Acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) occurs with a frequency of 3.5/1,000,000/year cases. AML patients have an invasion of diseased cells (blasts) medullary insufficiency, microenvironmental dysfunction, defects in the proliferation and function of the remaining normal cells, and global dysfunction of the immune system, and may present fatigue, fever, blotches on the body, and bone or joint pain. The diagnosis must be made based on clinical, morphological, immunophenotypical, molecular, and cytogenetic findings. Prognostic assessment can determine the choice of treatment for the patient, and it depends on many different patient-related factors. Traditionally, the karyotype has been used as the principal prognostic factor in *de novo* leukemias. The conventional treatment for AML is divided between induction and consolidation. The future of AML treatment, particularly in cases of more reserved prognoses, should count on the assistance of genomics with panels of genetic changes or sequencing, new drugs, and targeted therapy. This paper describes the various alternatives available and under investigation, in Brazil and worldwide.

1. Introduction

Acute myeloid leukemia (AML) occurs with a frequency of 3.5 cases per million people per year, and its incidence increases with age. This increase is significant after 60 years of age and the average age of occurrence is around 65 years of age.[1-3] In Brazil, according to the National Cancer Institute (Inca), there are an estimated 8000 to 9000 cases per year.[4]

It is known that the occurrence of AML can be linked to different factors, such as ionizing radiation, exposure to chemical products, prior exposure to chemotherapy drugs, genetic factors, congenital diseases, and medullary failure syndromes. But, in most cases, these factors are not identified.[5]

From a physiological perspective, the effects of the disease occur through the invasion of diseased cells (blasts), medullary insufficiency, microenvironmental dysfunction, defects in the proliferation and function of the remaining normal cells, and global dysfunction of the immune system. The disease is characterized, at the end, by peripheral cytopenias and invasion by blastic cells. [6]

2. Diagnosis

The diagnosis must be made based on clinical, morphological, immunophenotypical, molecular, and cytogenetic findings.[7] Clinically, patients may present fatigue, fever, blotches on the body, and bone or joint pain. The physical exam should check for paleness, mucocutaneous bleeding, fever, and visceromegalies. More rarely, patients may experience infiltration of the skin, gums, extramedullary tumors, and signs of infiltration of the central nervous system, manifested as headache or paralysis of the cranial nerves.[6]

For a morphological analysis, counts of 200 leukocytes in the peripheral blood and 500 nucleated cells in the bone marrow are recommended. According to World Health Organization criteria, when the number of blasts is equal to or greater than 20% it is considered to be AML, except in cases of t(15:17), t(8:21), inversion of chromosome 16, or t(16:16).[7]

The objectives of immunophenotyping are to analyze cell lines, to characterize the state of cell maturation, and to detect anomalous immunophenotypical expressions that could be useful in monitoring minimal residual disease. **Table 1** displays the principal markers for a diagnosis of AML.[8-10]

Various cytogenetic changes can occur in AML, among which monosomal and complex changes are described as very serious. **Chart 1** shows the cytogenetic changes of special interest in AMLs.[8-10]

We usually classify AMLs as *de novo* or secondary to myelodysplasia and/or chemotherapy or radiotherapy,

the latter having a worse prognosis. The French-American-British (FAB) classification was used for many years, taking only morphological characteristics into account and dividing the AMLs into M0, M1, M2, M3, M4, M5, M6, and M7 (**Table 2**), while the World Health Organization classification of 2008 is currently in effect and is based on risk (**Chart 2**).[11]

3. Prognostic factors

In AML, perhaps one of the most important steps is the prognostic assessment, because this can determine the choice of treatment for the patient. Several studies have been published in this regard and they usually consider patient-related factors, such as performance status, age, comorbidities, the existence of a compatible donor, factors related to the biology of the disease such as chromosomal changes, response to therapy, secondary versus *de novo* leukemia, morphology (FAB classification), immunophenotype, genetic-molecular factors, and even factors related to the environment, such as socioeconomic resources and conditions.

Traditionally, the karyotype has been used as the principal prognostic factor in *de novo* leukemias. **Table 3** separates the cases into favorable, unfavorable, and intermediate prognoses according to karyotype.[8-10] Keeping in mind that more than 40% of the AMLs have a normal karyotype and knowing about the variability of their evolution in these patients, the description of genetic-molecular factors and mutations, such as NPM1 in 55% of cases, FLT3-ITD (40%), MLL-PTD (6%), NRAS (8-10%), CEBPA (10%), and FLT3-TKD (6%), can contribute to the improvement of prognostic measurements.[12-15] Of these, FLT3-ITD, NPM1, and CBPA are the ones that have been more often used in clinical practice.[12-15] The presence of the c-Kit mutation has also been used in order to demonstrate cases of t (8:21) or inv(16) with poor prognosis.[16 15]

Therefore, a normal karyotype with negative FLT3 and positive NPM1 and those with CBPA and with inv16, t(8:21) without mutation of the c-Kit are considered to be factors for good patient prognosis. The others are considered to be of an intermediate or unfavorable prognosis.[16] The European Leukemia Net[17] then went on the classify AMLs as having favorable, intermediate I, intermediate II, and unfavorable prognoses, as displayed in **Table 4**. In our service, we have adopted the algorithm in **Figure 1** for an indication of consolidation chemotherapy with autologous or allogeneic bone marrow transplants. We take the c-Kit into account for cases of t(8;21), inv(16), or t(16;16).

New changes have been identified and their value in the clinical management of AML has been studied: mutations of TET2, ASXL1, IDH1 and IDH2, PHF6, and DNMT3A.[18-22] It

appears that the mutation of IDH2 R140, but not of IDH2 R172 or IDH1, was associated with an improvement in overall survival. Mutations of ASXL1 and PHF6, on the other hand, were associated with a worsening of overall survival.[18-22] Recently, a meta-analysis of studies including more than 4500 patients with AML showed that DNMT3A is associated with subtypes M4 and M5 and that it was an independent adverse marker. The authors of that study recommended incorporating this mutation in the decision algorithms for patients with AML.[23]

4. Treatment

The conventional treatment for AML is divided between induction and consolidation. The conventional induction treatment with anthracycline and cytarabine has been in use for more than 40 years. Studies with the addition of other drugs or increased doses of cytarabine have not reported any increase in remission rates, which ranged from 60 to 80%.[24,25] The anthracyclines traditionally used are idarubicin and daunorubicin.[24,25] In consolidation therapy, the use of two to four cycles of cytarabine in high doses, autologous transplants, and allogeneic transplants have been used depending on the prognosis of the patient.[26,27]

The future of AML treatment, particularly in cases of more reserved prognoses, should count on the assistance of genomics with panels of genetic changes or sequencing, new drugs, and targeted therapy. Today, some of these strategies are already being used in clinical studies, such as the use of FLT3 inhibitors, ATRA (all-trans retinoic acid) together with chemotherapy in the presence of NPM1, the use of hypomethylation agents for secondary leukemias and in elderly patients, histone deacetylase inhibitors, AKT/m Tor inhibitors, clofarabine, apoptopic agents, such as genasense, and MDR modulators, such as zosuquidar.[27,28]

5. Acute myeloid leukemia

Acute myeloid leukemia, designated as FAB classification subtype M3, accounts for between 10% and 15% of AMLs. Morphologically, it is characterized by the presence of abnormal promyelocytes, with eccentric nuclei and granulations in the cytoplasm, as well as numerous Auer rods in bundle formations. Immunophenotypically, there is high expression of myelomonocytic antigens (CD13, CD15 and CD33) and the absence of expression of monocytic antigens (CD14, including My4, Leu M3, and Mo2) and HLA-DR. The presence of the t(15:17) (q22: q21) translocation occurs in practically all cases and results in the fusion of the PML and RARa genes.[29]

Clinically, acute promyelocytic leukemia is characterized by disseminated intravascular coagulation,

with hemorrhage being the main cause of death in these patients. Treatment has changed significantly with the advent of ATRA associated with chemotherapy.[29,30]

The Spanish group (PETHEMA) developed a treatment protocol showing the importance of anthracyclines in combination with ATRA in the evolution and cure of the disease and established a risk classification based on leukocyte and platelet counts, providing individualized therapy for each case with good chances of a cure.[31]

Conventional treatment consists of induction, consolidation, and maintenance. Arsenic trioxide (ATO) is often used in patients suffering from recurrent disease, but there are several studies that combine ATRA and ATO in the first line, mainly for low-risk cases, with outcomes similar to those from the use of ATRA and chemotherapy.[32]

6. Leukemias in the elderly

Normally, AML treatment outcomes in the elderly are very bad. Many times these patients have compromised performance status, a high incidence of minimum residual disease following treatment, unfavorable cytogenetics, high treatment-related mortality, higher incidence of induction failure, shorter remissions, and shorter overall survival.[33,34]

The aging population, which in most cases reaches an advanced age under the proper clinical conditions, is forcing the world to adapt to more aggressive treatment strategies. Adequate geriatric evaluation, comorbidity rates, and less toxic treatment schemes are making curative treatment possible for these patients.[35]

In induction, we must consider the standard 3 + 7 (anthracycline + cytarabine) regimen, with remission rates of 60%, always remembering that progression-free survival is short (5 to 10 months) and that remission is maintained for more than 2 years in less than 10% of cases.[36-38] It is important to consider using hypomethylating agents, such as 5-azacytidine and decitabine, mainly in more fragile patients or those with secondary leukemias. Of note here are the studies with high doses of decitabine and rates of complete remission of around 50%.[39]

In the consolidation of elderly patients, those with favorable prognoses should be considered, i.e., those who do not present t(15;17), t(8:21), and inv16, with negative c-Kit, or normal karyotype with negative FLT3 and positive NPM1. They should be submitted to consolidations with intermediate doses of cytarabine, with 1 to 1.5 g every 12 hours for 3 days or an autologous transplant. Those with unfavorable prognoses and a good overall geriatric assessment should be selected (if under 80 years of age) for non-myeloablative or reduced intensity transplants.[40-42]

7. Acute myeloid leukemia and bone marrow transplants in Brazil

Data from several Brazilian authors report discouraging outcomes from the treatment of AML in Brazil.[43] [41] Since 2005, the Brazilian Bone Marrow Transplant Society (SBTMO) has encouraged multicenter studies and the evaluation of various treatment centers in Brazil with the goal of monitoring and trying to improve bone marrow transplant outcomes.

Several initiatives can be highlighted here. Between 2005 and 2007, data from 1289 patients in 17 treatment centers in São Paulo, Rio de Janeiro, Paraná, Pernambuco, and Rio Grande do Sul were evaluated retrospectively. Transplants for AML accounted for 16% of the transplants performed in Brazil, and in the same period, the data of the Center for International Blood and Marrow Transplant Research (CIBMTR) was 27%. The results were partially published and can be seen in **Figures 2** and **3** for allogeneic and autologous transplants, respectively. The conditioning regimens were mainly busulfan and cyclophosphamide, busulfan and melphalan, and TBI (total body irradiation) and cyclophosphamide.[44]

In this study, there was no prognostic cytogenetic classification and in general the autologous transplant had interesting results very similar to those of the allogeneic transplant. This is an interesting piece of information and should be analyzed within the context that, at least during this period, Brazil followed the more European than North American trend to value this type of procedure. The similarity of the survival data between the two transplant modalities was explained by higher mortality in the autologous transplants from recurrence (60%) than from toxicity (40%), with the percentages reversed for allogeneic transplants.[44.]

The Brazilian post-transplant survival data were subsequently validated by Marcelo Pasquini of the CIBMTR, who published the curves for early, intermediate, and advanced disease from the Brazilian data forwarded to the CIBMTR, as shown in **Figure 4**.[*]

From 2007 to 2012, several initiatives in Brazil in this area deserve mention:

- Experience with the use of oral or intravenous busulfan in association with fludarabine;[45,46]
- A study to evaluate how patients with AML were treated in Brazil;[47]
- A study of elderly patients with bone marrow transplants with a regimen of reduced toxicity;[48]

[*] Pasquini M. Resultados de transplantes de medulla óssea de centros de transplante brasileiros através dos registros do Center for International Blood and Marrow Transplant Research (CIBMTR). [Presented during the Brazilian Bone Marrow Transplant Conference-SBTMO 2007].

- Two consensuses (2009 and 2012) on bone marrow transplants from the Brazilian Bone Marrow Transplant Society.[49,50]

The group known as the "Conexão Caipira", led by the Centro de Transplantes de Jaú, showed that the use of oral busulfan and fludarabine produced a survival curve of 64% in two years (**Figure 5**).[45] The results with intravenous busulfan and fludarabine, mainly in elderly patients, were published by our group, showing their effectiveness (**Figure 6)** and low toxicity (**Figure 7)**.[46]

With the objective of evaluating the conditions under which patients with AML are diagnosed and treated in Brazil, a questionnaire was sent to the Brazilian treatment centers. The results obtained showed that:[47]

- The centers considered to be references and that perform bone marrow transplants use diagnostic and prognostic methods for more than 90% of their patients, while in other places, cytogenetics and immunophenotyping for diagnosis are lacking, with only 50% of the institutions that treat patients but do not perform transplants using these methodologies;
- In relation to molecular studies, the PML/RARa was conducted or forwarded for execution by more than 80% of the institutions consulted, while the FLT3 by 45% and the NPM1 by less than 15% of them;
- Induction therapy with cytarabine and idarubicin was used by 36% of the institutions, and daunorubacin and cytarabine by 64% of them;
- In consolidation, 91% of the institutions indicated allotransplant for intermediate risk and 100% for high risk patients, when a donor was identified;
- In low risk patients, 70% were consolidated with high doses of cytarabine and 30% with bone marrow autotransplants.

Transplants in the elderly are becoming an increasingly more common practice in Brazil. The first Brazilian initiative was conducted by our institution in association with the MD Anderson Cancer Center, in a study published in 2011, the results of which showed significant survival in patients between 60 and 80 years of age.[48] The two-year survival of treated patients reached 71% in those in first remission, 44% in those in second remission, and 32% in those with active disease. Interestingly, we found that 58% died from recurring disease and not from the toxicity of the transplant (42%).[48]

The work of the Latin-American consortium, coordinated by Professor Eduardo Rego, in collaboration with the American Hematology Association, to improve the outcomes of acute promyelocytic leukemias, was a significant milestone in the area of

leukemia in Brazil. Using a protocol from a Spanish risk-based classification (PETHEMA) and from agile and centralized molecular diagnosis laboratories, it achieved outcomes in developing countries similar to those observed at the international level, as shown in **Figure 8**.[51]

Finally, during the period from 2012 to 2015, initiatives such as targeted-dose busulfan, improvements in our diagnostic/prognostic tools, the 2015 Consensus of the Brazilian Society of Bone Marrow Transplants (SBTMO), consolidating the consensuses of 2009 and 2012 and as yet unpublished,[49,50] and the design of a protocol to evaluate the autotransplant versus chemotherapy in the consolidation of low-risk patients, demonstrated that specialists continue to be active in Brazil, always seeking better diagnostic, prognostic, and therapeutic conditions.

The SBTMO held expert meetings in 2009, 2012, and 2015 to establish a consensus for bone marrow transplants in Brazil around indications, conditioning regimens, prophylaxis, and treatment of the graft-versus-host disease. The key recommendations for AML were:[50,52]

1. To base indications of allogeneic transplants, autologous transplants, and consolidation chemotherapy in the first remission on the prognostic factors of the patients. Thus, patients with intermediate and unfavorable prognoses would be directed to allogeneic transplants, while those with favorable prognoses to consolidation chemotherapy with high doses of cytarabine or autologous transplant;
2. In spite of the knowledge that, in the second remission or in advanced or even refractory disease, the transplant has a worse prognosis, they accept it under these conditions;
3. The main conditioning regimens recommended would be busulfan and cyclophosphamide and busulfan and fludarabine, and whenever possible, busulfan dosing would be recommended;
4. Transplants in the elderly are indicated in patients in good overall condition and with few comorbidities, preferably with an extensive geriatric assessment.

Achieving the proper levels of busulfan seems to influence the outcome of transplants. Therefore, doses in the area under the curve (AUC) of 6000 μMol.min can be used in patients with more aggressive diseases, younger patients, or even those with active disease, while doses of 4000 μMol.min are ideal in transplants of low toxicity used in patients with myelofibrosis, in AML with comorbidities, and in the elderly.[53]

In Brazil, dosing of busulfan is not routinely performed. It is indicated especially when using oral busulfan, where absorption is erratic. However,

few treatment centers have the HPLC (high performance or pressure liquid chromatography) or UPLC (ultra performance) technology to perform the dosing. With the goal of trying to obtain dosing prior to the transplants that would be capable of predicting the dose to be administered to patients, the doctoral thesis of Iracema Esteves was conducted with technology developed at Einstein through the Ministry of Health's PROADI program.[53] A prior dosing was performed from 48 hours to 15 days before the transplant, comparing it with the dosing during the procedure. She showed that this strategy was feasible for the use of intravenous busulfan, but not accurate for oral use of the drug.[53]

Another project conducted with PROADI resources was the implementation of FLT3, NPM1, CEBPA, and c-Kit prognostic molecular tests in Brazil and making them available to both public and private services. We were able to observe that the incidence of FLT3, NPM1, and CEBPA in patients with normal karyotypes were 19%, 17%, and 1%, respectively. The c-Kit mutation among cases of t(8:21) or chromosome 16 inversion was 3%. Using cytogenetic tools and these molecular findings, we were able to classify out patients according to the European Leukemia Net,[54] as shown in **Figure 9**.

Based on the excellent results obtained in the acute promyelocytic leukemia consortium and on the fact that, for Brazil, the autologous transplant may be a more suitable consolidation strategy for low-risk patients, a group of Brazilian researchers, led by Eduardo Rego, intends to conduct a multicenter study to evaluate the best consolidation strategy, i.e., chemotherapy with high doses of cytarabine *versus* an autotransplant of hematopoietic stem cells.

After conducting a retrospective review of the literature regarding the results obtained in the treatment of AML and the efforts made in Brazil towards this end, the scientific community is hopeful that we will continue to achieve better results. We should remember, however, that in our country there exist enormous inequalities and often unfavorable conditions, causing distortions that should gain the attention of our authorities so they can be corrected.

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Table 1. Principal immunophenotypical markers for acute myeloid leukemia

Myeloid precursors	CD34, CD38, CD117, CD133, HLA-DR
Granulocytic line	CD13, CD 15, CD 16, CD33, CD65, MPOc
Monocytic line	CD11c, CD14, CD64, CD4, CD11b, CD36, lisozime
Megakaryocytic line	CD41, CD61, CD42
Erythroid line	CD235a (glicoforine A)

Chart 1. Principal cytogenetic changes found in acute myeloid leukemia (AML)

t(8;21)(q22;q22); *RUNX1-RUNX1T1:* 5% of AML cases
inv(16)(p13.1q22); t(16;16)(p13.1;q22); CBFB-MYH11*:* 5-8% of AML cases
t(15;17)(q22;q12); *PML-RARA:* 5-8% of AML cases
t(9;11)(p22;q23); *MLLT3-MLL:* 9-12% (children); 2% (adults)
t(6;9)(p23;q34); inv(3)(q21q26.2); t(3;3)(q21;q26.2); t(1;22)(p13;q13)

Table 2. FAB (French-American-British) classification based on cytology in seven subtypes of acute myeloid leukemia (AML)

M1	AML without maturation (more than 90% myeloid blasts, with less than 10% of maturing myeloid elements)
M2	AML with maturation (more than 30% blasts, up to 89% with more than 10% abnormal cells, from promyelocytic to more mature cells)
M3	Acute promyelocytic leukemia
M4	Acute myelomonocytic leukemia (the myeloid blasts must exceed 30% of the non-erythroid nucleated medullary cells, with 20% to 80% of them being monoblasts)
M5	Acute monocytic leukemia (more than 30% of the non-erythroid nucleated medullary cells are blasts, with more than 80% being monocyte precursors)
M6	Acute erythroleukemia or Di Guglielmo's syndrome (more than 50% of the nucleated elements of the medulla must be erythroblasts and more than 30% of the non-erythroid elements must be myeloid blasts)
M7	Acute megakaryocytic leukemia

Chart 2. World Health Organization (WHO) classification for acute leukemias[11]

Acute myeloid leukemia and related cancers
Acute myeloid leukemia (AML) with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) ou t(16;16)(p13.1;q22); *CBFB-MYH11*
AML with t(15;17)(q22;q12); *PML-RARA*
AML with t(9;11)(p22;q23); *MLLT3-MLL*
AML with t(6;9)(p23;q34); *DEK-NUP214*
AML with inv(3)(q21q26.2) ou t(3;3)(q21;q26.2); *RPN1-EVI1*
AML (megacarioblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
Acute myelogenous leukemia related with myelodysplasia transformation
Acute myeloid leukemia without other specific classification:
AML with minimal differentiation
AML without maturation
LMA with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemia
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Panmielose with acute myelofibrosis
Myeloid sarcoma
Myelogenous proliferation related with Down syndrome
Myelopoiesis transient abnormal
Myelogenous leukemia associated with Down syndrome
Blastic plasmacytoid neoplasia of dendritic cells

Table 3. Prognosis of acute myeloid leukemia (AML) by karyotype

Favorable prognosis	t(15;17)
	t(8;21)
	Inv(16) and t(16:16)
Intermediate prognosis	normal karyotype
	t(9;11), -y, +8, +6, del 12(p)
Unfavorable prognosis	t(6;9), -7, -5
	complex changes

Table 4. European Leukemia Net prognostic classification[17]

Genetic group	**Changes**
Favorable	t(8:21)(q22;q22),RUN X1-RUNX1T1
	Inv(16)(p13.1q22) or t(16;16) (p13.1;q22); CBFB- MYH11
	NPM1 mutated without FLT3-ITD (normal karyotype)
	CEBPA mutated (normal karyotype)
Intermediate I	NPM1 e FLT3-ITD mutated (normal karyotype)
	NPM1 e FLT3 negativos (normal karyotype)
	NPM1 negative and FLT3 positive (normal karyotype)
Intermediate II	t(9:11) (p22;q23); MLLT3-MLL
	Cytogenetic findings not classified as favorable or adverse
Adverse	Inv(3) (q21q26.2) or t(3;3) (q21:q26.2); RPN1- EVI1
	t(6;9)(p23;q34); DEK-NUP 214
	t(v;11) (v;q23); rearrangement MLL
	-5 or del (5q)
	-7
	anl (17p)
	complex karyotype

Figure 1. Consolidation therapy for patients with acute myeloid leukemia (AML)

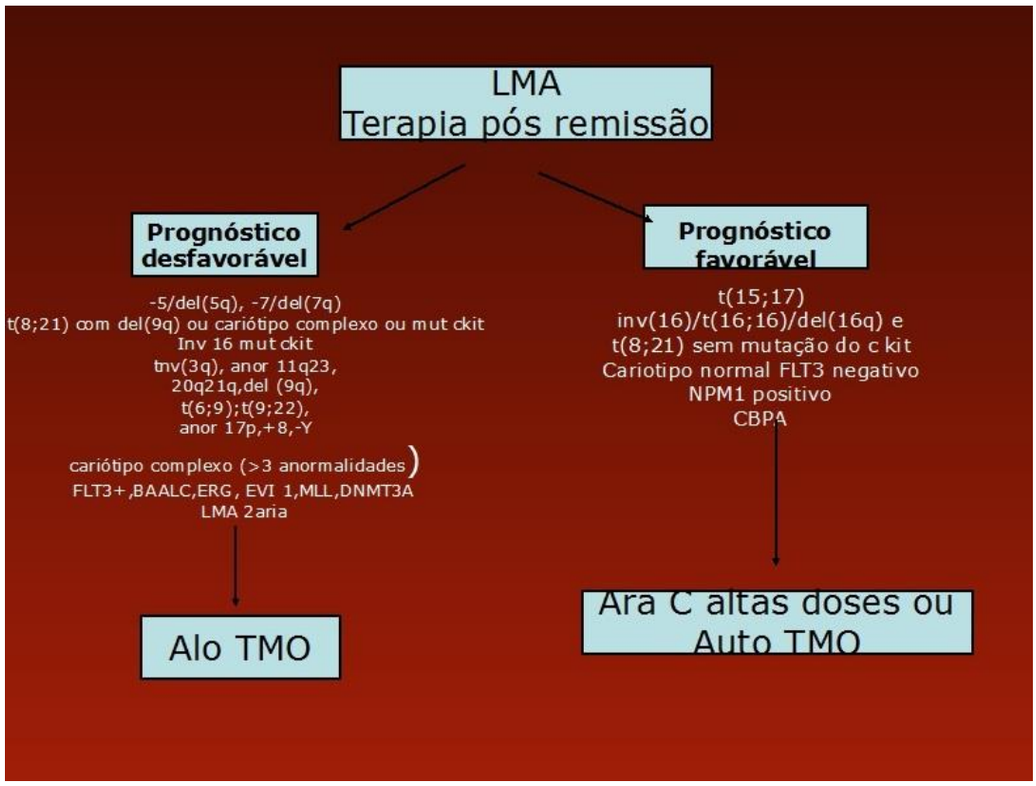
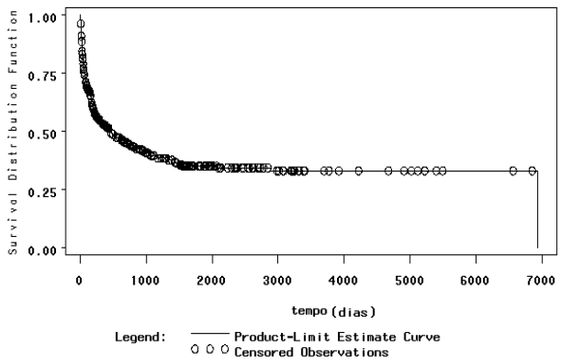


Figure 2. Allogeneic transplants in Brazil. Overall survival, survival by first remission, second remission, and advanced disease and by *de novo* or secondary disease.



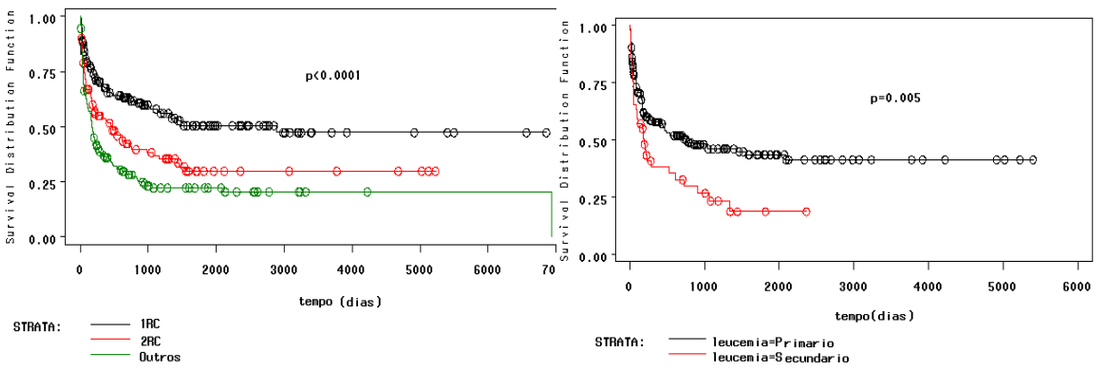


Figure 3. Autologous transplants in Brazil. Overall survival, survival by first remission, second remission, and advanced disease and by *de novo* or secondary disease.

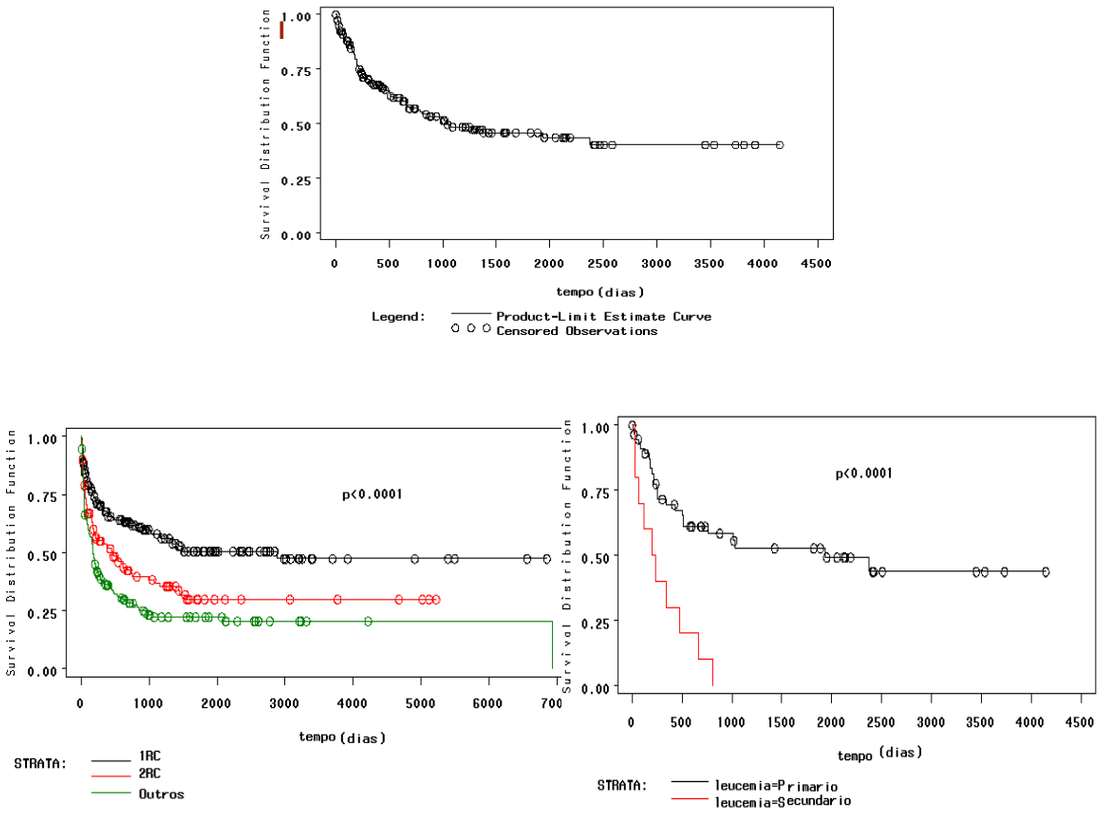


Figure 4. Data presented by Marcelo Pasquini, of the Center for International Blood and Marrow Transplant Research (CIBMTR), classifying the transplants of Brazilian centers that submitted data to the international registry.

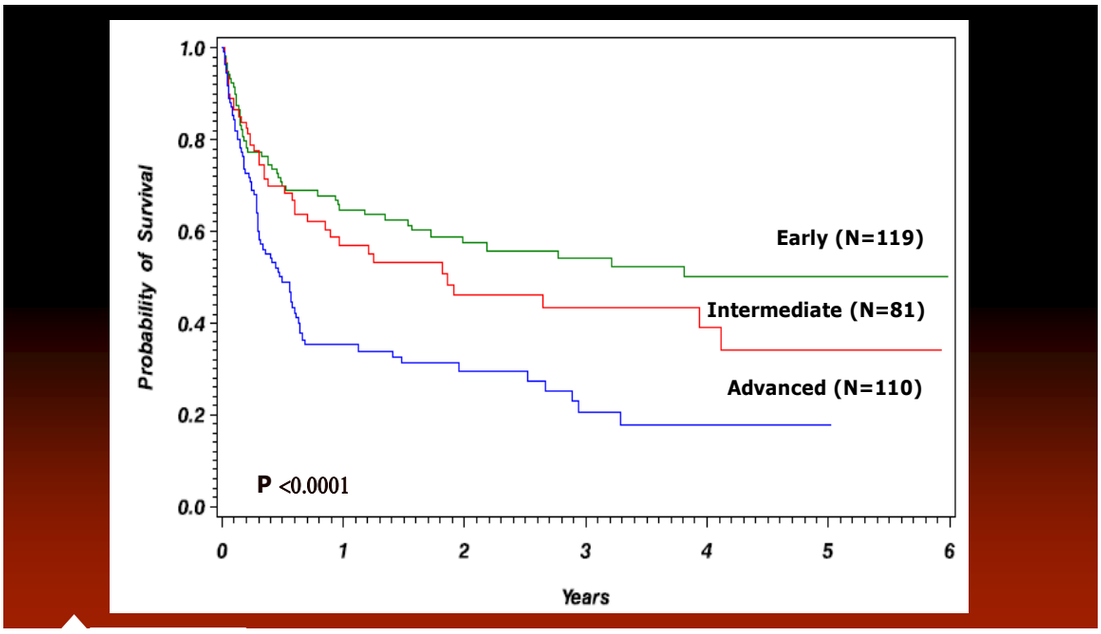


Figure 5. Survival curve with the use of oral busulfan and fludarabine in a group study directed by the Centro de Transplantes de Jaú.

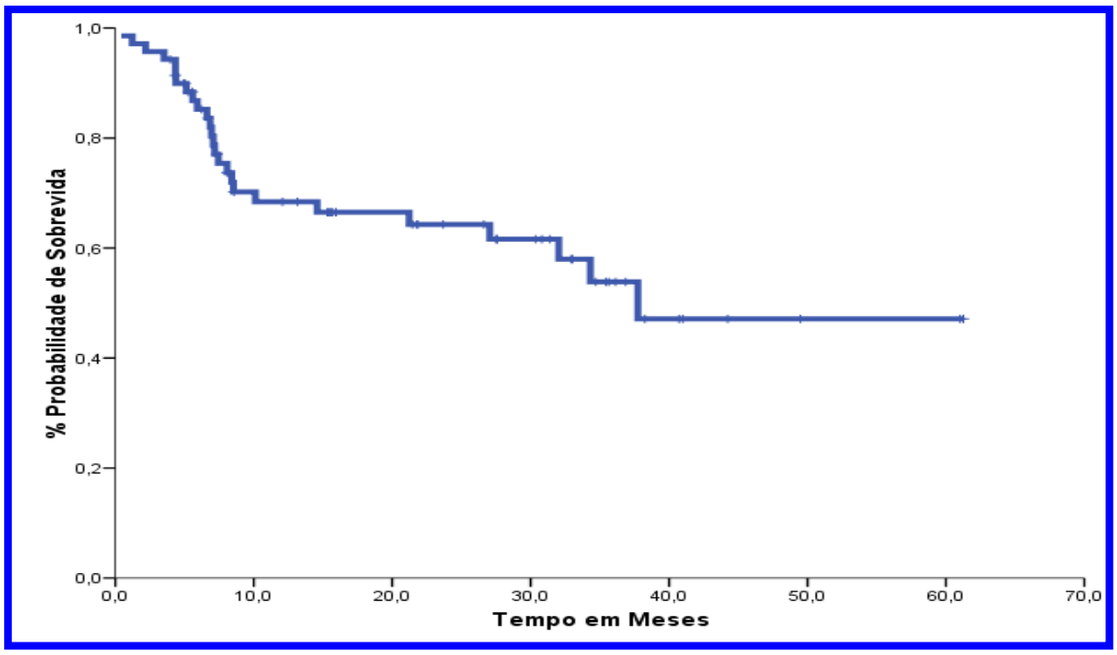


Figure 6. Overall survival with intravenous busulfan (BU) and fludarabine (FLU) in patients with acute myeloid leukemia (AML).

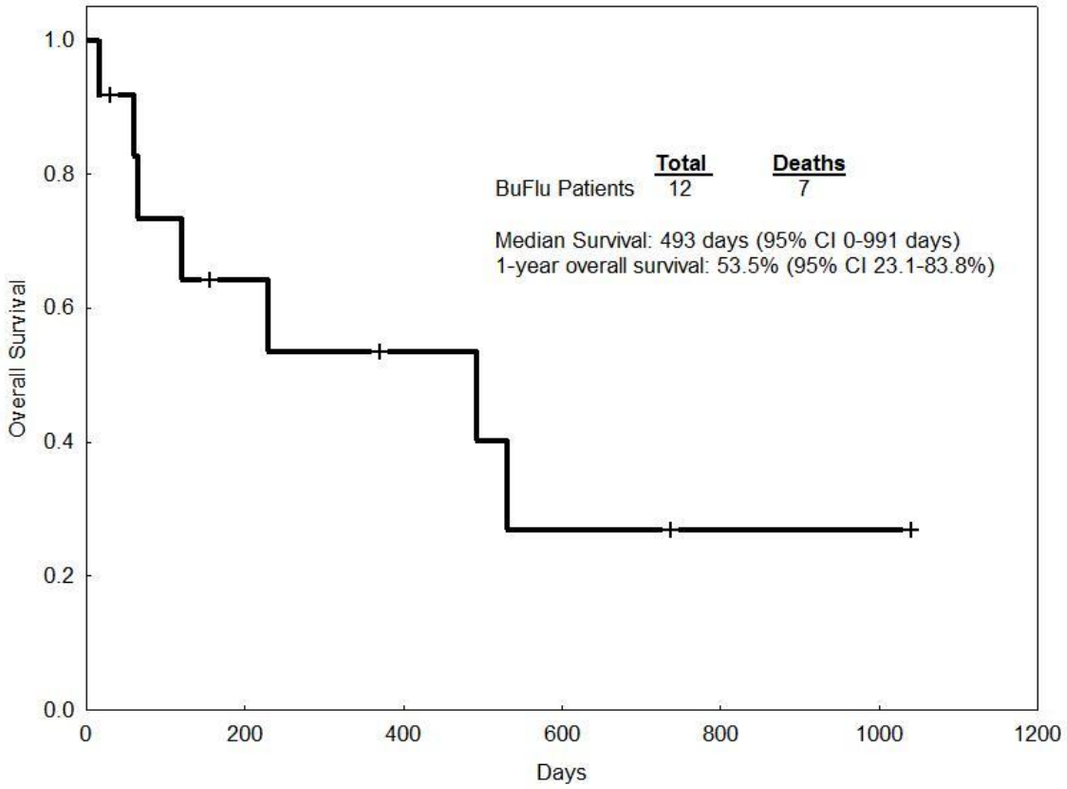


Figure 7. Main adverse effects from the use of busulfan (BU) and fludarabine (FLU) conditioning, showing few serious changes

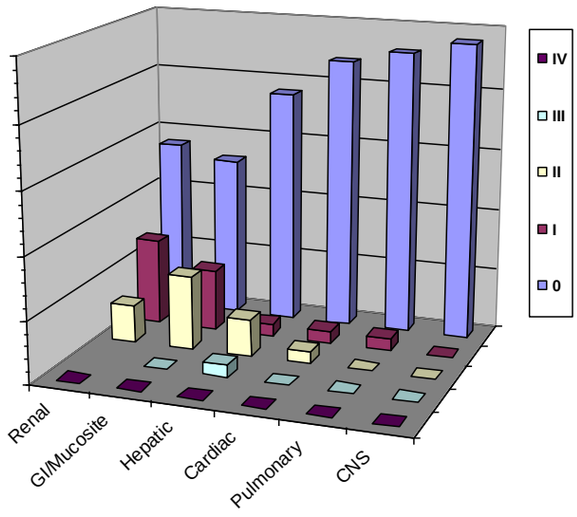


Figure 8. A: Overall survival and disease-free survival. B: Cumulative incidence of recurrence and deaths unrelated to recurrence

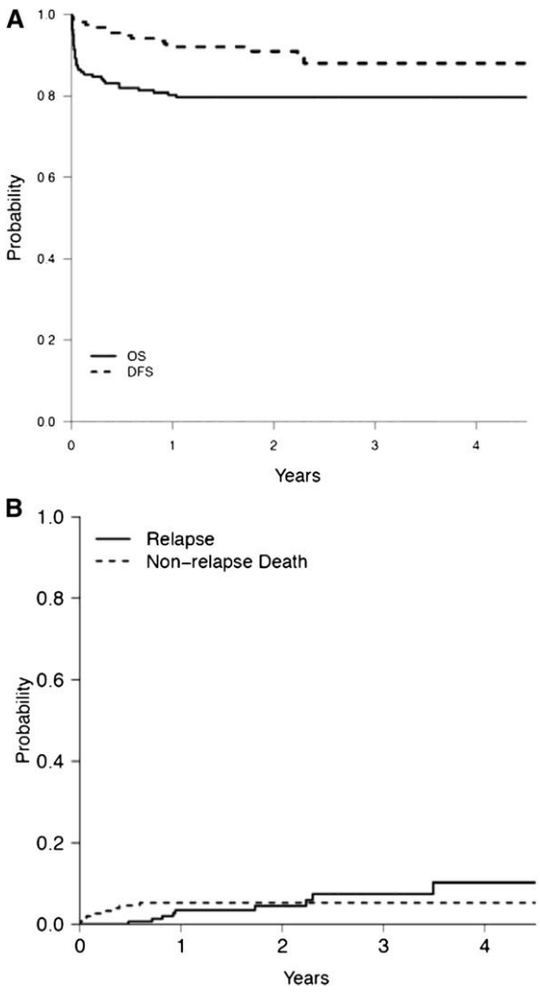


Figure 9. European Leukemia Net risk classification in 100 patients with acute myeloid leukemia (AML) studied in the Brazil AML project.