EMBO Molecular Medicine

Modelling, Optimization and Comparable Efficacy of T cells and HSC Gene Editing for treating HIGM1

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DOI: 10.15252/emmm.202013545

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Review Timeline:

Submission Date:	6th Oct 20
Editorial Decision:	8th Oct 20
Revision Received:	13th Nov 20
Editorial Decision:	1st Dec 20
Revision Received:	8th Dec 20
Accepted:	10th Dec 20

Editor: Zeljko Durdevic

Transaction Report:

(Note: Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at EMBO Molecular Medicine. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published. With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 8th Oct 2020

8th Oct 2020

Dear Dr. Genovese,

Thank you for submitting your manuscript to EMBO Molecular Medicine. I have now carefully read your manuscript and discussed it with the other members of our editorial team. In addition, I have also sought external advice on the study from an expert in the field. I am pleased to inform you that we find your manuscript suitable for publication in EMBO Molecular Medicine pending the appropriate revision.

Further consideration of your manuscript will depend on addressing the following points:
- Please revise aims of the study in such way that they are supported by the data presented.
- Please provide more detailed demonstration of gene editing efficiency in stem memory T cells (TSCM). The phenotype definition of TSCM should be demonstrated using more stringent phenotype panel, including CD95, CD11a or other known markers.
- Please perform cellular CD40L and NGFR co-staining to exclude leak expression of CD40L-NGFR construct.
- Please provide a rational for selecting the construct with the HBB rather than the EF1a splice acceptor.
- Please correct: Reference made to Fig1B in the results section (pg5) does not relate to the data shown in Fig1B.
- Please discuss possible cell doses needed for a therapeutic outcome and clinical utility of the depletion strategy using Cetuximab in regard to the in vitro results.
- Please discuss limitations of the study in regard to a) the missing proof-of-concept that autologous gene edited HSC and/or T cell infusion rescues HIGM1 mouse model, and b) the lack of long-term follow-up study to evaluate the efficiency and safety of the therapy.

Additional experiments that further strengthen the main conclusions of the study are of course appreciated. We would welcome the submission of a revised version within three months for further consideration. However, we realize that the current situation is exceptional on the account of the COVID-19/SARS-CoV-2 pandemic. Please let us know if you require longer to complete the revision.

***** Advice from external expert *****

7 Oct. 2020

I think you should publish this paper. It represents a solid approach to gene editing therapy and appropriate in my opinion for publication in EMBO Molecular Medicine.

Modeling, Optimization and Comparable Efficacy of T cells and Hematopoietic Stem Cells Gene Editing for Treating Hyper IgM Syndrome

We would like to thank the Editor and the Reviewers for their careful and positive evaluation of our manuscript, and the willingness of the Editor to consider a revised version for publication in EMBO Molecular Medicine as an Article. We now provide a revised version of the manuscript according to the Editor requests, including new data and additional clarifications on some of the previously reported findings.

We addressed all the indicated requests in the point-by-point reply below and all changes in the revised manuscript are highlighted in yellow to facilitate the revision.

We hope that the revised version of our study is now suitable for publication and thank the Editor for his consideration.

Further consideration of your manuscript will depend on addressing the following points:

- Please revise aims of the study in such way that they are supported by the data presented.
The aims of the study as well as the limitations imposed by using wild-type cells as surrogate of functional edited cells in the mouse model (see also last point below) were better highlighted in the new introduction and discussion sessions to ensure that all aims are fully supported by the presented data.

- Please, provide more detailed demonstration of gene editing efficiency in stem memory T cells (TSCM). The phenotype definition of TSCM should be demonstrated using more stringent phenotype panel, including CD95, CD11a or other known markers.
We thank the reviewer for highlighting this point. In our original manuscript, we used the minimum panel of surface markers that allows identifying TSCM on in vitro stimulated cells, which operatively may not contain any more naïve cells (Cieri et al., 2013). In order to provide a more stringent demonstration of gene editing efficiency in TSCM, we now expanded our analyses by including the CD95 and CD45RO markers, thus confirm the full TSCM identity as CD95+CCR7+CD45RO+CD62L+ CD45RA+ cells.

CD95 marker distinguishes naïve cells (CD95-) vs TSCM cells (CD95+).
CCR7 marker distinguishes TSCM and CM cells (CCR7+) vs EM and TEMRA cells (CCR7-).
CD45RO marker distinguishes naïve (or circulating, resting TSCM cells) (CD45RO-) vs cultured activated naïve-derived TSCM cells (CD45RO+).
CD62L marker distinguishes TSCM and CM cells (CD62L+) vs EM and TEMRA cells (CD62L-).
CD45RA marker distinguishes TSCM cells (CD45RA+) vs CM cells (CD45RA-).

We used the gating strategy reported in Cieri et al., 2013: in the CD95+ population (almost all the stimulated, live cells were CD95+), we first gated CCR7+ CD45RO+ cells and then CD62L+CD45RA+ cells. Inside the latter double-positive population, we measured NGFR expression as surrogate of gene editing efficiency and compared it with that of total live CD4+ T cells (see new Fig. EV2 B, C). Even by using this more stringent analysis, we observed a mean of 35% gene editing efficiency in the TSCM subpopulation, thus confirming the observation reported in the original manuscript.

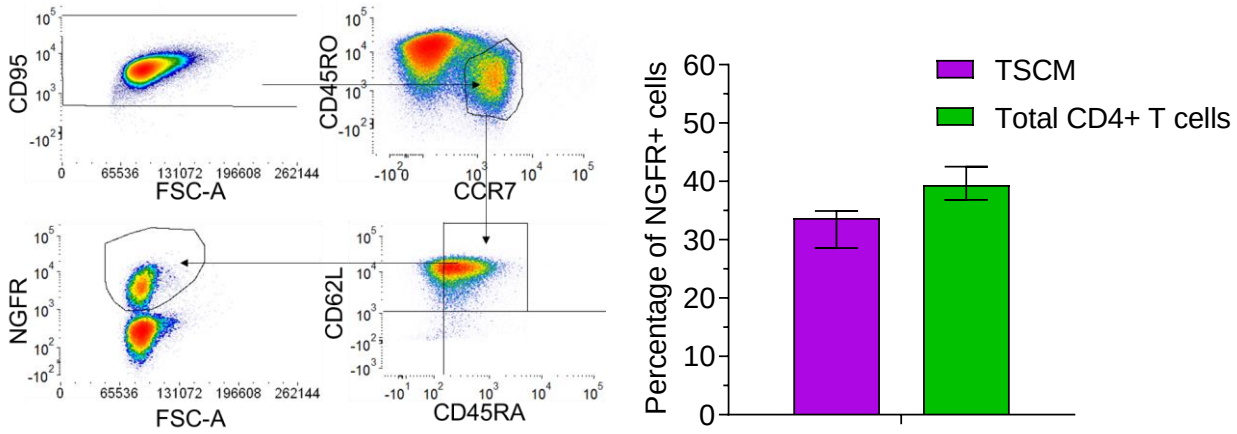

Figure EV2 B, C. ***B*** *Representative plots showing more detailed characterization of edited TSCM cells, defined as CD95+CCR7+CD45RO+CD62L+CD45RA+.* ***C*** *Percentage of NGFR+ cells within TSCM cell subpopulation (CD95+CCR7+CD45RO+CD62L+CD45RA+) or within total live cells, 17 days after CD40LG editing of healthy male donor (HD; n=3) derived CD4+ T cells, measured by FACS analysis.*

- Please, perform cellular CD40L and NGFR co-staining to exclude leak expression of CD40L-NGFR construct.

As noted in the Reviewer's comment, NGFR expression in edited cells using the selection cassette "showed basal levels of protein expression despite its expression being linked and regulated by the CD40L control elements", which does not allow surface CD40L translocation in absence of T cell stimulation. We hypothesize that this may be due to the physiologically regulated surface exposure of CD40L through trafficking and storage in different secretory compartments than NGFR. To confirm this, and ensuring there is no 'leakiness' in our vector design, we performed intracellular vs surface co-staining of both CD40L and NGFR in absence of stimulation (see new Fig. EV2 F, G). This analysis confirmed that all NGFR+ cells have a CD40L intracellular reservoir in absence of stimulation, which is similar than those measured in unedited and NGFR- cells (please, note that these latter populations contain also cells that are physiologically CD40L negative, thus, as expected, the mean fluorescent intensity in the NGFR+ fraction appears to be slightly higher). As we explain in the manuscript, because upon cell activation CD40L is translocated to the membrane by regulated secretion, its surface expression level might be restored to physiological levels once the stores have been replenished above a certain threshold. These results support the previously reported findings (Casamayor-Palleja et al., 1995; Koguchi et al., 2007) that a second layer of regulation is necessary to mediate CD40L surface translocation, thus further ensuring regulated expression control of CD40L after gene correction with our strategy.

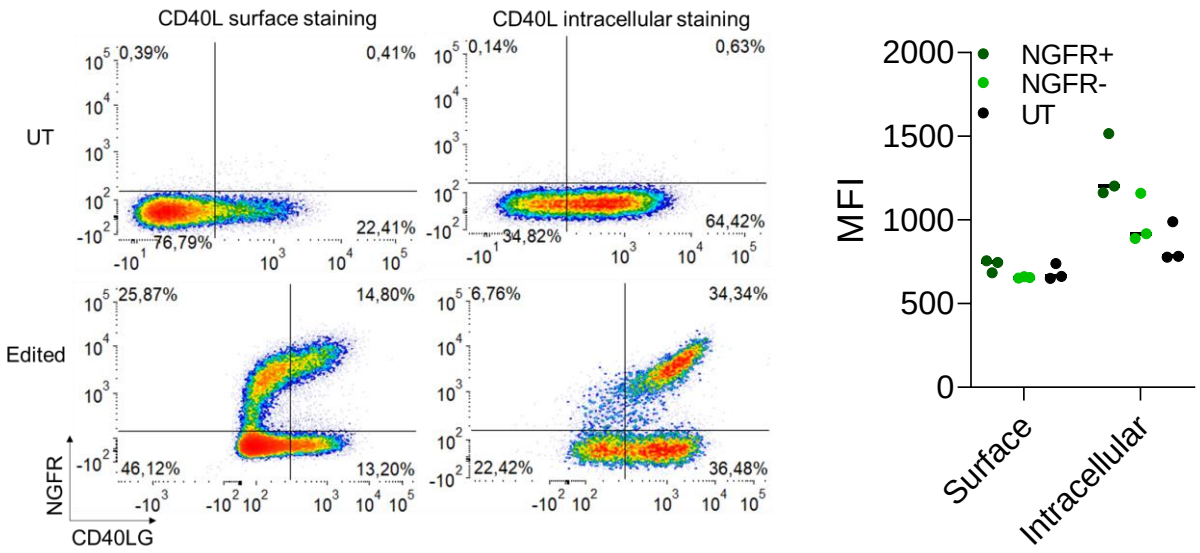

Figure EV2 F, G. ***F*** *Representative plots showing CD40L expression after surface (left) or intracellular staining (right) in UT or bulk edited CD4+ T cells derived from male HD in absence of Pma/Ionomycin stimulation.* ***G*** *CD40L expression measured by MFI after surface or intracellular staining in UT or bulk edited CD4+ T cells derived from male HD in absence of Pma/Ionomycin stimulation (n=3).*

- Please provide a rational for selecting the construct with the HBB rather than the EF1a splice acceptor.

To ensure regulated and physiological expression of the CD40LG after editing, we tested different configuration of the donor template on T cells from male donors and used PMA/Ionomycin stimulation to induce CD40L expression. To facilitate this screening, the donor templates were delivered to the cells by integrase defective lentiviral vector (IDLV) or dsODN. Since these delivery vehicles will not need long time-consuming vector production, they allow rapid testing of different template configuration. However, they are not optimal in terms of editing efficiency, especially if compared with AAV6, thus selection of the best performing candidates was performed only based on the expression of the edited CD40LG gene. Since all the splice acceptor tested allowed efficient and complete splice trapping of the endogenous transcript, we selected the HBB because was the first available for AAV6 production. In the revised manuscript, we added more detailed information about the selection process of the template configuration on the legend to Fig EV1.

- Please correct: Reference made to Fig1B in the results section (pg5) does not relate to the data shown in Fig1B.

We thank the Reviewer for spotting this mislabeling. We amended it in the revised manuscript.

- Please discuss possible cell doses needed for a therapeutic outcome and clinical utility of the depletion strategy using Cetuximab in regard to the in vitro results.

We thank the reviewer for highlighting these interesting points, which we both addressed in the revised text and discussion:

1. "*The T cell dose used for mouse modeling represents the upper range of cells/kg used in a previous trial with gene edited CD4 T cells (Tebas et al., 2014) and might exceed the doses of pathogen specific adoptive T cell therapies used in HSCT settings (Icheva et al., 2013). While care must be taken when translating results from experimental mouse models to the clinical setting, we should acknowledge that humans, as all wild animals, are exposed to commensal and pathogenic microbes throughout their lives, and this microbiome has a*

profound impact on immune system development, competence and overall health. The use of laboratory mice housed under specific pathogen-free (SPF) conditions is important to improve experimental consistency, but leaves the mice with an underdeveloped immune system (Huggins et al., 2019), thus possibly underestimating the level of immune response to an antigenic challenge predicted for the human setting. Indeed, previous sporadic reports of patients with genetic mosaicism, either an allogeneic HSCT patient with low engraftment (Petrovic et al., 2009) or female carriers with skewed X inactivation in the blood (Hollenbaugh et al., 1994), would support our contention that even low frequencies percentages of CD40L proficient cells, achieved by either T cell or HSPC therapy, are sufficient to provide substantial immune protection. A dose escalation design of a T cell therapy trial will allow safe testing of these predictions in the clinical setting."

2. *"In vivo depletion of hEGFRt-expressing cells by Cetuximab relies on antibody-dependent cellular cytotoxicity (ADCC), which also requires functional NK cells (Lee et al., 2011). Since ADCC on human cells is difficult to be assessed in xenotransplantation experiments with immunodeficient mice, we explored an in vitro immunotoxin-based strategy to evaluate if edited cells carrying hEGFRt were amenable to antibody-mediated depletion (Palchaudhuri et al., 2016). By culturing edited T cells in the presence of Cetuximab conjugated to the protein synthesis inhibitor toxin saporin (Cetuximab-SAP) or of antibody and toxin alone as controls, we observed substantial depletion (~50%) of hEGFRt-expressing lymphocytes at both doses tested (Fig 3C and D). While the decreased internalization rate of our modified hEGFRt is likely reducing the efficacy of immunotoxin treatment, these data suggest that hEGFRt is a suitable candidate both for in vitro selection and in vivo depletion of CD40LG edited cells".*

   …

   "The use of our optimized hEGFRt marker allows coupling selection with the possibility to deplete the transplanted cell product by treatment with a clinically approved monoclonal antibody which, based on the broad clinical experience in tumor therapies, is associated with only minor side effects, such as skin rash (Hansel et al., 2010; Peréz-Soler and Saltz, 2005). While our investigation on human cells remains limited in providing direct evidences of T cell killing, due to the lack of effector cells on xenogeneic models for assessing antibody-dependent cellular cytotoxicity (Shultz et al., 1995; Verma et al., 2017), previous studies performed in full mouse settings have already proved effective depletion from both blood and solid organs of T cell expressing hEGFRt within 4 days after Cetuximab administration (Paszkiewicz et al., 2016; Wang et al., 2011). Indeed, this strategy is already under investigation in several clinical trials as safety control of T cell-mediated cancer immunotherapy (Yu et al., 2019). Nevertheless, since the depletion by Cetuximab remains a relatively slow process, further studies will be necessary to assess whether this approach would also be suitable for controlling more acute adverse events related to T cell administration, such as the cytokine release syndrome reported in some patients after the infusion of activated CD8 T cells.".

- Please discuss limitations of the study in regard to a) the missing proof-of-concept that autologous gene edited HSC and/or T cell infusion rescues HIGM1 mouse model, and b) the lack of long-term follow-up study to evaluate the efficiency and safety of the therapy.

We thank the Reviewer and Editor for highlighting these points.

a. The disease rescue experiments in the mouse model were performed using competitive transplants with wild type cells used as surrogate of the corrected cells instead than bona fide gene edited mouse cells. Yet, since the gene editing procedures on mouse HSC or T cells would require significantly different culture protocols and procedures than those

developed and here optimized for clinically ready protocols for human cells, experiments with murine edited cells would provide only limited value in the perspective of future clinical translation. Indeed, the use of specie-specific reagents and the expectedly low efficiency of gene editing achieved on mouse cells would provide limited information on the long-term safety profile of the genetic manipulation on human cells and constrain transplantation studies. Nevertheless, whether edited human cells fully recapitulate upon transplantation the function and long-term persistence of healthy donor cells will have to be determined in clinical studies.

b. On the contrary, our experiments with mouse cells allow us showing that transferred wild-type T cells can engraft and persist at long-term follow-up in the mouse model (up to 178 days in naïve T cell experiments, up to 219 days in activated T cell experiments, up to 311 days in P. Murina T cell experiment). The impact of gene editing on the fitness of T cells and, consequently, their long-term persistence still need to be clarified. Despite long-term follow-up studies have demonstrated persistence of gene-modified T cells in all memory and effector T cell compartments for up to 14 years (Oliveira et al, 2015) and clinical trials have shown persistence of NHEJ-edited T cells for several months in humans (Tebas et al, 2014; Stadtmauer et al, 2020), no specific studies have been carried out on HDR-edited T cells so far. Nevertheless, if functional immune reconstitution would decrease over time, repeated administrations of the same or a new edited cell product could be performed to prolong therapeutic efficacy.

We have now included these considerations in the discussion our revised manuscript.

1st Dec 2020

Dear Prof. Genovese,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) In the main manuscript file, please do the following:
- Correct/answer the track changes suggested by our data editors by working from the attached/uploaded document.

***** Advice from external expert *****

22 Nov. 2020

I think they have addressed the points adequately.

2nd Authors' Response to Reviewers 8th Dec 2020

The authors performed the requested changes.

2nd Revision - Editorial Decision 10th Dec 2020

10th Dec 2020

Dear Prof. Genovese,

We are pleased to inform you that your manuscript is accepted for publication.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Pietro Genovese
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number:

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For human studies, sample size for each experiment was determined by the availability of human-derived donor cells, which is constrained especially in the case of patient-derived material, being HIGM1 an ultra-rare genetic disease. Whenever possible we aimed to reach at least 5 replicates per group, considered adeguate for carrying out nonparametric statistical comparisons. Number of biological replicates (different donors) is specified for each experiment in figure legends. All attempts at replication were successful. Inferential techniques were applied in presence of adequate sample sizes with respect to the type of methodology, otherwise only descriptive statistics are reported.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For animal studies, sample size corresponds to the minimal quantity of mice necessary to obtain scientific reliable data, on the basis of preliminary results, published data and power analysis (at least 5 replicates per group). Whenever needed, complete experiments were repeated up to three times. Number of biological replicates is specified for each experiment in figure legends. All attempts at replication were successful.Inferential techniques were applied in presence of adequate sample sizes with respect to the type of methodology, otherwise only descriptive statistics are reported.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	For in vivo experiments, failure during injection, confirmed by graft failure in recipient animals led to exclusion of that mouse from the experimental group. For gene editing efficiency analysis (ddPCR) on blood samples, single time points referring to 7 different mice were excluded because of insufficient blood material availability. All these criteria were pre-established. All observations included in the study were used in the statistical analysis, except if they resulted to be outliers for the linear (LME) or nonlinear mixed-effects (NLME) analyses or they belonged to a group with a sample size not sufficient to perform group comparisons. The number of outliers removed in each LME or NLME analysis is described in the Appendix Supplementary Statistical Methods. In the Figure Legend and, depending on the type of analysis, also in the Appendix Supplementary Statistical Methods, it is specified when a group was not considered in a comparison among groups because of the small sample size. No other data or sample were excluded from analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Mice were randomly distributed to each experimental group.
For animal studies, include a statement about randomization even if no randomization was used.	Mice were randomly distributed to each experimental group.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No blinding was done.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
5. For every figure, are statistical tests justified as appropriate?	Inferential techniques were carried out whenever appropriate sample size was available, otherwise descriptive statistics were reported. When the statistical analyses were performed, the most appropriate method was chosen depending on the assumptions satisfied by the data.

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Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	All standard correlation analysis and comparison between groups were performed with nonparametric statistical methods, that do not require the assumption of normality or other assumptions except that the data are at least ordinal. Longitudinal data were analyzed with appropriate linear (LME) or nonlinear mixed-effects (NLME) models which require the assumption of normality of the residuals of the model. This assumption was verified for each model through the q-q plot of the residuals of the full model. Thus, when necessary, to meet this assumption, an adequate transformation of the dependent variable was used and/or few observations (outliers) were excluded from the analysis. This is specified for each analysis in the Appendix Supplementary Statistical Methods.
Is there an estimate of variation within each group of data?	In each panel of each Figure, a measure of variability was shown, whenever it was appropriate, as SEM, IQR or Range.
Is the variance similar between the groups that are being statistically compared?	All standard comparisons between groups were performed with nonparametric statistical methods, that do not require the assumption of equal variance among groups. All the other comparison between groups were performed with mixed-effects models (LME or NLME), in which the heterogeneity can be appropriately accounted with respect to the different sources of variation by appropriately setting the random effects. Moreover, diagnostic check of residuals of the models allowed to verify model assumptions.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	See clone number and vendor in Appendix Table S4.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	C57Bl/6 Ly45.1 and C57Bl/6 Ly45.2 mice (6-10 weeks old, male) were purchased from Charles River Laboratory, Cd40lg-/- mice (B6.129S2-Cd40lgtm1Imx/J) (8 weeks old, male) and NOD-SCID-IL2Rg/ (NSG) mice (7-10 weeks old, male) were purchased from The Jackson Laboratory. C57Bl/6 Ly45.1 or C57Bl/6 Ly45.1/Ly45.2 obtained by crossing C57Bl/6 Ly45.2 and C57Bl/6 Ly45.1 mice at the San Raffaele Scientific Institute animal research facility, were used as donors for adoptive T cell transfer and HSPC transplant into Cd40lg-/- mice. All the mice were maintained in specific-pathogen-free (SPF) animal breeding department.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were designed and performed with the approval of the Animal Care and Use Committee of the San Raffaele Hospital (IACUC #749, #818) and communicated to the Ministry of Health and local authorities according to Italian law.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Compliance to ARRIVE guidelines is confirmed.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	San Raffaele Ethical Committee for patient-derived materials (Tiget-09 protocol).
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Buffy coats were obtained in accordance with the Declaration of Helsinki, as anonymized residues of blood donations, used upon signature of specific institutional informed consent for blood product donation by healthy blood donors. As regards HIGM1 patients' samples, referring physicians were responsible for the collection of informed consent for biological samples' collection and anonymized biological sample/data sharing from their own patients, according to local research
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Supplementary documents provided for central datasets present in main figures.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	