

The dynamics of hematopoiesis over the human lifespan

Corresponding Author: Dr R. Grant Rowe

Version 0:

Decision Letter:

1st Feb 2024

Dear Grant,

Your Resource, "The dynamics of hematopoiesis over the human lifespan", has now been seen by 3 reviewers. As you will see from their comments below, although the reviewers find your work of considerable potential interest, they have raised a number of concerns. We are interested in the possibility of publishing your paper in Nature Methods, but would like to consider your response to these concerns before we reach a final decision on publication.

We therefore invite you to revise your manuscript to address these concerns. We agree with the reviewers that more robust validation is necessary to support the conclusions of the analysis but we are going to overrule the request for validation in mouse experiments and need for mechanistic insight.

I think it would be valuable to set up a zoom chat to discuss the revision plan. How about sometime in the week of Feb 12? Please let me know.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your paper:

- * include a point-by-point response to the reviewers and to any editorial suggestions
- * please underline/highlight any additions to the text or areas with other significant changes to facilitate review of the revised manuscript
- * address the points listed described below to conform to our open science requirements
- * ensure it complies with our general format requirements as set out in our guide to authors at www.nature.com/naturemethods
- * resubmit all the necessary files electronically by using the link below to access your home page

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We hope to receive your revised paper within 12 weeks. If you cannot send it within this time, please let us know. In this event, we will still be happy to reconsider your paper at a later date so long as nothing similar has been accepted for publication at Nature Methods or published elsewhere.

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Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to consider your work.

Sincerely,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor
Nature Methods

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

The resource manuscript submitted by Li et al demonstrated how hematopoietic stem cells (HSCs) adjust their lineage output throughout a person's lifetime to support age-related physiological changes via single cell analysis. The researchers profiled individual transcriptome states of human HSPCs from gestation to aging, revealing the gene expression networks that govern age-specific differentiation of HSCs and the dynamics of fate decisions and lineage priming over time. They identified a fetal-specific HSC state with engraftment and multilineage capacity. They also proposed CD69 as a novel marker for fetal HSC transcriptional programs. Finally, they mapped acute myeloid leukaemia (AML) scRNA transcriptomes against their atlas, and proposed that their dataset could potentially be utilized for disease prognosis.

Detailed comments and suggestions for improvement are provided below,

Major concerns

1. While the challenge to establish HSPC data on human lifespan is meaningful, the essential approach in this manuscript is an established comparison method between multiple datasets of scRNA sequences. In this point, is it possible to emphasize high-impact novel procedures or significant improvements?
2. Were gene expressions on chromosome X and Y excluded or included? How about race? As researchers utilized samples from different individuals, the normalization of major genetic background factors would be indispensable. If they haven't, is it possible to show these genetic backgrounds do not affect the classifications. If they had, it is recommended to exclude or compensate for these factors and describe it in the method.
3. To enhance the impact worth for the public resource in Nature Methods journal, how about integrating to the scDNA-methylation profile? DNA-methylation is also reported to be a biomarker which is related to age, development and diseases, and a more stable factor compared to gene expressions. There are a few whole genome methylation analysis data in HSPC at this moment [Farlik, et al. Cell Stem Cell. 19(6):808-822 (2016), Li, et al. Cell Discov 7, 86 (2021)].
4. The significance of High mixed age score and High TF score in AML prognosis is also interesting. However, validation using single dataset does not provide strong evidence, as there are several publicly available AML datasets. Are they reproducible in the other datasets in AML? How about the relationship between classical prognostic factors in AML such as patient age, FAB-classification, FLT3-ITD, TP53mut, NPM1mut, CEBPAmut and, DNMT3Amut in the multiple variate analysis? Moreover, MDS, a pre-cancerous status of AML, is well-known to be associated with aging. Is it possible to connect normal hematopoiesis, MDS, and AML sequences, and/or predict the progression of MDS using the proposed scoring system?
5. The characterization of CD69 seems to be in its initial stages. Given that CD69 expression has been noted in mouse hematopoietic stem cells (HSCs), it would be valuable for the authors to investigate its expression in murine models. This could involve examining whether there is a dynamic change in CD69 expression from fetal liver to neonatal bone marrow HSCs. Additionally, it would be beneficial to compare the reconstitution capabilities of CD69+ and CD69- fetal or adult murine HSCs to assess any differences.
6. In Figure 6E, the fetal liver sample at Week 20 not only shows a different pattern of CD34 expression but also a distinct proportion of CD34+ cells compared to the postnatal samples. Given these differences, it would be beneficial for the author to elaborate on the validity of comparing the range of CD69 mean fluorescence intensity in this specific context.
7. The AML study raises concerns due to its perceived vagueness, stemming from a weak association with the transcriptional programs identified in this research. An exploration of differences in subtypes or genotypes within each AML cohort is crucial

for a more nuanced understanding. This revision aims to elucidate these distinctions and foster a clearer linkage between the AML study and the identified transcriptional programs.

8. In Figure 7B, it is important to ascertain the proportion of elderly cohorts within the "mixed age" category, particularly those exhibiting transcriptional program patterns that differentiate them from the "elderly enriched" category. Should such distinct patterns be identified, a comprehensive elucidation on the prevalent subtypes of AML among these elderly patients within the "mixed age" group becomes essential.

9. What conclusion can be drawn from connecting the dataset of lymphocytic leukemia patients with the transcriptome dataset? Is the level of association seen in lymphocytic leukemia patients comparable to that observed in AML patients? It is beneficial to clarify the implications and differences in the association between lymphocytic leukemia patients and the transcriptome dataset, particularly in comparison to AML patients.

Minor concerns

1. The recorded cell numbers for the majority of donors are generally below 3000 cells, which is deemed as relatively low. Specifically, in some cases involving adult bone marrow donors, fewer than 1000 cells are captured (e.g., 737, 580), resulting in a less well-defined resolution at individual level.

2. What are the standards or requirements used to define "healthy donors" who are over 50 years old in this study?

3. Can the authors provide further elucidation on the interpretation of the lineage dominance score depicted in Figure 5B? Specifically, it would be

helpful to have additional clarifications regarding what defines a high or low score in the violin plots. Additionally, could the authors expound on how this score is derived from the six lineages illustrated in Figure 5A? A more detailed explanation of the criteria for scoring, as well as the relationship between the score and the depicted lineages, would enhance the reader's understanding of the presented data.

4. Authors mentioned that they performed random forest classification not only AML but also B-ALL. What about the results of B-ALL?

5. With elder and mixed age score, as it is derived from original datasets, it should be validated whether it can predict the age of healthy donor in other datasets of hematopoietic cells. With TF score, how did authors choose the seven TFs? From Figure 7F, it seems there are several more enriched TFs in the volcano plots.

6. The scRNA-seq data of multiple life stages is unique point of this research. In this regard, is it possible to perform trajectory and pseudo-time analysis, as authors regard these datasets consistent continuous process?

Reviewer #2:

Remarks to the Author:

This work has focused on the characterization of transcriptomes at single cell level of human hematopoietic progenitors and stem cells (HSPC) at different stages of development and life. The collection of these samples is not minor and represents a comparable new dataset of embryonic to adult/aged life. The authors generate a new complete scRNAseq dataset (CD34+) from 26 donors covering 22 timepoints divided into 7 discrete phases (1st trimester – 3x, 2nd trimester – 6x, perinatal – 2x, childhood- 2x, adolescent- 2x, adult- 7x and elderly- 4x). A total of 58,041 cell were available for testing after QC. They used sound methods for exploring lineage fate probabilities that correlate with observed data. They used cNMF method to detect underlying cellular activities (GEPs) which further relate to unilineage program. With the first methods, authors were able to conduct fate predictions in a more general fashion (myeloid, lymphoid and ery/meg) while with the second, they define a more specific scheme for differential hematopoietic lineage branching.

Another important aspect of this work is the identification and functional validation of a fetal-specific HSC marker (CD69) that enriches for cells with engraftment capacity. They finally use this information to segregate AML patients by the comparison of their transcriptome and associate an elderly pattern of expression to a good prognostic factor. In turn, samples that do not show this elderly pattern are used to identify some transcription factors that are predictive of poor prognosis.

In general, the work contains very interesting data and some novel concepts. However, my main concern is that for the most part, the work remains mainly descriptive, even if the description is outstanding. They do not demonstrate the functional relevance of their predictions, except for the CD69 marker enriched in fetal HSCs. They do not demonstrate the potential for MPPs or for HSCs of specific time point nor the importance in leukemia of the TF identified in AML).

Other main concerns are outlined below:

There is an important limitation by considering 'only' 26 donors (9 of them prior to birth) for covering the whole lifespan. Is this really representative? I recognize the effort in collecting these human samples, but the title is too ambitious when referring to hematopoiesis over the human lifespan or human hematopoietic atlas. I would recommend to use a more specific title focused in the prediction of cell fate or leukemia prognosis which, for me, is the most novel aspect.

If they want to keep this general title, they should validate the findings about the predominant GEP at different stages in other independent published data, especially for childhood and adolescents that have fewer cells. I understand that they cannot compare all the integrated datasets (as they try at the beginning), but they can test the prevalence of specific GEPs independently in these datasets. Would they find the same patterns of lineage segregation as in Fig 4C?

Authors include the 17-yr donor in adulthood phase. Shouldn't this donor be considered as adolescent together with the 10-yr and 12-yr donors? (Figure 1D)

Additionally, this 17-yr donor shows an abrupt cell type freq difference compared to adjacent timepoints (12yr and 25-yr) (Figure 2A). Could this result be explained by the limited number of cells (737 cells) for this donor compared to the others? In fact, the low number of samples in adolescents and childhood is a problem for several conclusions.

Several figures are overloaded with panels that are not necessary or hard to follow. For example, Figure 2A represents values based on the 7 discrete phases they have previously defined and discussed in the paper and could be suppl. In Fig 2C, StatOT plots seem to show much less cells with designated states for childhood and adolescence (or it is a visual effect because cells are more compacted in StatOT)? What can we interpret from these plots? The centroid values are pretty similar except for childhood and adolescence. I would recommend to move it to Suppl. And just show Fig2D, as a summary of results.

Fig 2B – Authors included in the manuscript, as conclusions drawn from this figure, 'PCA supportive of the seven age designations'. Could the authors explain better their conclusions about this PCA? For instance, PC1 seems to capture childhood and adolescence vs others (clearly driven by Lymphoid programs) and PC3 seems to differentiate between stages up to birth (included) and the rest. PC2 is unclear.

Fig 2E. Range of reported adjusted p-values is extremely low (down to 10^{-300} !!). Although, mathematically it can be legitimately correct, these values are not biologically reasonable (or out of interpretation). Color scheme for this plot can focus on significant or non-significant or a more interpretable range such as (>0.05 , <0.0001 , <0.001 ...). Since heatmaps are symmetric, the authors could use just the lower or upper section to avoid redundancy.

One of the key aspects of NMF is to select the number of components (in this case, GEPs) K. In Supp Fig S4, the authors select $k=35$ (trade-off between error and stability). They assess components independence (although this is not a requirement for NMF, it is for ICA) with a Euclidean distance metric. Have they assessed the correlation among the 35 GEPs (Pearson and/or Spearman) as they show in Fig4? I wonder if part of the 35 GEPs are similar enough so they could be merged? Additionally, isn't any other useful info from the other 29 GEPs (not associated with unilineage state). Which is the overlap degree between the unilineage related GEPs genes (top 50 genes) with the marker genes for the associated clusters (Fig.1B) i.e. Mono/DC?

Fig 4C. First row is the same for all (HSC) so it does not seem meaningful. They seem to not detect any difference in HSCs, but in the next figure they do. So this is confusing. I would focus this figure in MPP and progenitors, maybe some HSC data from Fig 5 could be moved here. Fig 5A is far too difficult to understand (looks pretty, but not very intuitive), could the authors find another way to represent how they find that HSCs are all primed to specific lineages? More importantly, does figure 5A mean that all HSCs are biased to a single specific lineage? That is not what other functional data suggests when single cells are transplanted.

Figure 7 is indeed important. However, only one discovery cohort is used and not validation in other cohorts are performed for the elderly signature nor the prediction potential of the TF identified as prognostic tool. This must be performed for this data to be meaningful.

Minor concerns:

Fig3C. Missing legend for color scheme for the strength scale.

Sentence in line 204 ('an auxiliary lymphoid program was activated during childhood and adolescence'). Isn't this what they have already detected with the PBA/StatOT analysis Fig2c?

Fig4A is hard to interpret. Could the authors indicate which pairwise comparisons are significant? Maybe they could leave in white (not coloured) the non-significant ones. Could this figure be combined with panel B? Both are showing the same results.

Fig 4B is difficult to visualize due to the dot sizes. Since only the negative correlated cases are of interest, maybe figures can only show the negative axis section to zoom in values and then, directly add the labels of the significant comparisons (instead of listing below). This figure as it is may go to suppl. Additionally, is a negative correlation of -0.1 strong enough (biologically speaking) - although they are statistically significant -?

Fig 6D. In the elderly GEP, ribosomal genes are highly enriched. Any interpretation?

Fig 6H. There are very few animals in the 500 group, but the % of chimerism from 150 cells seems to be very low compared to the 50 cell group.

Fig7A. Could you plot the heatmap cells 'more square-shape' (they are vertical rectangles).

Fig 7E. Have you explored a multivariate analysis with age at diagnosis, blast % and ELN2017 (+ age score)?

Related to Fig7F. Have been those seven TFs previously associated with AML?

Reviewer #3:

Remarks to the Author:

Li et al describe an extensive single cell RNA-seq (scRNA-seq) characterization of human hematopoietic progenitors that spans a range of human ages. CD34+ cells were evaluated from humans as young as 10 weeks EGA to 77 years old. The authors show that lineage priming changes with age, consistent with prior observations. Peak lymphoid priming coincides with perinatal and pediatric ages. The authors used consensus non-negative matrix factorization (cNMF) to resolve lineage specific gene expression programs (GEPs). They then use the GEPs to draw several conclusions regarding age-specific patterns of bifurcation to the hematopoietic hierarchy and age-specific HSC priming. They argue that old HSCs tend to be less primed to a given lineage than fetal HSCs but that the priming that does occur in old HSCs tends to be more directed towards a specific lineage. The authors also identify a GEP that defines fetal HSCs and show that one member of this program, CD69, can enrich for functional HSCs in the fetal liver. Finally, they show that AML cells can differentially express genes that associate with fetal, perinatal, pediatric, adult or elderly HSPC states. AML that preferentially express genes or transcription factors that associate with elderly identity tend to have favorable outcomes, irrespective of the age of the patient.

This is a comprehensive data set. While the authors acknowledge that several single cell atlases of human HSPCs already exist to describe hematopoiesis at different ages, they correctly note that their study uniquely captures the full span of human development and aging. The authors have applied a number of sophisticated computational tools to analyze the data, and some of the conclusions (e.g. CD69 enrichment of fetal HSCs) are backed by functional assays. The data set will add significant value to the field. Overall, the manuscript and abstract are clear, and relevant literature are appropriately credited.

The paper does have some notable weaknesses, as discussed below.

Major criticisms:

1. Several of the claims are based on informatics alone with little or no supporting evidence to help establish a) whether the analytic approach is robust, or b) whether the findings are biologically important. For example:

- Figure 4 operates on the premise that anticorrelations between lineage GEPs imply bifurcations in the hematopoietic hierarchy. These anticorrelations are most evident at the committed progenitor level, and they change with age. For example, lymphoid, granulocyte and erythroid programs are more exclusive of one another in fetal progenitors. The programs become less exclusive in childhood, more exclusive in adulthood, then completely non-exclusive in elderly progenitors.

It is hard to know what to make of this without more mechanistic insight or experimental validation. Are there truly bipotent lymphoid-megakaryocyte primed progenitors in the first trimester that are not present (or are encompassed by multipotent progenitors) in childhood, but then are present again in adulthood, but then disappear again with old age, as Figure 4C indicates? Does lineage restriction within the CD34+ population really fluctuate like this with age, or is this an artifact?

The authors should back this model with functional data. They could also test whether similar patterns are evident in some of the other published data sets (e.g., the Roy et al data set).

- Figure 5 also makes claims that are difficult to interpret. The lineage dominance score and the GEP usage score are both contrived values. There is no frame of reference to know whether the statistically significant difference between elderly and adult or first trimester lineage dominance scores means anything, functionally. Likewise, there is no frame of reference to know whether a change in GEP usage means anything. At minimum, it would be helpful to see how the scores in HSCs compare to those of committed progenitors. For example, if a lymphoid primed progenitor at a given age has a log transformed lineage dominance score of ~10, then the small differences between the fetal, adult and elderly HSC scores might not mean much biologically despite statistical significance.

- The distinction between the “elderly enriched” and “mixed age” groups of AML patients in Figure 7 does not look compelling. Across the entire data set, there appears to be some enrichment for fetal genes in the AML, essentially no enrichment of perinatal genes, some enrichment of pediatric genes, low enrichment of adult genes and then varying degrees of high enrichment for elderly genes. This does not make intuitive sense, and it requires some explanation. The survival differences do offer a degree of reassurance, but the data still raise the question of whether age signatures are capturing some other dependent variable (e.g., age-related differences in stemness).

2. The fetal GEP in Figure 6 does not contain genes that have been canonically associated with fetal identity, such as LIN28B, HMGA2, IGF2BP3, etc. Other studies of human fetal hematopoiesis (e.g., the cited Calvanese et al. and Roy et al. papers) clearly identified these genes. What accounts for the discrepancy? It's concerning that the computational approach taken to identify a fetal GEP would miss several well-established fetal genes.

Minor comment:

1. Figure 5A would benefit from better clarity. After several reads it was clear that the brown insets to each pie piece were aggregates of lines of individual cells. However, this was not intuitive, even with the legend. Perhaps an inset magnifying part of one plot to show the lines of individual cells could be helpful.

Author Rebuttal letter:

Responses to Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

The resource manuscript submitted by Li et al demonstrated how hematopoietic stem cells (HSCs) adjust their lineage output throughout a person's lifetime to support age-related physiological changes via single cell analysis. The researchers profiled individual transcriptome states of human HSPCs from gestation to aging, revealing the gene expression networks that govern age-specific differentiation of HSCs and the dynamics of fate decisions and lineage priming over time. They identified a fetal-specific HSC state with engraftment and multilineage capacity. They also proposed CD69 as a novel marker for fetal HSC transcriptional programs. Finally, they mapped acute myeloid leukaemia (AML) scRNA transcriptomes against their atlas, and proposed that their dataset could potentially be utilized for disease prognosis.

Detailed comments and suggestions for improvement are provided below,

Major concerns

1. While the challenge to establish HSPC data on human lifespan is meaningful, the essential approach in this manuscript is an established comparison method between multiple datasets of scRNA sequences. In this point, is it possible to emphasize high-impact novel procedures or significant improvements?

Thank you. We have revised throughout (mainly in the introduction and discussion) to more clearly demonstrate how our novel approaches have supported our new age-dependent models of lineage fate choices and cell states.

2. Were gene expressions on chromosome X and Y excluded or included? How about race? As researchers utilized samples from different individuals, the normalization of major genetic background factors would be indispensable. If they haven't, is it possible to show these genetic backgrounds do not affect the classifications. If they had, it is recommended to exclude or compensate for these factors and describe it in the method.

The reviewer brings up an excellent point that we carefully considered when beginning our analysis. We purposefully did not exclude genes from the X and Y chromosome, as we could not a priori conclude that genes from the X and Y chromosome do not play a role in the regulation of human hematopoiesis. To exclude these genes from analysis would have decreased the discovery potential of our dataset and also removed X and Y chromosome genes known to play important roles in hematopoiesis (GATA1, etc.). To address the reviewer's comment regarding X and Y chromosome genes, we have now included in new Extended Data Figure 2 an analysis showing that less than 5% of all variable genes in the study were located on the X or Y chromosome, and furthermore, genes on the X and Y chromosomes make up less than 5% of genes marking nearly every human age. Given that 5% of genes in the human genome are located on the X and Y chromosomes, these results indicate that sex-based expression differences from X and Y chromosome genes are not overrepresented in driving the variation observed in our dataset. Regarding racial differences and genetic heterogeneity, this is certainly a major issue facing all of the human single cell atlases that have been published recently. Because race demographics are not consistently provided by the several diverse sources of our donors, there is no possible way for us to normalize for racial genetic background. Because few, or even nearly none, of the existing human single cell atlases truly addresses genetic heterogeneity from racial differences, we posit that methods for doing this from single cell RNA data will be an incredibly valuable approach that should be the focus of future investigations by the field but are outside the scope of this particular paper.

3. To enhance the impact worth for the public resource in Nature Methods journal, how about integrating to the scDNA-methylation profile? DNA-methylation is also reported to be a biomarker which is related to age, development and diseases, and a more stable factor compared to gene expressions. There are a few whole genome methylation analysis data in HSPC at this moment [Farlik, et al. Cell Stem Cell. 19(6):808-822 (2016), Li, et al. Cell Discov 7, 86 (2021)].

This is an outstanding point by the reviewer and has led us to further mechanistic insight into the age-related differences we have identified in hematopoiesis. Based on the reviewer's comment, we applied the age-specific HSC methylation data from Farlik et al. to our fetal-HSC-GEP in new Extended Data Figure 8, identifying increased DNA methylation surrounding the transcriptional start site of genes driving the fetal-HSC-

GEP as a potential mechanism for silencing of the fetal-HSC-GEP in postnatal bone marrow. We have revised to incorporate and discuss this finding.

4. The significance of High mixed age score and High TF score in AML prognosis is also interesting. However, validation using single dataset does not provide strong evidence, as there are several publicly available AML datasets. Are they reproducible in the other datasets in AML? How about the relationship between classical prognostic factors in AML such as patient age, FAB-classification, FLT3-ITD, TP53mut, NPM1mut, CEBPAmut and, DNMT3Amut in the multiple variate analysis? Moreover, MDS, a pre-cancerous status of AML, is well-known to be associated with aging. Is it possible to connect normal hematopoiesis, MDS, and AML sequences, and/or predict the progression of MDS using the proposed scoring system?

We thank the reviewer for these valuable comments to refine the data we present regarding AML prognosis. As requested, we sought to validate our transcriptome age score and TF score in the independent TCGA AML dataset. The transcriptome age score also significantly segregates survival outcomes in the TCGA dataset, and while the TF score trends towards segregating survival outcomes, the TCGA dataset is inadequately powered to demonstrate significance at a threshold of $p=0.05$. Moreover, as requested, we have also now included additional covariate analysis. We previously had already presented data in Figure 7 demonstrating patient age is not associated with which transcriptome age cluster the patient's AML segregates into, but have now added Cox proportional hazard analysis of FAB classification, FLT3-ITD, TP53, NPM1, CEBPA, and DNMT3A mutations. We additionally included Cox proportional hazard analysis of IDH and RAS mutations. These data are all presented in new Extended Data Figure 9. Lastly, regarding the reviewer's MDS comment- we believe that study of MDS and the biological sequence of transformation into AML is clearly an important next step for our studies. However, assessment of the specific sequence of molecular events during malignant transformation along the continuum from MDS to AML, and how this maps with transcriptome age, is not the focus of this current manuscript and could be the focus of an additional paper. We have, however, provided data in Extended Data Figure 9 demonstrating transcriptome age score does not significantly segregate with absence or presence of MDS prior to AML diagnosis (i.e., secondary AML).

5. The characterization of CD69 seems to be in its initial stages. Given that CD69 expression has been noted in mouse hematopoietic stem cells (HSCs), it would be valuable for the authors to investigate its expression in murine models. This could involve examining whether there is a dynamic change in CD69 expression from fetal liver to neonatal bone marrow HSCs. Additionally, it would be beneficial to compare the reconstitution capabilities of CD69+ and CD69- fetal or adult murine HSCs to assess any differences.

Thank you. We indeed previously examined CD69 expression in mouse fetal vs young adult HSPC populations. These data are now more clearly presented in Extended Data Figure 7A-B. We have revised the text to clarify and emphasize this.

6. In Figure 6E, the fetal liver sample at Week 20 not only shows a different pattern of CD34 expression but also a distinct proportion of CD34+ cells compared to the postnatal samples. Given these differences, it would be beneficial for the author to elaborate on the validity of comparing the range of CD69 mean fluorescence intensity in this specific context. Thank you. We typically observe slightly differing CD34/CD38 profiles on HSPCs of different ages. We believe that this is reflective of their different age states and tissue of origin rather than genetic backgrounds as we routinely observe differences in HSPC surface markers even in inbred mouse strains (see Extended Data Figure 7A/B). We agree that CD69 expression appears to be a continuum rather than discrete populations. We have therefore elaborated on our gating and sorting strategy, and our validation of this strategy via demonstration of the functional differences in Figures 6F-H. We have revised the text accordingly.

7. The AML study raises concerns due to its perceived vagueness, stemming from a weak association with the transcriptional programs identified in this research. An exploration of differences in subtypes or genotypes within each AML cohort is crucial for a more nuanced understanding. This revision aims to elucidate these distinctions and foster a clearer linkage between the AML study and the identified transcriptional programs.

We thank the reviewer for this suggestion. As requested, in new Extended Data Figure 9, we have provided more granular analysis of AML genetic subtypes and assessed their association with transcriptome age. These additional genetic subtypes assessed by Cox proportional hazard analysis include FLT3-ITD, TP53, NPM1, CEBPA, DNMT3A, IDH, and RAS mutations. We have presented and discussed these new results and

their potential significance.

8. In Figure 7B, it is important to ascertain the proportion of elderly cohorts within the "mixed age" category, particularly those exhibiting transcriptional program patterns that differentiate them from the "elderly enriched" category. Should such distinct patterns be identified, a comprehensive elucidation on the prevalent subtypes of AML among these elderly patients within the "mixed age" group becomes essential.

We apologize that this was not clear in our original text and figure. Indeed, we also felt this was important and had already analyzed the proportion of elderly patients within both of the transcriptome age categories and did not observe biased segregation. These data are now more clearly presented in Figure 7A.

9. What conclusion can be drawn from connecting the dataset of lymphocytic leukemia patients with the transcriptome dataset? Is the level of association seen in lymphocytic leukemia patients comparable to that observed in AML patients? It is beneficial to clarify the implications and differences in the association between lymphocytic leukemia patients and the transcriptome dataset, particularly in comparison to AML patients.

Thank you. We have expanded our discussion of the acute leukemia classification in Extended Data Figure 9A in both the results and discussion section to address this concern.

Minor concerns

1. The recorded cell numbers for the majority of donors are generally below 3000 cells, which is deemed as relatively low. Specifically, in some cases involving adult bone marrow donors, fewer than 1000 cells are captured (e.g., 737, 580), resulting in a less well-defined resolution at individual level.

We have noted and discussed this limitation in the discussion.

2. What are the standards or requirements used to define "healthy donors" who are over 50 years old in this study?

Thank you. We have revised the methods to state these criteria.

3. Can the authors provide further elucidation on the interpretation of the lineage dominance score depicted in Figure 5B? Specifically, it would be helpful to have additional clarifications regarding what defines a high or low score in the violin plots. Additionally, could the authors expound on how this score is derived from the six lineages illustrated in Figure 5A? A more detailed explanation of the criteria for scoring, as well as the relationship between the score and the depicted lineages, would enhance the reader's understanding of the presented data.

Thank you. We have expanded on the description and interpretation of these data in the results section, as requested. This section has been markedly expanded and deepened. We have also added further detail in the methods section.

4. Authors mentioned that they performed random forest classification not only AML but also B-ALL. What about the results of B-ALL?

Thank you. We have corrected the text to reflect that our B-ALL analysis was only classifying single leukemia cells into HSPC states identified by our atlas.

5. With elder and mixed age score, as it is derived from original datasets, it should be validated whether it can predict the age of healthy donor in other datasets of hematopoietic cells. With TF score, how did authors choose the seven TFs? From Figure 7F, it seems there are several more enriched TFs in the volcano plots.

We have revised to more clearly state that we utilized all transcription factors identified among the differentially expressed transcripts between the high- or low-composite score AMLs. The volcano plots do not only show differentially expressed TFs, but rather show all differentially expressed genes.

6. The scRNA-seq data of multiple life stages is unique point of this research. In this regard, is it possible to perform trajectory and pseudo-time analysis, as authors regard these datasets consistent continuous process?

This is an excellent suggestion. In future studies, we plan to perform trajectory analysis on the axis of real time to relate stem cell populations to downstream progenitors at later time points. This would require alterations to existing algorithms for trajectory reconstruction, and so we plan to do this in a future publication. We have added this future direction to the discussion.

Reviewer #2:

Remarks to the Author:

This work has focused on the characterization of transcriptomes at single cell level of human hematopoietic progenitors and stem cells (HSPC) at different stages of development and life. The collection of these samples is not minor and represents a comparable new dataset of embryonic to adult/aged life. The authors generate a new complete scRNAseq dataset (CD34+) from 26 donors covering 22 timepoints divided into 7 discrete phases (1st trimester – 3x, 2nd trimester – 6x, perinatal – 2x, childhood- 2x, adolescent- 2x, adult- 7x and elderly- 4x). A total of 58,041 cell were available for testing after QC. They used sound methods for exploring lineage fate probabilities that correlate with observed data. They used cNMF method to detect underlying cellular activities (GEPs) which further relate to unilineage program. With the first methods, authors were able to conduct fate predictions in a more general fashion (myeloid, lymphoid and ery/meg) while with the second, they define a more specific scheme for differential hematopoietic lineage branching.

Another important aspect of this work is the identification and functional validation of a fetal-specific HSC marker (CD69) that enriches for cells with engraftment capacity. They finally use this information to segregate AML patients by the comparison of their transcriptome and associate an elderly pattern of expression to a good prognostic factor. In turn, samples that do not show this elderly pattern are used to identify some transcription factors that are predictive of poor prognosis.

In general, the work contains very interesting data and some novel concepts. However, my main concern is that for the most part, the work remains mainly descriptive, even if the description is outstanding. They do not demonstrate the functional relevance of their predictions, except for the CD69 marker enriched in fetal HSCs. They do not demonstrate the potential for MPPs or for HSCs of specific time point nor the importance in leukemia of the TF identified in AML).

We thank the reviewer for this compliment. Guided by specific feedback from this reviewer and others, we now present several lines of validation compared to the previous version of the manuscript, which includes in silico validation of key findings in independent -omics datasets as well as experimental validation of lineage potential and biases in key HSC and progenitor age states.

Other main concerns are outlined below:

There is an important limitation by considering 'only' 26 donors (9 of them prior to birth) for covering the whole lifespan. Is this really representative? I recognize the effort in collecting these human samples, but the title is too ambitious when referring to hematopoiesis over the human lifespan or human hematopoietic atlas. I would recommend to use a more specific title focused in the prediction of cell fate or leukemia prognosis which, for me, is the most novel aspect.

If they want to keep this general title, they should validate the findings about the predominant GEP at different stages in other independent published data, especially for childhood and adolescents that have fewer cells. I understand that they cannot compare all the integrated datasets (as they try at the beginning), but they can test the prevalence of specific GEPs independently in these datasets. Would they find the same patterns of lineage segregation as in Fig 4C?

Thank you. We have added the limitation of donor number in the discussion. We have expanded the description of the GSEA analysis of the lineage GEPs where we show that these GEPs bear signatures consistent with the assigned lineage identified in independent datasets (new Extended Data Figure 5D). We have also expanded the discussion of new Extended Data Figure 8A-C where we show that the fetal HSC GEP is present in multiple independent human fetal HSPC datasets (in cells from both fetal liver and bone marrow) and is fetal-biased. We have revised the results and discussion related to these findings.

We also have further gone on to show that the lineage GEPs also are able to predict age-specific branching in an independent dataset that comes closest to matching the lifetime age range we profile and found that branching outcomes in the independent study closely parallel the age-specific branching outcomes we find in our study. These data are presented in new Extended Data Figure 6B.

Authors include the 17-yr donor in adulthood phase. Shouldn't this donor be considered as

adolescent together with the 10-yr and 12-yr donors? (Figure 1D)
Additionally, this 17-yr donor shows an abrupt cell type freq difference compared to adjacent timepoints (12yr and 25-yr) (Figure 2A). Could this result be explained by the limited number of cells (737 cells) for this donor compared to the others? In fact, the low number of samples in adolescents and childhood is a problem for several conclusions. Thank you. We have expanded on the limitations of this study in the Discussion, including variation and cell numbers and overall quantity of donors. Our classification of the 17-year-old specimen as young adult is based on PCA analysis (Figure 2B) where this specimen clusters more closely with young adult specimens. It is also apparent in Figure 2A that the pediatric lymphoid predominance appears to abate by this stage of life. Based on this very useful feedback and the feedback below, we have expanded our presentation of the PCA results in Figure 2B.

Several figures are overloaded with panels that are not necessary or hard to follow. For example, Figure 2A represents values based on the 7 discrete phases they have previously defined and discussed in the paper and could be suppl. In Fig 2C. StatOT plots seem to show much less cells with designated stated for childhood and adolescence (or it is a visual effect because cells are more compacted in StatOT)? What can we interpret from these plots? The centroid values are pretty similar except for childhood and adolescence. I would recommend to move it to Supl. And just show Fig2D, as a summary of results.

We appreciate this comment and in our revised manuscript have simplified the main figures as much as possible while maintaining presentation of the data required to support our main conclusions.

Fig 2B – Authors included in the manuscript, as conclusions drawn from this figure, ‘PCA supportive of the seven age designations’. Could the authors explain better their conclusions about this PCA? For instance, PC1 seems to capture childhood and adolescence vs others (clearly driven by Lymphoid programs) and PC3 seems to differentiate between stages up to birth (included) and the rest. PC2 is unclear.

Thank you – this is an excellent suggestion and we have done this in response to this comment and the comment above.

Fig 2E. Range of reported adjusted p-values is extremely low (down to 10^{-300} !!). Although, mathematically it can be legitimately correct, these values are not biologically reasonable (or out of interpretation). Color scheme for this plot can focus on significant or non-significant or a more interpretable range such as (>0.05 , <0.0001 , <0.001 ...). Since heatmaps are symmetric, the authors could use just the lower or upper section to avoid redundancy.

Thank you. We have revised as requested.

One of the key aspects of NMF is to select the number of components (in this case, GEPs) K. In Supp Fig S4, the authors select $k=35$ (trade-off between error and stability). They assess components independence (although this is not a requirement for NMF, it is for ICA) with a Euclidean distance metric. Have they assessed the correlation among the 35 GEPs (Pearson and/or Spearman) as they show in Fig4? I wonder if part of the 35 GEPs are similar enough so they could be merged? Additionally, isn't any other useful info from the other 29 GEPs (not associated with unilineage state). Which is the overlap degree between the unilineage related GEPs genes (top 50 genes) with the marker genes for the associated clusters (Fig.1B) i.e. Mono/DC?

Thank you for these suggestions. We have performed Spearman correlation of the GEPs and present this data in new Extended Data Figure 5C. Based on the correlation results, there were not actually any GEPs that could reasonably be merged. We now additionally have included in the text our criteria for identifying GEPs with relevant biology beyond unilineage commitment, provide full data for the contribution of every single gene to each GEP as well as marker genes for associated lineage clusters in new Extended Data Table 1, and have added to our text discussing these points.

Fig 4C. First row is the same for all (HSC) so it does not seem meaningful. They seem to not detect any difference in HSCs, but in the next figure they do. So this is confusing. I would focus this figure in MPP and progenitors, maybe some HSC data from Fig 5 could be moved here. Fig 5A is far too difficult to understand (looks pretty, but not very intuitive), could the authors find another way to represent how they find that HSCs are all primed to specific lineages? More importantly, does figure 5A means that all HSCs are biased to a single specific lineage? That is not what other functional data suggests when single cells are transplanted.

Thank you. Based on this helpful comment and feedback from reviewer 1, we have

expanded and clarified our presentation of Figure 5A. We do not mean to suggest that HSCs have unilineage outcomes – in our revision, we note that in Figure 5B, at all ages, there are subsets of HSCs that appear to be highly unilineage primed and others that seem to be more balanced – we have revised the results section to describe this more clearly. We believe that even among the highly primed HSCs that these are biases – in single cell HSC outcome validation studies, unilineage outcomes in HSCs are very rare – even highly biased clones show multilineage outcome, just with a dominant lineage present (new Extended Data Figure 6C-E). We have revised extensively to clarify this interpretation in the Results and Discussion.

Figure 7 is indeed important. However, only one discovery cohort is used and not validation in other cohorts are performed for the elderly signature nor the prediction potential of the TF identified as prognostic tool. This must be performed for this data to be meaningful.

We agree very much with this point. To this end, we have now validated the transcriptome age score in the TCGA AML dataset and find that it significantly predicts survival. We have also assessed our TF score in the TCGA AML dataset and found that while survival trends for the TF score match the BeatAML dataset, the lower power of the TCGA dataset (N=151 in TCGA, N=257 in BeatAML) does not allow to make a significant conclusion at a p-value threshold of 0.05 (TCGA only powered to yield $p=0.07$). These results are presented in new Extended Data Figure 9D-E.

Minor concerns:

Fig3C. Missing legend for color scheme for the strength scale.

We thank the reviewer for pointing out that the color scheme and strength scale as previously displayed were not clear. We have emphasized this in the updated Figure and Legend.

Sentence in line 204 ('an auxiliary lymphoid program was activated during childhood and adolescence'). Isn't this what they have already detected with the PBA/StatOT analysis Fig2c?

Thank you. The PBA/StatOT analysis did not define age-specific programs of lineage commitment – this was done using the Sobolev norm analysis in Figure 3C. We have revised the results section to clarify.

Fig4A is hard to interpret. Could the authors indicate which pairwise comparisons are significant? Maybe they could leave in white (not coloured) the non-significant ones. Could this figure be combined with panel B? Both are showing the same results.

We thank the reviewer for this good suggestion and as such have denoted the significant anti-correlations with an asterisk (*) as these are the comparisons from which we derive lineage branching later on. We also have edited the Figure Legend text to indicate non-significant pairwise comparisons are denoted in gray.

Fig 4B is difficult to visualize due to the dot sizes. Since only the negative correlated cases are of interest, maybe figures can only show the negative axis section to zoom in values and then, directly add the labels of the significant comparisons (instead of listing below). This figure as it is may go to suppl. Additionally, is a negative correlation of -0.1 strong enough (biologically speaking) - although they are statistically significant -?

This is a good point by the reviewer. To ease the viewing and interpretation of the main figure, we have moved these plots to new Extended Data Figure 6A. Additionally, regarding the strength of negative correlations, in our analysis of independent studies we actually found an even stronger significance of these negative correlations (Extended Data Figure 6B), providing validation that indeed we were not overly aggressive in identifying negative correlations.

Fig 6D. In the elderly GEP, ribosomal genes are highly enriched. Any interpretation? Thank you for noticing this and providing us the opportunity to address this observation. It is well established that the function of HSCs is sensitive to alterations in protein synthesis and translation (e.g., PMID 36803603 and 24670665) and there are stage-specific alterations in protein synthesis in mouse HSCs (PMID 33440178). Dysregulation of proteostasis can trigger hematologic malignancies, which is relevant to aging (PMID 36603185). We have expanded our presentation of the elderly HSC GEP to note the ribosomal gene expression and these key points and have now included these references.

Fig 6H. There are very few animals in the 500 group, but the % of chimerism from 150 cells seems to be very low compared to the 50 cell group.

Thank you. When nearing limiting dilution (cell dose of 50 or 150 cells with a measured engrafting cell efficiency of 1/92) it would be expected that chimerism could be variable due to cell-to-cell variation in output upon engraftment – we are essentially looking at single HSC output at these doses. We have noted this effect in the revised results section.

Fig7A. Could you plot the heatmap cells 'more square-shape' (they are vertical rectangles).

We have vertically shrunk the heatmap cells as requested but have not made them fully square shapes as that would not allow enough space for clearly labeling each age group on the left side of the heatmap

Fig 7E. Have you explored a multivariate analysis with age at diagnosis, blast % and ELN2017 (+ age score)?

Indeed, based on several of the reviewer comments, we have now added a significantly greater number of additional variates, most notably including seven of the most consequential genetic subtypes of AML, as well as FAB classification status.

Related to Fig7F. Have been those seven TFs previously associated with AML?

Thank you. We have revised the results section to provide references supporting previous association of a subset of these factors with AML, but lack of previous association for the remainder of these factors.

Reviewer #3:

Remarks to the Author:

Li et al describe an extensive single cell RNA-seq (scRNA-seq) characterization of human hematopoietic progenitors that spans a range of human ages. CD34+ cells were evaluated from humans as young as 10 weeks EGA to 77 years old. The authors show that lineage priming changes with age, consistent with prior observations. Peak lymphoid priming coincides with perinatal and pediatric ages. The authors used consensus non-negative matrix factorization (cNMF) to resolve lineage specific gene expression programs (GEPs). They then use the GEPs to draw several conclusions regarding age-specific patterns of bifurcation to the hematopoietic hierarchy and age-specific HSC priming. They argue that old HSCs tend to be less primed to a given lineage than fetal HSCs but that the priming that does occur in old HSCs tends to be more directed towards a specific lineage. The authors also identify a GEP that defines fetal HSCs and show that one member of this program, CD69, can enrich for functional HSCs in the fetal liver. Finally, they show that AML cells can differentially express genes that associate with fetal, perinatal, pediatric, adult or elderly HSPC states. AML that preferentially express genes or transcription factors that associate with elderly identity tend to have favorable outcomes, irrespective of the age of the patient.

This is a comprehensive data set. While the authors acknowledge that several single cell atlases of human HSPCs already exist to describe hematopoiesis at different ages, they correctly note that their study uniquely captures the full span of human development and aging. The authors have applied a number of sophisticated computational tools to analyze the data, and some of the conclusions (e.g. CD69 enrichment of fetal HSCs) are backed by functional assays. The data set will add significant value to the field. Overall, the manuscript and abstract are clear, and relevant literature are appropriately credited.

The paper does have some notable weaknesses, as discussed below.

Major criticisms:

1. Several of the claims are based on informatics alone with little or no supporting evidence to help establish a) whether the analytic approach is robust, or b) whether the findings are biologically important. For example:

- Figure 4 operates on the premise that anticorrelations between lineage GEPs imply bifurcations in the hematopoietic hierarchy. These anticorrelations are most evident at the committed progenitor level, and they change with age. For example, lymphoid, granulocyte and erythroid programs are more exclusive of one another in fetal progenitors. The programs become less exclusive in childhood, more exclusive in adulthood, then completely

non-exclusive in elderly progenitors.

It is hard to know what to make of this without more mechanistic insight or experimental validation. Are there truly bipotent lymphoid-megakaryocyte primed progenitors in the first trimester that are not present (or are encompassed by multipotent progenitors) in childhood, but then are present again in adulthood, but then disappear again with old age, as Figure 4C indicates? Does lineage restriction within the CD34+ population really fluctuate like this with age, or is this an artifact?

The authors should back this model with functional data. They could also test whether similar patterns are evident in some of the other published data sets (e.g., the Roy et al data set).

This is an excellent suggestion by the reviewer for validation of GEP conclusions using independent datasets. We have now additionally shown that the lineage GEPs also are able to predict age-specific patterns of fate decisions in an independent data (Roy et al) that comes closest to matching the lifetime age range we profile. We find that lineage branching outcomes in the independent study closely match the age-specific lineage branching outcomes we find in our study. These data are presented in new Extended Data Figure 6B. We additionally have also performed experimental validation and present data in new Figure 4C demonstrating the transition from adult to elderly progenitor cells is indeed accompanied by decreased lineage restriction in clonal outgrowth assays.

- Figure 5 also makes claims that are difficult to interpret. The lineage dominance score and the GEP usage score are both contrived values. There is no frame of reference to know whether the statistically significant difference between elderly and adult or first trimester lineage dominance scores means anything, functionally. Likewise, there is no frame of reference to know whether a change in GEP usage means anything. At minimum, it would be helpful to see how the scores in HSCs compare to those of committed progenitors. For example, if a lymphoid primed progenitor at a given age has a log transformed lineage dominance score of ~10, then the small differences between the fetal, adult and elderly HSC scores might not mean much biologically despite statistical significance.

This is an excellent point by the reviewer. We now add data in new Extended Data Figure 6C-E regarding how we trialed two methodologies for calculating unilineage dominance score (a division-based method and a subtraction-based method) and provide functional validation from HSC clonal outgrowth assays that the division-based unilineage dominance score more closely reflects HSC lineage biases from experimental data. Additionally, in new Extended Data Figure 6F we also provide comparisons of unilineage dominance score in HSCs vs progenitors for both division-based and subtraction-based methods and find that the subtraction-based method is more appropriate for quantifying overall magnitude of lineage commitment, highlighting differences in overall lineage commitment between HSCs and progenitors. We have enhanced the text to discuss gene expression reasons for why one methodology performs better at identifying lineage bias in HSCs, and the other methodology performs better at identifying differences in magnitude of lineage commitment between cells.

- The distinction between the “elderly enriched” and “mixed age” groups of AML patients in Figure 7 does not look compelling. Across the entire data set, there appears to be some enrichment for fetal genes in the AML, essentially no enrichment of perinatal genes, some enrichment of pediatric genes, low enrichment of adult genes and then varying degrees of high enrichment for elderly genes. This does not make intuitive sense, and it requires some explanation. The survival differences do offer a degree of reassurance, but the data still raise the question of whether age signatures are capturing some other dependent variable (e.g., age-related differences in stemness).

Thank you. Here, we used an unbiased hierarchical clustering approach as described, finding that these AMLs fell into two dominant classes – one with predominantly elderly signatures, and one with a lower elderly signature with gain of pediatric and fetal signatures. We used ‘mixed’ age to describe this latter category for simplicity, but we have revised to better describe the differences in signatures. Additionally, the hierarchical clustering was not meant to serve as a definitive final classifier, but more so was the hypothesis-generation tool to develop the more refined transcriptome age score, which we observe much stronger significance in predicting AML survival. We have additionally gone on to show that transcriptome age score also predicts survival in an independent AML cohort, as shows in new Extended Data Figure 9D.

2. The fetal GEP in Figure 6 does not contain genes that have been canonically associated with fetal identity, such as LIN28B, HMGA2, IGF2BP3, etc. Other studies of human fetal hematopoiesis (e.g., the cited Calvanese et al. and Roy et al. papers) clearly identified these genes. What accounts for the discrepancy? It's concerning that the computational approach taken to identify a fetal GEP would miss several well-established fetal genes.

This is an important distinction that we are glad the reviewer has brought up. These genes are not HSC-specific so would not go into a HSC-focused GEP. We have however added data into new Extended Data Figure 2D showing that LIN28B, HMGA2, and IGF2BP3 are all biased towards fetal expression in our whole dataset.

Minor comment:

1. Figure 5A would benefit from better clarity. After several reads it was clear that the brown insets to each pie piece were aggregates of lines of individual cells. However, this was not intuitive, even with the legend. Perhaps an inset magnifying part of one plot to show the lines of individual cells could be helpful.

This is a good suggestion and we have generated a magnified inset as requested.

Version 1:

Decision Letter:

Our ref: NMETH-RS54596A

26th Jun 2024

Dear Grant,

Thank you for submitting your revised manuscript "The dynamics of hematopoiesis over the human lifespan" (NMETH-RS54596A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Methods, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines. Please revise the paper as we discussed yesterday highlighting the validation and discussing the variability. In the discussion, I would recommend adding a few paragraphs on the limitations of the study.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements within two weeks or so. Please do not upload the final materials and make any revisions until you receive this additional information from us.

TRANSPARENT PEER REVIEW

Nature Methods offers a transparent peer review option for new original research manuscripts submitted from 17th February 2021. We encourage increased transparency in peer review by publishing the reviewer comments, author rebuttal letters and editorial decision letters if the authors agree. Such peer review material is made available as a supplementary peer review file. **Please state in the cover letter 'I wish to participate in transparent peer review' if you want to opt in, or 'I do not wish to participate in transparent peer review' if you don't.** Failure to state your preference will result in delays in accepting your manuscript for publication.

Please note: we allow redactions to authors' rebuttal and reviewer comments in the interest of confidentiality. If you are concerned about the release of confidential data, please let us know specifically what information you would like to have removed. Please note that we cannot incorporate redactions for any other reasons. Reviewer names will be published in the peer review files if the reviewer signed the comments to authors, or if reviewers explicitly agree to release their name. For more information, please refer to our [FAQ](https://www.nature.com/documents/nr-transparent-peer-review.pdf).

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IMPORTANT: Non-corresponding authors do not have to link their ORCIDs but are encouraged to do so. Please note that it will not be possible to add/modify ORCIDs at proof. Thus, please let your co-authors know that if they wish to have their ORCID added to the paper they must follow the procedure described in the following link prior to acceptance:
<https://www.springernature.com/gp/researchers/orcid/orcid-for-nature-research>

Thank you again for your interest in Nature Methods. Please do not hesitate to contact me if you have any questions. We will be in touch again soon.

Sincerely,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor

Reviewer #2 (Remarks to the Author):

We thank the authors for trying to address the raised concerns during the first revision, however they have not thoroughly revised all the comments. In addition, it is hard to follow all the changes since no tracking has been done.

I asked them to validate the findings with other datasets which they have done, and I appreciate that important addition. However, functional validation remains very poor, and only added in figure 4C showing the variety of cells formed from adult or elderly progenitors. This is an interesting experiment, but it has certainly too little details for the readers to be able to understand. What is 1,2,3,4,5 types of colonies why do not use the initials for the colony types? It would be more useful. Also it was performed from 2 donors in 2 different ages, this is quite poor, and the error bars are pretty big. Individual values as dots could be shown and more detail of the experiment should be added in the figure legend or text. In general, there are overstatements (such in the conclusion of figure 4C) all over the manuscript that should be toned down.

Some of other comments that have not been addressed or understood:

"The comment about Fig2A referred to a modification in the figure: instead of showing individuals (each age) represent trend lines (i.e. loess curve) along the 7 discrete phases you are using along the paper. The current figure could be included in supplementary. Or at least indicate in the same figure each phase start/end points."

About the PCA (Fig2B): "I appreciate that authors included interpretation from PCA. Nevertheless, PC2 interpretation has to be taken with caution since elderly and adult are together with samples from other stages and the same interpretation you are giving to elderly (partial similarity to fetal/perinatal) you can do it with adult (partial similarity with fetal). Maybe you could change the sentence (line 156) '...is supportive..' to 'may/might be supportive' "

Previous Fig7E: "I appreciate you performed multivariate analysis (Extended Fig 9). Nevertheless, could you conduct that analysis with all the variables simultaneously (not in pairs)? If not possible with all variables, assess which ones should be included."

Fig7F: "I appreciate you revised the literature searching for an association between the 7 TFs and AML. In the text you mention (line 408) '... only some of which...'. Could you be more specific and explicitly mentioned which ones? Additionally, I wonder if these 7 TFs are really driving the transcriptional landscape of the high- or low- composite score AMLs or if they are really orchestrating a relevant transcriptional pattern. Have you conducted a gene regulatory network analysis or similar to include evidence on this assumption? I also wonder if you would obtain the same result (significant difference in K-M curves, Fig7G) with other gene sets (also differentially expressed). Have you tried to generate n random sets of 4 down + 3 up DEGs - as a negative control for your 7 TFs finding? "

Reviewer #3 (Remarks to the Author):

The authors have done a reasonable job of responding to my comments, and to comments from the other reviewers. Though largely descriptive, this manuscript presents a valuable resource for the field.

With that said, my criticisms regarding figure 4 remain inadequately addressed. My skepticism centered on the temporal fluctuations in lineage bias that they claimed to observe in the committed progenitors, particularly the lack of bias in perinatal/childhood progenitors that was not evident at either earlier (1st and 2nd trimester) or later (adolescent/adult) ages. The comparison to the Roy et al data shown in Extended Figure 6B does not validate this apparent loss of bias in the perinatal/childhood period, perhaps because corresponding timepoints were not evaluated in the Roy data set. Functional validation would have resolved this concern, but they only evaluated lineage output in adult and elderly samples.

This figure is not vital to the overall impact of the paper. If they cannot validate their findings across the age ranges, they should narrow their claims.

Minor comment: Extended Fig 6B does not appear to be cited in the text.

Author Rebuttal letter:

Thank you. After discussing with our handling editor, we identified the most important points for revision that we have now addressed. We now present responses to the specific points needed to be addressed below.

Reviewers' Comments:

Reviewer #2:

Remarks to the Author:

We thank the authors for trying to address the raised concerns during the first revision, however they have not thoroughly revised all the comments. In addition, it is hard to follow all the changes since no tracking has been done.

I asked them to validate the findings with other datasets which they have done, and I appreciate that important addition. However, functional validation remains very poor, and only added in figure 4C showing the variety of cells formed from adult or elderly progenitors. This is an interesting experiment, but it has certainly too little details for the readers to be able to understand. What is 1,2,3,4,5 types of colonies why do not use the initials for the colony types? It would be more useful. Also it was performed from 2 donors in 2 different ages, this is quite poor, and the error bars are pretty big. Individual values as dots could be shown and more detail of the experiment should be added in the figure legend or text. In general, there are overstatements (such in the conclusion of figure 4C) all over the manuscript that should be toned down.

We have added the specific experimental colony outcomes to the supplemental table.

The reviewer also appears to have missed that additional experimental validation was not solely provided in Figure 4, but also for data focusing on HSC fate decisions in Extended Data Figure 6D-E. We have revised the manuscript to more clearly highlight these data.

Lastly, we made an effort to tone down conclusions throughout, although only the specific example of Figure 4C is provided.

Some of other comments that have not been addressed or understood:

"The comment about Fig2A referred to a modification in the figure: instead of showing individuals (each age) represent trend lines (i.e. loess curve) along the 7 discrete phases you are using along the paper. The current figure could be included in supplementary. Or at least indicate in the same figure each phase start/end points."

Overruled on discussion with the editor.

About the PCA (Fig2B): "I appreciate that authors included interpretation from PCA. Nevertheless, PC2 interpretation has to be taken with caution since elderly and adult are together with samples from other stages and the same interpretation you are giving to elderly (partial similarity to fetal/perinatal) you can do it with adult (partial similarity with fetal). Maybe you could change the sentence (line 156) '..is supportive..' to 'may/might be supportive' "

We have adjusted the language around our PCA interpretation.

Previous Fig7E: "I appreciate you performed multivariate analysis (Extended Fig 9). Nevertheless, could you conduct that analysis with all the variables simultaneously (not in pairs)? If not possible with all variables, assess which ones should be included."

Overruled on discussion with the editor.

Fig7F: "I appreciate you revised the literature searching for an association between the 7 TFs and AML. In the text you mention (line 408) '.. only some of which...'. Could you be more specific and explicitly mentioned which ones? Additionally, I wonder if these 7 TFs are really driving the transcriptional landscape of the high- or low- composite score AMLs or if they are really orchestrating a relevant transcriptional pattern. Have you conducted a gene regulatory network analysis or similar to include evidence on this assumption? I also wonder if you would obtain the same result (significant difference in K-M curves, Fig7G) with other gene sets (also differentially expressed). Have you tried to generate n random sets of 4 down + 3 up DEGs - as a negative control for your 7 TFs finding? "

We have revised to be more explicit as to which TFs have known or unknown association with AML.

Reviewer #3:

Remarks to the Author:

The authors have done a reasonable job of responding to my comments, and to comments from the other reviewers. Though largely descriptive, this manuscript presents a valuable resource for the field.

With that said, my criticisms regarding figure 4 remain inadequately addressed. My skepticism centered on the temporal fluctuations in lineage bias that they claimed to observe in the committed progenitors, particularly the lack of bias in perinatal/childhood progenitors that was not evident at either earlier (1st and 2nd trimester) or later (adolescent/adult) ages. The comparison to the Roy et al data shown in Extended Figure 6B does not validate this apparent loss of bias in the perinatal/childhood period, perhaps because corresponding timepoints were not evaluated in the Roy data set. Functional validation would have resolved this concern, but they only evaluated lineage output in adult and elderly samples.

This figure is not vital to the overall impact of the paper. If they cannot validate their findings across the age ranges, they should narrow their claims.

We have adjusted our claims around Figure 4 as suggested by the reviewer.

Minor comment: Extended Fig 6B does not appear to be cited in the text.

This is a helpful observation as it appears we did not insert the reference to Extended Fig 6B in the text – this is done now although figure order is changed slightly to conform to publishing guidelines.

Version 2:

Decision Letter:

19th Sep 2024

Dear Grant,

I am pleased to inform you that your Resource, "The dynamics of hematopoiesis over the human lifespan", has now been accepted for publication in *Nature Methods*. The received and accepted dates will be 29th Nov 2023 and 19th Sep 2024. This note is intended to let you know what to expect from us over the next month or so, and to let you know where to address any further questions.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to *Nature Methods* style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required. It is extremely important that you let us know now whether you will be difficult to contact over the next month. If this is the case, we ask that you send us the contact information (email, phone and fax) of someone who will be able to check the proofs and deal with any last-minute problems.

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Please feel free to contact me if you have questions about any of these points.

Best regards,
Madhura

Madhura Mukhopadhyay, PhD
Senior Editor
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