Successful translation and future prospects of TALEN editing for leukaemia patients

Abstract:

In this editorial, we briefly summarize the current role of transcription activator-like effector nucleases (TALENs) editing in the creation of universal chimeric antigen receptor (CAR) T cells for the treatment of leukaemia. We explore the targets for TALENs in the creation of these universal CAR T cells, as well as the first clinical successes of these therapies. We briefly discuss the way the field of genetic engineering in translational medicine is moving forward and give a comparison between the latest gene editing techniques that are currently in clinical trials and/or are being developed for clinical use.

Keywords: Adoptive cell therapy, CRISPR, gene editing, immunotherapy, leukaemia, TALEN, universal CAR T cell

Main Text:

In recent years CAR T cell therapies have emerged as powerful targeted immunotherapies. They have had impressive success in the treatment of different types of B-cell leukaemias, with early phase trials of CAR T cell therapy against the B cell antigen CD19 (CAR19) accomplishing disease remission in a significant proportion of treated patients [1]. The process for the generation of these autologous CAR T cells requires leukapheresis for the isolation of the T cells, followed by genetic engineering to express the desired chimeric antigen receptor (CAR) against one or more B cell antigens (e.g. CD19, CD22) on their surface. Finally, these CAR T cells are expanded and, after the patient has been lymphodepleted using combination chemotherapy, they are reinfused. Because these are bespoke patient-specific therapies, the manufacturing of these cells has proven to be

expensive, time-consuming, and in some cases technically very difficult, especially in patients that have been extensively treated and rendered lymphopenic. Due to these difficulties, there has been marked interest in generating an "off-the-shelf", universal CAR T cell that could be derived from unrelated donors. There are two major barriers in this regard, one involves the recognition of the patient's human leucocyte antigens (HLA) by the native T cell receptor of the CAR T cells (potentially causing transfusional graft-versus-host disease (GvHD), which often manifests with cytopenias and is potentially life threatening), and the other involves the foreign HLA expressed by the CAR T cells being recognized by the immune system of the patient (hence rejection of the CAR T cells).

In order to address the former and to render the CAR T cells non-alloreactive, it is now possible to disrupt expression of the T cell receptor (TCR) on CAR T cells by targeting the TCRalpha constant (TRAC) gene via TALEN edition [2]. One possible approach to the latter is to disrupt expression of CD52, the target of the lympholytic depleting monoclonal antibody alemtuzumab [2,3]. By rendering CAR T cells resistant to the effects of alemtuzumab, it is possible to establish transient engraftment in a patient whose immune system has been depleted with this drug. These universal CAR T cells could be further improved by using TALEN-mediated edition to target different immune checkpoints such as PD-1 or CTLA-4, so as to enhance efficacy by conveying resistance to inhibition mediated via these inhibitory pathways [4].

Clinical application of these 'universal' CAR T cells is already underway. In 2015 the first-in-human experience of this approach (UCART19) was demonstrated in an infant with relapsed refractory $CD19^{+}$ B cell acute lymphoblastic leukaemia (B-ALL) [5]. Although

results of such early experiences must be interpreted with caution, the child achieved engraftment and expansion of the third party cells and attained molecular remission. Notably, the child did develop a skin rash consistent with GvHD with concurrent development of profound cytopenias/aplasia, suggesting that this first generation product still had alloreactive potential, and required a salvage allogeneic stem cell transplantation (allo-SCT). Subsequent trials with this product in both paediatric (NCT02808442) and adult (NCT02746952) patients are being performed in the context of bridging patients to an allogeneic transplant, and further refinement will likely be necessary to abrogate the residual alloreactivity and allow application as a stand-alone therapy [3].

The same platform technology can be used to target other surface antigens, e.g. UCART123 has begun phase I trials for both acute myeloid leukaemia (AML) (NCT03190278) and blastic plasmacytoid dendritic cell neoplasm (BPDCN) (NCT03203369). UCART123 cells target CD123, the alpha-chain of the interleukin-3 receptor, which is expressed on blasts and leukaemia stem cells on the majority of AML patients, but also potentially on other non-malignant target cells. UCART123 cells have been shown to eliminate AML cells *in vivo* and improve overall survival in patient-derived xenograft (PDX) mice [6]. Unfortunately, after the BPDCN trial recorded a fatality in the first patient treated, the U.S. Food and Drug Administration (FDA) placed holds on both of the clinical trials for UCART123 [7]. These were lifted after revisions were made to the protocols of both trials, and currently the researchers are awaiting IRB's approval on the amended protocols to resume patient enrolment [8]. Further experience will help to define the nature of the original toxicity, and whether CD123 is a viable clinical target.

That these TALEN-edited T cells are already in early clinical trials illustrates how rapidly the field of genetic engineering has progressed in the last decade. The field has advanced from the use of zinc finger nucleases to TALENs and, more recently, to clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 reagents for the generation of double stranded DNA cleavage for the disruption of genes of interest. CRISPR-Cas9 reagents have superseded TALENs in preclinical work because of their ease of design, versatility, low cost, and their high efficiency. In the clinical setting there are still potential advantages associated with the use of TALENs, notably their high degree of precision in target gene editing, and it currently remains unclear whether one technology will emerge as clearly superior. Trials are now beginning to evaluate CRISPR-edited T cells [9]. One such trial is using CRISPR-edited universal CD19-specific CAR T (UCART019) cells for the treatment of relapsed or refractory $CD19^{+}$ leukaemia and lymphoma (NCT03166878). It will be important to incorporate the improvements that have been developed when using these reagents to maximize efficiency and minimize off-target effects [10,11]. It is of interest to note that the role of TALEN editing in clinical trials has been mostly restricted to the creation of universal CAR T cells for the treatment of leukaemias. This is in contrast to the role of CRISPR-Cas9 editing, which has also been used in the generation of PD-1 knockout autologous lymphocytes for the treatment of different types of solid malignancies, such as non-small cell lung cancer (NCT02793856), renal cell carcinoma (NCT02867332), prostate cancer (NCT02867345), and bladder cancer (NCT02863913).

With the inclusion of TALENs and CRISPR-Cas9 in the toolkit for targeted editing, the field of genetic engineering has evolved incredibly quickly, reaching early clinical trials at an impressive speed. However, one of the central concerns regarding the use of genetic

engineering in clinical work has been the assessment of specificity. This is perhaps better documented for TALENs, although multiplex gene editing platforms raise a greater possibility of translocation events. Systems for the detection of off-targets of CRISPR editing have been developed and are constantly being improved upon [12,13]. Looking forward, we predict that TALEN-based editing will continue to be explored in the clinical setting because of its precision and control, at least until CRISPR editing is proven to be as specific, at which point CRISPR editing may prove more popular given its ease-of-use, affordability, and multiplex capabilities [14]. In the future, we can expect to see the development of a wider range of these therapeutic genetic engineering tools, with the incorporation of multiplex edition already the next step to be accomplished [14]. These early clinical trials are paving the way for gene-editing to shape the future generation of T cell therapies, with exciting possibilities as yet unknown.

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