

A Phase I Study Evaluating the Safety and Persistence of Allorestricted WT1-TCR Gene Modified Autologous T Cells in Patients With High-Risk Myeloid Malignancies Unsuitable for Allogeneic Stem Cell Transplantation

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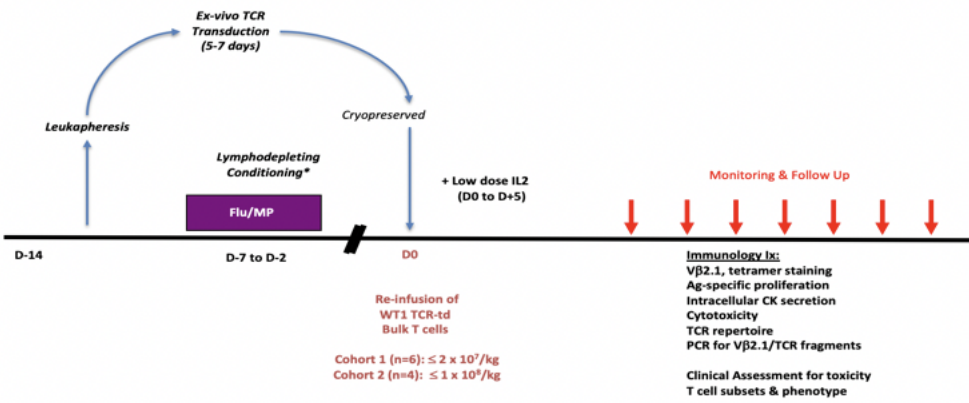
Background: Patients with acute myeloid leukemia (AML), myelodysplasia (MDS) or tyrosine kinase inhibitor resistant chronic myeloid leukemia (CML) who are unsuitable for consolidative allogeneic stem cell transplantation (alloSCT) have high relapse rates following chemotherapy. Wilms' tumor 1 (WT1) is highly expressed in the majority of acute myeloid leukemias (AML) and in many subtypes of myelodysplasia (MDS) as well as other hematological and solid tumors. WT1 is an intracellular antigen, which makes it difficult to target using current Chimeric Antigen Receptor (CAR)-T cell technologies. The use of genetically modified T cells expressing WT1-specific α/β T cell receptors can re-direct T cell specificity via the recognition of intracellular peptides presented by MHC molecules on the malignant cell surface. Phase I clinical trials of WT1-TCR gene-modified T cells have been conducted in the settings of relapsed disease and post-alloSCT and preliminary data suggests this treatment approach is safe and potentially clinically effective in these cohorts (Tawara *et al.* Blood. 2017;130(18):1985-94; Chapuis *et al.*, Nat Med. 2019;25(7):1064-72).

Methods: We report a phase I/II safety and dose escalation study evaluating WT1-TCR gene-modified autologous T cells in HLA-A*0201 positive patients with AML, MDS and CML, unsuitable for alloSCT (NCT02550535) (Fig 1A). Patient T cells were harvested by leucapheresis and transduced with a retroviral vector construct encoding the codon optimised variable and constant α and β chains of the human pWT126-specific TCR separated by a self-cleaving 2A sequence (Fig 1B). Bulk transduced T cells were analysed by flow cytometry (CD3, CD8 and V β 2.1) prior to infusion and at regular intervals post-infusion. A quantitative PCR assay was developed to identify WT1-TCR expressing T cells in the peripheral blood post infusion. Patients received minimal conditioning with fludarabine and methylprednisolone prior to transfer of transduced T cells. All subjects were followed for a minimum of 12 months or until death.

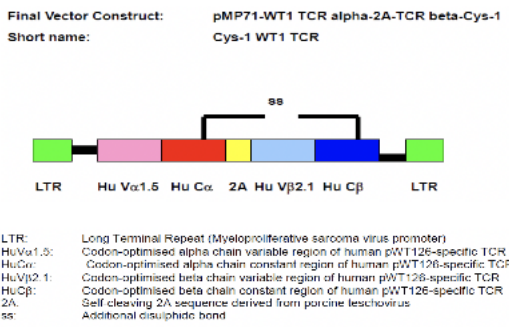
Results: A total of 10 patients (6 AML, 3 MDS and 1 TKI- resistant CML) were recruited. The mean age was 71.3 years (range 64-75) and all had high risk disease (by cytogenetic or clinical criteria). All AML patients were in complete morphological remission at the time of trial entry, whilst MDS patients had $\leq 15\%$ blasts on bone marrow examination. All 10 patients received the gene-modified T cells in dose escalation cohorts (seven patients received $\leq 2 \times 10^7$ /kg and three patients received $\leq 1 \times 10^8$ /kg bulk WT1 TCR transduced cells). No adverse events directly attributable to the investigational product were recorded apart from one possible cytokine release syndrome, which was managed without tocilizumab. Transferred T cells demonstrated *in vivo* proliferation commensurate with maintenance of functional capacity despite *ex vivo* manipulation (Fig 1C and 1D). The TCR-transduced T cells were detectable in all patients at 28 days and in 7 patients persisted throughout the study period (Fig 1E). All 6 AML patients were alive at last follow up (median 12 months; range 7-12.8 months). The 3 patients with MDS had a median survival of 3 months (range 2.1-3.96 months) post T cell infusion. 2 died from progressive disease and one from other causes. 2 patients discontinued the study early due to disease progression.

Conclusions: This is the second reported phase I/II clinical trial of autologous WT1-TCR gene-modified T cells for treatment of AML and MDS in a high-risk cohort of patients not suitable for alloSCT. We have shown that the WT1-TCR T cells demonstrated a strong safety profile without detectable on-target, off-tumour toxicity and no severe adverse events in the ten patients treated. An important cause of treatment failure for adoptive cellular therapies is the lack of persistence of transferred T cells leading to loss of disease specific effects. We demonstrated that autologous WT1-TCR T cells proliferated *in vivo* and persisted for many months. Recent work within our group (in press) has shown that TCRs modified to include key framework residues, show increased TCR expression and functional improvement. These modifications could be incorporated into future studies to improve efficacy. This data supports the rationale for a larger, phase II trial of WT1-TCR T cells in myeloid malignancies in patients for whom alloSCT is not appropriate, in order to assess clinical efficacy.

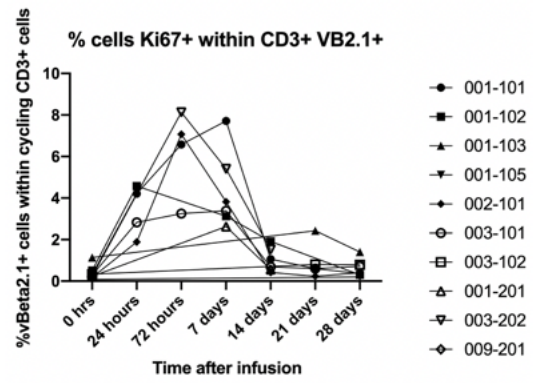
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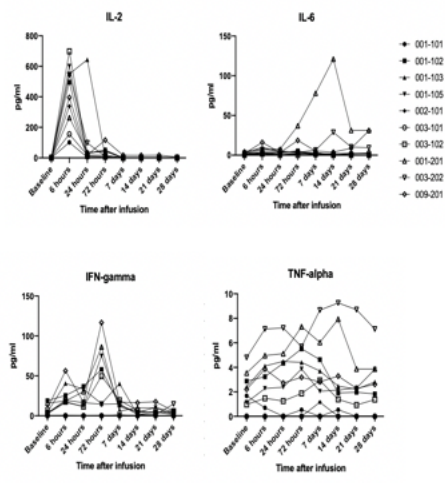
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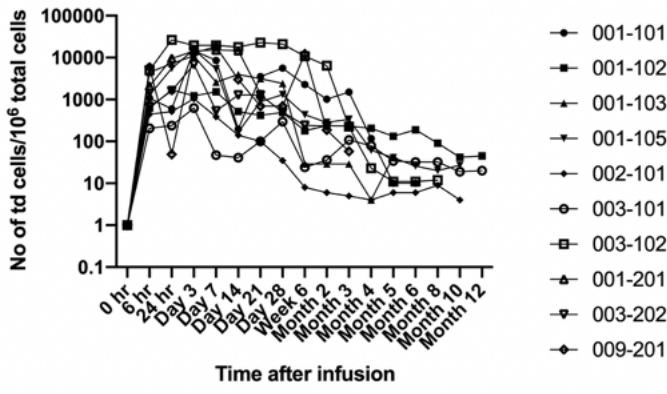


D



E

Persistence of WT1-TCR gene-modified cells: qRT-PCR



A: Trial schematic: Once successful manufacture of the WT1 TCR-transduced T cells was confirmed, the subject underwent a lymphodepleting conditioning regimen for five days consisting of fludarabine 30 mg/m² administered intravenously for 5 days and methylprednisolone 500 mg administered intravenously for 1 day. **B:** MP71 retroviral vector consisting of codon optimised variable and constant alpha chains of the human pWT126-specific TCR and the codon optimised variable and constant beta chains of the human pWT126-specific TCR separated by a self-cleaving 2A sequence and flanked by long terminal repeats. An additional disulphide bond between the constant regions was added to enhance preferential pairing and reduce mispairing with endogenous TCR chains. **C:** % of transduced cells Ki67+ showing that gene-modified cells proliferated in vivo. **D:** Cytokine (IL-2, IL-6, IFN-γ, TNF-α) profiles in all subjects in first 28 days following infusion of WT+ TCR transduced T cells. **E:** qRT-PCR data showing TCR transduced T cells were detectable in all patients at 28 days and in 7 patients persisted throughout the study period.