

Wilms' tumour antigen 1 Immunity via DNA fusion gene vaccination in haematological malignancies by intramuscular injection followed by intramuscular electroporation: a Phase II non-randomised clinical trial (WIN)

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Scientific summary

The WIN Phase II non-randomised clinical trial

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Scientific summary

Background

For chronic myeloid leukaemia (CML), tyrosine kinase inhibition offers significant clinical benefit. Over 85% of imatinib-treated patients with chronic-phase CML (CML-CP) achieve a complete cytogenetic response (CCyR), but the majority of patients have a persisting molecular disease, as assessed by quantitative polymerase chain reaction (qPCR) analysis for the *BCR-ABL* (breakpoint cluster region-Abelson murine leukaemia viral oncogene homolog 1) transcripts, and almost all will relapse following imatinib withdrawal. Functional leukaemia CD34+ (cluster of differentiation 34-positive) progenitor cells have been identified in such patients in CCyR, suggesting the presence of a reservoir of leukaemia cells resistant to the tyrosine kinase inhibitor (TKI). Furthermore, the durability of these responses has not yet been established. The immunological effect of allogeneic stem cell transplantation (alloSCT) and donor lymphocyte infusions (DLIs) suggests that an approach based on the amplification of the patient's own immune response to the disease could add to the responses seen after treatment with TKIs. Based on our own previous data we argue here that vaccinating against Wilms' tumour antigen 1 (*WT1*) using deoxyribonucleic acid (DNA) vaccination is an attractive choice for delivering this immune attack. *WT1* is significantly overexpressed in all CD34+ subpopulations in CML, encompassing the most primitive haematopoietic stem cell to the most mature cells, which escape control by imatinib and previous data suggest that active immunotherapy holds significant promise by the induction of tumour antigen-specific CD8+ (cluster of differentiation 8-positive) T lymphocytes (T cells) without adding toxicity.

Acute myeloid leukaemia (AML) is a disease of older adults, with a median age of 68 years, and an incidence of 8–12 per 100,000 of the population. Advances in the understanding of the pathophysiology of AML have not yet led to major improvements in disease-free and overall survival of adults with this disease. Only approximately one-third of adults aged between 18 and 60 years who are diagnosed with AML can be cured; disease-free survival is rare and current therapy is devastating in older adults. Treatment of AML involves chemotherapy and remission rates are high – up to 85%; however, remissions are often short lived, and in > 70% of patients the disease progresses and leads to death within 2 years. In patients with AML, *WT1* has been established as a marker for minimal residual disease. Additionally, based on data from a number of studies, *WT1* gene expression has been suggested to carry adverse prognostic implications in AML. As in CML, peptide vaccination has been tested with some success and the data support that active immunotherapy other than alloSCT holds significant promise by the induction of tumour antigen-specific CD8+ T cells without added toxicity.

The purpose of the trial was to build on an established programme of DNA fusion gene vaccination delivered by intramuscular injection, and exploiting this unique experience with electroporation, to induce durable immune responses with the aim of controlling disease by precision attack of the tumour by CD8+ T cells. DNA fusion vaccines were initially developed to treat B-lymphocyte malignancies, which showed that fusion of the microbial sequence, fragment C from the tetanus toxin (FrC) to the idiotypic tumour antigen, provided the T-cell help required to induce humoral and CD4+ (cluster of differentiation 4-positive) T-cell responses in preclinical models. An important development has been electroporation, which has dramatically increased DNA vaccine performance in mice and rhesus macaques and this has been included in a previous clinical trial in patients with prostate cancer where clear evidence for amplification of antibody and CD4+ T-cell responses in patients was found. For the induction of CD8+ T-cell responses, the vaccine design was modified by reducing the FrC sequence to a single domain (p.DOM). This decreased the potential for peptide competition but retained the major histocompatibility complex class II-restricted peptide p30. An epitope-specific sequence was then inserted at the C-terminus of FrC to aid processing/presentation.

The preclinical data predict a response in humans. For patients with relapsed prostate cancer, a p.DOM epitope design incorporating a peptide sequence from prostate-specific membrane antigen [Gene Therapy Advisory Committee (GTAC) 089] has induced high levels of epitope-specific interferon gamma, producing CD8+ T-cell responses in 67% (10/15) of patients. This was the first ever trial to exploit delivery of DNA by electroporation; this approach was found to be safe and readily accepted by patients. Responses were robust and persistent over many months to the end of follow-up at 18 months. The clinical effectiveness of the p.DOM epitope design for the treatment of myeloid malignancies has been explored based on these clinical results.

The Wilms' tumour antigen 1 (*WT1*) gene has emerged as one of the most promising targets for immunotherapy of haematological malignancies, including CML, AML and myelodysplastic syndromes. It is also a potential target for the treatment of solid tumours. Despite its ubiquitous expression during embryogenesis, *WT1* expression in normal individuals is limited to renal podocytes, gonadal cells and a small proportion of CD34+ cells, in which expression is significantly lower (10- to 100-fold). This could raise a concern about autoimmunity but, reassuringly, the available data document selectivity of attack against tumour cells, sparing the CD34+ cells and without any evidence of renal or other autoimmune toxicity in murine models or patients. We and others have tested WT1 peptide vaccines both in preclinical models and in clinical trials. The latter data document that T-cell responses can be induced in patients and confirm the presence of an expandable CD8+ T-cell repertoire. Importantly, the ability of peptide vaccines to induce measurable clinical responses has been documented. However, a key problem with class I-restricted peptide vaccines is the inability of this approach to provide linked CD4+ T-cell help, crucial for the maintenance of tumour antigen-specific CD8+ T-cell populations. In the clinic, this is visible through poor persistence of the detected CD8+ responses.

Preclinically, in a previous study, three domain 1 from fragment C of tetanus toxin (DOM) epitope vaccines were evaluated, each encoding a different, previously described, WT1-derived, human leucocyte antigen A2 (HLA A2)-restricted peptide. All were able to induce CD8+ T-cell responses in 'humanised', and presumably tolerised, mice expressing HLA A2 and these killed human WT1-positive (WT1+), HHD+ leukaemia cells ex vivo. A direct comparison with a WT1 peptide vaccine (plus T-cell help and adjuvant) showed a clear superiority of the DNA fusion vaccine. In parallel, we showed that low numbers of human WT1 peptide-specific T cells could be expanded in vitro to kill human leucocyte antigen A2-positive (HLA A2+) (WT1+) leukaemia cells. WT1 peptide 37 (WT1-37) and WT1 peptide 126 (WT1-126) were selected for current studies. We have already documented clinically the ability of p.DOM epitope vaccines to induce cytotoxic T cells and anticipate that dual attack against more than one epitope will provide added clinical benefit. Vaccination with p.DOM-WT1-37 and p.DOM-WT1-126 into different locations will allow us to avoid antigenic competition. Given the clear effect on the response to the FrC portion of the vaccine in the prostate trial, electroporation was used as a delivery strategy.

Objectives

The aim of the trial was to bring together substantial preclinical and clinical expertise to exploit the advantages of DNA fusion vaccines to form the basis for larger, randomised studies.

The objectives were to evaluate:

1. molecular response in patients with CML (i.e. transcript levels of *BCR-ABL* and *WT1*) and AML (i.e. transcript levels of *WT1*)
2. time to disease progression, 2-year survival rate (patients with AML)
3. correlation of molecular responses with immunological responses.

Methods

Trial design

This was a non-randomised, open-label, single-dose-level Phase II trial in two patient groups (CML and AML) based on HLA A2 genotype. HLA A2+ patients were vaccinated with two DNA vaccines: (1) p.DOM–WT1-37 (epitope sequence: VLDFAPPGA); and (2) p.DOM–WT1-126 (epitope sequence: RMFPNAPYL). Patients with HLA A2-negative genotype were not vaccinated and formed the control group. The original trial design followed Simon's optimal Phase II trial design (Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;**10**:1–10). This was changed to A'Hern's single-stage design during the course of the trial (A'Hern RP. Sample size tables for single-stage phase II designs. *Stat Med* 2001;**20**:859–66), which was endorsed by the trial's independent oversight committees.

Participants

Chronic myeloid leukaemia patients with Philadelphia chromosome-positive CML in chronic phase, in CCyR but with detectable *BCR–ABL* transcripts and maintained the CCyR on TKI monotherapy for a minimum of 24 months, were considered for the trial.

Acute myeloid leukaemia patients with WT1+ AML in complete remission (CR) post chemotherapy or AML in morphological CR with incomplete blood count recovery, defined as patients who fulfil all of the criteria for CR except for residual neutropenia (< 1000/μl) or thrombocytopenia (< 100,000/μl).

Eligibility criteria included age ≥ 18 years, a World Health Organization status 0 or 1, a haemoglobin level of > 100 g/l, a creatinine level of < 1.5× upper limit of normal, liver function tests < 1.5× upper limit of normal; a lymphocyte count ≥ 1.0 × 10¹¹/l; and normal clotting. These criteria were used to select patients in the three centres (Southampton, Hammersmith and Exeter) that the trial was conducted in.

Main outcome measures

For the CML treatment group, the primary outcome was molecular response of *BCR–ABL* and *WT1* [major or minor response or complete molecular response (CMR)]. For the AML treatment group, the primary outcome was time to disease progression and the secondary outcomes were molecular response of *WT1* transcript levels, immune responses to *WT1* and DOM, toxicity, CML time to disease progression, next treatment and survival, AML 2-year survival, *WT1* molecular response, overall survival and safety profile [toxicity according to Common Terminology Criteria for Adverse Events (CTCAE), version 4, and pain assessment after vaccination]. Immunological responses were key secondary outcomes for both CML and AML treatment groups.

Trial procedures

Principal investigators identified potential eligible patients from their existing patient population. Patients who were interested in participating in the trial were provided with the patient information leaflet and signed the informed consent form prior to enrolment into the trial.

For the intervention group, the DNA vaccine was administered six times every 4 weeks followed by a further six vaccinations every 3 months to a maximum of 24 months, at the dosing amounts as follows:

- p.DOM–WT1-37: 1 mg/dose/vaccine
- p.DOM–WT1-126: 1 mg/dose/vaccine.

Patients were followed up as outpatients from the start of treatment to 3 years or withdrawal from the trial.

Statistical methods

Baseline characteristics and molecular data were summarised by HLA A2 status, together with reasons for premature withdrawals from treatment and premature withdrawals from the trial.

The primary outcome of the trial was molecular response of *BCR-ABL* (major or minor response or CMR), which was summarised by HLA A2 status. Secondary outcomes included *WT1* response (major or minor response or CMR) and time to disease progression, time to death and time to next treatment, which were analysed using unadjusted Cox proportional hazards models and by producing Kaplan–Meier survival curves.

Toxicity was assessed according to CTCAE, version 4.0, and pain assessment information was summarised in terms of the median and worst pain recorded immediately after and 48 hours post vaccination.

Results

In the 12 patients evaluated, the vaccine and electroporation were safe, with no new or unexpected side effects. The evaluation of patients for adverse events of special interest (heart, bone marrow and renal) did not reveal any safety concerns. No significant difference in the frequency of side effects between the two trial groups was seen. Side effects related to vaccination site reaction were more common in the HLA A2+ group. In the HLA A2+ cohort, one major molecular *BCR-ABL* response was seen at week 8. In the unvaccinated cohort, a molecular response was detected at week 23. Two *WT1* molecular responses were seen in the vaccinated group and one was seen in the unvaccinated group.

At an immunological level, the vaccine performed as expected. Positive responses to the tetanus-derived component of the vaccine were detected in all (10/10; 100%) evaluable vaccinated patients, providing confirmation of successful vaccine delivery. Immunological responses to the antigen-specific (*WT1*) component of the vaccine were measured using the validated *WT1* tetramer assay to measure T cells that were reactive with the vaccine target, *WT1*. *WT1*-specific T cells were detected for 7 out of 10 (70%) evaluable vaccinated patients. This included responses to *WT1*-37 in 6 out of 10 (60%) and to *WT1*-126 in 2 out of 10 (20%) evaluable patients. The immunological analyses for the *WT1* Immunity via DNA (WIN) trial provide evidence to show that the combined p.DOM-*WT1* vaccines can stimulate measurable immune responses against both the DOM and the *WT1* components in CML patients in the chronic phase while being treated with imatinib.

The study met its primary decision-making target with one major molecular response in *BCR-ABL* transcript levels and, in parallel, new data emerged illustrating that immune responses detected in the blood appear to evolve by 6 months post vaccination in most patients. The early onset of the major molecular response suggests that this response is due to the vaccination. In parallel, a *WT1* molecular response was observed.

The study did not complete recruitment and there were multiple hurdles that contributed to this failure.

This is disappointing as the robust induction of FrC responses (10/10 evaluable patients) and *WT1* T-cell responses in 7 out of 10 evaluable patients support that the preclinical data link to immunological outcomes as predicted. Overall, the data confirmed the immunogenicity and safety of the vaccine.

Recommendations for future research

The observation of a molecular response in 1 out of 12 patients suggests that the rate of clinical benefit in patients on imatinib in CML in chronic phase is low. Although immune results support effective delivery of the vaccine and stimulation of the expected immune response, we do not intend to further assess the vaccine approach in this clinical context. Evaluation of the p.DOM-*WT1* vaccine in patients with AML remains attractive, but it is unlikely to be feasible at this time. A combination of the DNA vaccine approach with strategies to expand T-cell responses with immunomodulatory antibodies is in development.

Trial registration

This trial is registered as EudraCT 2009-017340-14 and ISRCTN62678383.

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