Hypothesis

Purging Autologous HSCs via Detection of Clonal Mutations

Michael Renteln *

Posted Date: 2 October 2024

doi: 10.20944/preprints202410.0163.v1

Keywords: purging; autologous bone marrow transplant; hematopoietic stem cells; dual adenoviral transduction; *ex vivo* and *in vivo* treatments; personalized oncolytic vector

 doi:10.20944/preprints202410.0163.v1

Hypothesis

Purging Autologous HSCs via Detection of Clonal Mutations

Michael Renteln

Affiliation 1; mrenteln@gmail.com

Abstract: Allogeneic hematopoietic stem cell (HSC) transplants are usually used in cases where patients have active cancerous illness in their bone marrow, but allogeneic transplants can cause graft vs. host disease. If autologous HSCs could be purged of cancer cells, they could then be used for transplantation purposes without concern. It is still controversial whether purging is necessary, but it certainly would alleviate concerns. A novel method of purging is discussed herein that does not require chemotherapy and is targeted for each patient. It could be applied either *in vitro* or *in vivo*.

Keywords: Purging; autologous bone marrow transplant; hematopoietic stem cells; dual adenoviral transduction; *ex vivo* and *in vivo* treatments; personalized oncolytic vector

Introduction

A non-genotoxic, targeted method of purging hematopoietic stem cells (HSCs) that can overcome resistance could allow for safer autologous HSC transplantation [1].

Allogeneic HSC transplant is possible, but it often leads to graft vs. host disease[2].

If the patient's blood cancer cells in circulation, as well as the lymph nodes and bone marrow, are sequenced[3], clonal mutations can be identified - i.e., mutations that are contained in all of their cancer cells. These mutations can be detected with molecular switches, and thus the cells can be eliminated using the effector module of said switches[4–6].

This process can be done *ex vivo*, using transfection or transduction. It can also be done *in vivo* via mobilization of patient HSCs and dual adenoviral vector transduction[7,8].

Purging Strategy

First, cancer cells in the patient's bloodstream, lymph nodes, and bone marrow would be sequenced to determine his or her clonal mutations. The vector that is delivered to patient HSCs *ex vivo* or i*n vivo* would have very broad tropism. It would be programmed to express CRISPRa modules that upregulate the expression of clonally mutated genes. It would also encode switches that can recognize the clonally mutated mRNA sequences and respond via activation of a toxin module that eliminates the cell [4–6]. One example of such a switch is RADAR, a system developed recently that can even detect point mutations in mRNA[9,10].

As described previously, CRISPRa expression could be halted via detection of the clonally mutated transcript somewhere other than the mutation site, as this would prevent long-term activation of the gene in non-cancerous cells [6].

Blood Cancer Elimination Strategy

The vector would also base edit the *cd45* gene of the patient's harvested HSCs so that anti-CD45 chimeric antigen receptor (CAR) T-cells can be intravenously administered to eliminate the remainder of the patient's blood cancer cells[11]. The CAR T-cells can be purged as well if necessary, and should also have a base edited *cd45* gene themselves to avoid fratricide. For acute myeloid leukemia, instead of *cd45*, a combination of *flt3*, *cd123*, and *kit* epitope editing can be exploited[12]. The CAR T-cells could potentially be off-the-shelf, precluding tumor cell contamination issues[13,14].

 doi:10.20944/preprints202410.0163.v1

They could be eliminated after treatment and re-administered later if necessary[15]. An immunotoxin targeting CD45 could be used instead of CAR T-cells[16]. It was demonstrated that the base edited *cd45* gene product for the immunotoxin referenced here[17] was more biophysically optimal than the one generated earlier for CAR T-cell targeting[11]. In either case, an anti-CD45 immunotoxin could be used to select HSCs base edited *ex vivo*. However, with regard to *in vivo* administration, repeated injections may be required for immunotoxins, as opposed to autologous CAR T-cells, which can persist in circulation for long periods of time. Finally, healthy HSCs with an edited *cd45* gene, i.e., after selection, would be infused intravenously or selected *in vivo*. With regard to the *ex vivo* approach, treated cells could possibly be expanded *in vitro* if necessary [8,18]. In the latter case, a mixture of unedited and edited HSCs may home to the bone marrow. However, the edited versions would be selected over time and amplify. The edited HSCs would eventually reconstitute all the immune cell compartments.

Conclusions

As opposed to other purging strategies, this one would likely prevent escape variant issues caused by the decreased expression of a small number of cell surface proteins, as the vector would have very broad tropism. Additionally, it may be more resilient with regard to resistance to the cytotoxic element of the purge process, as a potent toxin targeting a necessary cellular component such as a ribosome-inactivating protein can be activated in response to clonal mutation detection.

The aforementioned purging strategy could ensure that autologous HSCs can be used in the context of blood cancer treatments, avoiding the issue of graft vs. host disease.

This clonal mutation targeting strategy can be used in the context of solid tumors as well, using oncolytic vectors that require detection of said mutations for replication and hyper-virulence[4–6].

Funding: None.

Conflicts of interest: None.

References

1. Ishitsuka K, Nishikii H, Kimura T, et al. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood-mobilized stem cells. Experimental Hematology 2024;131:104138; doi: 10.1016/j.exphem.2023.104138.
2. Malard F, Holler E, Sandmaier BM, et al. Acute graft-versus-host disease. Nat Rev Dis Primers 2023;9(1):1–18; doi: 10.1038/s41572-023-00438-1.
3. Landau DA, Carter SL, Getz G, et al. Clonal evolution in hematologic malignancies and therapeutic implications. Leukemia 2014;28(1):34–43; doi: 10.1038/leu.2013.248.
4. Renteln M. Conditional replication of oncolytic viruses based on detection of oncogenic mRNA. Gene Ther 2018;25(1):1–3; doi: 10.1038/gt.2017.99.
5. Renteln MA. Promoting Oncolytic Vector Replication with Switches that Detect Ubiquitous Mutations. CCTR 2024;20(1):40–52; doi: 10.2174/1573394719666230502110244.
6. Renteln M. Targeting Clonal Mutations with Synthetic Microbes. 2024; doi: 10.20944/preprints202308.1536.v11.
7. Wang H, Georgakopoulou A, Zhang W, et al. HDAd6/35++ - A new helper-dependent adenovirus vector platform for in vivo transduction of hematopoietic stem cells. Mol Ther Methods Clin Dev 2023;29:213–226; doi: 10.1016/j.omtm.2023.03.008.
8. Wang H, Georgakopoulou A, Nizamis E, et al. Auto-expansion of in vivo HDAd-transduced hematopoietic stem cells by constitutive expression of tHMGA2. Molecular Therapy Methods & Clinical Development 2024;32(3); doi: 10.1016/j.omtm.2024.101319.
9. Kaseniit KE, Katz N, Kolber NS, et al. Modular, programmable RNA sensing using ADAR editing in living cells. Nat Biotechnol 2023;41(4):482–487; doi: 10.1038/s41587-022-01493-x.
10. Gayet RV, Ilia K, Razavi S, et al. Autocatalytic base editing for RNA-responsive translational control. Nat Commun 2023;14(1):1339; doi: 10.1038/s41467-023-36851-z.

11. Wellhausen N, O'Connell RP, Lesch S, et al. Epitope base editing CD45 in hematopoietic cells enables universal blood cancer immune therapy. Sci Transl Med 2023;15(714):eadi1145; doi: 10.1126/scitranslmed.adi1145.
12. Casirati G, Cosentino A, Mucci A, et al. Epitope editing enables targeted immunotherapy of acute myeloid leukaemia. Nature 2023;621(7978):404–414; doi: 10.1038/s41586-023-06496-5.
13. Wang X, Cabrera FG, Sharp KL, et al. Engineering Tolerance toward Allogeneic CAR-T Cells by Regulation of MHC Surface Expression with Human Herpes Virus-8 Proteins. Molecular Therapy 2021;29(2):718–733; doi: 10.1016/j.ymthe.2020.10.019.
14. Jo S, Das S, Williams A, et al. Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing. Nat Commun 2022;13(1):3453; doi: 10.1038/s41467-022-30896-2.
15. Stavrou M, Philip B, Traynor-White C, et al. A Rapamycin-Activated Caspase 9-Based Suicide Gene. Mol Ther 2018;26(5):1266–1276; doi: 10.1016/j.ymthe.2018.03.001.
16. Hayal TB, Wu C, Abraham D, et al. The Impact of CD45-Antibody-Drug Conjugate Conditioning on Clonal Dynamics and Immune Tolerance Post HSPC Transplantation in Rhesus Macaques. Blood 2023;142:3419; doi: 10.1182/blood-2023-182066.
17. Garaudé S, Marone R, Lepore R, et al. Selective haematological cancer eradication with preserved haematopoiesis. Nature 2024;630(8017):728–735; doi: 10.1038/s41586-024-07456-3.
18. Meaker GA, Wilkinson AC. Ex vivo hematopoietic stem cell expansion technologies: recent progress, applications, and open questions. Experimental Hematology 2024;130; doi: 10.1016/j.exphem.2023.12.001.