Hypothesis

Purging Autologous HSCs via Detection of Clonal Mutations

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Posted Date: 3 October 2024

doi: 10.20944/preprints202410.0163.v2

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Purging Autologous HSCs via Detection of Clonal Mutations

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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.

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Introduction

Allogeneic hematopoietic stem cell (HSC) transplantation can be used to treat blood cancers, but it often leads to graft vs. host disease [1]. Autologous HST transplantation would be better, and mobilization of HSCs can now be done much more effectively than in the past [2]. However, the harvested patient cells could be contaminated to some extent with their tumor cells. A targeted method of purging HSCs could allow for safer autologous HSC transplantation [3].

If the patient's blood cancer cells in circulation, as well as the lymph nodes and bone marrow, are sequenced [4], 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 the cells can be eliminated using the effector module of said switches [5–7].

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 [8]. In this in vivo system, one adenoviral genome expresses Flpe recombinase and a hyperactive transposase to circularize and randomly insert the transgene cassette in the second into the HSC genome.

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[4]. After isolating mobilized CD_{34}^{+} cells from a patient's bloodstream, an ex vivo vector could target CD34 for cell entry[2]. Here, the early enrichment strategy for CD_{34}^{+} HSCs containing the gene vector, either as an integrated element or S/MAR-containing plasmid [9], would rely on epitope base editing of the CD45 gene [10]. Epitope base editing would shield HSCs containing the therapeutic DNA from an immunotoxin and be important for cancer therapy purposes [11–13].

After purging and *CD45* base editing, a replicating plasmid can be destroyed via CRISPR/Cas9 targeting of multiple plasmid loci. In the case of an integrated vector, a recombinase could excise the therapeutic DNA.

For in vivo purposes, mobilized HSCs would be targeted by the dual adenoviral vectors that can bind CD34[8]. Here, *CD45* base editing would also be effected. Display of self-peptide based on CD47 on the adenoviral capsids [14,15] may preclude or reduce the need for innate immune suppression.

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Protein engineering approaches could shelter transduced HSCs from the adaptive immune system [16,17]. Alternatively, MHC class I and natural killer cell inhibitor proteins could be expressed by the vector during this process [18]. Moreover, zinc finger base editors may be less immunogenic than TALE or CRISPR base editors [19,20]. The CRISPRa and base editor modules at least could be regulated by a recently developed, tetracycline-regulated RNA switch, as RNA elements would be non-immunogenic [21].

A recombinase could also be utilized here for excision of the integrated transgene cassette after purging and *CD45* base editing.

The ex vivo vector or adenoviral genome-derived transgene cassette would encode switches that can recognize the patient's clonally mutated mRNA sequences and respond via activation of a toxin module that eliminates the cell [5–7]. One example of a type of switch that could accomplish this is the reprogrammable ADAR sensor "RADAR", a system developed recently that can even detect point mutations in mRNA [22,23]. It would also be programmed to express CRISPRa modules that upregulate the expression of the patient's clonally mutated genes.

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 [7]. This site could be recognized by an RNA-binding "proximity" switch based on Pumby modules [6,24].

Blood Cancer Elimination Strategy

Base editing of the CD45 gene in the patient's HSCs would allow anti-CD45 chimeric antigen receptor (CAR) T-cells to be intravenously administered and eliminate the remainder of the patient's blood cancer cells [25]. The CAR T-cells can be purged as well if necessary, and should have a base edited CD45 gene themselves to avoid fratricide. For acute myeloid leukemia, instead of *CD45* epitope editing, a combination of *FLT3*, *CD123*, and *KIT* epitope editing can be exploited [26]. The CAR T-cells could potentially be off-the-shelf, precluding tumor cell contamination issues [18,27]. They could be eliminated after treatment and re-administered later if necessary [28]. An immunotoxin targeting CD45 could be used instead of CAR T-cells [29]. It was demonstrated that the base edited CD45 gene product for the immunotoxin cross-referenced here [10] was more biophysically optimal than the one generated earlier for CAR T-cell targeting [25]. However, repeated injections may be required for immunotoxins, as opposed to autologous CAR T-cells, which can persist in circulation for long periods of time.

Conclusion

The purging strategy described in this piece could ensure that anti-CD45 immunotherapy involving base editing of patient HSCs can be employed to treat blood cancer without generating $CD34^+$ cancer cells resistant to the therapy.

In the near future, multiplexed epitope editing of universal leukocyte markers other than CD45 could be performed to avoid the survival of escape variants.

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[5,6,7]. It may even be applicable to hematological malignancies like multiple myeloma, which forms solid tumors. Whether it can help treat others depends in part on whether it can efficiently reach cells in the bone marrow after intravenous injection.

Funding: None.

Conflicts of interest: None.

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