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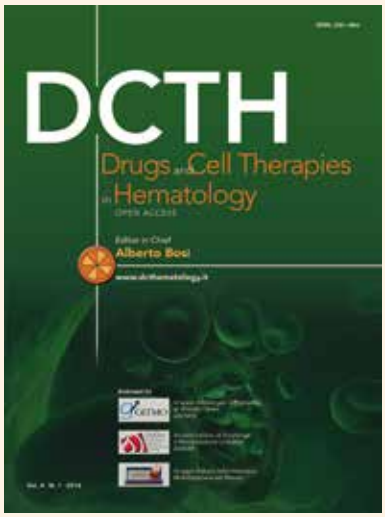

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REVIEW

Improving conditioning regimen prior to allogeneic hematopoietic stem cell transplantation in acute leukaemia

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SUMMARY

Allogeneic hematopoietic stem cell transplantation (HSCT) is an increasingly offered treatment option in the management of adult acute leukemia. The major causes of treatment failure remain disease relapse and treatment toxicity.
The advent of reduced-intensity conditioning regimens, coupled with expansion in alternative donor stem cell sources, has dramatically increased the number of patients with acute leukemia in whom allogeneic HSCT can be considered a potential treatment option. At the same time, a significant proportion of patients who proceed to transplantation are still destined to die of either procedure-related toxicity or disease relapse. In this context, optimization of both patient selection and conditioning regimen design is central to improving outcome.
This review presents an overview of important advances in the development of both novel conditioning regimens and post-transplantation strategies aimed at reducing the risk of disease relapse and improving the outcome.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only cure for several patients with high-risk hematological malignancies. The 2013 activity survey of the European Society of Blood and Marrow Transplantation (EBMT), describing the status of HSCT in Europe and affiliated countries, reported a record number of 39,209 HSCT in 34,809 patents (14,950 allogeneic, 43%); main indications were leukemias, 11,190 (32%; 96% allogeneic). Notable in this year's survey is the increase in the use of allogeneic HSCT and the increasing use of alternative donor transplants, where an impressive trend for more haploidentical HSCT has been observed (1).

The advent of reduced-intensity conditioning (RIC) regimens, coupled with expansion in alternative donor

Key words: conditioning regimen, allogeneic HSCT, acute leukemia, treosulfan.

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stem cell sources, has dramatically increased the number of patients with acute leukemia in whom allogeneic HSCT can be considered a potential treatment option. Whereas the introduction into clinical practice of less toxic chemotherapeutic agents, new antimicrobials, and more effective graft-versus-host disease (GvHD) therapies has significantly reduced the treatment-related mortality of allogeneic HSCT over the last decades, the mortality due to disease recurrence remained largely unchanged (2).
Allogeneic HSCT still represents an ideal platform to develop and validate innovative therapeutic approaches. However, approaches for reducing relapse, such as more intensive conditioning regimens, could also increase toxicities without improving overall outcomes (3). Given its attendant toxicity, the choice of which patients have the potential to benefit from an allograft now assumes even greater importance, and approaches for relapse reduction, including conditioning regimen design, need to be carefully studied in prospective clinical trials.

CONDITIONING PARADIGM SHIFT

The therapeutic effect of allogeneic HSCT is mediated by both the administration of high-dose chemo-radiotherapy and the induction of the graft-versus-leukemia (GVL) effect by immune competent cells in the graft (4). The condition regimen given prior to HSCT has two aims; suppression of host immunity to allow donor cell engraftment and ablation of the underlying malignancy. Bacigalupo et al. defined conditioning regimen intensity based on the expected duration and reversibility of cytopenia after HSCT. Myeloablative conditioning (MAC) results in irreversible cytopenia in most patients and stem cell support after HSCT is required. Truly non myeloablative (NMA) regimens cause minimal cytopenia and can theoretically be given without stem cell support. RIC regimens cause profound cytopenia and should be given with stem cells, but cytopenia may not be irreversible. Outcome after HSCT is determined mostly by the ability to eradicate leukemia and to survive transplant-related complications such as organ toxicities, infections, and the complications of acute and chronic GvHD (5). A perfect preparative regimen for patients with acute leukemia undergoing allogeneic HSCT would eliminate leukemia, provide adequate immunosuppression to guarantee engraftment, and have negligible toxicity. Lacking perfection, we are forced to select among a variety of imperfect choices, considering various tradeoffs between regimens and how these might be influenced by other factors, including GvHD and patient-specific variables (6, 7).
Historically the emphasis of the therapeutic effect of HSCT was on the conditioning regimen, with the thought that the more intensive the regimen, is more effective. Either busulfan (Bu) or total body irradiation (TBI) is a major component of most currently used preparative regimens. With either agent, as doses increases, relapse rates decrease (8). The combination of cyclophosphamide (Cy, total 120 mg/kg) and TBI (fractionated, 12 Gy) emerged as the standard TBI-based conditioning. Attempts to increase radiation dose further, beyond this range have failed to improve survival

in acute myeloid leukemia (AML) (9). The combination of high-dose Bu and Cy (BuCy) was developed in the 1980s as an alternative regimen for patients who were unable to receive TBI and as an attempt to reduce toxicity (10). Historically, Bu was only available as an oral preparation. Bu tablets are associated with erratic intestinal absorption and a large inter-patient variability. The intravenous formulation allows 100% bioavailability, within the standard therapeutic doses of 0.8-3.2 mg/kg (11). Conditioning dose-intensity is highly correlated with outcome after HSCT. In 2013, the EBMT reported results of a retrospective analysis and found that relapse rate increased from 23% with myeloablative conditioning (MA) to 39% in RIC transplantations (12). In the setting of ablative transplantations, although higher dose TBI is associated with reduced relapse rates, it is also associated with increased non-relapse mortality (NRM).

Similarly, in several retrospective studies, comparing MA to RIC regimens, NRM rates tended to be higher with higher dose regimens, especially during the first 100 days after transplantation (7, 12, 13). In general, higher dose regimens have been associated with increased transfusion requirements, increased incidence of idiopathic interstitial pneumonia and sinusoidal obstruction syndrome, and more bacterial infections (14-16). However, the incidence of viral and fungal disease does not appear to be influenced to a major degree by the intensity of the preparative regimen (14). Controversy exists as to the relative merits of BuCy and Cy/TBI in SCT for AML. Several randomized studies as well as large registry retrospective studies compared the two regimens, but reported conflicting results (17-19). Thus, BuCy and Cy/TBI are the standard MAC regimens in AML with comparable outcomes, especially in recent years when using intravenous Bu.

However, in recent years, the paradigm has shifted to optimization of the therapeutic impact of the GVL effect as opposed to just the cytotoxic effects of the conditioning regimen alone (20). A plethora of non myeloablative (NMA) and RIC conditioning regimens were introduced trying to reduce transplant-related toxicities and allow HSCT in elderly and medically infirm patients. In addition, reduced-toxicity myeloablative regimens (RTC) based on fludarabine and myeloablative alkylating-agent doses were designed to allow safer administration of dose-intensive therapy (6).

The Seattle group developed the prototype of NMA conditioning using a single fraction of 2 Gy TBI with or without fludarabine and post grafting immune suppression with calcineurin inhibitor and mycophenolate mofetil (MMF). This regimen relies almost completely on GVL as the dose intensity effect on leukemia cells is minimal. The 5 year survival was 33%. The 1 year and 5 year NRM was 16% and 26%, respectively. Patients with chronic GvHD had reduced relapse risk (21).

Earlier studies showed that outcomes after RIC are dependent on disease status, with patients in complete remission (CR) having better outcomes than patients with active disease (22). In patients transplanted with active disease, studies have shown survival rates of 0% due to the very high risk of relapse after fludarabine/busulfan conditioning (23). Because the therapeutic effect of

RIC transplant relies on the GVL effect as opposed to ablative pre-transplant chemotherapy, the risk of relapse is higher and it has been clearly shown that patients with active leukemia have a higher risk of relapse and worse overall survival (OS) after HSCT (24).
These studies suggest that optimizing disease status in the pre-transplant period is likely critical to optimizing outcomes in the post-transplant period. To achieve a reduction in relapse risk, investigators are now looking at ways to optimize dose intensity while safely minimizing NRM.
Investigators previously looked at the use of 3 days of Bu and found that the results were similar to those achieved with 4 days of the alkylating agent (25).
A prospective, phase 2, multicenter trial recently assessed the efficacy of a RIC/reduced toxicity conditioning regimen of fludarabine plus anti-thymocyte globulin (ATG) plus a higher dose of intravenous Bu (FB3) for a total dose of 390 mg/m^2 in patients with high-risk malignancies not eligible for a fully ablative MAC transplant.
At 2 years, OS and LFS rates were 62% and 50%, respectively, with a cumulative incidence of disease progression of 44% at 2 years and NRM of 11%.
This study showed that increasing the anti-tumor efficacy of the reduced-toxicity conditioning regimen with FB3 was effective while limiting toxicity (26).
This area of investigation will likely continue to be of interest in terms of optimizing transplant outcomes. Another important area of investigation to optimize transplant outcomes, especially in high-risk situations, has been the sequential use of intensive chemotherapy followed by a RIC allograft. Schmid et al. previously described a regimen of fludarabine, (4 x 30 mg/m^2), cytarabine (4x2 g/m^2), and amsacrine (4x100 mg/m^2), followed 4 days later by a RIC regimen of 4 Gy TBI, Cy (80-100 mg/m^2), and ATG. This regimen was initially developed in patients with refractory disease with promising results (27). It was, therefore, evaluated in 23 patients with high-risk AML in first CR, producing long-term remissions, thus warranting further investigation (28).

TREOSULFAN - BASED CONDITIONING: SAN RAFFAELE EXPERIENCE

Treosulfan (L-treitol-1,4-methanesulfonate) is a prodrug of a bifunctional alkylating cytotoxic agent and has been initially used as an antineoplastic agent for ovarian carcinoma. In recent years, it has been increasingly applied to pediatric and adult patients with hematological malignancies as, and has been shown to be associated with low risk of organ toxicity and treatment-related mortality combined with effective immunosuppressive and cytotoxic properties (29-31). In the Hematology and Bone Marrow Transplantation Unit of the San Raffaele Scientific Institute, we investigated the benefits of treosulfan-containing regimens in different settings.
In a clinical trial (TrRaMM protocol, Eudract 2007-5477-54), we investigated the feasibility of a sirolimus-based GvHD prophylaxis to allow the infusion of unmanipulated peripheral blood stem cells (PBSCs) grafts from haploidentical donors in patients affected by high-risk hematological malignancies (32). Patients received myeloablative conditioning based on Treosulfan

14 g/m^2 days -6 through -4 and Fludarabine 30 mg/m^2 days -6 through -2. GvHD prophylaxis was based on ATG fresenius (10 mg/kg) on days -4 through -2, sirolimus (orally, monitored twice a week to achieve a target plasma level of 8-15 ng/ml) from day -1 and MMF (15 mg/kg tid orally or i.v.) from day 0. Rituximab was administered in a single 500 mg dose on day -1, as secondary prophylaxis of EBV reactivation and as an additional agent to deplete B cells *in vivo* and potentially reduce the incidence of chronic GvHD. Donor PBSCs were mobilized with G-CSF and infused without any *ex vivo* manipulation. Patients achieved rapid immune reconstitutions toward T-regulatory cells. Despite all these efforts, acute and chronic GvHD still represent crucial complications of haploidentical HSCT. Cumulative incidence of grade II–IV acute GvHD was 35±9% at day 150 after HSCT. Overall cumulative incidence chronic GvHD was 47±11% at 2 years. Overall, in the TrRaMM trial, the infusion of the unmanipulated haploidentical PBSC graft translated in a rate of GvHD that paralleled the observations in mismatched unrelated donor (MUD) based on PBSC graft and a standard calcineurin inhibitor-based GvHD prophylaxis (33).

In the last years, haploidentical HSCT has gained considerable attention worldwide, due to the development of new promising tools to prevent GvHD, such as the post-transplant administration of high-dose cyclophosphamide (34, 35); based on these two innovations, the number of transplants performed using unmanipulated haploidentical grafts has grown.

In this context, we decided to investigate in a cohort of 40 high-risk hematological patients the feasibility of PBSC grafts within a PTCy and sirolimus-based GvHD prophylaxis (Sir-PTCy) (36). Myeloablative conditioning consisted of treosulfan (14 g/m^2/day) on days -6 to -4, fludarabine (30 mg/m^2/day) on days -6 to -2, and melphalan (70 mg/m^2/day) on days -2 and -1, followed by T-replete G-CSF-mobilized PBSCs. Post-grafting immunosuppression consisted of PT-Cy (50 mg/kg/day) on days 3 and 4, followed by MMF for 30 days, and sirolimus for 3 months. Donor engraftment occurred in all patients with full donor chimerism achieved by day 30. Post-HSCT recovery of lymphocyte subsets was broad and fast. Cumulative incidences of grade II-IV and III-IV acute GvHD were 15% and 7.5%, respectively, and were associated with a significant early increase in circulating regulatory T cells at day 15 post HSCT, with values <5% being predictive of subsequent GvHD occurrence. The 1-year cumulative incidence of chronic GvHD was 20%. NRM at 100 days and 1 year was 12% and 17%, respectively. With a median follow-up for living patients of 15 months, the estimated 1-year overall and disease-free survival (DFS) was 56% and 48%, respectively. Outcomes were more favorable in patients transplanted in CR (1-year DFS 71%) vs patients transplanted with active disease (DFS 34%; P=0.01). These results suggest that myeloablative haploidentical HSCT with PBSC and Sir-PTCy is a feasible treatment option in advanced patients.

Therefore, treosulfan-based conditioning resulted a safe and effective approach for adult patients with hematological malignancies, with promising results also in advanced diseases.

CONCLUSION

The most common cause of death in treated patients with AML is therapy-resistant disease. Approximately 50% of patients under the age of 60 and 90% of patients over the age of 60 with non-acute promyelocytic AML relapse from initial therapy (37).

Overall outcome is determined by the net effect of these opposing effects as may be predicted by patient age, comorbidities and disease status at transplantation. Retrospective comparative trials showed that while outcome may be similar with the various regimens in patients given HSCT in remission, NMA/RIC are inferior when HSCT is given in advanced disease, due to high relapse risk. Therefore, the status of disease at HSCT is an important factor in predicting outcome and selecting the conditioning regimen.

Options for decreasing the risks of relapse and progression are limited thus far (3). The use of more intense conditioning regimens would increase the risk of toxicity in elderly patients and those with comorbidities and, moreover, offer no benefit to the majority of current patients who did not experience relapse.

The challenge of reducing the risk of disease relapse after allograft remains a particularly stubborn problem, but emerging and varied strategies focusing on either augmentation of the antitumor activity of the conditioning regimen, without increasing its toxicity, or enhancement of a GVL effect hold promise. Pivotal to improving transplantation outcome will be the incorporation of advances in our understanding of the biology of disease relapse into transplantation schedules, coupled with the prospective assessment of novel treatment strategies in early phase clinical trials and subsequently appropriately powered randomized studies. Well-tolerated targeted drugs or antibodies that are not curative on their own could set the stage for curative GVL effects.

Major efforts should be directed toward methods that control progression of malignant disease early after HSCT and more effectively prevent clinically significant GvHD (38). The use of ATG has been recently shown to lower the incidence of chronic GvHD among patients in complete remission from acute leukemia, who received PBSC from an HLA-identical sibling (39).

The transplant approach has rapidly changed from a one regimen that fits all to multiple potential regimens and a patient tailored approach. Regimen dose intensity can vary from high-dose myeloablative (now almost completely abandoned due to excess NRM) to standard MAC (such as standard Cy/TBI and BuCy), to various RIC and NMA regimens (20). A search for modifications in the conditioning regimens to increase dose intensity as well as addition of novel therapies in the conditioning regimen represent areas of burgeoning interest to improve long term outcomes. Recently, patients 40-65 years with acute myeloid leukemia were randomly assigned to receive intravenous busulfan plus cyclophosphamide or busulfan plus fludarabine (40). Busulfan plus fludarabine was associated with lower transplant-related mortality, while maintaining a potent antileukaemic activity.

The author declares that there is no conflict of interest.

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REVIEW DCTH - 1·2016 - 14-21

FLT3 inhibitors in acute myeloid leukemia

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SUMMARY

Acute myeloid leukemia (AML) is the most frequent acute leukemia in adults, and has an unfavourable prognosis. Over the past decade, significant progress has been made in the understanding of the cytogenetic and molecular determinants of AML pathogenesis. One such advance is the identification of recurring mutations in the FMS-like tyrosine kinase 3 gene (FLT3); currently, this marker signifies a poorer prognosis, but also identifies an important target for therapy. FLT3 kinase inhibitors have now undergone clinical evaluations in phase I, II and III clinical trials; this review aims to highlight the role of FLT3 inhibitors as single agents and in combination with other anti-leukemia agents.

INTRODUCTION

While cure rates for acute myeloid leukemia (AML) have improved over the past decades, survival remains suboptimal; five years survival for patients under 60 years old is only 40% (1). In 20 % to 30% of AML patients, mutations in the FMS-like tyrosine kinase 3 (*FLT3*) occur, leading to internal tamed duplication in the juxtamembrane domain of the receptor (*FLT3/ITD*) (2). *FLT3/IDT* dictates a particularly poor clinical outcome (3); in particular, a high ratio of the mutant *FLT3/ITD* allele compared to the *FLT3* wild type (WT) allele (allelic burden) has been associated with worse survival and decreased complete remission (CR) in response to conventional chemotherapy in newly diagnosed AML patients (4). This ratio can change during the course of disease and patients with relapsed disease have a higher allelic burden (5). Lastly, the length of the ITD is variable and a longer ITD has been associated with a worse clinical prognosis (6). The prognostic implications of *FLT3/D835* point mutations found at diagnosis, comprising approximately 7% of cases, are not yet well established (7).

As a result of the poor response of patients with *FLT3-ITD* mutations to standard cytotoxic agents, many groups worldwide have investigated a targeted approach involving kinase inhibitors directed against *FLT3* mutated AMLs.

The first generation of FLT3 inhibitors (*Tabella 1*) (sorafenib, midostaurin, sunitinib, lestaurtinib) were TKIs developed for the treatment of solid tumours; these drugs were initially designed to inhibit other kinases but were incidentally found to be active against FLT3.

Key words: Acute myeloid Leukemia, FLT3 mutation, FLT3 inhibitors.

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TABLE 1 • FLT3 inhibitors.

First-generation	Sorafenib (BAY43-9006) Midostaurin (PKC412) Sunitinib (SU11248) Lestaurtinib (CEP-701)
Second-generation	Quizartinib (AC220) Crenolanib (CP-868596) PLX3397

By their very nature, therefore, these drugs inhibited multiple kinases along FLT3, but often carried significant toxicity. These agents generally failed to show robust and sustainable responses as single agents in phase I/II trials in various groups of relapsed or refractory AML. However, newer agents, such as quizartinib, crenolanib and PLX3397, have now been developed for the specific purpose of treating patients with *FLT3/ITD* AML.

FLT3 inhibitors have been tested in various trials for *FLT3/ITD* AML, but an incomplete FLT3 inhibition *in vivo* has mainly been reported (8). A profound, sustained inhibition of FLT3 activity is necessary to induce a cytotoxic effect (9), and most of the agents studied have many off-target effects at the doses necessary for complete FLT3 inhibition. Because of the relative lack of *in vivo* potency of these agents, clearance of bone marrow blasts is rarely achieved with use as a single agent, so they have been, and continue to be, studied in combination with conventional chemotherapy.

SORAFENIB

Sorafenib is a TKI with activity against several different kinases, including RAF kinase, VEGFR, c-Kit, and FLT3. Sorafenib is approved by the FDA for the treatment of hepatocellular cancer, renal cell carcinoma, and thyroid cancer. In a phase I study, Crump et al. (10) evaluated sorafenib for patients with relapsed myelodysplastic syndrome (MDS) or AML older than 65 years; a dose of 400 mg twice daily was not well tolerated in this study and, among 42 patients, only one Complete Remission (CR) was observed in a patient with *FLT3/ITD* mutated AML. In another phase I clinical trial, by Borthakur et al. (11), 50 AML patients were treated with two different dose schedules of sorafenib (once or twice daily for 5 days per week, weekly for 21 days for 1 cycle, or in the second schedule once or twice daily for 14 days every 21 days). CR or CRp were demonstrated in 5 patients (10%), all with FLT3/ITD; a significant reduction in bone marrow and/or peripheral blood blasts was seen in an additional 17 (34%) patients. The use of sorafenib in the post-transplant setting was further examined by Metzelder et al. (12); in this study, 29 patients (45%) had had a prior allogeneic stem cell transplant. They reported that patients who received sorafenib after relapse following an allogeneic stem cell transplant developed sorafenib resistance less commonly and significantly later in their disease course. Additionally, a durable remission occurred mostly in patients who had undergone a prior allogeneic stem cell transplant. The authors concluded that sorafenib results in durable remission in some *FLT3-ITD* mutated patients who relapsed post-transplant, and that there may be a synergy between sorafenib and the immunological effects of an allogeneic stem cell transplant. Several studies in AML patients combining sorafenib with conventional chemotherapy or with hypomethylating agents have been published. In a phase I/II trial, sorafenib

400 mg twice daily was combined with idarubicin and cytarabine in patients under the age of 65 (13). Of 51 patients treated in a phase II study, 38 patients (75%) achieved CR, including 14 of 15 patients (93%) with mutated *FLT3*. The most common grade 3 or higher toxicities reported as possibly related to sorafenib during induction chemotherapy were elevated transaminases, bilirubin and diarrhea.

Ravandi et al. (14) reported their data from a study combining sorafenib with 5-azacytidine in 43 patients with relapsed AML; forty patients (90%) had *FLT3* mutations. The overall response rate was reported as 46% and the most commonly noted side effect was fatigue, in 47% of patients. The authors hypothesised that the combination of 5-azacytidine with sorafenib may be associated with less resistance due to promoting lower levels of FLT3 ligand than cytotoxic intensive chemotherapy. Recently, in a multicentre, phase II, randomized trial (15), 276 AML patients received up to two cycles of standard 3+7 induction chemotherapy followed by two consolidation cycles with intermediate dose cytarabine; patients were randomly assigned to receive either sorafenib or placebo. Sorafenib (400 mg twice daily) was administered between cycles and as maintenance for 12 months. With a median follow-up of 36 months, median EFS was 9 months in the placebo group versus 21 months in the sorafenib group, corresponding to a 3 year EFS of 22% in the placebo group versus 40% in the sorafenib group. Among 46 patients with *FLT3/ITD* mutations, whereas no clear pattern was apparent for EFS (median 5 months in the sorafenib group *vs* 6 months in the placebo group), a better median relapse-free survival (18 months vs 6 months) and overall survival (median not reached vs 19 months) was seen in the sorafenib group.

MIDOSTAURIN (PK412)

Midostaurin is a multi-targeted indolocarbazole with activity against VEGF, KIT, PDGFR, and FLT3. Of note, midostaurin is equally active against *ITD*-mutated and *TKD*-mutated *FLT3*. In a phase II study (16), 60 patients with *FLT3* wild-type AML and 35 patients with *FLT-3* mutated AML were randomly assigned to receive midostaurin at a dose of 50 mg or 100 mg twice daily. A reduction in peripheral blasts or bone marrow blasts by 50% or greater was obtained in 71% patients with mutated *FLT3*, compared to 42% of patients with wild-type *FLT3*. The drug was well tolerated and the authors proposed further studies with this active agent in combination with chemotherapy for *FLT3*-mutated AML.

A phase Ib study examined three different dose schedules of midostaurin combined with daunorubicin and cytarabine followed by high dose cytarabine maintenance in patients with newly diagnosed AML (17). A 50 mg twice daily dosing schedule was better tolerated than the 100 mg twice daily regimen. Of the 40 patients receiving 50 mg twice daily, 45% discontinued therapy. In this cohort, there was an 80% CR rate and this trial served as a pilot for a randomized phase 3 study of conventional chemotherapy and midostaurin for newly diagnosed adult *FLT3*-mutated AML. In the RATIFY study (18), 717 *FLT-3* AML patients (555 *FLT3/ITD* and 162 *FLT3/TKD*) received induction chemotherapy with daunorubicin

and cytarabine plus midostaurin or placebo followed by four consolidation cycles with high-dose cytarabine plus midostaurin or placebo and maintenance with midostaurin or placebo. The CR rate was 59% in the midostaurin group and 54% in the placebo group but the addition of midostaurin to standard chemotherapy significantly improved EFS and OS compared to the placebo group.

SUNITINIB

Sunitinib is an oral multi-target kinase inhibitor with activity against FLT3, PDGFR, and c-Kit. Two phase I studies have examined the use of sunitinib in *FLT3/ITD* AML. The first of these studies enrolled 29 patients with relapsed/refractory AML or considered unfit for standard chemotherapy (19); five patients have *FLT3* mutations. A significant FLT3 inhibition in vivo was seen in 50% of patients receiving 200 mg and higher doses of sunitinib. Another phase I study (20) examined 15 refractory AML patients receiving sunitinib for 4 week cycles followed by either a 2 or 1 week rest period. All four patients with *FLT3* mutations had morphologic or partial responses compared with 2 of 10 evaluable patients with wild-type *FLT3*. Responses, although longer in patients with mutated *FLT3*, were of short duration. The authors concluded that monotherapy with sunitinib induced partial, short duration remissions in AML. Recently, a phase I/II trial (21) evaluated the role of sunitinib as maintenance therapy after intensive induction and consolidation chemotherapy in 22 AML elderly patients with activating *FLT3* mutations. Thirteen of 22 patients (59%) (8/14 with *FLT3*-internal tandem duplication and 5/8 with *FLT3*-tyrosine kinase domain) achieved CR/CR with incomplete blood count recovery. Of note, four of five analysed patients with relapse during maintenance therapy lost their initial *FLT3* mutation, suggesting outgrowth of *FLT3* wild-type subclones.

LESTAURTINIB

Lestaurtinib was derived from indolocarbazole and is structurally very similar to midostaurin. Early phase I/II trial results demonstrated transient reductions in peripheral and bone marrow blasts and, furthermore, that these clinical outcomes were strongly correlated with a robust suppression of FLT3 phosphorylation *in vivo* (22, 23). These results prompted a large, multicentre phase III clinical trial evaluating lestaurtinib in combination with chemotherapy in relapsed/refractory patients (24). In this study, 224 patients with first relapsed AML harbouring *FLT3* mutations were randomly assigned to chemotherapy (MEC or high-dose AraC) alone, or a combination of chemotherapy and lestaurtinib. The CR/CRp rate in the combination group (16%) was not statistically better than that in the chemotherapy group (21%).

QUIZARTINIB (AC220)

Quizartinib demonstrated enhanced potency and selectivity for FLT3 *in vitro*. The IC_{50} of quizartinib for FLT3 inhibition in plasma is 18 nM in contrast to the various first-generation FLT3 inhibitors, all of which are associated with IC_{50} measurements of greater than 400 nM in patients plasma (25,26). A phase

I study with 76 patients suffering from refractory/relapsed AML revealed an acceptable toxicity profile and enhanced clinical activity in *FLT3* patients (26); side effects included gastrointestinal upset, reversible QT prolongation, and myelosuppression. Partial or complete responses were noted in 30% of study participants: 53% of *FLT3-ITD* patients responded versus 14% of *FLT3*-wild type patients. These results were followed by a phase II study to assess quizartinib efficacy in two cohorts of patients. The first cohort consisted of 133 patients over 60 years of age with relapsed or refractory AML (25); quizartinib was noted to be particularly effective in patients harbouring *FLT3/ITD* mutations, who demonstrated a 54% composite complete remission rate compared with 31% in the *FLT3*-wild type group. These results constitute the highest degree of single-agent efficacy for elderly patients with refractory/relapsed AML. Moreover, in a number of patients (8%) it constituted a successful bridge to hematopoietic stem cell transplantation. The second cohort (27) consisted of 137 younger patients (median age 55 years) who had relapsed or were refractory to second-line treatment or hematopoietic stem cell transplantation. *FLT3-ITD* patients again demonstrated a higher composite remission rate: 44% versus 34% in the *FLT3*-wild type subset. Response to quizartinib consisted of clearance of peripheral blood blasts and reduction of bone marrow blasts; because most patients remained platelet and red cell transfusion-dependent, the responses were not considered classic working group remission but, rather, complete remission with incomplete count recovery. The reduction in bone marrow blasts count induced by this drug without systemic toxicity allowed 47/137 (35%) cohort 2 patients to undergo allogeneic transplantation, resulting in a significant number of long-term survivors from this very poor-risk group. Another significant finding that emerged from this trial was that patients achieving a response to quizartinib often developed resistance-conferring point mutations, most commonly at D835 and less frequently at a "gatekeeper" residue, phenylalanine 691 (F691) (28). Although these results provided further biologic evidence that *FLT3/ITD* mutations were true drivers of this disease, it was obviously very problematic clinically and has spurred development of new FLT3 inhibitors with activity against these new mutants.

CRENOLANIB

Crenolanib is a potent inhibitor of FLT3. Originally designed to inhibit PDGFR, crenolanib was found to inhibit FLT3 in both mutant and wild-type cell at similar plasma concentrations to quizartinib (29). Furthermore, crenolanib demonstrated no QTc prolongation in patients and less inhibition of cKIT *in vitro,* which will perhaps correlate with a reduced myelosuppression in vivo. Crenolanib has demonstrated efficacy against tumor cell lines and primary blasts that have developed D835 activating mutations and resistance to quizartinib (29,30).

PLX3397

PLX3397 is a novel, potent, specific inhibitor of *FLT3-ITD* mutant AML. In leukemic cell lines, the agent was shown to

selectively inhibit *FLT3-ITD* variants (31). PLX3397 was also effective against primary AML samples harbouring *FLT3-ITD* mutations in cell culture, and inhibited cell growth in a dose-dependent fashion, while no significant effects were noted in *FLT3* wild-type samples at equivalent doses (31).

CONCLUSIONS

The research into FLT3 inhibitors remains promising, as it marks an important aspect of drug development for patients with AML. Historically, patients with *FLT3-ITD* mutations have done extremely poorly with standard therapy, and the current recommendation is the use of chemotherapy aimed at achieving complete remission, followed by an allogeneic stem cell transplantation in first remission. However, as many patients are unable to undergo stem cell transplantation due to more advanced age and the presence of comorbidity, incorporation of targeted therapy with FLT3 inhibitors is a promising strategy. Acquired resistance to FLT3 inhibitors continues to present a significant challenge; understanding the molecular mechanism driving resistance and overcoming these obstacles to target inhibition will be central to the success of these agents.

Non-specific, first-generation FLT3 inhibitors demonstrated therapy-related toxicities, and the acquisition of resistance. Recently, a new generation of FLT3 inhibitors has demonstrated safety, increased potency, and a high degree of specificity.

Strategies for the optimum utilization of FLT3 inhibitors include use with conventional chemotherapy in induction regimens, as maintenance therapy, in relapsed/refractory patients as a bridge to transplantation, and after stem cell transplantation.

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REVIEW DCTH - 1·2016 - 22-30

Tyrosine kinase inhibitors in adult Philadelphia chromosome positive acute lymphoblastic leukemia: before, after, or instead of allogeneic stem cell transplantation

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SUMMARY

With the advent of Tyrosine Kinase Inhibitors (TKIs) targeting BCR-ABL1, the outcomes of Ph+ ALL improved substantially.

Although allogeneic stem cell transplantation (alloHSCT) in first complete remission remains the consolidation therapy of choice in Ph+ ALL, the remarkable improvement of the quality of the hematologic remission achieved by the combined treatment with chemotherapy and TKIs is now challenging the absolute indication to perform an allogeneic transplant in these patients. Indeed, recent studies have demonstrated the possibility of long-term survival without a transplant in patients achieving a deep molecular remission.

In this regard, monitoring minimal residual disease (MRD) with the aim to identify patients with a more favorable outcome who possibly may be cured without alloHSCT is very important. Moreover, patients failing the achievement of a deep molecular remission should be considered for innovative targeted therapies, such as second and third-generation TKIs or immunotherapy, able to achieve a sustained MRD negative status. Post-transplant TKIs administration is another crucial point under investigation, since leukemia relapse remains a major cause of treatment failure after alloHSCT.

Key words: acute Lymphoblastic Leukemia, Allogeneic transplantation, Minimal Residual Disease (MRD).

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INTRODUCTION

For decades, Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) marked the most unfavorable subgroup of adult ALL with an overall survival observed in unselected series of patients less than 20%, even when allogeneic hematopoietic stem cell transplantation (alloHSCT)

was offered (1, 2). In recent years, the combination of tyrosine kinase inhibitors (TKIs) with the standard ALL chemotherapy has substantially improved the complete response rates (3-7), increasing the proportion of patients to whom an alloHSCT transplantation can be offered in first complete remission (CR1) (3-5). Although alloHSCT still represents the only curative option for these patients, recent studies have demonstrated the possibility of long-term survival without a transplant in patients achieving a deep molecular remission after treatment with combination of TKIs and chemotherapy (8, 9). In this regard, monitoring minimal residual disease (MRD) with the aim to identify patients with a more favorable outcome who possibly may be cured without alloHSCT is very important and a matter of intensive clinical investigation.

In this review we will focus on these relevant issues for the management of adult Ph+ ALL.

TKIS IN FRONT-LINE THERAPY

The prognosis of Ph+ ALL patients has significantly improved upon the incorporation of TKIs in the standard ALL therapy. A positive impact of combination of TKIs with standard chemotherapy as front-line therapy has been reported early after its therapeutic use in both adults (3-5, 7, 10) and children (11, 12). The use of refined programs with first/second generation TKIs and chemotherapy together with alloHSCT allow up to 50% of all patients to be cured (3-5, 13), that represents a significant improvement compared to the pre-imatinib era (2, 6, 14). The German Multicenter Acute Lymphoblastic Leukemia (GMALL) published results obtained in 92 patients with newly diagnosed Ph+ALL treated with imatinib administered concurrent to or alternating with induction and consolidation chemotherapy in whom was obtained a CR rate of 95%, a 2 year OS rate of 36% and the majority of patients (77%) underwent alloHSCT in CR1 (15). The Northern Italian Leukemia Group (NILG) reported a CR rate of 92% in 59 patients with a 5 year OS and DFS of 38% and 39%, respectively, compared with 23% and 15%, respectively of controls in the pre-imatinib era (6). More recently, Fielding et al. demonstrated a significant enhancement of long-term outcomes for a large series of adult patients who were prospectively treated in 2 sequential cohorts with an imatinib-containing protocol. The CR rate was 92% in the imatinib cohort *vs* 82% in the pre-imatinib cohort, while the 4 year OS rate was 38% compared with 22% in the pre-imatinib cohort (7). Similar results were reported by the Japanase Adult Leukemia Study Group that compared alloHSCT outcomes between 542 patients who received imatinib before alloHSCT during the initial complete remission period (imatinib cohort) and 196 patients who did not receive imatinib (non-imatinib cohort). The 5 year OS was significantly higher in the imatinib cohort than in the non-imatinib cohort (59% vs 38%; p<.001) (13). Finally, the European Group for Blood and Marrow Transplantation (EBMT) examined the retrospective data of 473 *de novo* Ph+ ALL patients undergoing first-line treatment followed by alloHSCT (16). This study represents the largest multicenter analysis carried out on Ph+

ALL adult patients and confirms that the introduction of TKIs pre and post alloHSCT significantly improved outcomes of Ph+ ALL patients, reducing disease recurrence (16).

It is worth noting that, although TKIs administration along with chemotherapy is able to raise CR rates up to 95%, early deaths can occur in about 5% of patients (3, 4, 6, 7). In this regard, to investigate if a less aggressive regimen during induction is effective and safe, sparing the toxicity of intensive treatments, the GIMEMA and GMALL groups, separately, evaluated the use of TKIs alone or in combination with steroids. These protocols demonstrated that almost all patients achieved hematologic CR, with a remarkable low toxicity profile even in older patients (17-19). These results were useful for underlining the concept that in TKIs era intensive chemotherapy during induction may be harmful, but unfortunately they showed also that TKIs may not be sufficient as monotherapy to produce long-term responses. Indeed, late relapses were observed and most of them were associated with a highly resistant phenotype, such as the presence of T315I mutation (17, 20, 21). Recently Chalandon et al. (22) compared high doses of the tyrosine kinase inhibitor imatinib combined with reduced-intensity chemotherapy to standard imatinib/hyper CVAD therapy (arm B) in 268 adults with Ph+ ALL. The less-intensive arm, with fewer induction deaths yielded a significantly higher hematologic CR rate and a similar major molecular response rate after the second course.

The 5 year EFS and OS rates were similar between the 2 arms. Moreover, upfront alloHSCT was associated with a significant benefit in relapse-free survival in patients randomized to receive imatinib combined with either less-in-

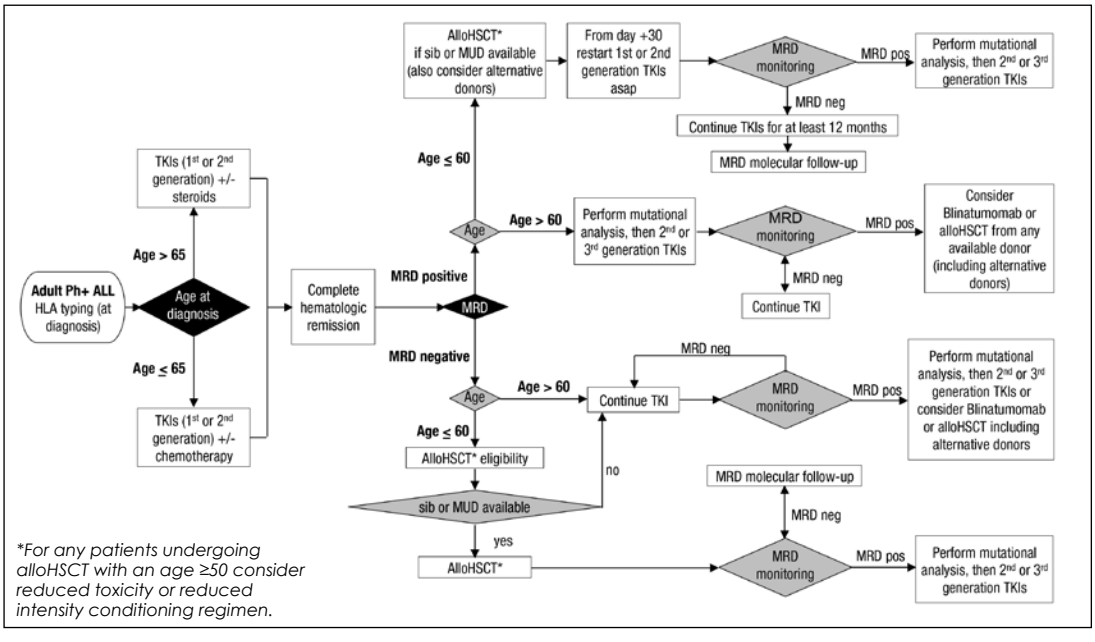

FIGURE 1 • A possible algorithm for the treatment of adult Ph+ ALL.

tensive chemotherapy or a more intense regimen.
In conclusion, the available data suggest that incorporation of TKIs into standard ALL-type chemotherapy should be considered as a mainstay treatment of Ph+ ALL patients because it increases initial remission rates, reduces early death rate with a beneficial impact in eligibility for alloHSCT and also improves outcome after transplant. Ongoing efforts include testing in clinical trial whether or not lower intensity regimens in combination with TKIs are effective and safe, sparing the toxicity of more intensive regimens.

TKIS AND MINIMAL RESIDUAL DISEASE (MRD) BEFORE TRANSPLANT

The molecular monitoring of the *BCR-ABL1* gene rearrangement during treatment of Ph+ ALL represents an ideal application of MRD driven therapies in human cancers due to high specificity of the molecular analysis and the use of target specific drugs such as TKIs. Therefore, the levels of *BCR-ABL1* reduction during treatment indicate the responsiveness to treatments, while persistence or any increase of the molecular signal is associated to mechanisms of resistance, such as the presence of T315I mutation and more in general, to a pending treatment failure. To date, there is a general agreement that MRD positivity in Ph+ ALL is a major risk factor for relapse and poor outcomes. Lee *et al.* showed that an early reduction *BCR-ABL1* identify a subgroup of Ph+ ALL transplants at lower risk of relapse (23). A recent study showed that a negative MRD status after three months and beyond of a TKIs based chemotherapy program was associated with a low risk of relapse and an OS above 60% at 3-5 years in patients excluded from alloHSCT as first-line therapy (8). Moreover, a more recent phase 2 trial that examined the combination of chemotherapy with ponatinib, showed that long-term disease free survival (DFS) was not affected by alloHSCT in patients achieving MRD negative status (9). These interesting results confirm the importance of obtaining a sustained molecular CR that increases the chance of cure and allows to identify a subgroup of patients who may be possibly cured without alloHSCT.
A second relevant issue for the therapeutic decision is the prognostic value of measurable levels of MRD at time of conditioning before transplantation. In childhood and adult ALL patients, previous studies provided reasonable support to suggest an inferior outcome of patients undergoing alloHSCT with measurable level of MRD at time of conditioning (24-26). Recently, the Japan Society for Hematopoietic Cell Transplantation retrospectively analyzed data form of 432 adult Ph+ ALL patients (27). The authors reported that incidence of relapse in MRD negative patients at transplant was significantly lower compared to patients transplanted in MRD positivity (19% *vs* 29%, respectively). The OS was also significantly better in patients transplanted in MRD negativity than in patients transplanted in MRD positivity (67% *vs* 55%) (27). In this respect, we analyzed the prognostic value of the MRD level at time of conditioning in newly diagnosed Ph+ ALL patients enrolled into 2 consecutive prospective clinical trials conducted within

NILG group (28). According to the study design, all patients were treated front-line with chemotherapy and imatinib and in CR1 were eligible to perform an alloHSCT. In this prospective cohort, we observed that MRD negativity at time of conditioning was associated with a significant benefit in terms of reduction of the relapse risk at 5 years that declines from 39% to 8%. Nonetheless, as a consequence of the still relevant non relapse mortality and the post-transplant use of TKIs, the improvement of LFS and OS was not statistically significant. Overall, our results reinforce the notion that patients undergoing alloHSCT with measurable level of MRD show an inferior outcome after transplant, at least in terms of relapse incidence and this information is certainly useful for an effective HSCT planning. In this respect, Bachanova et al. showed that reduced intensity conditioning (RIC) alloHSCT may represent a viable alternative to myeloablative conditioning (MAC) alloHSCT for patients with Ph+ ALL at lowest risk of relapse, such as those that have achieved a MRD negative status, reducing their risk of non-relapse mortality (29). Moreover, patients failing the achievement of a deep molecular CR should be considered for newer experimental treatment strategies able to achieve a sustained MRD negative status before transplantation.

TKIS FOR PREVENTING RELAPSE AFTER TRANSPLANT

Post-transplant TKIs administration is another crucial point under investigation, since leukemia relapse remains a major cause of treatment failure after alloHSCT in Ph+ ALL (16). In a prospective, randomized multicenter trial, the tolerability and efficacy of post-transplant imatinib administered either prophylactically or following detection of MRD was investigated. Prophylactic imatinib significantly reduced the incidence of molecular recurrence after alloHSCT compared with MRD-triggered imatinib but the overall survival after transplantation proved equally very high (80 *vs* 75%) (30). More recently, Shimoni and co-workers explored the use of nilotinib for the prevention of relapse after alloHSCT in advanced-phase CML and Ph+ ALL. Patients were given prophylactic nilotinib maintenance, which was started at a median of 38 days after transplantation. Most patients achieved or maintained a complete molecular response, and only 1 of them later relapsed. With a median follow-up of 46 months, for the recipients on nilotinib maintenance, the 2 year OS and PFS rates were 69% and 56%, respectively (31). It is worth noting that in both experiences the high efficacy of treatment in preventing relapse was hampered by a high rate of early discontinuation due to poor tolerability, mostly gastrointestinal and hepatic (30, 31). Since treatment with TKIs after alloHSCT can be difficult to tolerate and may be followed by a high rate of early discontinuation, a careful risk assessment of patient at highest risk of relapse may lead to timely initiation of an effective treatment with TKIs and to avoid it, if not strictly necessary. In this regard, our opinion is to limit TKIs prophylactic use to patients at high risk of relapse, such as MRD positive patients before transplant and to apply a MRD-driven strategy in MRD negative patients.

CONCLUSIONS AND FUTURE PERSPECTIVES

Recent results showed that the incorporation of TKIs into the standard chemotherapy has substantially improved the outcomes of Ph+ ALL. Patients achieving a complete molecular remission have a better outcome, due to a significant decreased risk of leukemia relapse (24-28), and in some of these patients the paradigm of myeloablative alloHSCT, as a mandatory post-remission therapy in adult Ph+ ALL, is no longer supported by the available results (8, 9). Accordingly, an accurate evaluation of MRD values is crucial and should guide the clinical decision making process. This implies that for improving the clinical outcome of Ph+ ALL with or without allotransplant in CR1, patients not obtaining a complete molecular remission with standard treatments should be considered for newer experimental treatment strategies able to achieve a convincing molecular CR.

In patients failing to achieve a complete molecular remission with imatinib or second generation TKIs, ponatinib (a third generation TKI) may offer the possibility to target this objective. Indeed, it has been recently shown its ability to overcome the pharmacologic resistance mediated by some mutations of the BCR/ABL protein, such as the T315I, and achieve impressive frequency of molecular CR (9). In this context, other recent innovative therapies are represented by blinatumomab, the first member of a novel class of T cell-engaging, bispecific single-chain (BiTE) antibodies (it engages T cells for redirected lysis of CD19+ target cells) and the chimeric antigen receptor (CAR) modified T cells. For the treatment of minimal residual disease, blinatumomab showed very encouraging results in terms of high percentage of MRD response rate, that translates into a favorable relapse free survival (32, 33) of refractory B precursor ALL, including Ph+ cases (34).

Indeed, the first pilot trial conducted in MRD positive ALL resulted in 80% of patients achieving a MRD negativity. Nine of 20 patients received alloHSCT after blinatumomab treatment and 65% of these were in hematologic CR at a median follow-up of 33 months. According to these promising results and to its manageable toxicity profile (35), a short period treatment with blinatumomab in patients with MRD positivity before alloHSCT could have the potential therapeutic to achieve a convincing complete molecular remission and, thus, to allow an improved cure rate after transplantation.

This hypothesis needs to be evaluated and warrants the planning of specifically *ad hoc* designed clinical trials. The potential use of blinatumomab after alloHSCT also holds promise (36), but the quality of the immune reconstitution after transplantation remain a key point for an effective use of this drug in this setting (37).

Finally, CAR-modified T cells have shown exciting results in highly refractory populations (38, 39). However, their clinical use as a pre-emptive strategy of leukemia relapse is at this time limited by the costs and the high technical complexity for the clinical grade manufacturing of these genetically modified cells as well as by the significant toxicity mainly related to the massive cytokine release following their *in vivo* infusion (38, 40).

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New drugs in haematopoietic stem cell transplantation for paediatric acute lymphoblastic leukaemia

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SUMMARY

Patients relapsing after transplantation are at dismal prognosis and are eligible for experimental approaches. In the last decade novel strategies have been investigated in the setting of post-transplant relapse. A second transplantation is offered, often from an haploidentical related donor, regardless of the previous donor type. Compared with selective T-cell depletion techniques, the use of post-transplant cyclophosphamide is a relatively easier tool, which increased its reproducibility. The role of new drugs, such as blinatumomab, or of novel approaches, such as chimeric antigen receptor modified T cells (CAR-T) after post-transplant relapse are under investigation.

We report three clinical paediatric cases who relapsed after transplant and were treated by means of novel strategies.

INTRODUCTION

The overall cure-rate of acute lymphoblastic leukaemia (ALL) in children is as high as 85% with conventional chemotherapy (1-3). However, approximately 10% of the patients in first remission (CR1), who are at very high-risk of relapse, mostly due to failure to achieve morphological remission after induction or poor molecular response after consolidation, and most of the patients in second remission (CR2) are eligible for hematopoietic stem cell transplantation (HSCT) (1, 2, 4, 5).

The most frequent cause of transplant failure is disease relapse, which occurs in 25% of the patients and remains a therapeutical challenge (4, 5). Patients who relapse after HSCT have an extremely dismal prognosis and are eligible for experimental approaches (6).

A subsequent HSCT after post-transplant relapse was up-to-now the only curative option, which could rescue approximately 10% of the children, in case hematologic remission could be achieved (6). Additional chemotherapy *per se* would be of limited benefit, due to multi-drug resistance, which progressively increases at each treat-

Key words: acute lymphoblastic leukaemia, childhood, hematopoietic stem cell transplantation.

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ment line, and may be associated with fatal toxicity. Therefore, new therapeutic strategies with lower toxicity profile are warranted (7).

Clone-specific markers for the detection of minimal residual disease (MRD) are available in most (>90%) ALL patients (1, 2). In patients undergoing HSCT, MRD positivity at the time of transplant is associated with a 5-fold higher incidence of relapse, as compared with MRD negative patients (8-10). Persistence or recurrence of detectable leukemic cells after transplant was strongly associated with imminent disease relapse; the risk of relapse was 2.5-fold higher in patients becoming positive in the first three months and 8-fold higher in those becoming positive between six and 12 months after transplant (8).

Post-transplant MRD monitoring, becoming a routine approach in many centers and groups, may allow early tapering of immunosuppression and cell therapy planning in the attempt to enhance the graft versus leukemia effect (GvL) and prevent disease relapse (8-10).

Second HSCT after relapse is a therapeutic option; regardless of the type of donor used for the first transplant, an haploidentical donor is frequently chosen, due to the prompt availability of a parent for virtually all children and adolescents. Moreover, post-transplant cyclophosphamide (PT CY) is a relatively easy tools, compared with evolving strategies of selective T-cell depletion, therefore haploidentical HSCT is increasingly used worldwide (11).

The novel class of bi-specific single-chain antibody construct (BiTE) blinatumomab and chimeric antigen receptors (CARs), synthetic receptors that mediate antigen recognition, T cell activation and co-stimulation to augment T cell functionality and persistence, are currently under investigation (11-15).

Trials are underway with anti-CD19 chimeric antigen receptor T cells in B-lineage ALL that will help define the role of chimeric antigen receptor T cells as a rescue strategy for patients relapsing after HCT (15-19).

We report three children affected with ALL, relapsing after first HSCT, who were treated by means of novel approaches.

CASE 1

A 7-year old child, diagnosed with common-ALL, negative for clonal abnormalities and central nervous system involvement, was enrolled in the AIEOP BFM ALL 2009 protocol, high-risk arm, on the basis of his slow early response (high MRD level after induction) (1). Isolated bone marrow relapse occurred 32 months after diagnosis, which is intermediate risk (S2) according to the BFM Rez stratification. Treatment according to AIEOP REC 2003 Protocol, 5 months after relapse, included HSCT from his HLA-identical sister, after conditioning with total body irradiation (TBI), etoposide (VP-16) and graft-versus-host disease (GvHD) prophylaxis with cyclosporine (CyA) only; no methotrexate (MTX) was given in the attempt to enhance a potential graft-versus-leukemia effect, since his MRD at transplant was $1.2x10^{-3}$.

The child experienced skin and liver grade II GvHD, requiring methyl-prednisolone (mPDN). Seven months after HSCT, MRD positivization, $<1x10^{-4}$, was detected, progressively increasing in the following weeks. Nine months after HSCT his MRD level was $9.5x10^{-4}$, de-

spite the discontinuation of immunosuppression.
An haploidentical HSCT was performed 10 months after the first transplant; his mother served as a donor, stem cell source was bone marrow (BM), conditioning included thiotepa (TT), treosulfan (Treo), fludarabine (Flu) and GvHD prophylaxis CyA, micophenolate mofetil (MMF), and cyclophosphamide (Cy 50 mg/kg day +3, +4).
Five months after second transplant, the patient experienced a further MRD positivization and was enrolled in the CIK2 protocol, which includes two donor lymphocyte infusions (DLI) and three cytokine-induced killer cell infusions (CIK). MRD negativization and severe GvHD occurred after the first DLI dose. The patient is still in remission, with limited skin cGvHD, despite a further MRD positivization, waiting for his third CIK, 10 months off second transplant, 5 months after MRD positivization and 5 years after his primary disease diagnosis. In case disease level increased up to 5% marrow blasts, the boy would be eligible for CAR-T cell therapy.

CASE 2

A 9-year old boy, diagnosed with ALL B-I and treated according to AIEOP R 2006 protocol, presented with combined bone marrow and right testis relapse 33 months after diagnosis and was treated according to AIEOP LLA REC 2003 protocol, which included monolateral orchiectomy and a matched unrelated donor HSCT, complicated by grade II GvHD gut and liver. Twelve months after HSCT, the boy presented with a second left testis relapse and 3 months later with a bone marrow relapse, which was treated with chemotherapy (steroid, vincristine, FLAG-D, FLA-G), followed, 5 months later, by a further relapse in his bone marrow.
One blinatumomab cycle was followed by a new remission status and, after a second cycle, 3 months after his fourth relapse, he could undergo a second HSCT from his father, with bone marrow as stem cell source, TT-Treo-Flu as conditioning regimen and CyA, MMF, PT CY as GvHD prophylaxis.
His MRD was $7x10^{-3}$ pre-HSCT, became negative up to 5 months after transplant, when a positivity $<1x10^{-4}$ was detected. The adolescent was enrolled in the CIK2 protocol (see above). His MRD increased but remained always $<1x10^{-4}$ so far and subsequently achieved negativity after the first CIK infusion. The patient is now 1 month off his last CIK, 5 months after his MRD positivization, 10 months after his second transplant and 6 years after his primary disease diagnosis. Even in case his level of disease increased up to morphological relapse, blinatumomab is an exclusion criterion for enrollment in any CAR-T trial.

CASE 3

An 8 year old girl, diagnosed at birth with thalassemia major and transplanted at 3 years from her HLA-identical sister after conditioning with TT-Treo-Flu and GvHD prophylaxis with CyA and MTX. Despite 7 DLI, she experienced secondary rejection and full autologous reconstitution 12 months after transplant. In the third after transplant, she was diagnosed with t(9;22) positive acute lymphoblastic leukaemia and was treated according to EsPhALL (induction + block HR-1). Her disease achieved morphological remission but

her TCR MRD and BCR/ABL transcript never became negative.
Five months after ALL diagnosis, she underwent a second bone marrow transplant from another HLA-identical donor, after conditioning with TT-Treo-Flu and GvHD prophylaxis with CyA, MTX, with positive MRD at HSCT (BCR/ABL 178 copies/10^4ABL copies; MRD <$5x10^{-4}$). The post-HSCT course was uneventful, three-lineage engraftment and full donor chimerism were achieved and imatinib was started in the second month after HSCT, due to persistent t(9;22) positivity both in peripheral blood and bone marrow, despite MRD TCR/Ig negativity.
Twelve months after her second HSCT, she presented with varicella and overt relapse as bi-phenotipic t(9;22) ALL (BM blasts 90%) and was enrolled in the phase I study with nilotinib as an only drug. Three months later she achieved a second morphological remission (BM blasts 3%; BCR-ABL 400/10.000 copies) and nilotinib was discontinued 3 months later.
In the following month the girl was then enrolled in the first generation CAR-T trial CD19TPALL. Patients relapsed after transplant were eligible for the prophylactic arm of the trial. The girl underwent her third HSCT from the same HLA-identical brother, as for her second bone marrow transplant, after conditioning with TT-Treo-Flu and GvHD prophylaxis with CyA, MTX. Post transplant treatment (4 months after 3rd HSCT) included lymphodepletion with fludarabin 30mg/m^2, transfusion of cytotoxic T-cells (CD19-zeta EBV-CTLs) and BLCL-Vaccination (irradiated donor-derived B-Lymphoblastoid Cell Line).
Six months after HSCT, one month after BLCL vaccination, MRD positivity was detected and overt relapse occurred five months later.
In the following five months imatinib, palliation and supportive care were provided, which did not compromise her quality of life, despite high leukemia burden, up to sudden death due to infection.

DISCUSSION AND CONCLUSIONS

Despite successful reduction of treatment-related mortality, relapse rates in children with high risk ALL undergoing HCT remains as high as 25% (5). Highest risk of relapse can now be identified by means of MRD monitoring before and after transplant (8).
The three cases of pediatric ALL, relapsing after transplantation, presented here, underwent a subsequent transplant which included a novel strategy. The first two patients underwent a second HSCT from an haploidentical parent and their GvHD prophylaxis included post-transplant cyclophosphamide and MMF (11).
According to the Baltimora experience, PT CY aims at selective depletion of alloreactive T-cells, leading to immunogenic tolerance by specific clonal killing of activated mature T-cells and preserving stem cells and resting memory T-cells essential for engraftment and immune reconstitution. According to reports in adults and preliminary reports in children and adolescents, PTCY HSCT seems associated with lower incidence of graft-versus-host disease and TRM, compared with other approaches in the haploidentical setting (11).
New agents and immuno-therapies may reduce the risk of relapse after HCT. Clinical trials to assess the effica-

cy of such novel therapies, employed either before or after transplant are under investigation (12-15).

Blinatumomab is a murine recombinant single-chain antibody construct combining both the binding specificity for the pan B-cell antigen CD19 and the ε chain of the T-cell receptor/CD3 complex on one polypeptide chain. It belongs to a new class of bispecific antibody constructs called bispecific T-cell engagers (BITE), which have been designed to direct T-effector memory cells towards target cells. The proximity induced by the BITE® triggers target cell-specific cytotoxicity, which closely resembles standard cytotoxic T lymphocyte (CTL) activation. This T-cell-mediated target-specific killing is the therapeutic mechanism of action of blinatumomab (12-15).

Paediatric B-cell precursor ALL blasts show a stable and sufficient expression of CD19 which makes B-lineage ALL a good target for immunotherapeutic approaches. Blinatumomab recruits and activates T-cells, by means of the TCR complex, and redirect them to lyse malignant and non-malignant cells expressing CD19, by enabling the formation of cytolytic synapses which is the prerequisite for leukemic cell lysis (12-15).

Trials are underway with anti-CD19 chimeric antigen receptor T cells in B-lineage ALL that will help define the role of chimeric antigen receptor T cells as a rescue strategy for patients relapsed after HCT.CAR-T cells are T-cells, transduced to express a chimeric antigen receptor, which includes an anti-CD19 antibody fragment fused to a T-cell intracellular signaling domain (16-19).

Chimeric antigen receptor-modified T-cells (CAR T-cells) with CD19 specificity (adoptive transfer of CD19ζ chimeric receptor transduced donor-derived EBV-specific cytotoxic T-lymphocytes (EBV-CTL) are first generation CAR-T, currently studied as a novel therapy for high-risk or relapsed B cell precursor ALL after HSCT (16).

Second-generation CAR-T cells also encode for a co-stimulatory domain, such as CD28 or members of the tumor necrosis factor receptor family, such as CD27, CD137 (4-1BB) and CD134 (OX40).

The co-stimulatory domains activate the CAR-T-cells, allowing for targeting and lysis of CD19+ cells (17-19).

Whether the implementation of post-transplant MRD monitoring, PT CY, blinatumomab and CAR-T will actually prevent post-transplant relapse or improve its prognosis should be addressed in randomized trials.

A COG trial for children with relapsed ALL who achieve CR after reinduction will include a randomization between blinatumomab and chemotherapy before HSCT, a strategy that is aimed at minimizing pre-HCT MRD and reduce relapse.

The phase II Novartis trial with CAR-T is concluded and results will be made available after the completion of one year of minimum potential follow-up for all the patients enrolled. A phase III trial with CAR-T is in its planning phase and will contribute to assess the role of this innovative approach.

Conflicts of interest

None.

Conference Presentation

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REVIEW

Correlation between *MYC* gene rearrangement and MYC protein expression suggests that MYC regulation is more complex than previously known

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SUMMARY

Since its discovery in the 1970's, MYC oncoprotein has been continuing to fascinate the scientific world and there is a growing interest in the role of MYC in the genesis and prognosis of cancer.
Initially MYC was identified as the cellular homologue of the MC29 transforming avian retrovirus. Shortly hereafter, additional related sequences were identified, suggesting that MYC might be part of a larger family of genes.
The constellation of MYC effects on genes involved in proliferation has led to the concept of MYC-driven lymphomas, that include Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), and lymphomas that share morphologic features of DLBCL and BL, officially termed B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCLU). Other lymphomas showing MYC over-expression comprises Plasmablastic lymphoma and Plasmacytoma, Double hit/triple hit lymphomas and Anaplastic lymphomas Kinase-positive Large B-cell Lymphoma.
MYC aberrations can be detected by standard cytogenetics, interphase fluorescence in situ hybridization (FISH), comparative genomic hybridization and most recently immunohistochemistry. By comparing expression profiles of MYC gene rearrangement and MYC protein expression has came up that MYC gene rearrangements do not necessarily correlate with MYC protein expression. In fact, by applying immunoistochemistry, the frequency of MYC protein expression appears much higher than what is detected by FISH standard method. Therefore, nowadays the key problem in the hematopathology field is to define the clinical impact of the double-expressor lymphoma status. The updated World Health Organization (WHO) of tumours of hematopoietic and lymphoid tissues asses that the status of double or triple lymphoma should rely only on molecular biology findings and not on immunohistochemistry results.

Key words: MYC gene dysregulation; MYC protein expression; Burkitt lymphoma.

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INTRODUCTION

The iconic history of the MYC oncoprotein encompasses three decades of intense scientific discovery. There is no question that *MYC* has been a pioneer, advancing our insight into the

molecular basis of cancer, as well as functioning as a model of several diverse biological processes and regulatory mechanisms.
By analyzing all the published articles on MYC, two major themes emerge: first, the fine-tuned regulation and numerous activities of MYC in normal cells; second, the role of MYC as oncoprotein when its regulation is lost (1).

Identification of MYC
In the late 1970's an avian acute leukemia virus (MC29) was shown to promote a spectrum of malignancies, including myelocytomas, sarcomas and carcinomas.
This ability to induce carcinomas was of particular interest. The transforming sequence of MC29 was identified as v-myc, and named myelocytomatosis for a resultant leukemia. v-myc was found to present in the cells as a 110 kDa v-gag-myc fusion.
Consistent with the notion that oncogenes could be stolen by retroviruses, the cellular homologue was identified soon after in normal cells from many species. The discovery of MYC further reinforced the startling realization that oncogenic transformation could be caused by the activation of a cellular gene (2-5).

The MYC family of transforming oncoproteins
MYC was first identified as the cellular homologue of the MC29 transforming avian retrovirus. A schematic representation of human *MYC* is presented in *Figure 1*. Soon after, additional related sequences were identified, suggesting that *MYC* might be part of a larger family of genes. Two family members, *MYCN* and *MYCL1*, were identified as a result of their amplifications in neuroblastoma and lung cancer respectively (6-11). While there is a clear role for all family members in tumorigenesis, there are some important differences that exist between family members. Specifically, *MYCL1* consistently promotes transformation to a lesser extent than the other two family members, and to date the mechanisms underlying this remain a question in the field. *MYCN*, on the other hand, has been shown to be functionally interchangeable with *MYC* in development through the generation of a knock-in mouse model (6).

MYC: structure and functions
All the genes belonging to the *MYC* proto-oncogene family are expressed in mammals. A forth gene, B-*MYC*, encodes a protein that shows significant homology to the N-terminus, but lacks

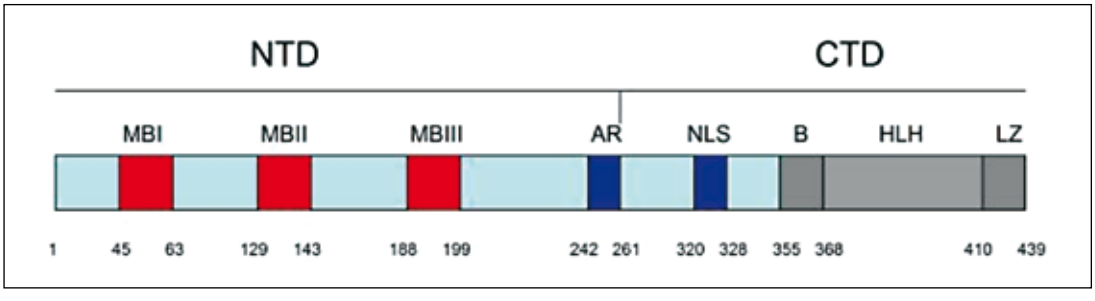

FIGURE 1 • The structural domains of human *MYC*.

essential domains in the C-terminus of the other MYC proteins, and its biology is poorly understood. The *MYC* gene is located on the human chromosome 8q24, and it consists in three exons. Translation at the AUG start codon nucleotide in the second exon produces a major 439 amino acid MYC protein (64 kDa). Alternative translation initiation start sites result in both longer and shorter forms of the protein.

A longer polypeptide of 67 kDa results from translation initiated 15 codons upstream of the AUG at a CUG codon (exon1) and the shorter one, 45-kDa polypeptide, results from an internal translation initiated.

The MYC protein is O-linked glycosylated and phosphorylated and these modifications may alter the protein half-life. As such, it is important to note that *MYC* mRNA and protein have a very short half-life (20-30 min) and are tightly regulated. *MYC* gene encodes for a transcription factor of the basic helix-loop-helix leucine zipper (b-HLH-LZ) superfamily. Traditionally the MYC protein is functionally referred to a N-terminal domain (NTD) and a C-terminal domain. The N-terminal, which is defined as amino acids 1-262, and the C-terminal, defined as residues 263-439, match respectively to the N-terminal transactivation domain (TAD) and C-terminal DNA-binding and basic-region/helix-loop-helix/leucine-zipper (BR/HLH/LZ) domain (1).

The N-terminus of *MYC* is the major regulatory region responsible for assembly of the transcriptional machinery. Within the N-terminus there are three highly conserved elements, known as *MYC* boxes I-III which, together with the C-terminal b-HLH-LZ, define the MYC family of proteins. Of these, MYC box I (MBI, from approximately amino acids 45-63) and MYC box II (MBII, from approximately amino acids 128-143) contain sequences highly conserved among the different MYC family of proteins throughout evolution. These two regions appear to be particularly important for the transcription activity of MYC; in fact, deletion of either MBI or MBII, diminishes the transcriptional activation potential 10-50 fold, respectively.

Specifically, although MBI is required for gene activation, deletion of this region only partially abolishes the transforming ability of MYC while an MBII deletion, which is essential for the ability of MYC to transform, drive cell proliferation and activate certain target genes, completely abolished it. MYC box II is not involved in the binding of MYC to Max or to DNA, but is required for activation and repression of most, but not all, MYC target genes. Recently, a third conserved region of MYC has been described, MYC box III (MBIII), that plays a role in transformation, lymphomagenesis and apoptosis.

MYC biological activities

Cell cycle and differentiation

There were several lines of evidence to suggest that *MYC* might play a role in cell cycle progression. Ectopic expression of *MYC* promotes growth factor independent proliferation (1, 12). Not unrelated to the ability to promote cell cycle progression, *MYC* expression also blocks differentiation. It was demonstrated by multiple groups and through a number of models that MYC down-regulation is essential for cells to exit the cell cycle and undergo differentiation. These abilities to promote cell proliferation and block differenti-

ation have natural associations with tumorigenesis and are features of aggressive disease (1).

Apoptosis

The ability of MYC to promote apoptosis provides a built-in safety mechanism to protect against inappropriate proliferation as a consequence of deregulated MYC (13-16). This finding also shed considerable light on the model of oncogene cooperation and how an anti-apoptotic protein such as BCL2 could cooperate with MYC to promote oncogenesis (17-19). Moreover, deregulation of MYC was shown to activate the tumor suppressor p53 through the upregulation of ARF (20-22). A loss of either of these tumor suppressors accelerated tumorigenesis in mouse models of oncogenic *MYC* (23-27). Further, MYC is also able to promote apoptosis through p53-independent mechanisms by influencing the balance between pro- and anti- apoptotic proteins in the cell (20, 28-34).

Transcriptional activation

The C-terminus of MYC was shown to contain both a helix-loop-helix (HLH) (35) and a leucine-zipper (LZ) domain (36) to which a MYC partner can binds, MAX (MYC associated factor X) (39). MAX was shown to be a constant and obligate partner for MYC, with consistent and abundant expression in both proliferating and quiescent cells, which was not altered in response to extracellular stimuli. It is, however, important to note that MAX also forms heterodimers with members of the MXD family, which thereby provides an additional mechanism to regulate MYC activity in the cell (40, 41). In non-transformed cells, the MYC protein appears to integrate environmental signals, in order to modulate a diverse, and sometimes opposing, group of biological activities, including proliferation, growth, apoptosis, energy metabolism and differentiation. MYC protein levels are induced or suppressed by virtually all signalling cascade bearing proliferative and anti-proliferative cues, respectively. Mitogen stimulation induces *MYC* as an immediate-early response gene, whose expression is essential and sufficient for the G1/S progression. *MYC* also plays a role in G2/M transition, making it one of the key players in cell cycle regulation. Abnormal or ectopic over-expression of MYC in primary cells activates a protective pathway through the induction of p16/p14ARF and the p53-dependent cell death pathway. Hence, normal cells that overexpress MYC are eliminated from the host organism through apoptosis, thereby protecting the organism from lethal neoplastic changes.

Transcriptional regulation

MYC can both activate and repress transcription of its target genes. MYC-Max heterodimers activate transcription by binding to E-box elements. The DNA binding of MYC and Max complexes is mediated by amino terminal 143 amino acids of c-MYC (TAD). Deletions within the N-terminal TAD, can greatly affect or abrogate the biological function of MYC, as its transactivation potential. The activation involves the recruitment of multiple coactivators and protein complexes to E-box elements. Coactivators include the Mediator complex, Positive Transcription Elongation Factor b (P-TEFb), the ATPases TIP48 and TIP49, and histone acetyltransferases such as CREB-bind-

ing protein (CBP) and p300, GCN5 and TIP60. Of note, GCN5 and TIP60 are bound to MYC indirectly through the TRRAP adaptor protein that interacts with MYC box II, TRRAP also recruits the p400 histone-exchange protein to MYC. Another way for MYC to activate target genes is by interaction with E3 ubiquitin ligase SCF^{SKP2} (SKP2) which recruits components of the APIS complex to E-box sequences. Transactivation by MYC is antagonized by Mad-Max and Mnt-Max heterodimers, which repress transcription by recruiting histone deacetylase complexes (HDACs) through the adaptor protein SIN3 (33,34) RNA pol II and SAP.

Interestingly, only a minority of the sites to which MYC and Max are bound *in vivo* have a consensus CACGTG sequence. Indeed, MYC-Max heterodimers are able to recognize non canonical sites, variations of the canonical E-box containing core TG or CG di-nucleotides. In addition, nucleotides immediately flanking the E-box, as well as methylation of CpG within the E-box, can affect MYC-Max binding. In those cases, is possible that MYC and Max are recruited to non-consensus binding sites through the interaction with other transcription factors. One example is offer by Miz1, which can recruit MYC and Max to core promoter sequence that lack a CACGTG sequence. The association Max-MYC allows the interactions with a number of additional transcription factors and co-factors that modulate transcriptional activation. One is TRAAP, which is a core subunit of the TIP60 and GCN5 histone acetyltransferase (HAT) complexes; MYC recruits HAT activity to its target genes and the recruitment depend on the integrity of MYC box II. Inhibition of TRAAP synthesis blocks MYC-mediated oncogenesis, establishing an essential role for TRAAP in MYC activity. TRAAP is also part of a complex containing the p400 E1A-binding protein, which is devoid of HAT activity, suggesting that MYC-TRAAP interaction has other roles in additional roles in addition to recruiting of HAT activity. Two other proteins bind to MYC box II independently of TRAAP; these are TIP48 and TIP49, two highly conserved hexameric ATPases, both involved in several chromatin remodelling complexes. Both proteins have ATP hydrolysing activity, as well as suspected helicase activity and have been shown to be required for the foci formation by MYC and Ras in a primary co-transformation assay. MYC box II is required for interaction with SKP2, of the E3 ubiquitin ligase, SCF^{SKP2}, and MYC recruits SKP2 to its target genes *in vivo*. Recruitment of SPK2 is required for the transactivation of several MYC target genes. Interestingly, not all MYC target genes require the integrity of MYC box II for activation, suggesting that there are other mechanisms of MYC-dependent activation. In the past *MYC* has been considered a "permissive factor", stimulating gene activity by creating a chromatin environment that is conducive for gene induction. It has been recently shown that MYC-driven transcriptional repression is critical for its oncogenic activity. However, less is known about how MYC represses transcription. Recent findings suggest the involvement of DNA methylatransferases enzymes (DNMTs) and histone deacetylase (HDAC) as possible cofactors in the *MYC*-mediated transcription repression. DNA methylation at CpG dinucleotides is the major epigenetic modification in mammals

and is known to be associated with transcriptional repression. The three active DNA CpG methylatransferases identified in mammals are DNMT1, DNMT3a and 3b. Whereas DNMT3a and 3b have been shown to be required for *de novo* methylation, DNMT1 appears to function primarily as a maintenance methylatransferase, restoring methylated cytosine following DNA replication. Several studies have shown that DNMTs can act as corepressors to silence gene expression that maintain chromatin in a compacted and silent state. Indeed, it was recently demonstrated the ability of MYC to repress the transcription through recruitment of DNMT3a, to the MYC-Miz1 complex, indicating that MYC-dependent gene repression could at least partly be mediated by methylation of its target promoters (1).

Novel biological activities

In recent years, additional biological activities of MYC have been characterized. The renewed interest in the Warburg effect and tumor cell metabolism has highlighted a new role for MYC. In addition to stimulating mitochondrial biogenesis, oncogenic levels of MYC have been shown to promote glutaminolysis (42-58). This increased glutamine metabolism fuels cell growth and proliferation, which are essential for tumor cells to thrive. It has been suggested that tumor cells become addicted to glutamine, which may provide opportunities for therapeutic intervention (44). Moreover, *MYC* gene can induce senescence in the context of the loss of other genes such as *WRN* or *CDK2* (59-63). The ability of *MYC* to block differentiation perhaps foreshadowed the recently uncovered role of *MYC* in regulating "stemness". Conditional knock-out mice have demonstrated an essential role for MYC in the normal developmental control of haematopoietic and neuronal stem cells (64,65). *MYC* has recently been identified as one of the four genes whose overexpression could re-program normal terminally differentiated fibroblasts into induced pluripotent stem (iPS) cells (66, 67). While it was later shown that *MYC* was dispensable for this process (68), it did underscore important implications for deregulated *MYC* in initiating and maintaining tumor stem cells. In fact the stem cell signature of undifferentiated and aggressive tumours has great similarity to the phenotypes of MYC-activated tumours (1).

Genetic mechanisms of MYC targets deregulation

MYC transcription factor is considered one of the most potent oncoproteins in human cancer. The consequence of MYC dysregulation is the alteration of important biological activities. Capable of acting as both a transcriptional activator and repressor, MYC controls the expression of a vast array of genes, together accounting for at least 10% of the human genome (69, 70). In general, genes targeted by MYC include mediators of metabolism, biosynthesis, DNA replication, apoptosis, and cell cycle progression (71) such that aberrant MYC expression is associated with uncontrolled cell growth, division, and metastasis (72) whereas loss or inhibition of MYC expression reduces growth, promotes differentiation, and sensitizes cells to DNA damage (73). Some of the most biologically important targets are thought to be cyclins and cyclin-dependent kinases (CDKs),

resulting in accelerated cell cycling (74) down-regulation of phosphatase and tensin homolog (*PTEN*) with consequent up-regulation of the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway (75) and stabilization of the proapoptotic protein and tumor suppressor p53 (76) which can bypass the apoptotic BCL2 program (77). Consistent with its potent growth promoting properties, MYC can drive oncogenic transformation, and deregulated MYC expression and activity is a hallmark of many human cancers. Indeed, by contrast to the highly regulated state of MYC and the absence of N-MYC and L-MYC expression in normal cells, cancer cells often harbour dysregulated expression of any one of these three *MYC* oncogenes.

The MYC transcriptional network also includes the direct regulation of a large number of microRNAs (miRs) with oncogenic or tumor suppressor function (78-80). MYC up-regulates the oncogenic miR 17-92 cluster that is commonly amplified in several subtypes of aggressive lymphomas (81, 82) and its oncogenic function is mediated in part by the down-regulation of PTEN, TP53, and E2F1, facilitating the activation of the PI3K/AKT pathway and the inhibition of apoptosis (83). MYC represses several miRs with tumor suppressor function by the recruitment of HDACs (84). These miRs include miR15a/16-1, miR26a, miR29, and miR34, which regulate crucial functions in neoplastic development such as apoptosis (miR15a/16-1 and miR34 targeting BCL2 and TP53, respectively), proliferation (miR29a targeting CDK6), or cell differentiation (miR26a targeting EZH2) (83, 85, 86). *MYC* itself is also negatively regulated by some miRs, such as miR34 and miR494 (85, 86). miR494 is in turn repressed by EZH2, creating a positive autoregulatory loop (MYC/miR26a/EZH2/miR494) that sustains the persistent expression of MYC and EZH2, promoting the malignant phenotype of cells (85). The interactions of the networks regulated by MYC and its target miRs are complex and suggest fine-tuning of different processes that may be targeted by new therapies (84, 87). Given the emerging pathogenetic role of miRNAs in the methylation status of cancer cells, this link between the regulatory function of *MYC* over miRNAs is particularly noteworthy.

The transformative capacity of *MYC* is often in concert with other oncogenes and viruses, including the rat sarcoma (*RAS*) oncogene (88) and Epstein-Barr virus (EBV) (89). The correlation between EBV and *MYC* is complex, because EBV-associated proteins in turn potentiate *MYC* activity (90).

Intriguingly, the gene profile transcriptionally regulated by MYC varies in different cell types with relatively little overlap (91). Two recent studies shed light on this puzzling observation, showing that, instead of activating a particular gene signature, MYC acts as an amplifier of the transcribed genes in a given cell by uploading to the promoters of active genes and enhancing their transcription (92, 93). MYC does not bind to promoters of silent genes and therefore acts as an activator of the preexisting transcription program. This function of MYC may be relevant to understanding the increased aggressiveness of tumors associated with other oncogenic events carrying *MYC* alterations and may offer perspectives for new therapies (92, 93).

Although the transcriptional role of *MYC* has been well described, there are also non-transcriptional functions that only recently have been appreciated, including regulation of mRNA translation and direct regulation of DNA replication. For instance, *MYC* directly promotes methyl cap formation on the 5' end of pre-mRNA for many genes, including cyclin D1 and CDK-9, and resulting in enhanced mRNA translation (94-100).

MECHANISMS OF MYC DEREGULATION IN CANCER

Building on the awareness that, unlike other proto-oncogenes, *MYC* activation was not a consequence of mutations in the coding sequence, research focused on identifying and understanding other modes of oncogenic activation. This led to the discovery of three mechanisms through which *MYC*, and in turn other oncogenes, could be deregulated and promote transformation: insertional mutagenesis, chromosomal translocation, and gene amplification. Combined, these findings led the way for the discovery and understanding of oncogenes and provided new paradigms for the genetic basis of cancers (1, 101-104).

Non-random chromosomal translocations had been observed in a number of malignancies, including Burkitt's lymphoma (BL) and Chronic Myeloid Leukemia. It was tempting to speculate that these translocations resulted in aberrant expression of the same proto-oncogenes identified in the acutely transforming retroviruses. The mapping of *MYC* to the long arm of chromosome 8 gave credence to this hypothesis (105, 106). Specifically, Burkitt's lymphomas had been characterized to contain reciprocal translocations between chromosome 8 and chromosomes 14, 2 or 22, which harbour immunoglobulin (Ig) heavy and light chain genes (107). It was then discovered that the cancer is driven by activated expression of *MYC* resulting from the translocation. One of the first MYC transgenic mice, Eμ-MYC, was developed to model Burkitt's lymphoma. Activated *MYC* expression was driven from the heavy chain enhancer (Eμ), leading to clonal B-cell lymphomas. Mouse plasmacytomas were similarly found to be a consequence of *MYC* translocation with the Ig heavy chain locus (108, 109).

It was well established that cancer cells contained a number of chromosomal abnormalities and contributions of these aberrations to cellular transformation were largely appreciated through the study of MYC (110-112).

Amplification of *MYC* and/or dysregulated expression is evident in many tumors including melanomas and carcinomas of the breast, prostate and colon. The deregulation of *MYC* plays a decisive role in lymphomagenesis, by driving the cells through the cell cycle. In fact, as a result of the translocation, the normal control mechanisms of *MYC* expression are disrupted, leading to constitutive expression of the protein throughout the cell cycle. Briefly, MYC protein is not only a potent inducer of proliferation, it also induces as a fail-safe mechanism, a large number of pro-apoptotic and inhibits expression of anti-apoptotic genes, thereby inducing apoptosis or predisposing cells to apoptosis. As a consequence, *MYC*-driven tumors usually have acquired additional genetic mutations or epigenetic modifications

that promote cell survival and shift the balance between proliferation and apoptosis towards proliferation. Importantly, a major development within the past decade has been the realization that *MYC* dysregulation is not restricted to gross genetic changes at the *MYC* locus, such as chromosomal translocation, insertional mutagenesis and gene amplification, but *MYC* can be deregulated by one of several mechanisms that target its expression and/or activity either directly or indirectly.

Deregulation of MYC in human lymphomas

The constellation of *MYC* effects on genes involved in proliferation has led to the concept of *MYC*-driven lymphomas (113). The classic *MYC*-driven lymphoma is BL, in which balanced rearrangements between chromosome 8 and either chromosome 14 (immunoglobulin (Ig) heavy chain), chromosome 22 (IgG lambda light chain), or chromosome 2 (IgG kappa light chain), lead to a highly proliferative lymphoid malignancy with a propensity for extranodal involvement, particularly in immunocompromised patients. According to the last WHO classification, other lymphomas commonly associated with *MYC* deregulation are diffuse large B-cell lymphoma (DLBCL), B-cell lymphoma, unclassifiable, with features intermediate between DLB-

TABLE 1 • Lymphoid neoplasms characterized by MYC deregulation.

	Clinic	Morphology	Immunohisto-chemistry	Molecular Biology
Burkitt lymphoma (BL)	Extranodal sites of children and young adults. 3 variants: endemic, sporadic, HIV associated	Cohesive medium-sized cells with coarse chromatin, in a starry sky pattern	The neoplastic cells express CD20, CD19, CD22, CD79a, CD10, BCL6 but are negative for BCL2, proliferative index (Ki 67): >95%	t(8;14) or variant t(2;8) or t(8;22). >90%
Diffuse large B-cell lymphoma (DLBCL)	Adult and elderly with enlarging mass in nodal and extranodal sites	Diffuse growth pattern with centroblastic, immunoblastic, anaplastic or mixed morphology	Pan-B cell antigens positive with expression of germinal center markers in a subset of cases; proliferative index (Ki 67): 30-40%	t(14;18); translocation involving 3q27; two groups identified by gene expression profile, the ABC and GC type
B cell lymphoma, unclassifiable	Older patients presenting with nodal and extranodal disease, usually in an advanced clinical stage	Diffuse growth pattern with intermediate-sized cells, some admixed larger cells, irregular nuclei with single prominent nucleoli	Expression of CD19, CD20, CD22, CD79a, CD10, BCL2, variably BCL6	t(8;14) and other t(qq24), complex karyotype, *MYC* rearrangement to IG and non-IG partner, often accompanied by *BCL2* or *BCL6* rearrangement

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	Clinic	Morphology	Immunohisto-chemistry	Molecular Biology
Plasmacytoma	Middle-aged patients, bone pain at site of involvement or pathological fracture. Soft tissue extension may produce a palpable mass	Poorly differentiated plasma cells (plasmablastic or anaplastic) with eccentric nuclei, and dispersed chromatin	Neoplastic plasma cells express CD79A, CD38, CD138 and monotypic cytoplasmatic immunoglobulins (Ig), they lack surface Ig	Immunoglobulin heavy and light chain genes rearrangement
Plasmablastic lymphoma	Aggressive neoplasm usually present in extranodal sites and frequently in the head and neck region in patients with different immunodeficiency states	Diffuse proliferation of large B cells with immunoblastic morphology, admix with small to intermediate-sized cells	Neoplastic cells express CD38, CD138, MUM1 and they are negative for CD20, PAX5, proliferative index (Ki-67): >90%.	*MYC* translocations are encountered in 40-50% of cases, usually with Igh loci, EBER positive in 40-70% of cases.
ALK-positive DLBCL	Young patients (30% occur in the pediatric age group) commonly with nodal disease	The tumor cells have an immunoblastic or plasmablastic appearance, sinusoidal infiltration is common	Neoplastic cells lack mature B-cell markers but express EMA, kappa or lambda light chain, (most often IgA); CD138, ALK	Up-regulation of the *ALK* gene is mainly due to the presence of t(2;17)(p23;q23)*CLTC* (clathrin)/*ALK*. Rare cases with t(2;5)(p23;q35)(*NPM-ALK*) translocation have also been reported

CL and BL (BCLU), Plasmablastic lymphoma (PBL), Plasmacytoma (PC) and Anaplastic lymphomas Kinase-positive Large B-cell Lymphoma. *Table 1* and *Figure 2* summarize the clinical, morphological, immunophenotypical and molecular biology features of the lymphoid neoplasms characterized by *MYC* deregulation. Apart from the typical characteristics of the above mentioned lymphomas, genetic abnormalities involving *MYC* results in a more aggressive phenotype of tumours cells and a poor prognosis, largely independent of other clinical and molecular risk factors (113).

Novel concept in MYC-related B-cell lymphomas

MYC aberrations can be detected by standard cytogenetics, interphase fluorescence *in situ* hybridization (FISH), comparative genomic hybridization and most recently immunohistochemistry (IHC) (114-116). In recent years, it has been well established that patients

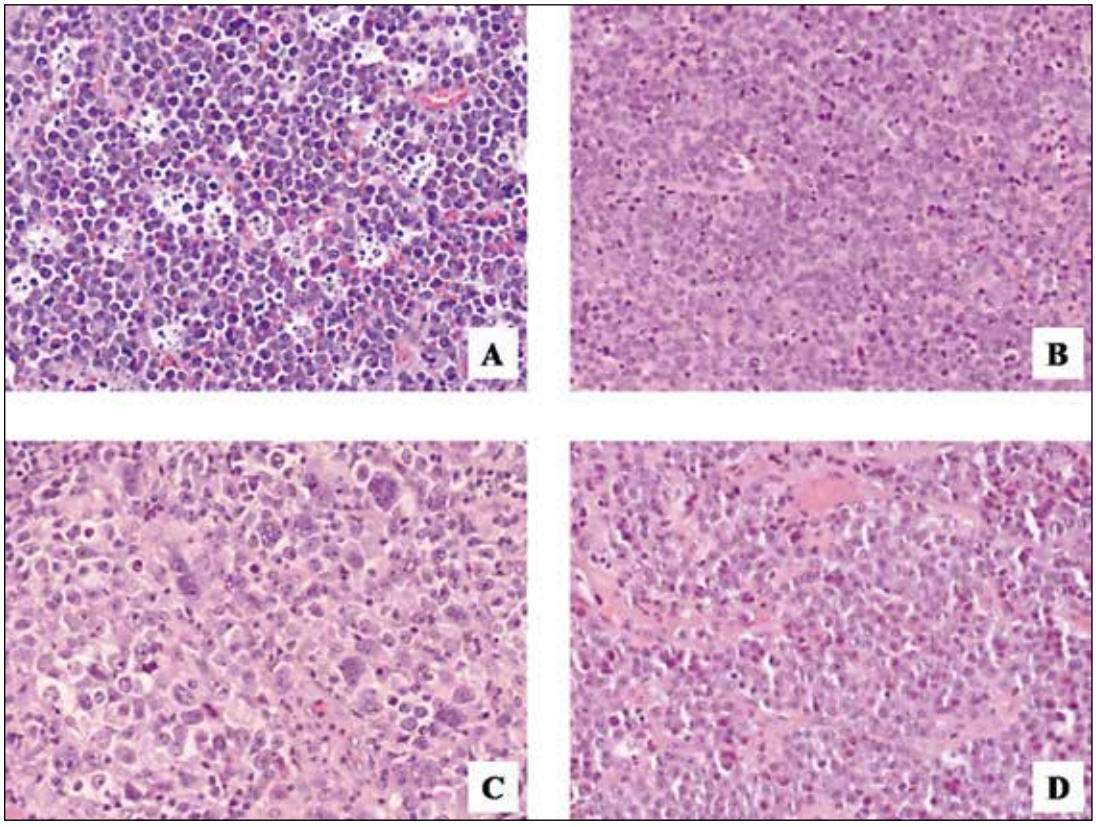

FIGURE 2 • Lymphoid neoplasms characterized by *MYC* deregulation (A: Burkitt lymphoma-BL; B: diffuse large B-cell lymphoma-DLBCL; C: B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL; D: plasmablastic lymphoma; haematoxylin and eosin, original magnification, O.M.: 20x).

harbouring FISH-detected gene-activating breaks in both *MYC* and *BCL-2*, suffer from poor response to standard therapy and have an adverse prognosis (117). Conventional cytogenetics or molecular biology method could be considered the gold standard for the identification of *MYC* rearrangements, because they identify the translocation patterns. From a technical stand-point, to test for the *MYC* rearrangement, break-apart probes and not dual-fusion are typically used to account for the non-*IGH* translocation partners of *MYC*. For *BCL-6* either break-apart or *IGH/BCL-2* dual fusion testing strategy could be used, whereas for *BCL-2* translocations break-apart probes are recommended (118). Cytogenetic analysis, however, is cumbersome, time-consuming and not routinely performed in the work-up of lymphomas at many centers. Moreover, FISH technology is generally not designed to detect genetic deregulation that affects gene expression on the transcriptional and translational levels. The availability of anti-MYC antibody (clone Ab 32072, dilution 1:200, Abcam-Cambridge, United Kingdom), amenable for use in formalin-fixed paraffin-embedded tissue offers a less costly and less laborious means of de-

tecting MYC over-expression and represents a key step forward in studying MYC-associated lymphomas (115).
By comparing expression profiles of *MYC* gene rearrangement and MYC protein expression emerged that *MYC* gene rearrangements do not necessarily correlate with MYC protein expression. In fact by applying immunohistochemistry, the frequency of MYC protein expression appears much higher than what is detected by FISH standard method (116). Therefore, nowadays the key problem in the hematopathology field is to define the clinical impact of the double/triple expressor status apart from the presence of genetic alterations characterizing the so-called double/triple hit (DH/TH) lymphomas. Although genotype controls phenotype because genes direct the products of proteins, there are proteins, in turn, that dictate virtually every reactions in the cells and thus are directly responsible for observable characteristics and effects. From a biological point of view, phenotype as the results of multiple cross-talks between genes, proteins and environment, is more relevant than genotype, proteins expression levels likely representing a more direct measure of the activity of a particular gene. Accordingly, assessing the expression of MYC, BCL-2 and BCL-6 by IHC is attractive because this should identify double/triple-expressing lymphoma patients whose disease are driven by increased MYC, and/or BCL-2, and/or BCL-6 activity secondary to a variety of mechanisms, not solely translocations (115-118).
However, there is a significative interpretative challenge to define the score of positivity for MYC, BCL-2, BCL-6 although Authors have used the threshold of greater than 40% for MYC and greater than 70% for BCL-2, respectively. Moreover, MYC staining may be heterogeneous, rendering accurate quantification wondering. In addition, many double-expressor lymphomas reflect DLBCL, for instance, activated B-cell (ABC) type of DLBCL, in which the MYC and BCL-2 are over-expressed by other mechanisms than translocations (119, 120).
For all these reasons, the updated WHO classification of tumours of hematopoietic and lymphoid tissues that will be published in the next future, suggests to limit the definition of DH/TH lymphoma only in the case in which the "hit" could be demonstrated by cytogenetic or molecular biology. Accordingly, it proposes a novel classification for high grade B-cell lymphomas, including: diffuse large B-cell lymphoma, not otherwise specified; high grade B-cell lymphomas, not otherwise specified; high grade B-cell lymphomas with *BCL-2* and/or *BCL-6* and *MYC* rearrangements. *Table 2* summarizes the clinical, morphological, immunophenotypical and molecular biology features of these novel subtypes.

MYC-TARGETED THERAPY

Because of its oncogenic properties in neoplastic cells, *MYC* has become an interesting and feasible target for novel therapies of a variety of human malignancies. However, MYC protein itself has generally been considered "undruggable" and the potential approaches have been directed at reducing its expression (121). It has been shown that several small molecules target the transcription of *MYC* gene directly or the MYC downstream path-

ways. Especially, G-rich region of *MYC* promoter has become a promising target. Many reports demonstrate that low-molecular weight compounds have a potential to be developed into therapeutic drugs in individualized cancer therapy. Even with the advances in the field of drug design and

TABLE 2 • Novel subtypes of high grade B-cell lymphomas included in the updated WHO classification of tumours of hematopoietic and lymphoid tissues.

	Clinic	Morphology	Immunohisto-chemistry	Molecular Biology
DLBCL, not otherwise specified (NOS)	Adult and elderly, nodal and extranodal sites	Large tumour cells with abundant cytoplasm, large nuclei and prominent nucleoli	Pan-B cell antigens positivity; Ki-67: 30-40%	ABC or GC type
High grade B-cell lymphoma, NOS	Elderly patients	Histologic appearance that resembles Burkitt lymphoma more than DLBCL; irregular nuclear contour; although many areas mimic DLBCL, the nuclear size remains small	Positivity for CD20, negativity for TdT, MUM1/IRF4; BCL6, CD10,Ki-67 variable; MYC expression depends on the presence of *MYC* translocation	Approximately 20-35% of the cases have a *MYC* breakpoint (with or without increased copy numbers or rarely amplification of 18q21 involving *BCL2*). The presence of *BCL2* and/or *BCL6* breakpoint in combination with *MYC* breakpoint should be excluded
High grade B-cell lymphoma, with *BCL2* and/or *BCL6* and *MYC* rearrangements/ translocations	Elderly patients, two peaks of incidence (30 and 70 years). Widespread disease, including involvement of lymph nodes with more than one extranodal site, bone marrow (59-94%) and CNS (45%-60%)	Fibrosis as well as starry sky macrophages may be (focally) present. The number of mitotic figures and apoptotic figures is highly variable. The nuclei have a variable size and contour. The cytoplasm is usually more abundant and less basophilic than in BL	Neoplastic cells are CD19, CD20, CD79a, PAX5, BCL2 positive and TdT negative. CD10 and BCL6 (75-90%) MUM1/IRF4 (20%)	In addition to the *MYC* rearrangement, all cases contain *BCL2* rearrangement at 18q21 and/or a *BCL6* rearrangement at 3q27. HGBL-DH often have complex karyotypes with many other structural and numerical abnormalities Sequencing studies reveal frequent *TP53* mutations mainly in the *MYC&BCL2* double hit cases, few *MYD88* mutations

HIV: Human Immunodeficiency Virus, ABC: Activated B-cells, GC: germinal center, EBER: Epstein-Barr encoding region, ALK: Anaplastic lymphoma kinase, CNS: central nervous system.

in the mechanisms underlying the MYC over-expression in tumor cells, it is still difficult to obtain highly specific and active anti-cancer drugs (121).

Several approaches may be employed to target MYC activities:

– *Blocking MYC activation:* one small molecule, 10058-F4 recognizes the MYC amino acid residues 402-412, which reside within the HLH-LZ domain inhibiting the MYC/MAX heterodimerization (122). Despite its success *in vitro* (cell cycle arrest and apoptosis) 10058-F4 did not prove to be effective in *in vivo* animal studies primarily because of its limiting PK/PD properties.

– *Inhibiting MYC-associated chromatin modifications:* a small molecule, named JQ1, was designed to BRD4 the first bromodomain of the BET family member BRD4. The molecule inhibits MYC pathway activation by targeting chromatin modifications associated with the process of MYC-mediated transcriptional activation. Thanks to encouraging results in *in vitro* models, further preclinical efficacy studies on BRD4 inhibition against a wider range of cancers with elevated MYC expression *via* different mechanisms are ongoing.

– *Exploiting MYC-dependent synthetic lethal interactions:* the essential role of MYC in both cancer and normal tissue development and homeostasis raises the concern that even if direct MYC inhibitors could be developed, they might be too toxic for clinical use. An alternative approach is to identify and target signalling pathways activated by MYC selectively in tumor cells but not in non-tumorigenic cells. Several Poly (ADP-ribose) polymerase (PARP) inhibitors are currently being evaluated in late phase clinical trials. PARP inhibitors thus serve as an important proof of concept that synthetic lethal approaches are clinically relevant and exploitable.

– *Targeting cell cycle kinases:* cancer cells with elevated MYC expression often exhibit highly proliferative and poorly differentiated phenotypes, suggesting that the MYC-activated cells are poised to continuously drive the cell cycle. It may also suggest that other cellular processes have had to adjust to accommodate such significant changes in cell physiology (123, 124). Based on these observations, a dinaciclib phase I trial using MYC expression and signalling as a clinical correlate biomarker of response has been initiated (ClinicalTrials.gov Identifier: NCT01676753). This is among the first trials in which a small molecule CDK inhibitor is used to determine whether MYC overexpressing cancers are selectively targeted. Among other CDKs, an interphase cell cycle kinase CDK2, was reported to be essential for the viability of neuroblastoma cells with *MYCN* amplification (125). CDK2-specific siRNAs and seliciclib (also known as roscovitine), a small molecule CDK inhibitor with higher specificity toward CDK2, 7, and 9, induced apoptosis in a panel of established neuroblastoma cell lines. The sensitivity to CDK2 inhibition was dependent on wild-type p53 and MYCN over-expression. Seliciclib was previously evaluated in phase I and II trials. The potential clinical efficacy of CDK2 inhibition has been controversial. Earlier genetics studies demonstrated that CDK2 was not essential for mammalian embryonic development *in vivo* or for the cell cycle progression of non tumorigenic as well as tumorigenic cells *in vitro* (126, 127). It was recently reported that specif-

ic small molecule inhibition of CDK2 kinase activity diminished cell cycle progression in non-transformed and MYC-transformed epithelial cells without induction of cell death (128, 129). Interestingly, CDK2 genetic depletion *via* siRNA in the same system resulted in accelerated cell proliferation, which was accompanied by the upregulation of CDK1 that has been shown to be capable of functionally compensating for any of the interphase CDKs (128). Whether CDK2 inhibitors will have a role for therapy of neuroblastomas or other MYC or MYCN-driven tumors remains to be determined. Mitosis regulators Aurora kinases A and B, which control mitotic spindle attachment and dynamics, have been targeted in MYC-deregulated cancer cells. It was reported that multiple Aurora selective small molecule inhibitors caused strong antitumorigenic effects-including cell cycle arrest, apoptosis, and autophagy-in model epithelial cells in a MYC-dependent manner (130). Small molecule Aurora kinase inhibitors were also effective in extending animal survival in multiple mouse models of MYC-induced lymphomas (130). More recently, an Aurora kinase small molecule inhibitor, alisertib, was found to increase animal survival in a mouse model of *MYCN*-driven neuroblastoma, in which Aurora kinase plays a key role in maintaining MYCN protein stability that is central to its tumorigenic activity (131). Alisertib is currently being evaluated in numerous phase I and II trials. Chk1-an essential kinase involved in DNA damage and cellular stress-responsive pathways-is another cell cycle-related kinase that has been targeted in MYC-deregulated cancer cells. The hypothesis is that highly proliferative MYC-driven cancer cells increase endogenous DNA damage from replicative stress, DNA replication fork collapse, or oxidative stress. A Chk1 checkpoint allows for repair of these insults and protects rapidly proliferating MYC-driven cells from these endogenous DNA damage insults (132).

– *Other possible target:* beyond the cell cycle, MYC has also been shown to regulate numerous additional signalling pathways critical for tumor development and maintenance. A current challenge is to identify additional synthetic lethal targets in these signalling pathways downstream of MYC. Among all the possible targets there are: small ubiquitin-related modifier (SUMO)-activating enzyme 1/2 (SAE1/2, a heterodimer complex), metabolism regulator enzymes, 5' AMP-activated kinase (AMPK)-related kinase 5 (ARK5) or AMPK itself (121).

A LOOK FORWARD

To overcome the conflicting data present in the literature on the correlation among *MYC* gene aberrations and MYC protein expression, we reviewed a total of 119 clinical, morphological and immunophenotypical typical BL cases and we checked the expression of MYC at both mRNA and protein level by respectively RT-PCR and immunohistochemistry. In addition, FISH analysis for *MYC*-translocation was also performed by using the available probes (dual-color break-apart probe). Different patterns of *MYC* gene translocation/MYC protein expression were identified:

– 99 cases bearing a translocation involving *MYC* gene expressed MYC at both mRNA and protein level (positivity

in almost 80% of neoplastic cells). This finding is in line with the data reported in the literature (133, 134).

– 10 cases in which a translocation involving *MYC* gene was not detectable, expressed MYC at both mRNA and protein level. It is known that none of the techniques currently used to diagnose genetic changes can unambiguously rule out all of *MYC* translocations (134). In fact this may be due to technical failure of FISH, as these cases may present with a very small excision of *MYC* and insertion of the gene into one of the *IG loci*, which is missed by the available probes. Another option is that the breakpoint is localized far outside the region covered by the currently available FISH probes. However, some observations suggest that mechanisms other than translocation may be responsible for elevated MYC protein expression in BL even in the absence of genomic rearrangements (134). Therefore, we investigated the microRNA expression profile of *MYC* translocation-positive and *MYC* translocation-negative BL cases in order to uncover possible differences at the molecular level. We found that *MYC* translocation - positive and negative - BL cases are slightly different in terms of microRNA and gene expression, and we validated our findings at the mRNA and protein levels. Interestingly, in *MYC* translocation-negative BLs we found overexpression of DNA methyltransferase (DNMT) family members, secondary to hypo-expression of hsa-miR-29 family. This finding suggests an alternative way for the activation of lymphomagenesis in these cases, based on global changes in methylation landscape, aberrant DNA hypermethylation, lack of epigenetic control on transcription of targeted genes, and increase of genomic instability. In addition, we observed the over-expression of another *MYC* family gene member, *MYCN* that may therefore represent an additional mechanism for malignant transformation (*Figure 3*). Our finding may be helpful to explain the pathogenetic mechanisms of tumours in which over-expression of *MYC* is independent of a chromosomal translocation or a gene amplification (134);

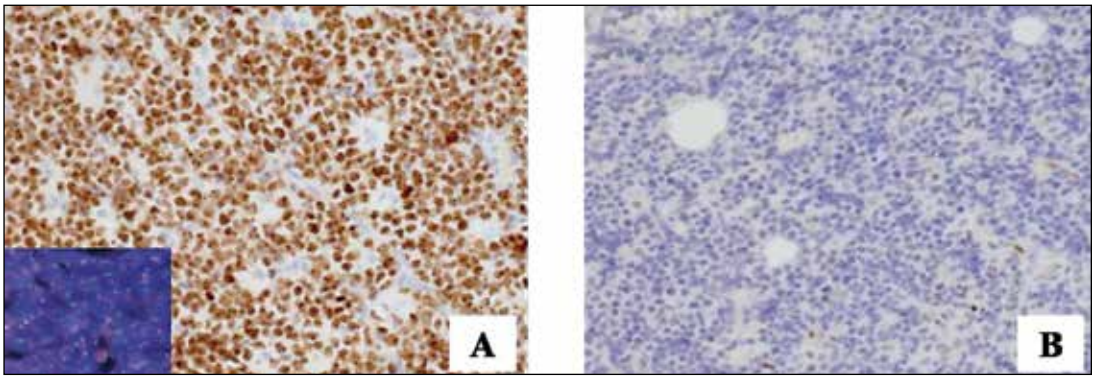

FIGURE 3 • Inverse correlation between MYC (A) and N-MYC (B) protein expression in a BL case carrying a *MYC/IGH* rearrangement (A inset) (A: MYC stain, B: N-MYC stain; A, inset: FISH analysis; A-B, O.M.: 20x).

– 10 cases showed *MYC* gene translocation but did not express MYC at protein level. To evaluate if the negativity for MYC protein was due to a defect in the transcription of *MYC* gene or in the assembling of the protein, RT-PCR for MYC mRNA was performed. We found that among the 10 cases not expressing MYC protein, 5 lacked also MYC mRNA. A study is ongoing in our laboratory to shed new light on how a *MYC* gene aberration does not result in MYC mRNA and protein over-expression.

Authorship

Wrote the paper: MRA, GLB, LL; made contributions to acquisition of histological images: AB; contributed in the field and fruitful discussion: RS; coordinated the work and gave final approval of the version to be published: MRA, LL. All authors read and approved the final manuscript.

Conflict of interest

The Authors declare that they have no competing interests.

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REVIEW

Consolidated and innovative approaches in lymphoma. Indolent non-follicular lymphomas

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SUMMARY

Non-follicular indolent NHLs are a diverse group of disorders with different presenting features, behaviour patterns, and treatment outcomes. A precise diagnosis can be difficult to achieve in a not inconsiderable number of cases. Specific diagnostic criteria are needed to more precisely define some of the rarer indolent tumours. Furthermore, universally accepted therapeutic guidelines do not exist and our current knowledge is largely based on retrospective analyses. New drugs with different mechanism of action have been introduced in recent years, showing notable activity in indolent non-follicular lymphomas. They include new monoclonal antibodies, such as obinutuzumab and new small molecules with targeted action, lenalidomide, ibrutinib and PI3K inhibitors being the most representative. Carefully designed, prospective clinical studies are needed to further assess therapeutic approaches for these indolent lymphomas.

INTRODUCTION

Indolent non-follicular non-Hodgkin lymphomas are a heterogeneous group of lymphoid malignancies with different presenting features, genetic characteristics, behaviour patterns and treatment outcomes. According to the World Health Organization (WHO) classification small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma (LPL) and marginal zone lymphoma (MZL) are the main subtypes. In addition, MZLs are further categorised into 3 clinicopathologic entities: nodal MZL, splenic MZL (SMZL) and extranodal MZL of the mucosa-associated lymphoid tissue (MALT). The present review will focus on marginal zone lymphomas.

Key words: Indolent non-follicular lymphomas, monoclonal antibodies, tyrosine kinase inhibitors, PI3K inhibitors.

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CONSOLIDATED ASPECTS

Together, MZLs represent 5-17% of all NHLs in adults. These lymphoma subtypes are heterogeneous and display different characteristics, with clinical

and biological variations depending on the organ in which the lymphoma arises. All three MZL subtypes have a tendency to disseminate, and can transform into aggressive diffuse large B-cell lymphoma. Many individuals who develop these MZLs have a history of chronic infections or autoimmune diseases.

MALT lymphoma

MALT lymphoma is the more common MZL subtype, accounting for 70% of them and constitutes the third most common type of NHL in western countries. These lymphomas originate in sites normally devoid of lymphoid tissues but, paradoxically, these affected sites accumulate B-cells in response to chronic infection or inflammation. Such infections include *Helicobacter pylori* (HP) and *Helicobacter heilmannii* in the stomach, *Borrelia burgdorferi* in the skin, *Chlamydia psittaci* in the ocular adnexal, *Campylobacter jejuni* in the small intestine and, recently, *Achromobacter (Alcaligenes) xylosidans* in the lung. Hepatitis C virus (HCV) and parasites have also been associated with MALT lymphomas.

Several genetic abnormalities can be found in MALT lymphomas. The most common structural cytogenetic abnormalities are trisomies 3 and 18, and the most common recurrent translocation is translocation t(11;18)(q21;q21) that can be found in 30-40% of gastric MALT lymphomas. Less frequent translocations are t(1;14)(p22;q32), t(14;18)(q32;q21), t(1;2)(p22;p12) and t(3;14)(p14.1;q32), among others.

Clinical features at presentations vary depending of the primary site of MALT lymphoma. Approximately, half of the cases arise in the stomach or the other half in other extranodal sites: skin, lung, salivary glands, ocular adnexa, etc. It is important to remind that up to 50% of patients with extra-gastric MALT lymphoma present with disseminated disease, but these figures vary according to the exhaustive of the work-up. Staging is challenging in MALT lymphomas, and in addition to standard lymphoma work-up, some additional specific studies should be performed based on the primary site of origin. The role of PET scans is controversial in MALT NHL and consequently, PET is not recommended for daily practice, but can be useful in selected cases.

The prognosis of patients with MALT lymphoma is excellent regardless of site of origin and treatment modality. In HP positive gastric MALT NHL, eradication of HP achieves complete remissions in 60-90% of patients, at a median time of 2-6 months. Treatment with doxycycline has also displayed an overall response rate of 65% in stage I ocular adnexa MALT lymphoma, showing higher responses among those in which Chlamydia was eradicated. Similarly, treatment of HCV-positive MALT lymphomas with interferon and ribavirin or with the newer protease inhibitor combinations(interferon-free) has led to complete responses of the lymphoma.

For patients with localised disease, surgery or radiotherapy has been used in the past. Gastrectomy is currently abandoned and its role is limited only to treat complications, such us perforation or haemorrhage. However, surgery might be reasonable used in other extranodal sites (skin and other sites). Radiotherapy of the stomach and the perigastric nodes is the treatment of choice in some centres, with

excellent local control of disease and low rate of local relapses.
Systemic treatment is usually reserved for patients with disseminated disease or for those relapsing to antibiotics, surgery or radiotherapy. Patients are usually treated according to the same principles as for other advanced-stage indolent lymphomas. Conventional chemotherapy (alkylating agents, purine nucleoside analogs, anthracyclines, etc.), alone or in combination, has demonstrated activity in gastric and extra-gastric MALT lymphomas, with response rates of over 70-90%. Rituximab has demonstrated activity in patients with (relapsed or *de novo*) gastric or extra-gastric MALT lymphoma. A randomised trial carried out by the International Extranodal Lymphoma Study Group (IELSG) has demonstrated that combination of chlorambucil plus rituximab achieves higher complete remission (CR) rates and better event free survival (EFS) than chlorambucil or rituximab alone. Recently, the GELTAMO group has shown that bendamustine plus rituximab is very active in first-line MALT lymphoma (gastric and extra-gastric). CR rate was 97% and EFS at 4 years was 88%. In addition, only 24% of patients needed more than four cycles of treatment, providing a favourable safety profile. Other novel agents have shown antitumor activity in MALT lymphomas and will be developed later.

Splenic MZL
Splenic MZLs generally occur in individuals about65-70 years of age and are rare, accounting for approximately 20% of all MZLs. This disease presents with splenomegaly, sometimes with loco regional lymph nodes, but rarely with peripheral lymph node involvement. Blood dissemination and bone marrow infiltration with small CD20+, CD5-, CD10-, CD103-, CD23-, CD43- lymphocytes is seen. Liver infiltration is reported in up to 90% of patients showing intrasinusoidal and nodular infiltrate in portal tracts.
Cytogenetic analyses have demonstrated chromosomal aberrations, being deletion of 7q32 and complete or partial trisomy 3 the most frequent abnormalities. In addition, other chromosomes frequently involved, in order of frequency, are: 1, 8, 6, 12, and 14. Recently, with the use of next generation sequencing, recurrent somatic mutations have been described in several genes: *NOTCH2, NOTCH1, SPEN, DTX1*, etc. Recently, *KLF2* mutation has been found in 42% of SMZLs, but it is rarely in other B-cell lymphomas.
Clinically, the disease evolves from asymptomatic lymphocytosis and/or splenomegaly with an indolent, but usually progressive course, often manifested with cytopenias due to bone marrow infiltration and hypersplenism. A monoclonal serum component is frequently seen as well as other autoimmune phenomena (autoimmune hemolytic anemia, immune thrombocytopenia, primary biliary cirrhosis, etc.). A high prevalence of HCV infection in SMZL has been reported, especially in endemic areas for HCV.
Various biological and clinical prognostic factors have been specifically proposed for this lymphoma subtype. The European SMZL Working group has recently developed the HPLL score (hemoglobin, platelet count, high LDH and extrahilar lymphadenopathy), providing a stratification system that allows the identification of three

groups of SMZL patients with different outcomes.

Since most patients are asymptomatic at diagnosis or have low tumour burden, they do not require immediate treatment for several years and can be managed with a "wait and watch" strategy. In patients with progressive and symptomatic disease, mainly due to hypersplenism, disseminated lymphadenopathy or severe bone marrow infiltration, treatment is required. Currently, treatment for SMZL is not standardized.

Classically, splenectomy was the first-line treatment for most of these patients, especially for those with cytopenia or symptomatic splenomegaly. Typically, this procedure rapidly improved clinical symptoms, with correction of cytopenias, particularly neutrophils and platelets, and reduction of circulating lymphoma cells.

Paradoxically, several cases of lymphoma progression in the bone marrow after splenectomy have been reported. Chemotherapy with alkylating agents or purine nucleoside analogs is active, although most of the series reported in the literature are retrospective and there are not randomised studies performed. Rituximab has been investigated in phase 2 studies, yielding better results than previously seen with chemotherapy alone.

Therefore, rituximab is becoming the treatment of choice for these patients. In addition, combination of rituximab with chemotherapy might be a sensible option for patients with more advanced disease or with adverse prognostic factors. Patients with anti-HCV antibodies and HCV RNA can achieve sustained hematologic responses with interferon treatment, alone or in association with ribavirin, or at the present time with the new combinations against HCV.

Nodal MZL

Nodal MZL is the less common MZL subtype, accounting for 10% of them. This nodal lymphoma shares morphologic characteristics of marginal zone cells, sometimes with lymphoplasmocytoid differentiation and a variable content of plasma cells, monocytoid B-cell and even medium to large cells. Although several recurrent clonal abnormalities have been identified, there is no characteristic cytogenetic profile in this lymphoma type. However, the absence of *MYD88* mutations in these lymphomas might be a useful tool to differentiate them from lymphoplasmacytic lymphomas that very commonly carry mutations in *MYD88*. Median age at diagnosis is about 55-65 years and there is a slight predominance of females. Disseminated nodal involvement occurs in 75% of cases, with bone marrow infiltration in approximately 21-62%of cases. Serum M-component is found in less than 10-15% of cases and cytopenias are rare.

The cause of nodal MZL is not known, but an association with HCV is also notorious. Nodal MZL is more aggressive than the other MZL subtypes and shows shorter progression free survival. However, by and large, overall survival at 5 year is about 56-69%, which is similar to that reported for the other MZLs subtypes.

Treatment recommendations are difficult, since there are limited data on nodal MZL. Treatment guidelines for nodal MZL have followed similar recommendation as follicular lymphomas. With the incorporation of rituximab to

lymphoma treatment, chemoimmunotherapy is the standard. Regimens with alkylating drugs such as CVP or CHOP or with purine nucleoside analogs have been used in the last decade. Recently, the STIL study has showed that combination of rituximab with bendamustine achieve good results in terms of responses and PFS. These results have been confirmed by the FIL in a phase 2 study, although the response rates for nodal MZL were inferior to those seen in LPL or SLL patients. Of interest, the safety of the combination was favourable. Other new agents with activity in nodal MZL will be discussed later.

INNOVATIVE ASPECTS

Indolent non-follicular B-cell non-Hodgkin's lymphomas generally experience a long-term survival but a subset of cases is characterized by a more aggressive behaviour (1). There is a growing understanding of the pathways involved in indolent lymphoma pathogenesis and of the role of microenvironment and several new agents are currently being tested in indolent follicular and non-follicular lymphomas (2). In this setting, here we report data from trials with new drugs including a significant portion of indolent non-follicular lymphomas.

Lenalidomide

Lenalidomide is an immunomodulatory agent that has been used as monotherapy or in combination with immunotherapy in phase 2 studies dedicated to indolent B-cell lymphomas (relapsed/refractory or previously untreated) (3). In patients with relapsed indolent lymphomas, single agent lenalidomide shows activity (4). However the combination of lenalidomide with rituximab (R2 scheme) impressively increased the response rates and the duration of response (5-8). A phase II trial including 27 patients with relapsed/refractory patients including 3 marginal zone lymphomas (MZL) (52% rituximab-refractory) demonstrated overall response rate (ORR) and complete response (CR) rates of 74% and 44%, respectively; median PFS was 12.4 months (7). R2 scheme has demonstrated excellent clinical activity in 110 (30 MZL) newly diagnosed patients with indolent lymphomas enrolled in a phase II trial: ORR and CR rates were 90% and 65%, respectively; in patients with MZL ORR was 89%, CR was 67% and 3-year progression-free survival (PFS) and overall survival (OS) were 87% and 100% respectively; toxicity was acceptable (8). In a recent trial from Fondazione Italiana Linfomi (FIL), 39 relapsed patients with indolent lymphomas (8 MZL) received R2 scheme (9): ORR was 75% and 2-year PFS and DFS were 75% and 100%, respectively for MZL. Recently, a phase I study on combination of bendamustine, lenalidomide, rituximab have been reported in 20 patients with B-cell malignancies (3 MZL) (10): neutropenia was the most common grade 3 and 4 toxicity but overall clinical activity was modest.

BTK inhibitors

Ibrutinib is a small-molecule irreversible inhibitor of Bruton's tyrosine kinase (BTK), a critical signalling kinase in the pathway activated by the B-cell receptor that is relevant in the survival and progression of some mature B-cell neoplasms. In a phase I study, ibrutinib was used in 56 patients with B-cell malignancies, including 4 patients with

MZL: CR was 16% (ORR 60%9, and the median PFS was 13.6 months (11). On the other hand, in a phase I/Ib trial in 48 patients (only 1 MZL case) with newly diagnosed or relapsed/refractory lymphoma, ibrutinib was combined with bendamustine and rituximab (12): ORR was 72% with a CR of 52%. In patients with relapse/refractory Waldenström's macroglobulinemia, ibrutinib monotherapy obtained an ORR of 90.5%, with higher response rates observed in patients with MYD88L256P than those with MYD88WT (13).

Data on combination of ibrutinib with rituximab are only available in mantle cell lymphoma (MCL) (14) and follicular lymphoma (FL) (15) while clinical trials with second-generation BTK inhibitors including acalabrutinib (ACP-196) (16), BGB-3111 (17), ONO-4059 (18) and CC-292 (19) are ongoing.

PI3K inhibitors

Idelalisib (CAL-101, GS-1101) is a potent inhibitor of PI3Kδ, a signalling pathway frequently hyperactive in B-cell lymphomas (20, 21). In phase I studies idelalisib had an acceptable safety profile and promising activity in patients with chronic lymphocytic leukemia (22) and indolent lymphomas (23). In a phase II single-arm study in 125 patients with relapsed/refractory indolent lymphomas, 15 patients with MZL were enrolled (24). All patients did not respond to rituximab combined with an alkylating agent or had relapsed within 6 months after this approach. Regarding response, ORR was 57%, CR was 6% and tumor reduction was reported in 90% of the patients; the median duration of response was 12.5 months. In an updated report, among the 15 patients with MZL, the ORR and CR rates were 47% and 7%, respectively; median PFS was 6.6 months and a median duration of response was 18.4 months (25). The most frequent adverse events were aminotransferase elevation, neutropenia, diarrhoea and pneumonia (26). Interestingly the combination of idelalisib with rituximab and lenalidomide resulted to be too toxic to be further developed (27).

Among other PI3K inhibitors, duvelisib (IPI-145) is an oral, dual p110δ/γ inhibitor (28): in a phase I study in 32 patients with relapsed/refractory indolent lymphomas ORR was 65% with 5 CR in FL (29). Other studies on new PI3K inhibitors are ongoing including INCB-40093 (30), TGR-1202 (31) and AMG-319 (32).

Obinutuzumab (GA101)

Obinutuzumab is a glycoengineered type II anti-CD20 monoclonal antibody characterized by an increased antibody-dependent cell-mediated cytotoxicity and reduced complement-dependent cytotoxicity (33). This molecule proved to be effective in relapsed/refractory indolent lymphomas but numbers of non-follicular cases were limited (34, 35). Preliminary data have been recently reported from the GADOLIN study (36): this is a phase III study comparing obinutuzumab plus bendamustine followed by obinutuzumab maintenance with bendamustine alone in rituximab-refractory patients with indolent lymphomas (non-follicular cases were 75 out of 396 patients). Responses were overlapping in 2 treatment arms but immunochemotherapy showed a better PFS but no advantage on OS. In the subset analysis PFS was significantly longer for FL in antibody arm and not significant for indolent non-follicular lymphomas but study was not

planned to find a difference in the non-follicular subset. A phase III study comparing 2 different immunochemotherapy schemes (chemotherapy plus rituximab vs chemotherapy plus obinutuzumab) is ongoing in indolent B-cell lymphomas (GALLIUM, NCT01332968). Regarding new combinations, in a phase I trial the combination of lenalidomide plus obinutuzumab has been tested in relapsed indolent lymphomas: ORR was 93% and CR was 27%. Toxicity was acceptable and similar to the R2 scheme (37).

Other drugs

Bortezomib is a proteasome inhibitor active as single agent in previously untreated and relapsed/refractory extranodal MZL of mucosa-associated tissue (MALT) (38-40). In a study on 32 patients with relapsed/refractory MALT lymphoma treated with bortezomib monotherapy, ORR was 48% (40). Patients with previously untreated indolent lymphomas (5 MZL) have been enrolled in a phase II trial of R-CHOP plus bortezomib (modified dose of vincristine) (41): in 29 patients ORR was 100% (CR 66%) and the 4-year PFS and OS were 83% and 93%, respectively. As expected, peripheral neuropathy was the most common adverse event. A phase II trial evaluated the activity and safety of everolimus in 30 patients with relapsed/refractory MZL (42): ORR was 25% and the median response duration was 6.8 months but 17 patients stopped treatment earlier due to toxicity.

Vorinostat is an orally histone deacetylase inhibitor approved by FDA for the treatment of T-cell lymphomas. In a phase II study vorinostat as single agent demonstrated activity in patients with relapsed/refractory FL and MZL: in MZL the ORR was 22% and median duration of response resulted of 27 months (43). Recently, vorinostat has been combined with rituximab in a phase II study (44): a total of 28 patients with untreated or relapsed/refractory FL, MZL or MCL were enrolled. ORR was 46% for all patients (67% for first-line patients and 41% for relapsed/refractory patients) and the median PFS was 29.2 months for the entire cohort.

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Elotuzumab: a new monoclonal antibody for multiple myeloma therapy

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SUMMARY

For decades, since its first description, multiple myeloma represented a hard challenge for clinicians due to the scarce results obtained with conventional chemotherapies. Autologous stem cells transplantation and novel drugs such as immunomodulating drugs (IMIDs) and proteasome inhibitors (PIs) demonstrated remarkable results and encouraged the search for new therapies. Elotuzumab is a first in class anti-CS1 monoclonal antibody showing good toxicity profile and promising efficacy data in association with standard treatment schedules.

INTRODUCTION

Multiple myeloma (MM) is characterized by an uncontrolled proliferation of neoplastic plasma cells in the bone marrow and, less frequently, in other sites. A significant role is played by the bone marrow microenvironment (represented by extracellular matrix, hematopoietic, endothelial and stromal cells, osteoblasts and osteoclasts) which produces cytokines, growth factors and adhesion molecules promoting survival of myeloma cells and causing the clinical features of the disease: anemia, bone lesions, hypercalcemia and renal failure.

In recent years the introduction of novel drugs, as PIs and IMIDs, led to a real improvement in patients prognosis and quality of life, but virtually all patients eventually relapse and become refractory to treatments.

Several efforts have been done to identify new molecular targets in order to combine drugs with different mechanisms of action: histone deacetylases, signal transduction pathways, cycline-dependent kinases and heat shock proteins have been studied as potential therapeutic targets in multiple myeloma. Less than one year ago, panobinostat was the first histone deacetilase inhibitor to obtain FDA approval for relapsed/refractory multiple myeloma (RRMM) patients in combination with bortezomib and dexamethasone. Moreover, new proteasome inhibitors (such as carfilzomib,

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ixazomib and oprozomib) and monoclonal antibodies (such as antiCD38 daratumumab, anti RANKL denosumab, antiCS1 elotuzumab and anti PD-1 pembrolizumab) are currently included in clinical trials. Carfilzomib was approved in July 2015 for RRMM patients in combination with lenalidomide and dexamethasone after one to three prior therapies. Recently, other three drugs had FDA approval: ixazomib was approved for the use in triple combination with lenalidomide and dexamethasone in RRMM after at least one prior therapy, daratumumab was approved as single agent for RRMM after at least three prior treatments, while elotuzumab had FDA approval as a "breakthrough therapy" for the use in combination with lenalidomide and dexamethasone in RRMM patients who have received one to three prior therapies.

Elotuzumab - mechanism of action

Monoclonal antibodies can act with various mechanisms for example interfering with receptor-ligand interaction, activating signaling transduction cascades, carrying cytotoxic agents, inducing cell killing by antibody-mediated cellular cytotoxicity or macrophage-mediated phagocytosis and complement-dependent cytotoxicity.

Elotuzumab is a first in class humanized IgG1 monoclonal antibody targeting surface glycoprotein CS1 (a member of the SLAM family receptors), which is physiologically expressed on normal plasma cells, NK cells, most CD8+ T cells, activated B cells, mature dendritic cells and few CD4+ T cells (1). High CS1 levels are also found on malignant plasma cells (2).

Preclinical studies suggested that elotuzumab could promote antibody-dependent cellular cytotoxicity mediated by NK cells (by targeting CS1 on MM cells) and also induce NK cells activation and cytokine release (by targeting CS1 on the NK cells surface which leads to the phosphorylation of ERK). Elotuzumab can also act as a homotypic adhesion receptor, promoting CS1-CS1 interactions for example between NK cells and MM cells resulting in increased cytotoxicity (3). Since CS1 seems to have a role in MM cells adhesion to bone marrow stromal cells, elotuzumab could also interfere with the proliferation and survival of the plasma cells sustained by their connections with the microenvironment (2, 4).

Phase I clinical trials

A phase I trial, conducted in RRMM patients and published in 2012, explored the tolerability of elotuzumab monotherapy administered at doses ranging from 0.5 to 20 mg/kg every 2 weeks. Patients had a median of 4.5 prior therapies. No maximum tolerated dose was identified up to the maximum planned dose of 20 mg/kg. Elotuzumab demonstrated poor activity as a single agent: 9 over 34 evaluable patients had stable disease and the remaining progressed. However it showed a good toxicity profile with most frequent adverse events consisting in grade 1-2 infusion reactions (5).

Another phase I trial was designed to study the combination of dose-escalated (2.5 to 20 mg/kg) elotuzumab with bortezomib in patients treated with 1 to 3 prior therapies; of 27 evaluable patients, 11 were already been treated with bortezomib. ORR was 48%, including 2 of 3 patients formerly refractory to bortezomib. The median

TTP was 9.5 moths (6), comparing to the 6.2 months of the APEX trial (phase III study of bortezomib monotherapy in RRMM patients) (7). Dose-escalated elotuzumab (5 to 20 mg/kg) was also tested in combination with lenalidomide and low-dose dexamethasone in a phase I study conducted by Lonial S et al. (8).
No dose limiting toxicities were observed up to the maximum planned dose of 20 mg/kg. Two patients experienced a severe infusion reaction during the first treatment cycle, one grade 4 anaphylactic reaction and one grade 3 stridor. The ORR was 82%; patients with prior exposure to lenalidomide were included and responses (one PR and one VGPR) were observed in two of six evaluable patients. The median TTP was not reached for 22 patients receiving 20 mg/kg after a median follow-up of 16.4 months.

Phase II-III clinical trials

The HuLuc63-1703 study (8) continued with phase II which included 73 lenalidomide-naïve patients treated with 1 to 3 prior therapies. They were randomly assigned to elotuzumab 10 mg/kg vs 20 mg/kg in association with lenalidomide and dexamethasone. All patients received a premedication regimen. Treatment continued until disease progression or unacceptable toxicity. The ORR was 92% in the 10 mg/kg arm and 76% in the 20 mg/kg arm (global 84%); 57% had at least a VGPR while 27% had a PR. The median time to response was approximately 1 months. Median PFS was 29 months. The most common adverse events were diarrhea (66%), muscle spasms (62%), fatigue (56%), constipation (51%), nausea (48%), and upper respiratory tract infections (47%), similar to those reported for the phase I study (8). Serious adverse events were experienced by 58% of patients and pneumonia represented the most common (12% of patients). Overall rate of infusion reactions was 11% (9).
A randomized multicenter phase III trial (ELOQUENT-2) of elotuzumab in combination with lenalidomide and dexamethasone started in June 2011 and results have been published after a median follow-up of 24.5 months (10). Six hundred and forty-six RRMM patients were randomly assigned in a 1:1 ratio to received either elotuzumab in combination with lenalidomide and dexamethasone or lenalidomide and dexamethasone alone until disease progression, unacceptable toxicity or withdrawal of consent. Less than 10% of patients were not lenalidomide-naïve. Elotuzumab was administered at the dose of 10 mg/kg on days 1, 8, 15, 22 of the first two 28-days cycles, then on days 1 and 15.
Extended 3 year follow-up data were recently presented to the 57th American Society of Hematology annual meeting in Orlando. Infusion reactions occurred in 11% of the patients in the elotuzumab group, mostly grade 1-2. The ORR was 79% in the elotuzumab group and 66% in the control group. Grade 3-4 adverse events included lymphopenia (78% in the elotuzumab vs 49% in the control arms, respectively), neutropenia (35% vs 44%), anemia (20% vs 21%), and thrombocytopenia (21% vs 20%).
Exposure-adjusted infection rates (incidence rate/100 person-years of exposure) were 196 and 193 in the elotuzumab and in the control arms, respectively. Median PFS was 19.4 months in the elotuzumab group and 14.9 in the

control group (p<0.001). A 27% of reduction in the risk of disease progression was observed in the elotuzumab group vs the control group with a TNT delay of 1 year (median 33 months vs 21 months). Focusing on cytogenetic high risk patients, median PFS was 21.2 vs 14.9 months in 17p deleted patients and 15.8 vs 5.5 months in t(4;14) patients (11). Elotuzumab was also studied in combination with bortezomib in a phase II multicenter randomized trial of 152 MM patients relapsed/refractory after 1 to 3 prior therapies (12). Patients were assigned to receive either elotuzumab combined with bortezomib and dexamethasone or bortezomib and dexamethasone alone. Elotuzumab was administered at 10 mg/kg iv weekly for 2 cycles then on days 1 and 11 from cycle 3 up to cycle 8, then on days 1 and 15 starting from cycle 9; bortezomib standard dose (initially iv then, after protocol amendment, subcutaneously) on days 1, 4, 8, 11 during the first 8 cycles then on days 1, 8, 15; dexamethasone at 20 mg orally on days with bortezomib and the day after (dose given at 8 mg orally plus 8 mg iv on elotuzumab days).
Preliminary data showed a significant difference in PFS rate between the two arms: 2-year PFS rate was 18% in the elotuzumab vs 10% in the control arm (PFS HR 0.75, 70% CI 0.62, 0.90). Discontinuation occurred mainly due to disease progression (56%). At last data cut-off (16 Apr 2015), 12% of patients treated with elotuzumab vs 3% of those treated without elotuzumab were still on therapy. Interestingly, early overall survival results also appeared to be in favor of elotuzumab. Grade 3-4 adverse events occurred respectively in 71% vs 60% of patients in the elotuzumab vs non-elotuzumab arm, mostly represented by thrombocytopenia (9% vs 17%) and infections (23% vs 15%). Incidence of infusion reactions was 5% all grade 1 or 2 (13).

Ongoing clinical trials

Based on available results from phase I and II clinical trials, elotuzumab seems to have minimal efficacy when used as a single agent. Moreover, the high complexity of MM pathophysiology, which is not yet entirely understood, makes it a preferable choice to use drugs combination strategies.
Several clinical trials are currently on going to explore the role of elotuzumab both in RRMM and in newly diagnosed MM patients (including two studies recruiting high-risk smoldering myeloma patients); 8 of these studies are still enrolling.
Among phase III trials, ELOQUENT-1 is investigating the efficacy of elotuzumab combined with lenalidomide and dexamethasone in newly diagnosed MM patients who are ineligible for autologous stem cell transplantation (ASCT).
Enrollment is closed but results are not available yet. Another phase III trial is including newly diagnosed patients eligible for ASCT to receive induction therapy with bortezomib combined with lenalidomide and dexamethasone, ASCT, consolidation with bortezomib, lenalidomide, dexamethasone with or without elotuzumab and maintenance with lenalidomide with or without elotuzumab.

CONCLUSIONS

The ideal monoclonal antibody should act against specific targets on the

neoplastic cells with limited toxicity toward other cells, promoting cell apoptosis or activating the immune response through several mechanisms. Elotuzumab could be usefully added to standard induction regimes or combined with chemotherapy in order to improve the quality and duration of responses; it could also be an option as a long term therapy in protocols providing a maintenance strategy. More data are needed to confirm the efficacy of elotuzumab in combination with proteasome inhibitors and/or IMIDs and to define how to integrate it with current treatment regimens.

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