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Targeted-delivery of chemotherapy (NANOLEUK) vs standard chemotherapy in acute myeloid leukemia: a proposed randomized, double-blinded, multicenter efficacy trial

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Abstract

Treatment protocols in acute myeloid leukemia (AML) have been associated with high rates of acute treatment-associated morbidity and mortality. Phase I trials of novel liposomal nanoparticle-based targeted delivery systems for chemotherapy (NANOLEUK) have shown safety in AML patients. We aim to determine that NANOLEUK improves survival and decreases adverse events as compared with the standard chemotherapy in AML patients.

We will conduct an international, multicenter, randomized controlled, double-blind, 24 month-long trial involving 224 patients recruited from 10 hematology-oncology centers in four countries (Brazil, Peru, Colombia and United States) diagnosed with de novo non-promyelocytic AML. The patients will be randomly assigned in a 1:1 ratio to receive 1) NANOLEUK (liposomal cytarabine and daunorubicin) or 2) standard chemotherapy. The study will use a central computer assisted block randomization scheme and an interactive web response system. The primary outcome will be median survival time at 12 months of follow-up. Secondary outcomes will include complete remission, adverse events, quality of life and hospitalization length.

We anticipate that the results will show improved median survival time at 12 months of follow-up in the NANOLEUK group, with a reduction in treatment-associated adverse events and improvement in quality of life.

This is the first randomized controlled trial to establish the efficacy of NANOLEUK compared with standard chemotherapy in AML patients. The efficacy and safety profile of NANOLEUK will have a broad clinical implication in AML treatment that should be confirmed in a follow-up phase III trial.

Keywords: Acute myeloid leukemia, complete remission, adverse events, targeted therapy, clinical trial, study design, nanoparticles.

Trial registration: This trial will be registered at www.clinicaltrials.gov

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INTRODUCTION

Acute myeloid leukemias (AML) are a group of hematologic malignancies characterized by hyper proliferation of neoplastic immature non-functioning progenitor cells, leading to a state of hyper catabolism, anemia, neutropenia and thrombocytopenia. They are the most frequent acute leukemias in adults, with around 20,830 new cases and 10,460 attributed-deaths in the United States in 2015, according to estimates from the American Cancer Society (American Cancer Society,

2015). Improving the treatment and outcomes of non-promyelocytic AML has been a continuous challenge; although advances in supportive therapy have resulted in improved survival, only 40% of patients under 65 years old are alive 5 years after diagnosis (Howlader, 2012).

Treatment protocols have remained constant over the past four decades; based on a standard therapy of 7 days of cytarabine and 3 days of daunorubicin, per cycle, these protocols are associated with high rates of toxicity, prolonged myelosuppression with an increased

incidence of life-threatening infections and hemorrhage, and severe mucositis. In fact, up to 20%-30% of patients with AML die during induction or consolidation therapy due to adverse events (Hengeveld, 2012).

Novel liposomal formulations promise significant advances in the treatment of AML, improving chemotherapy efficacy and safety, through targeted drug delivery. Doxorubicin and cytarabine-loaded nanoparticles have been shown to be safe and effective in AML both in preclinical studies and in a Phase I trial approved by the Ethics Review Board in Portugal (Pereira, personal communications, unpublished data).

NANOLEUK is a liposomal nanoparticle-based targeted-delivery system that integrates surface lipid layer-associated TargetA-ligand (TarAL) molecules that specifically bind to a receptor, TargetA (TarA), that is overexpressed on the surface of AML cancer cells (Kindler, 2010). After intravenous administration, NANOLEUK is internalized into the cancer cell by receptor-mediated endocytosis; fusion of the endosome with lysosomes decreases the pH of the milieu, which triggers the destabilization of the nanosystem and leads to the intracellular release of the encapsulated anti-leukemic chemotherapeutic drugs. This pH-sensitive nature of NANOLEUK allows control over both the location of delivery and the release rate of the transported agent (Fonseca, 2005). The expected increase in drug concentration inside the cancer cell, with simultaneous sparing of non-neoplastic tissues, is expected to significantly improve the efficacy and safety of treatment, while decreasing systemic toxicity and treatment-related mortality (Fang, 2014), as described for solid tumors such as breast cancer (Miller-Kleinhenz, 2015) or glioblastoma (Mu, 2015). Therefore, we intended to design a Phase II trial able to assess the clinical efficacy of liposomal-based chemotherapy delivery systems, such as NANOLEUK, in increasing overall survival time in AML treatment. Secondary objectives are toxicity, rates of complete remission, quality of life and length of hospitalization.

METHODS

Study Design

We will conduct a phase II, parallel, randomized, double-blinded, multicenter, international trial involving 10 hematology-oncology centers in four countries: Brazil, Peru, Colombia and the United States.

Eligibility

Our study will enroll male and female adult patients younger than 65 years of age diagnosed with de novo non-promyelocytic AML according to the World Health

Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues. (See Appendix). The exclusion of the elderly AML population - over 65 years of age, per the National Comprehensive Cancer Network (Hurria, 2014) - is based on their diminished eligibility for the intensive chemotherapy used as a comparator in this trial, and increased incidence of comorbidities and of severe treatment-related adverse events.

Exclusion criteria will be: severe comorbidities (Charlson Comorbidity Index >3, see Appendix), poor performance status (Eastern Cooperative Oncology Group staging ≥ 2 , see Appendix), renal failure (serum creatinine >2.0 mg/dL), left ventricular ejection fraction <50% (by multigated acquisition (MUGA) scanning or echocardiography), liver failure (total bilirubin >2.0 mg/dL or transaminases >3 \times upper limit of normal), previous or concurrent malignancy, AIDS criteria, overall life expectancy <6 months (unrelated to AML), lack of insurance coverage, simultaneous participation in another trial, diagnosis of underlying neuro-psychiatric disorders that would preclude the patient from giving informed consent.

Sampling and sample size calculation

We will use convenience sampling. To detect a difference of 30% in survival time between the 2 treatment arms with a two-sided 10% significance level and 80% power, a sample size of 100 patients per group will be needed. A 10% loss to follow up is anticipated; using a simple inflation method, an additional 12 patients per group will be needed, for a total of 112 patients per group. To recruit this number of patients, a 12-month enrollment period is anticipated.

Stata Statistical Software, Release 13 (StataCorp LP, College Station, TX, USA) was used for sample size calculations.

Recruitment

The recruitment period will take place over 12 months, between December 2016 and December 2017. All patients younger than 65 years of age, diagnosed with de novo non-promyelocytic AML and presenting to one of the ten hematology-oncology centers will be screened for study inclusion criteria by a research assistant and, if found eligible, will be asked by their attending hemato-oncologist to sign an informed consent form to participate.

The study will be approved by the local ethics committee at each participating institution and will be conducted in agreement with the Good Clinical Practice guidelines.

Randomization and allocation concealment

We will use a central computer-assisted block randomization with blocks of four. Eligible patients will be randomized 1:1 to receive standard treatment or NANOLEUK. In order to ensure concealment of the allocation process, we will use an interactive web response system (IWRS); the pharmacist will call the IWRS and will distribute to the patients from both treatment groups an identical-looking chemotherapy infusion bag, containing either standard therapy or NANOLEUK, according to the randomization scheme. Randomization data will be kept strictly confidential for the sponsor and investigators until the time of treatment unblinding.

Confidentiality will be guaranteed by unidentified acquisition of data using Redcap (Research Electronic Data Capture).

Blinding

Patients, physicians, adjudicators of outcome, data collectors and data analysts will be blinded to the treatment groups: standard therapy or NANOLEUK. The laboratory will provide identical infusion bags for both groups, to ensure the blinding of patients and physicians; the color of the intravenous fluid circulating through the infusion system and into the patient's central venous catheter is also identical in both treatment arms. Adequate measures will be taken throughout the entire duration of the trial to minimize any possible ascertainment bias in this study. These measures will include: intense efforts to maintain blinding of patients and physicians to the intervention until the end of the trial, to decrease the risk of co-intervention bias, efforts to maintain blinding of the outcome assessors to avoid observer bias and to ensure blinding of the data collectors and data analysts by coding the participants in the two groups until the statistical analysis is completed, to avoid selective reporting and fraud bias. Blinding of patients will be achieved by keeping the contact between in-trial subjects to a minimum. The trial participants will be admitted in different rooms and scheduled for outpatient follow up on different days. On the wards, patients are in isolation and cannot have contact with each other. In the rare event of unblinding due to an unforeseen patient emergency (such as an acute, severe reaction during the infusion), the principal investigator from the affected site will be responsible for reporting the event and informing the Data Monitoring Committee (DMC). The physicians involved in the inpatient and outpatient care will not be informed of the patient's group allocation. The trial will use an independent person to collect the data that will be unaware of the group allocation. An independent

statistician, not involved in the trial or randomization sequence, will prepare data reports for the DMC. The groups will also be labeled with nonidentifying terms (such as A and B). The adjudicators of outcome will not be aware of the patients' group allocation either.

Intervention

Upon signing the informed consent form, patients will be allocated to one of the treatment arms (standard or NANOLEUK) and will receive the following interventions:

1. Standard therapy: the group will receive induction therapy with commercially available daunorubicin (90 mg/m² intravenous bolus/day, on days D1-D3) and commercially available cytarabine (100 mg/m²/day as a continuous infusion on days D1-D7), starting as early as clinically possible within the first week after diagnosis, enrollment and randomization.

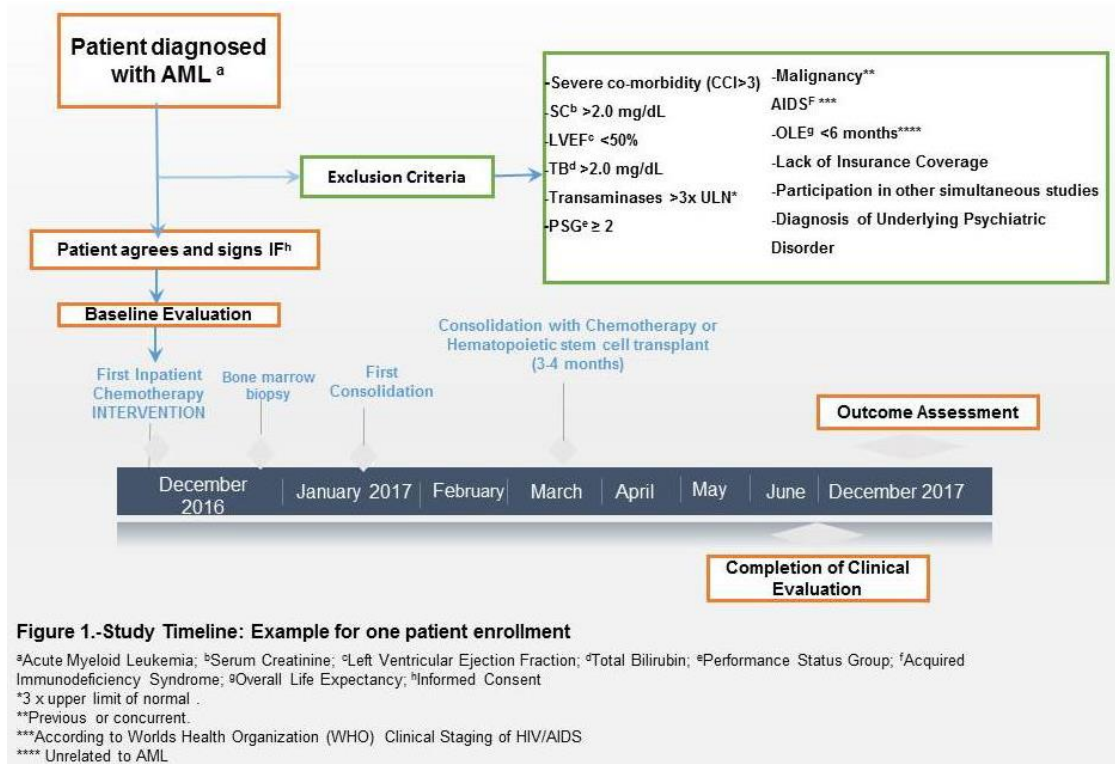
2. NANOLEUK therapy: the targeted treatment group will receive the same chemotherapeutic drugs, at the same doses, delivered inside a targeted liposomal nanoparticle (NANOLEUK); a dose reduction of 25% will be introduced if hand-foot syndrome develops. In the first three days of treatment, the liposomal nanoformulation will enclose both antineoplastic drugs (daunorubicin and cytarabine) and in the last four days, we will only load cytarabine into the liposomes.

Pretreatment with pharmacologic cytoreduction or leukapheresis will be admissible before induction treatment is started, in the case of severe hyperleukocytosis and/or leukostasis.

Endpoints

The primary endpoint will be median survival time at 12 months. Survival time at 12 months will be divided as 1. Event-free survival (EFS), 2. Relapse-free survival (RFS), 3. Overall survival (OS). Secondary endpoints will include Complete Remission (CR) or Complete Remission with incomplete blood count recovery (CRi); incidence of adverse events determined according to the Common Terminology Criteria for Adverse Events, version 4.0; quality of life, as measured by the Short Form Health Survey (SF-36) questionnaire (see Appendix); and length of hospitalization.

Event-free survival (EFS) will refer to the interval from randomization to 1) the date of the evaluation of response after the last induction cycle, if CR/CRi has not been achieved by that time, 2) the date of death, 3) the date of relapse, or 4) the end of the follow-up period. Relapse-free survival (RFS) will be defined as the time between the first date of objective CR/CRi and the date of relapse or death, or the end of the follow-up period. Overall survival (OS) will be defined as the time between



the date of randomization and the date of death or the end of the follow-up period. Complete Remission will be defined using the “Revised Criteria” of the International Working Group (IWG) for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia (see Appendix). Complete Remission with incomplete blood count recovery (CRi) will be defined as fulfilling all of the criteria for CR but without recovery of peripheral blood counts.

Length of hospitalization will be defined as the total number of days in the hospital from the first day of induction until the discharge day after the initial chemotherapy cycle.

Data collection

Data collection will be via an electronic data capture system. All captured data will be stored in a password-protected database, and the reports will be forwarded to the statistician for data analysis. We will have a centralized web-based registry; the principal investigators and authorized research coordinators will have access to the registry. Each participating site will have one research assistant responsible for data entry; the patient’s randomization code and the date of birth will be used as identifiers for each patient.

Study timeline

Once patients are diagnosed with AML and meet the inclusion criteria, they are invited to participate in the clinical trial (enrollment will occur continuously during 12 months). If they agree to participate, they will be randomized to be part of the experimental (NANOLEUK) or control group. Patients will be evaluated at baseline with an initial clinical exam, as well as MUGA scanning or echocardiography, ECG, chest X-ray, abdominal ultrasound, basic hematology and biochemical parameters, blood and urine cultures, and bone marrow trephine biopsy and aspirate, with cytogenetic and molecular studies, and start immediate inpatient chemotherapy (study intervention). All patients will receive a bone marrow biopsy around 2 weeks (Day 14) after the intervention, after recovery from the nadir, to assess remission. Patients who fail to achieve CR after induction will undergo re-induction treatment according to standard AML practice, using each center’s protocol for primary refractory patients, as well as physician and patient preference. Survival outcomes will be assessed 12 months after the intervention (see Figure 1).

Statistical analysis plan

Baseline characteristics

To assess for prognostic balance between the groups, patient-specific (age, gender, BMI, socio-economic status, smoking status, and number and type of concurrent comorbidities) and disease-specific (AML subtype, cytogenetic risk group, mutational status, and

central nervous system involvement) baseline characteristics will be recorded. For continuous variables expressed as mean + standard deviation with a normal Gaussian distribution, the comparison between groups will be made by using the student t test, or the Mann-Whitney test for a non-Gaussian distribution. For categorical variables expressed in frequencies, the chi-square test or its exact version will be used, when applicable, to make comparisons between the two groups.

Outcomes

The primary outcome will be median survival time after 12 months of follow-up. This will be plotted using Kaplan-Meier curves for EFS, RFS and OS; the curves for both groups will be compared using Cox proportional hazards. The secondary outcomes will be compared by chi-square or Fisher test for categorical variables; and Cox proportional hazards for explanatory variables; generalized estimating equations (GEE) will be used, as appropriate. The level of significance will be set at a two-sided alpha level of 0.10.

Withdrawal and losses to follow-up

Patients will be analyzed in the groups they were randomized to, through an intention-to-treat (ITT) approach, with the aim of preserving the prognostically balanced groups created through the randomization process.

Missing data

Missing covariates will be handled through maximum likelihood estimation, and missing outcome data will be accounted for by Cox proportional hazards.

Interim analysis

We will perform an interim analysis when half of patients are enrolled, to test efficacy and safety, using the Haybittle-Peto method with $\alpha=0.001$ at 50% enrollment and $\alpha=0.099$ at 100% enrollment.

Institutional Review Board Submission

We will file an Institutional Review Board (IRB) submission locally at each of the 10 participating centers. The research coordinator will be responsible for the IRB submission.

Trial Registration

After all the IRB approvals are obtained, the trial will be registered at www.clinicaltrials.gov.

Privacy and confidentiality

All researchers and ancillary staff involved in this study will be trained and certified to protect the privacy and confidentiality of the subjects' health information. Data

will be de-identified accordingly in electronic forms and other study-related material after the study is closed.

DISCUSSION

Potential limitations

Prognostically imbalanced groups: to assess for the possibility of an unbalanced distribution of patient-specific and disease-specific prognostic factors across the two treatment groups by chance alone, despite randomization, multivariate analyses will be performed to test the influence of covariates in the outcome and correct possible imbalances.

Potential unblinding and blinding assessments: if the NANOLEUK group experiences significantly less adverse events or will have significantly higher rates of CR, patients and the physicians might guess to which group the patients were assigned, which is a source of bias. Strict measures will be taken to maintain blinding of patients, physicians, data collectors and data analysts and adjudicators of outcome, as stated above in the Materials and Methods section. At the end of the study, both patients and physicians will be asked to answer a questionnaire to assess to which degree they were aware of the treatment assignments.

Drop-outs: due to the acute and rapidly fatal nature of the untreated disease, with an imperative for urgent inpatient treatment, and based on historical data from other AML trials, we expect a low rate of drop-outs; we predict that any drop-outs that do occur, will be potentially less frequent in the NANOLEUK group. Likewise, since subjects will be treated as inpatients, with exclusively nurse-administered intravenous medication, rates of adherence to treatment are expected to be near 100%.

Dosing: the choice of using the same doses of daunorubicin and cytarabine as free drugs and also when loaded inside the nanoparticles can be open to debate. Since we expect that clinical efficacy will increase with improved targeting of the drug and more specific delivery to leukemic cells, a dose-reduction could be proposed, helping to decrease drug-specific toxicity in addition to the improvement in the toxicity profile achieved by the sparing of non-neoplastic cells. However, ethical issues must be considered when proposing the reduction of the established dose, which has proved effective over the last four decades. On the other hand, consideration must also be given to the ethical requirement of reducing any unnecessary toxicity of a test drug. Therefore, we have opted to start with the same dose of daunorubicin and cytarabine as used in the standard established protocol, and introduce a very early dose-reduction of 25% if hand-

foot syndrome (palmar-plantar erythrodysesthesia) is found to occur. An alternative approach would be to design a multi-arm trial to compare alternative (full-dose and dose-reduced) protocols, although a larger sample would be necessary.

Applicability: the external validity of the study is slightly decreased, as there are certain exclusion criteria that will limit the applicability of the intervention to all patients with de novo non-promyelocytic AML.

Strengths

The overall risk of selection bias, ascertainment bias, observer bias, co-intervention bias and selective reporting bias for this proposed study is very low. Through the rigorous study design, we predict to start, continue and finish the study with same prognostically balanced groups, which will ensure a very high internal validity.

Future perspectives

The demonstration of the ability of NANOLEUK to reduce the effects of systemic chemotherapy on non-neoplastic cells, with a decrease in toxicity and treatment-related mortality, and increased early survival, will be a huge step forward in the treatment of AML, after the demonstration of NANOLEUK-based increased concentration of chemotherapy within the leukemic cell, and improved directed-cytotoxicity, in preclinical trials. This could potentially mark one of the first major improvements in treatment outcomes since the standard treatment protocols were introduced, four decades ago. These results will need to be confirmed in a large-scale multicentric Phase III trial, with prolonged follow-up, to capture deaths due to late relapse or late toxicity, in particular due to the long-term cardiotoxicity by anthracyclines. Consideration should be also given in future trials to administering consolidation cycles with NANOLEUK and to treating refractory or relapsed AML, patients older than 65 years of age, or patients with concurrent comorbidities that would normally exclude them from being able to receive standard chemotherapy.

Although results in this trial will be demonstrated for daunorubicin and cytarabine, the nature of this targeted nanoparticle allows for the loading of other chemotherapeutic agents and/or drug combinations; therefore, specific targeting of NANOLEUK to the leukemic cell will validate the possibility of using drugs or drug combinations that are highly active, but currently unused due to unacceptable systemic toxicity. This might be the beginning of a new research era in hematology-oncology field. Likewise, the use of NANOLEUK would potentially be expanded to the delivery of chemotherapy

in the treatment of other cancers with overexpression of the TarA surface receptor that is targeted by the NANOLEUK surface ligand.

Multiple Phase I/II trials should be designed to test different drug combinations, or resurrect drugs that were abandoned due to their severe toxic effects.

CONCLUSION

We anticipate that NANOLEUK will improve survival and reduce serious adverse events in patients with de novo non-promyelocytic AML when compared to standard chemotherapy. NANOLEUK will constitute a groundbreaking intervention that will revolutionize the treatment of AML, as it will have a tremendous positive impact in patients' clinical outcomes.

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Conflict of interest and financial disclosure

The authors followed the International Committee or Journal of Medical Journals Editors (ICMJE) form for disclosure of potential conflicts of interest. All listed

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