Author's response to reviews

Title: Hierarchical Cluster Analysis of Immunophenotype Classify AML Patients with NPM1 Gene Mutation into Two Groups with Distinct Prognosis

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Author's response to reviews: see over
Mr. Danrolf de Jesus and Dr. Elisabeth Nacheva

BMC Cancer

RE: MS 7145704047702166

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Dear Dr. Elisabeth Nacheva:

Thank you very much for the comments regarding our manuscript entitled “Hierarchical Cluster Analysis of Immunophenotype Classify AML Patients with NPM1 Gene Mutation into Two Groups with Distinct Prognosis” by Chen et al. According the editorial opinions, we revised our manuscript as below:

Reviewer Wu Depei:
Major Compulsory Revisions

1. It is hard to believe that 94 NPM1 mutated AML patients was recruited with informed consent for NPM1 mutations in this study from 1987 to 2007. NPM1 was found with prognostic significance in the past few years.

Answer: Thank you for the reviewer's comment. We revised the description as below. “The informed consents were collected from all living patient. The NPM1 mutation was retrospectively checked in part of patients. Cryopreserved samples were collected from the marrow bank according to the criteria of local ethics committee. This research was approved by the National Taiwan University Hospital Research Ethics Committee.”

2. The choice and intensity of treatment might serve as important values for survival, and the authors should clearly state the treatment of NPM1 mutated AML patients in this manuscript. Actually, idarubicin (12 mg/m2*3) plus cytarabine is of great toxicity in Asian patients, especially for the aged patients (the median age of 58 years in validation cohort). How about the TRM? How many patients received HSCT? The description of the treatment is unconvincing.

Answer: Although the progress of current science and target therapy, the golden standard chemotherapy in AML is Idarubicin (3) and cytarabine (7) chemotherapy for several decades. We revised and add the description in detail as below.” There were 31 patients received supportive care alone due to
old age and frailty. Sixty-three patients were treated with standard intensive chemotherapy. Eight patients died following induction chemotherapy, and Eleven patients received HSCT and their disease free survival was censored at the time of transplantation.

3. The cohort of 36 patients is not sufficient to test the conclusion. Answer: We enrolled the NPM1 patients as possible as we could in the following cohort in this study period.

Minor Essential Revisions

1. In Table 3, Cytogenetic should be evaluated as a prognostic factor. Answer: Thank you for the reviewer’s comment. We add the cytogenetic (Normokaryotype vs others) in this table.

2. Which exon of NPM1 were detected? exon 12? The authors should provide methods of gene mutations in detail. Answer: The analysis of mutation in NPM1 gene is exon 12. We revised described this part in detail in the manuscript. Analysis of NPM exon 12 mutation was done as described by Falini et al [8, 11]. Briefly, the final volume for PCR reaction was 35 µL containing 200 ng DNA, 200 nmol/L deoxynucleotide triphosphate, 2 mmol/L MgSO4, 140 nmol/L of each primer, and 1 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). PCR was done by heating at 95°C for 10 minutes, followed by 35 cycles of 95°C for 45 seconds, 49°C for 1 minute, and 72°C for 1 minute, with a final step for 10 minutes at 72°C. PCR products were electrophoresed on 2% agarose gels, purified and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit, which contained AmpliTaq DNA polymerase FS (Applied Biosystems), on an automated ABI-3100 Genetic Analyzer (Applied Biosystems). Abnormal sequencing results were confirmed by at least two repeated analyses.

Reviewer Cristina Mercucci:

I would suggest to merge figure 2 and 3. Answer: We merge figure 2 and 3 according to the reviewer’s comment.

As around 15% of NPM+ AML are associated with cytogenetic aberrations it would be interesting to know cytogenetic changes in this
series and their distribution in the two immunophenotypic subgroups.
Answer: The 108 NPM patients include 91 patients were normal karyotype, 12 with additional change, 5 were non-mitosis. There were 89 patients with complete cytogenetic data and cluster classification. 77 patients with normal karyotype (67 patients were the cluster one and 10 patients were cluster two), and twelve patients with additional cytogenetic changes (10 were cluster one and 2 were cluster 2). The p value is 0.662.

Table 3. CEBPA mutations need to be classified as mono- or bi- allelic.
Answer: Four patients have NPM1 mutation and concurrent with mono-allelic CEBPA mutation. We add this description in the Table.

In addition to addressing the referees's comments we require the following editorial points be addressed:

1. Copy Editing - After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

Answer: Aristine Cheng is a doctor who completes her medical education in the Cambridge University with fluent English speaking. She helps us for English editing in the manuscript.

Sincerely

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