questions. A common denominator nevertheless is the absolute need for international collaboration in this aggregate of rare diseases called ALL!

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