Commentary

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Intesive fludarabine-high dose cytarabine-idarubicin combination as induction therapy with risk-adapted consolidation may improve treatment efficacy in younger Acute Myeloid Leukemia (AML) patients: Rationales, evidences and future perspectives

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Summary **Acute Myeloid Leukemia (AML) is the commonest form of leukemia in the adults, with an incidence of 3-4 cases per 100,000 people/year. After the first description of the effective cytarabine + antracycline (3+7) induction regimen, in the last 3 decades, no effective targeted drug has been included in the standard treatment of AML. Many efforts of modifying 3+7 adding a third drug or increasing the dose of anthracycline, cytarabine or both did not lead to substantial improvements, mainly due to increased toxicity. Many in vitro and in vivo evidences suggested that fludarabine may increase efficacy of cytarabine through a synergistic effect. Considering the continuous improvements in supportive care and management of infectious complications the feasibility of more intensive induction strategies have increased and a renewed interest in fludarabine-containing induction strategies arose. The recent MRC AML 15 trial has shown that a fludarabine-containing induction, FLAG-Ida, resulted superior to conventional 3+7 in terms of complete remission rates, relapse incidence and survival, although only a minority of patients could complete the whole planned consolidation program due to an excessive hematological toxicity. Our group recently published a 10-year experience with a fludarabine-containing induction that slightly differed from the MRC one and resulted in good efficacy and higher feasibility. In this commentary we review the major evidences supporting the employ of a fludarabine-containing induction in AML, and discuss the future perspectives.**

Keywords: Acute myeloid leukemia (AML), fludarabine, high dose cytarabine

Acute myeloid leukemia (AML) has an incidence of 3-4 cases per 100,000 people/year and is the commonest form of acute leukemia in the adults (*1,2*).

The development of AML is a multi-step process linked to the progressive accumulation of mutations in a multipotent stem cell. According to a hierarchical model, different mutations occur during leukemogenesis, with founder mutations usually affecting genes involved in the epigenetic machinery (*1,2*).

Intensive induction chemotherapy followed by a consolidation treatment for patients achieving hematological complete remission represents the backbone of AML treatment (*3*).

In the last three decades no effective new drugs have been introduced for AML treatment, with the exception of gemtuzumab-ozogamicin, whose potential benefit for AML patients has not been completely elucidated (*1*).

Standard induction therapy for younger AML patients is still based on a combination of daunorubicin and cytarabine. The rational for testing alternative

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regimens including higher dosage cytarabine (Ara-C), a different anthracycline as idarubicin or a third drug such as and fludarabine and etoposide is to try to reduce the rate of treatment failure. Idarubicin is the anthracycline derivative that is less sensitive to P-glycoprotein (PgP) and more toxic to multi-drug resistance (MDR) cells (*4*). Fludarabine inhibits various cellular metabolic pathways such as DNA, RNA and protein synthesis (*5*). It has been demonstrated that the combination of Fludarabine with Cytarabine results in a synergistic effect on myeloid cells (*6*): *in vitro* studies have shown that fludarabine is able to enhance the concentration of Ara-C triphosphate (Ara-CTP), the active metabolite of Ara-C, in leukemic blasts (*7*) and to inhibit DNA repair mechanisms, providing a rationale for combination therapy with DNA-damaging agents (idarubicin and mitoxantrone) (*8*). The combination of fludarabine plus cytarabine (± anthracycline) provided interesting results when it was first tested on relapsed and refractory AML patients (*9*). Furthermore, fludarabine is reported to be toxic to MDR cells (*5,6*), with the potential of counteract the poor prognostic value of Pgp expression (*10*).

In vitro, the efficacy of fludarabine-cytarabine combination has been improved by adding granulocyte colony stimulating factor (G-CSF) that seems to be able to recruit quiescent leukemic cells into the S-phase of the cell cycle, thus rendering them more sensitive to cycle-specific drugs. G-CSF may also increase the formation of the active metabolite of fludarabine, F-Ara-ATP, and Ara-CTP (*11*).

However, the first studies designed to improve standard 3+7 induction either by increasing or modifying the anthracycline or cytarabine dosing failed to produce better results, probably because of an excess of toxicity (*12,13*). In 1991 our group started investigating induction strategies based on fludarabine-containing regimens in relapsed/refractory and high-risk patients (*14,15*). We showed that a regimen including only one cycle of fludarabine, cytarabine, idarubicin and G-CSF (FLAG-Ida) was effective, well tolerated and improved the feasibility of stem cell transplantation in younger, untreated, de novo AML patients (*16*). Moreover, the continuous improvements in supportive care and management of infectious complications contributed to increase the feasibility of more intensive inductions (*17*). In 2004, we modified the original schedule by omitting G-CSF priming (FLAI), adding a second induction course with cytarabine and idarubicin in order to increase efficacy, and we improved the risk-oriented consolidation (*18*).

In the recent MRC AML15, standard 3+7 with or without etoposide was compared to a fludarabine containing induction which consisted in two identical courses of FLAG-Ida (*19*). CR rate after the first course, relapse risk and survival were better in the FLAG-Ida arm, however, due to higher myelosuppression, only a minority of patients were able to complete the whole planned consolidation therapy (*19*). However, it has to be noted that even for patients who received only the two FLAG-Ida courses without any consolidation, outcome was equivalent to patients receiving 3+7 double induction plus the full planned high dose Ara-C consolidation (*19*).

On the contrary, according to our FLAI-5 induction, fludarabine is administered only in the first course only and idarubicin dose is increased from 10 mg/sqm to 12 mg/sqm in the second course. This strategy may reduces the myelosuppression and the incomplete haematological recovery rate, allowing the majority of patients to complete the scheduled therapy in a timely manner, without jeopardizing the efficacy (*18*).

Consolidation therapy, comprehending allogeneic stem cell transplantation (allo-SCT) or chemotherapy alone, for patients achieving hematological complete remission is fundamental in order to prevent leukemia relapse (*1,7,20*). In the MRC trial, as well as in our experience, consolidation therapy following fludarabine containing induction did indeed improve outcome(*18,19*), however, the concern of therapy-related toxicity is not negligible.

The consolidation strategy is generally chosen after the evaluation of disease-related factors (*i.e.* risk assessment according to clinical and biological features at diagnosis and response to induction) and patient-related factors (*i.e.* performance status, comorbidities, infectious complications) (*1,7,20*) and the correct identification of patients who may benefit from early allo-SCT consolidation is fundamental in order to maximize treatment efficacy (*21*).

Allo-SCT increases the chance of disease cure, through the immunological control of residual disease eventually surviving after the intensive conditioning regimen (*7,20*). In the last decade a deep reduction in transplant related mortality has been achieved thanks to the development of new strategies to prevent Graft versus Host Disease, and through a better management of early and late transplant related complications (*22*). The use of unconventional hematopoietic stem cells sources, as matched unrelated donors and cord blood and the introduction of new transplant procedures designed for the utilization of haploidentical donors have largely increased the feasibility of allo-SCT for patients lacking a sibling matched donor.(*22-24*) Moreover reduced-intensity conditioning regimens allowed older or frail patients in hematological remission to benefit from the transplant associated immunological control of leukemia (*7,20*).

In poor-risk AML patients according to standard risk classification allo-SCT in CR1 is widely recommended. On the contrary the role of allo-SCT for patients belonging to intermediate-risk groups is still matter of debate, considering the therapy-related mortality (TRM) and long term complications related to Graft

versus Host Disease and immunosuppression (*1,7,20*). As a general indication, according to ELN, allo-SCT should be performed in CR1 if an advantage in LFS of at least 10% can be deduced from the individual risk of relapse and non-relapse mortality (*20*). The prognostic role of pre-transplant minimal residual disease (MRD) assessment before allo-SCT in predicting relapse risk and overall survival, have been reported by several groups (*25*). A deep MRD evaluation through high sensitivity techniques as multicolor-flow cytometry or real- time PCR, should be routinely employed to improve patients risk stratification, identifying those who may benefit from a more aggressive consolidation strategy (*25*).

Following the landmark study by the CALGB (*26*), high-doses of Cytarabine (HD-AR-C) have become the standard consolidation chemotherapy for younger patients not undergoing allo-SCT. However, since different studies led to conflicting results, a consensus has not been reached about the usefulness of higher versus intermediate doses of Cytarabine and the number of consolidation cycles which have to be administered (*1,7*). The possible explanation of these discrepancies is that cytarabine was given in the context of different drug combinations, with different infusion rate and following different inductions schedules. The recent MRC trial, who applied two or three courses of higher dose Cytarabine (3 g/sqm bid days 1,3,5) showed that overall consolidation chemotherapy did improve survival, but there was no significant difference between two or three cycles (*19*). In our experience we applied consolidation chemotherapy with lower doses of Cytarabine (2 g/sqm daily days 1 to 4) and confirmed that survival was improved by consolidation chemotherapy in patients not undergoing allo-SCT in first CR; however, we found that 3 or more cycles were better than 2 (*18*). This discrepancy with the MRC trial may be explained by the difference in the Cytarabine dose, with an higher number of cycles needed in our experience to reach the same cumulative dose (*18,19*).

The timing and the intensity of consolidation therapy in the next year will probably be guided by MRD assessment: many groups have highlighted the importance of MRD clearance in AML and how the persistence of MRD is strongly linked to relapse risk. However there is still no consensus on how and when MRD should be assessed and which therapeutic decision should be taken after MRD evaluation (*25*).

Therefore, until specific MRD-driven strategies will be developed, after intensive fludarabine-containing regimen, two or three courses of high dose Cytarabine should be the standard consolidation, at least for younger patients not undergoing allo-SCT.

As a future perspective, the good results of more intensive induction strategies such as FLAI could be further improved with the addition of innovative therapies. Following the observation that the synergistic effect observed with the combination fludarabine-cytarabine could be mostly related to an increased availability of ara-CTP into the leukemic blasts, a modification on cytarabine chemical structure was attempted. Elacytarabine is the elaidic acid ester derivative of cytarabine, designed to enter cells independently of nucleoside transporters. Phase I/II trials were encouraging although subsequent clinical studies did not confirm superiority of elacytarabine as monotherapy in relapsed/refractory AML, compared with standard of care (*27*). Perhaps, elacytarabine should be further tested in earlier stage of disease and in combination with fludarabine or other conventional AML drugs. Many groups have shown that low-dose Gemtuzumab Ozogamicin can be safely incorporated in modern induction therapy and may improve the outcome, at least for non high-risk patients (*28*). Moreover, recent evidence suggest that the addition of the multi-kinase inhibitor midostaurin to conventional 3+7 induction improves the outcome in FLT-ITD mutated AMLs (*29*). Since fludarabine containing induction seems to partially overwhelm the negative prognostic impact of FLT3-ITD, at least when concomitant NPM1 mutation is present (*30*), there is a strong rationale for incorporating midostaurin in fludarabine containing regimen as well.

In conclusion, thanks to the great improvement in the supportive care, nowadays more intensive fludarabine-containing induction represents the most effective therapy at least for younger AML patients. A risk-oriented strategy is of great importance in order to maximize the adherence to therapy and to tailor consolidation to the individual risk, minimizing treatment related-toxicity. In the next years, the incorporation of targeted molecules and the implementation of minimal residual disease-driven choices will further contribute to improve the overall good results achieved.

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