# Longitudinal MRD evaluation in AML with NPM1 mutation: from definition of molecular relapse to MRD- driven salvage approach

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# Longitudinal MRD evaluation in AML with *NPM1* mutation: from definition of molecular relapse to MRD-driven salvage approach

**Running Title: MRD-directed therapy in AML with mutated *NPM1***

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ABSTRACT

Acute Myeloid Leukemia with mutated *NPM1* has been recognized as a distinct entity. Although the high prognostic value of RT-PCR based minimal residual disease (MRD) assessment in this setting is clear, the time from first MRD positivity to overt relapse can be extremely variable and a definition of molecular relapse (Mol-relapse) is lacking.

The aim of the present study was to give a definition of Mol-relapse and to evaluate the feasibility and efficacy of an MRD-directed salvage therapy.

Forty-two consecutive AML patients with *NPM1* mutation were included. We defined Mol-relapse as the reoccurrence of *NPM1* mutation confirmed in 3 consecutive BM samples, with a total increase of *NPM1* expression levels of at least 2 logarithms.

Starting from January 2015, all patients meeting Mol-relapse criteria were included in a prospective clinical trial and treated with MRD directed therapy. At the time of Mol-relapse disease burden was significantly lower if compared to that observed in patients at overt relapse ($p<0.03$). Both hematological and non-hematological toxicities were significantly lower than d in patients being treated with the same therapy for overt relapse. After a median follow-up of 19 months all patients are alive and in MRD negative CR.

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To the Editor,

Acute Myeloid Leukemia with mutated *NPM1*(NPM-AML) has been recognized by WHO 2016 classification as a distinct entity commonly associated with favorable prognosis. In 2017 European Leukemia Net (ELN) classification NPM-AML is included in low or intermediate-risk group according to *FLT3-ITD* mutational status (Dohner *et al*, 2017).

Many groups have reported that the clearance of minimal residual disease (MRD), assessed by real time PCR for *NPM1* expression level, is crucial to achieve long-term disease free survival (Kronke *et al*, 2011, **Ivey *et al* NEJM 2016**). In addition, detection of *NPM1* mutation during follow up is associated with an high risk of hematologic relapse (Hem-relapse). However, the interval between first *NPM1* reoccurrence to Hem-relapse can be extremely variable, **as reported in large trials (Schnittger *et al*., Blood 2009;Ommen*et al.,* Blood 2010; Kronke *et al.,* JCO 2011). Furthermore**, a clinically **applicable** definition of molecular relapse (Mol-relapse) is lacking, **as in the recent ELN definition (Schuuruis *et al*, Blood 2018) the precise timing between follow-up samples, to confirm a positivity, is not given. Moreover,** no data at all are available on the feasibility and efficacy of a MRD-driven salvage therapy.

Herein, we analyzed the correlation between *NPM1* MRD kinetics and Hem-Relapse in a cohort of homogeneously, intensively treated young AML patients, in order to give an operational definition of Mol-relapse and to evaluate the feasibility and efficacy of an MRD-directed salvage therapy.

From January 2004 to January 2014, 42 consecutive younger (<65 years old)normal karyotype AML patients with *NPM1* mutation have been treated in our center. Median age was 50 (range 29-61), *FLT3-ITD* mutation was detected in 17 patients (41%), with high allelic burden in 8/14 evaluated patients (57%). All patients received fludarabine-cytarabine-idarubicine containing induction (FLAI) and achieved hematological CR, followed by high-dose cytarabine-based consolidation, according to our policy (Guolo *et al*, 2016). At the end of the program all patients were *NPM1*-MRD negative and were included in a longitudinal observational MRD study.

MRD assessment was scheduled on bone marrow (BM) samples every 3 months for the first 5 years of follow up. Patients who displayed *NPM1* reoccurrence during follow up were re-assessed after 15-30 days. The correlation between MRD detection and Hem-Relapse was analyzed in order to provide an operative definition of Mol-relapse.

*NPM1* mutations (A,B,C,D) were evaluated on BM samples using Mutant Quant® Standard from Ipsogen (Marseille, France) as previously reported (Miglino *et al*, 2012). All Real-Time PCR were performed on DNA Engine 2 (Opticon®, MJ Research®). All statistical analyses were performed as recommended by Delgado *et al* (2014), using IBM SPSS® v22, Debian (Linux) version.

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In patients treated between 2004-2014 (cohort 1), our conventional approach for relapsed patients consisted of two courses of MEC regimen administered at Hem-Relapse, followed by allo-SCT when feasible (Guolo *et al*, 2016).

In cohort 1 Hem-relapse occurred in 8 patients, after a median of 24 months (range 14-52); **3 of them had concomitant *FLT3-ITD* mutation and two had low-level positive MRD after induction course 2**. Three-year overall survival (OS) of cohort 1 was 60.4% (median not reached). The only variables significantly related to a shorter survival were unsatisfactory *NPM1* clearance after FLAI (*NPM1* MRD reduction <3.5 logs, p<0.01) (Miglino *et al*, 2012) and Hem-relapse (p<0.001). All patients with Hem-relapse proceeded to MEC salvage treatment. Second CR rate was 63% (5/8 patients). One patient died of infection before response evaluation and 2 were refractory. At Hem-relapse, median *NPM1* expression levels was 13276. After two MEC, complete *NPM1* MRD clearance was achieved in 2/8 relapsing patients (25%).

In all cases *NPM1* MRD reoccurrence anticipated Hem-Relapse. The median time from NPM MRD reoccurrence to Hem-Relapse was 4.5 months but the range was rather wide (1-8.4 months).

By considering ~~**consecutive samples and**~~ the trend of MRD increase **in consecutive samples**, we defined Mol-relapse as the reoccurrence of *NPM1* mutation confirmed in 3 consecutive BM samples, with an **overall** ~~**total**~~ increase of *NPM1* expression levels of at least 2 logarithms. **Our definition of Mol-relapse is similar to that recently provided by ELN, but includes more stringent timings for re-assessment.** Median *NPM1* expression level at Mol-relapse was 1875. All patients with Mol-relapse defined according to the aforementioned criteria progressed to overt Hem-Relapse within 3 months from the third BM sample (range 0.5-3) and no patient experienced hematological relapse before fulfilling Mol-relapse criteria.

Starting from January 2015, all patients with Mol-relapse were included in a prospective clinical trial and treated with MRD-directed therapy, consisting of one course of MEC (cohort 2). **Among 19 NPM-AML patient who were treated in our Institution after January 2015, 4 showed Mol-relapse and were therefore included in the prospective study. None of these patients had concomitant *FLT3-ITD* mutation. Overall, patients in cohort 1 and 2 had comparable clinical and molecular features.** At the time of Mol-relapse disease burden was significantly lower if compared to that observed in patients at overt Hem-relapse (*NPM1* expression levels of 1217 vs 13276, respectively, p<0.03, Fig. 1).

All patients receiving salvage treatment at Mol-relapse achieved complete MRD clearance and then proceeded to allo-SCT. After a median follow-up of 19 months all patients are alive and in MRD negative CR at the time of analysis. **With the limitation of the cohort size, survival of patients receiving MRD-directed therapy compares favorably with that of patients receiving MEC salvage at Hem-relapse (Median OS not reached vs 12 months, p<0.05).**

Both hematological and non-hematological toxicities of patients receiving MRD-directed therapy were significantly lower than those observed in patients treated with MEC for overt Hem-Relapse. ~~**In particular, significantly shorter neutropenic period was observed in patient treated with MRD-directed therapy**~~

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~~which lead to a lower risk of infectious complications.~~ **The neutropenic period, defined as median number of days with ANC <0.5 x $10^6$/l, was significantly shorter if compared to that following the same therapy at leukemic relapse (10 and 17 days, respectively, p<0.05), resulting in a lower risk of infectious complications.**

Our study provides the first evidence that the application of standardized molecular relapse criteria, based on the logarithmic increase of MRD burden, is able to identify patients with impending Hem-Relapse, and can rationally drive the administration of early salvage therapy. MRD-driven salvage approach has become part of the current clinical practice in acute lymphoblastic leukemia (Theunissen *et al*, 2017) and in acute promyelocytic leukemia (Diverio *et al*, 1998). NPM-AML is a good model for MRD-driven therapy as *NPM1* mutations are stable during disease history and molecular NPM1 monitoring is now widely employed (Burke *et al*, 2016). However, no information is at the present available on management of NPM-AML patients still in hematological CR but displaying persistence or reoccurrence of *NPM1* mutation. Our experience shows that MRD-driven salvage therapy is feasible and effective in NPM-AML. Due to the low disease burden and normal blood cell count at the time of Mol-relapse toxicity is reduced and, more importantly, a single course of MEC is able to achieve MRD negative status in all patients. As already reported (Guolo *et al,* 2017), complete MRD clearance before allo-SCT deeply affects the outcome after transplant and should be the aim of a modern rescue approaches (Bloomfield *et al*, 2018).

**Conflict of Interest**

Authors declare that they have no conflict of interest to disclose

**Acknowledgements:**

F.G., P.M. and M.C. designed research

M.G. and RM.L. supervised research

M.M. and N.C. performed all molecular analysis

A.C., M.C., F.B. and A.T. collected and revised the data

F.G. and P.M. performed all statistical analysis

F.G., P.M., R.C, M.C and RM.L. wrote the paper

M.G. and RM.L. revised the final version of manuscript

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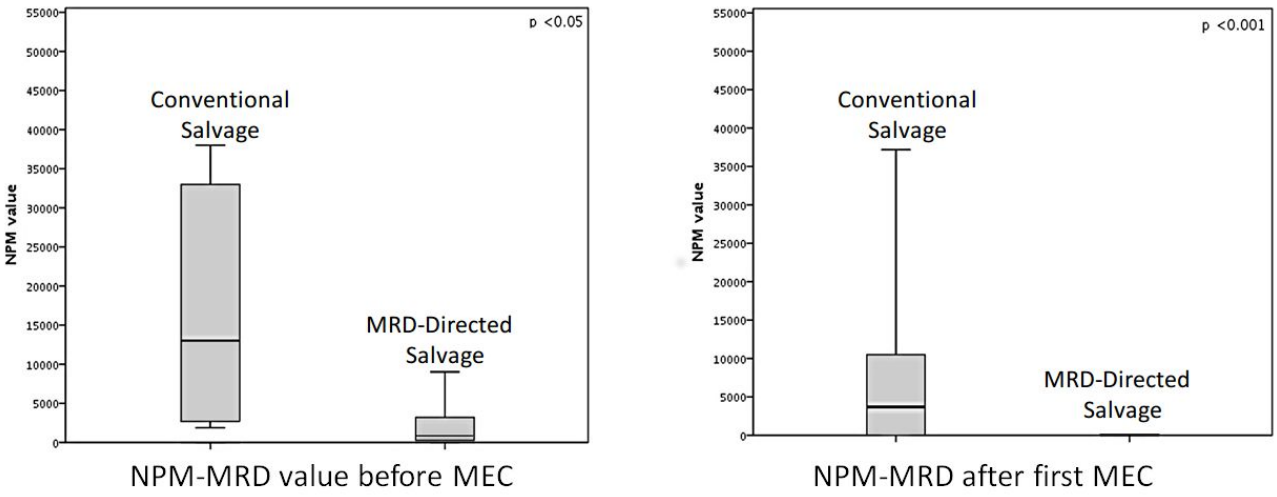
**Figure 1:** Pre and post therapy disease burden in patient treated for MRD relapse compared to patients treated for hematological relapse

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