The authors thank Reviewer 2 for insightful comments and careful analysis of our manuscript. As a matter of fact, we had considered several of the Reviewer's remarks before assembling the manuscript, which allows us to provide additional information and to amend the manuscript as follows.

Re: Based on the variability observed in testing RRM2 and TFRC expression in leukemic cells (not surprising, in my opinion, since they are primary cells), the authors refer to proliferation-prone samples (patient), but ultimately they do not associate this predicted fast proliferating phenotype to an iron condition, nor authors investigate the cell oxidative state.

We agree the large variability of gene expression in leukemic blasts had to be expected. We did not strictly associate the seemingly larger proliferation potential of these blasts to a specific change of iron homeostasis as compared to non-leukemic cells, but the data provided in Figures 2E, 3A,B possibly D, and 4B show that the expression of the ferritin subunits and transferrin receptor, and the IRP activity are all larger in leukemic cells. This set of features points to a transcriptional response which should lead to a larger iron input and storage in cancer cells, but, counterintuitively, also to a translational regulation associated with the apparent lack of intra-cellular iron availability. Overall these observations do define a different iron condition between leukemic and non-leukemic blasts, noting in addition that increased induced heme oxygenase in the leukemic ones (Figure 3D) releases iron previously inserted into hemoproteins. We added a sentence at the end of the 4th paragraph of the Discussion to further highlight the likely distinct iron condition in AML compared to hematopoietic progenitors.

Investigation of the cell oxidative state Actually, an extensive overlap exists between the samples studied here and those reported in a previous work (Mondet et al. Haematologica 104, e293, 2019). One early conclusion from the latter study was that the basal levels of reactive oxygen species did not display significant differences among these samples. Only when challenged with effectors of NOX or the mitochondrial respiratory chain did differences appear, and these were broken down according to the genetic abnormalities of the leukemic cells. However, given the limited number of samples falling in each category of genetic abnormalities, significant correlations with the presently reported data could not be established. This is why we avoided introducing elements of discussion about the oxidative status in the present report.

Re iron levels are missing.

Total iron levels in cells are not very informative as they include a wealth of intra-cellular species among which the most reactive ones are a minority. Over the last couple of decades a method measuring the iron concentration being displaced by chelators and reported by fluorescent indicators has become popular, but what is actually measured this way remains a matter of controversy. It is true that these measurements have sometimes been correlated with properties of interest, such as the sensitivity to

oxidative stress, in the literature. We did not have such data, and we'd like to recall here that what is often named LIP (labile iron pool) is orders of magnitude larger than what is the active iron concentration we previously defined as the FIC (functional iron concentration) in the Pourcelot et al. 2015 reference quoted in the manuscript. The FIC is the iron-associated factor that determines the proliferation rate of cells, as we demonstrated in the above reference. Therefore our opinion is that opening a discussion on any real or supposed intracellular iron concentration may contribute to lose the message of the present report by diverting the discussion on subjects that may hide the undisputable molecular properties we base our analysis on.

Re: For example: do cells with high TFRC expression correlate with cells with high or low PDK1 or LDH1?

We did try to extract correlations of the kind the reviewer suggests with the data at hand, but we failed to get clear ones that deserved being introduced in the manuscript. For instance, a vague linear relationship may be claimed between expressions of PDK1 and TFRC {[PDK1 mRNA] = 0.00675 + (0.0434 * [TFRC])} but with $R^2 = 0.408$, and without selective clustering of samples belonging to partitions 1 and 2 of Figure 4C. The only apparent feature of the plot is that, if the control samples are added, they all fall in the lower left part of the plot, a result that, in our views, is already shown in a clearer way in Figures 2E and S2A.

Re: Which group of cells is more proliferating/resistant to drugs.

Here, the relatively small number of patients is a stringent limit, considering that a part of them could not receive the induction therapy. Among the others, the only observation we can provide is that the leukemic blasts that were resistant to the treatment belonged to partition 1, i.e. to the group with the higher IRP activity and the lower ferritin-H concentration (Figure 4D,E). The survival of these patients was short, but we are reluctant to associate resistance to apparent functional iron shortage and limited storage because of the small number of such patients for which data are available.

Re: Proliferation studies and drug testing comparing AML cells with different iron signatures coming from different patients are needed in my opinion to fulfill the message.

We fully agree with the reviewer. Again, since the number of patients we had access to and for which we were able to gather extensive data is small, the purpose of our manuscript is to provide a line of study relying on molecular signatures that the present observations allowed us to establish. The clinical consequences may be significant when more data are available, but we cannot claim we have reached definitive conclusions at this stage.

Re: Moreover, is there any correlation between iron signature and patient outcome?

In a similar way as for our answer above, the available numbers are small with the additional problem that the clinical follow-up is incomplete for some patients. It means

we cannot precisely calculate the overall survival. In the population we studied, the longest living patients belonged to partition 1 and were AML4, but the outcome seemed mixed (relatively short and long overall survival) in both partitions. This is why we avoided pushing the idea that our conclusions might have a general interest to adjust the treatment of all AML patients. We rather seek to alert on the molecular aspects of iron homeostasis to be considered when a specific treatment, such as one trying to induce ferroptosis of leukemic blasts, is envisioned by clinicians.

Minor concerns:

Re: Figure legends could be condensed as well as the discussion

An additional paragraph, now 4.6, was added to the Material and Methods section to describe the way the data were plotted and these details were removed from the legends. Also, the nature of the statistical comparisons were shifted to the text and only the P values were kept in the legends.

In addition, Discussion has been shortened by removing part of the 1^{rst} sentence of the 4th paragraph, and by rewriting and shortening the 5th and 6th paragraphs (new versions highlighted in green).

Re: It's not clear how gene expression values are plotted in the graphs. Multiple measurements made on an individual primary sample should be used to generate a mean value referred to that sample. Then, mean values should be used to graph results and make statistical comparisons.

What the Reviewer describes is what we did to report our results. The first step is explicitly described in the legend of Figure 3C, the only place it is directly shown. To comply with the Reviewer's suggestion, we added a sentence at the end of paragraph 4.5 to detail how Forest plots were constructed.

Re: Figure 2. A discrepancy emerges between the text and the figure legend in terms of cell proliferation. In the text the authors refer to leukemic cells as non-proliferating, while in the figure legend the increase of proliferation after iron addition respect to no iron is reported as statistically significant.

The Reviewer refers to panel A, right bars, and the sentence 'With Tf-iron supply, leukemic blasts did not expand (***Figure 2A***)' in the text. The misunderstanding arises from the fact that our sentence was written with the amount of seeded cells for comparison (the rightmost lane is below this 100% reference value), whereas the Reviewer understood that we were comparing growth with or without transferrin for which the difference is significant. Therefore, we replaced the unclear sentence (l.136 submitted version) by a new one: 'Addition of saturating Tf-iron significantly limited the loss of viability of leukemic blasts, but the blast population still decreased between seeding and 3 days after *ex vivo* culture (***Figure 2A***)'.

Figure S2. The reported differences in PDK1 and LDH1 gene expression between controls and AML cells is not sufficient in my opinion to ultimately refer for a shift in cellular metabolism.

Again, we agree with the Reviewer, and we reported these data because a good deal of the scientists interested in these questions (see one comment of Reviewer 1 for instance) considers the decrease of oxidative phosphorylation and increase of glycolysis as a general and large effect (a hallmark) occurring in all cancer cells. The reality is subtler, as often developed in several critical recent papers and reviews, such as the Pavlova reference quoted in the manuscript. This is why we tried to show the data for the present samples, taking care of not being too affirmative (l.203-205 of submitted version: 'may be…an indication…; l.349 'signs of metabolic shift toward glycolysis'). We slightly reworded these sentences by changing them to 'might be … an indication' and 'signs of a possible metabolic shift…' to more clearly stress that these observations suggest rather than demonstrate.

Moreover, a discrepancy emerges again between the text and figure legend in terms of statistically significance of the results.

We did not detect, or did not understand, the discrepancy mentioned by the Reviewer. The differences between leukemic and non-leukemic cells for expression of both PDK1 and LDHA are significant and labeled with 3 stars in the figure and its legend, and this is summarized in the text to suggest metabolic changes (see specific response above).

Figure 4. Assuming that samples initiating with P are leukemic cells (please, add a reference in the legend), it's not clear why authors refer to a higher IRE-binding activity in leukemic cells than in controls (starting with N?). Moreover, IRP1 and IRP2 are not predicted to co-migrate in RNA shift assay of human samples?

Here we have neglected introducing our internal nomenclature to designate the patients, thinking it was not needed. However this led to confusion for the Reviewer: we apologize. All labels beginning by P or N are for patients, either with large amounts (P = positive) or low amounts (N = negative) of the CD34 surface marker. Figure 4A was aimed at showing the variability of the amount of IRP activity in leukemic blasts, and there are no non-leukemic samples on this blot. Non-leukemic samples were studied in this respect in a previous study (Pourcelot et al. 2015) but they were not of use in the analysis illustrated in Figure 4C-E. We tried to clarify this point in the legend, and also in that of Figure 3C.

Regarding the respective migrations of human IRP1 and IRP2, the Reviewer is correct with the conventional RNA-shift assay in which they co-migrate, in contrast to other species such as mouse. However, we and others, *e.g.* Tong and Rouault, Cell Metab 3, 199-210, 2006, showed that it was possible to separate the human molecular forms by adjusting the acrylamide % and by extending the migration. This is why we described the method in some details in the relevant section (4.4), despite the fact that it was published before. An illustration of the obtained raw data is in Figure 4A, in which the

recombinant forms, that correspond exactly at the native ones (see quoted references), migrate differently.