EFFICACY AND MECHANISM EVALUATION

VOLUME 3 ISSUE 3 APRIL 2016
ISSN 2050-4365

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DOI 10.3310/eme03030

National Institute for Health Research

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Declared competing interests of authors: none

Published April 2016
DOI: 10.3310/eme03030

This report should be referenced as follows:

Ottensmeier C, Bowers M, Hamid D, Maishman T, Regan S, Wood W, *et al*. 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). *Efficacy Mech Eval* 2016;**3**(3).

Efficacy and Mechanism Evaluation

ISSN 2050-4365 (Print)

ISSN 2050-4373 (Online)

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: nihredit@southampton.ac.uk

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This report

The research reported in this issue of the journal was funded by the EME programme as project number 08/99/24. The contractual start date was in January 2011. The final report began editorial review in March 2015 and was accepted for publication in October 2015. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The EME editors and production house have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the final report document. However, they do not accept liability for damages or losses arising from material published in this report.

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Abstract

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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Background: In the UK almost 7000 people are diagnosed with leukaemia each year, but despite continuing advances in diagnosis and treatment with new drugs, such as the tyrosine kinase inhibitors, the majority of these patients will eventually die from their disease. Until quite recently, the only treatment to offer the possibility of long-term disease-free survival was allogeneic stem cell transplantation. However, this carries a substantial risk of mortality and is available to only a minority of patients.

Objectives: The aim of the study was to test the hypothesis that molecular and clinical responses, induced by T lymphocytes (T cells), can be predicted by increases in the number of CD8+ (cluster of differentiation 8-positive) T cells specific for the vaccine-encoded T-cell epitopes. This project also aimed to build on the established programme of deoxyribonucleic acid (DNA) fusion-gene vaccination delivered by intramuscular injection, exploiting a 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.

Method: A non-randomised, open-label, single-dose-level Phase II clinical trial in two patient groups [chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML)] on stable doses of imatinib. Human leucocyte antigen A2-positive (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). The HLA A2-negative patients formed an unvaccinated control group. The sample size for the HLA A2+ group was originally determined following 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.

Results: The study included 12 patients with CML who were vaccinated and nine patients with CML who were unvaccinated as the control group. Both the vaccines and the electroporation were safe, with no new or unexpected toxicities. The evaluation adverse events of special interest (heart, bone marrow, renal) did not reveal safety concerns. Two *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral oncogene homolog 1) responses were observed, both of which were defined as a major response, with one in each group. Two Wilms' tumour antigen 1 (*WT1*) molecular responses were observed in the vaccinated group and one was observed in the control group. At an immunological level, the vaccine performed as expected.

Conclusions: The study met its primary decision-making target with one major molecular response in *BCR–ABL* transcript levels. Overall, the data showed, in this clinical setting, the immunogenicity and safety of the vaccine.

Limitations: The study did not complete recruitment and there were multiple hurdles that contributed to this failure. This is disappointing given the robust induction immune responses against *WT1* T-cell responses in 7 out of 10 evaluable patients.

Future work: Evaluation of the p.DOM–WT1 vaccines in AML remains attractive clinically, but it is unlikely to be feasible at this time. Combination of the DNA vaccine approach with strategies to expand T-cell responses with immunomodulatory antibodies is in development.

Funding details: This project was funded by the Efficacy and Mechanism Evaluation (EME) programme, a Medical Research Council (MRC) and National Institute for Health Research (NIHR) partnership, and Bloodwise.

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Wilms' tumour antigen 1 peptide 126 Peptide from Wilms' tumour antigen 1 with the amino acid sequence RMFPNAPYL.

List of abbreviations

ABL	Abelson murine leukaemia viral oncogene homolog 1
AE	adverse event
alloSCT	allogeneic stem cell transplantation
AML	acute myeloid leukaemia
BCR	breakpoint cluster region
BCR–ABL	breakpoint cluster region–Abelson murine leukaemia viral oncogene homolog 1
CCyR	complete cytogenetic response
CD34+	cluster of differentiation 34 positive
CD4+	cluster of differentiation 4 positive
CD8+	cluster of differentiation 8 positive
CK	creatine kinase
CML	chronic myeloid leukaemia
CML-CP	chronic-phase chronic myeloid leukaemia
CMR	complete molecular response
CONSORT	Consolidated Standards Of Reporting Trials
CR	complete remission
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T lymphocyte
DLI	donor lymphocyte infusion
DMEC	Data Monitoring and Ethics Committee
DNA	deoxyribonucleic acid
DOM	domain 1 from fragment C of tetanus toxin
ECG	electrocardiogram
ECHO	echocardiogram
ELISA	enzyme-linked immunosorbent assay
EME	Efficacy and Mechanism Evaluation
FBC	full blood count
FrC	fragment C from tetanus toxin
GTAC	Gene Therapy Advisory Committee
GUS	β-glucuronidase
GVL	graft versus leukaemia
HIV	human immunodeficiency virus
HLA A2	human leucocyte antigen A2
HLA A2–	human leucocyte antigen A2 negative
HLA A2+	human leucocyte antigen A2 positive
HSC	haematopoietic stem cell
IFN-γ	interferon gamma
ISRCTN	International Standard Randomised Controlled Trial Register
ITT	intention to treat
MHRA	Medicines and Healthcare products Regulatory Agency
MRD	minimal residual disease
NIHR	National Institute for Health Research
pCRF	paper case report form
p.DOM	plasmid domain 1 from fragment C of tetanus toxin
PFS	progression-free survival
PI	principal investigator
PSMA	prostate-specific membrane antigen
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
SAE	serious adverse event

SCTU	Southampton Clinical Trials Unit	WT1	Wilms' tumour antigen 1
T cell	T lymphocyte	WT1-126	Wilms' tumour antigen 1 peptide 126
TKI	tyrosine kinase inhibitor	WT1-37	Wilms' tumour antigen 1 peptide 37
WHO	World Health Organization	WT1+	Wilms' tumour antigen 1 positive
WIN	Wilms' tumour antigen 1 (WT1) Immunity via DNA trial		

Plain English summary

The Wilms' tumour antigen 1 (*WT1*) Immunity via deoxyribonucleic acid (DNA) (WIN) study tested a possible vaccine treatment for patients with chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML). The treatment is directed at a molecule called WT1, which helps the survival of leukaemia cells. The vaccine also contains a small piece of genetic information from the tetanus bacterium, *Clostridium tetani*, which, linked to the gene element from *WT1*, is designed to boost the immune system to make white blood cells (lymphocytes) that can see and kill leukaemia cells. During the study, two types of molecules in the blood that are markers for leukaemia were measured: one is *WT1* itself, the other is called *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral oncogene homolog 1). The vaccine was administered with a new type of injection called electroporation, designed to make the vaccine work better.

Twelve patients with CML were vaccinated. It was disappointing that the study was not completed for various reasons, the main one being slow recruitment. The levels of *BCR–ABL* were found to be reduced in one patient, whereas the levels of *WT1* were reduced in another patient. The vaccine was well tolerated and there were no safety concerns. Immune response was evaluable in 10 patients. All responded to the tetanus component of the vaccine; 70% also made immune responses [CD8+ (cluster of differentiation 8)-positive T lymphocytes] to *WT1*. The vaccine had stimulated immune responses as we had hoped. Evaluation of the p.DOM–WT1 vaccines in AML remains clinically attractive and combination of the DNA vaccine with booster strategies is in development in the laboratory.

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 ≥ 1.0×10^{11}/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.

Funding

This project was funded by the Efficacy and Mechanism Evaluation (EME) programme, a Medical Research Council (MRC) and National Institute for Health Research (NIHR) partnership, and Bloodwise.

Chapter 1 Background

Importance of the health problem to the NHS

In the UK, 7279 patients were diagnosed with leukaemia in 2005,[1] but despite continuing advances in diagnosis and treatment the majority of these individuals will eventually die from their disease.[2]

Summary of current evidence

Chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a clonal disease of the haematopoietic stem cells (HSCs) in which a reciprocal translocation, t(9;22)(q34;q11), known as the Philadelphia chromosome, results in a fusion gene, *BCR–ABL* (breakpoint cluster region–Abelson murine leukaemia viral oncogene homolog 1), which in turn expresses an activated tyrosine kinase, and is regarded as the initiating lesion of CML.[3,4] Until quite recently, the only treatment to offer the possibility of long-term disease-free survival was allogeneic stem cell transplantation (alloSCT), the 'curative' effect of which is mediated in large part through the alloimmune graft-versus-leukaemia (GVL) effect.[5] However, alloSCT carries a substantial risk of mortality and is only available to a minority of patients. Tyrosine kinase inhibitors (TKIs), notably imatinib, have replaced alloSCT as first-line therapy for CML owing to their lower toxicity and impressive efficacy. Although > 85% of imatinib-treated patients with chronic-phase CML (CML-CP) achieve a complete cytogenetic response (CCyR), the majority of patients have persisting molecular disease, as assessed by quantitative polymerase chain reaction (qPCR) for *BCR–ABL* transcripts, and almost all will relapse following imatinib withdrawal.[6,7] Functional leukaemia CD34+ (cluster of differentiation 34-positive) progenitor cells have been identified in such patients within CCyR, suggesting the presence of a reservoir of leukaemia cells resistant to TKIs.[8] Furthermore, the durability of these responses has not yet been established. In contrast, long-term survivors of alloSCT very rarely have any detectable molecular disease, indicating that all leukaemia cells must be susceptible to immune destruction (GVL effect). Therefore, novel strategies to eradicate quiescent CML stem cells are required, especially because these cells provide a reservoir for disease relapse.

The immunological effect of 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 the TKI.

Based on our own data we argue here that vaccinating against the Wilms' tumour antigen 1 (*WT1*), using deoxyribonucleic acid (DNA) vaccination, is an attractive choice for delivering this immune attack.[9,10] The validity of *WT1*, as a target for immunotherapy in CML, was recently shown in work published by Yong *et al.* [in John Barrett's group at the National Institutes of Health (USA)].[11] This group studied the expression of leukaemia-associated antigens, including *WT1*, within the CD34+ primitive stem cell and committed progenitor cell pools in CML patients. *WT1* is significantly overexpressed in all CD34+ subpopulations in CML encompassing the most primitive HSCs to the most mature cells,[11] which escape control by imatinib. Taken in the context of these clinical data and that from other groups, which show that even suboptimal vaccination with peptide can have clinical effects,[11,12] these data strongly suggest that active immunotherapy other than allotransplantation holds significant promise by the induction of tumour antigen-specific CD8+ (cluster of differentiation 8-positive) T lymphocytes (T cells) without adding toxicity.

Clearly it is critical to choose the best clinical setting in which to vaccinate, as previous data have shown that the effect of TKIs as a drug class on the immune system is variable,[13] and can be either suppressive or stimulatory. For imatinib specifically, in vivo data show that it can be immunostimulatory, supporting our proposed trial, both in murine[14,15] as well as human[16–18] studies. Furthermore, Wang *et al.*[14] demonstrated that in vivo treatment with imatinib not only prevented the induction of tolerance, while preserving responsiveness to a subsequent immunisation, but, critically, enhanced vaccine efficacy. In patients, low-frequency CD8+ T-cell responses to four leukaemia-associated antigens (ABL kinase, proteinase 3, telomerase and WT1) were detected in CML patients on imatinib and show the immune system's ability to respond to leukaemia-associated antigens in the presence of imatinib.[19] It is therefore unsurprising that two vaccine studies using BCR–ABL peptides in patients with CML treated with imatinib clearly demonstrated the successful induction of CD8+ and CD4+ (cluster of differentiation 4-positive) T cells against the vaccine, even with a suboptimal peptide vaccine approach.[20,21] Bocchia *et al.*[20] found that antileukaemia T-cell responses could be stimulated after vaccination in 9 out of 14 patients. In the EPIC trial, T-cell responses to CD4+ T-cell responses against the vaccine were seen in all patients and 14 of 19 patients developed T-cell responses to BCR–ABL peptides.[21] Prospective analysis of immune responses to vaccination against influenza A (H1N1, 2009 strain) and *Streptococcus pneumoniae* (Klein 1884) Chester 1901 in 50 CML-CP patients treated with imatinib (Glivec®, Novartis Pharmaceuticals UK Ltd), dasatinib (Sprycel®, Bristol-Myers Squibb) or nilotinib (Tasigna®, Novartis Pharmaceuticals UK Ltd) and 15 healthy controls was recently performed.[22] Significant CD8+ and CD4+ T-cell responses against flu were induced in patients with CML-CP on TKIs following vaccination and there was no significant difference in the vaccine-induced T-cell response between CML-CP patients on TKIs and healthy controls (manuscript in preparation). These data strongly support that vaccination of patients on stable doses of imatinib will induce immune responses.

Acute myeloid leukaemia

Acute myeloid leukaemia (AML) is a disease of older adults with a median age of 68 years[23] and an incidence of 8–12 per 100,000 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 about one-third of those 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 with high remission rates in up to 85% of patients; however, remissions are often short lived and > 70% of patients will progress and die from their disease within 2 years (see *Figure 1*).[24] Treatment also causes significant morbidity and mortality. AlloSCT from a compatible donor carries a 20–75% chance of long-term disease-free survival depending on whether the transplant is performed in remission or with residual disease. Death from relapse is the most common cause of treatment failure following transplant. At this point, a minority of patients respond to chemotherapy and DLIs, but remission rates are around 15% with only a fraction being durable.[17,18] There is, therefore, a need to devise better treatments for AML.

In AML, *WT1* has been established as a marker for minimal residual disease (MRD).[25] Additionally, *WT1* gene expression has been suggested to carry adverse prognostic implications in AML based on data from a number of studies.[26,27] A recent trial by the European LeukemiaNet defined and standardised a *WT1* real-time qPCR assay as a marker for MRD monitoring and risk stratification in AML.[28] We intend to exploit this for the proposed trial of *WT1* vaccination. As in CML, peptide vaccination has been tested with some success[12,29–33] and the data support that active immunotherapy other than allotransplantation 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 the disease by precision attack of the tumour by CD8+ T cells. The aim of the trial was to evaluate an identical vaccine strategy in two parallel settings with the purpose of identifying the most promising context for eventual Phase III testing. The hypothesis was that molecular and clinical responses, induced by T cells can be predicted by increases in the number of CD8+ T cells, specific for the vaccine-encoded T-cell epitopes.

Trialling two patient groups will maximise the knowledge gained from this vaccine trial. Patients with CML will allow a direct and objective assessment of the antileukaemia effect of vaccination at the molecular level by *BCR–ABL* and *WT1* monitoring. Patients with AML offer a difficult challenge to haematologists. The advantage of including this patient group is twofold: (1) the antileukaemia effect of vaccination can be assessed objectively by measuring *WT1* gene expression levels; and (2), more importantly, data can be collected on the highly clinically relevant question of whether or not vaccination will prevent relapse in this patient group.

Selection of patients for vaccine therapy

Novel therapies are often first introduced in patient groups who have failed all conventional treatment options and have far advanced or metastatic disease. This strategy is inappropriate for vaccine treatments, which depend upon an intact well-functioning immune system, known to be severely impaired in advanced cancers. The cohort to be studied here has therefore been chosen to reflect this conclusion.

Immunotherapy in haematological malignancies targeting Wilms' tumour antigen 1

DNA fusion vaccines were initially developed to treat B-cell malignancies,[34] which showed that fusion of the microbial sequence, fragment C from the tetanus toxin (FrC) to idiotypic tumour antigen provided the T-cell help required to induce humoral[35] and CD4+ T-cell responses in preclinical models.[36] Early clinical testing was undertaken in a Phase I/II dose escalation trial [LIFTT trial; Gene Therapy Advisory Committee (GTAC) 029A], with individual idiotypic DNA fusion vaccines to treat patients with follicular lymphoma. The vaccine was safe and 14 out of 18 patients showed an antibody and/or CD4+ T-cell responses against the FrC portion of the fusion gene. Encouragingly, 6 out of 16 patients showed responses to the tumour-specific idiotypic antigen (manuscript in preparation). There was no evidence of a dose response for doses ranging from 500 µg/dose to 2500 µg/dose.[9] Overall, however, the levels of response were relatively low and improvements were sought.

An important development has been electroporation, which has been shown to dramatically increase DNA vaccine performance in mice[37] and rhesus macaques,[38] and this method of delivery was used in our clinical trial in patients with prostate cancer. We found clear evidence for amplification of antibody and CD4+ T-cell responses in patients.[39] For induction of CD8+ T-cell responses, the vaccine design was modified by reducing the FrC sequence to a single domain (p.DOM; plasmid domain 1 from FrC). This decreased the potential for peptide competition but retained the major histocompatibility complex class II-restricted peptide p30.[39] An epitope-specific sequence was then inserted at the C-terminus of FrC to aid processing/presentation. In multiple models, this p.DOM epitope design (*Figure 1a*) was able to induce high levels of epitope-specific CD8+ T cells.[9]

Importantly, provision of high levels of T-cell help enables induction of immune responses in tolerant settings.[9,39]

The preclinical data appear to predict response in humans.[10] For patients with relapsed prostate cancer, a p.DOM epitope design incorporating a peptide sequence from the prostate-specific membrane antigen (PSMA; GTAC 089) has induced high levels of epitope-specific interferon gamma (IFN-γ), producing CD8+ T-cell responses in 67% (10/15) of patients.[40] Data from the 10 patients in the group receiving the lowest dose levels of DNA and DNA/electroporation are shown in *Figure 1b*. This was the first ever trial to exploit delivery of DNA by electroporation and it was found this approach was safe and readily accepted by patients.[10] Responses were robust and persistent over many months to the end of follow-up in the trial at 18 months (*Figure 1b*).

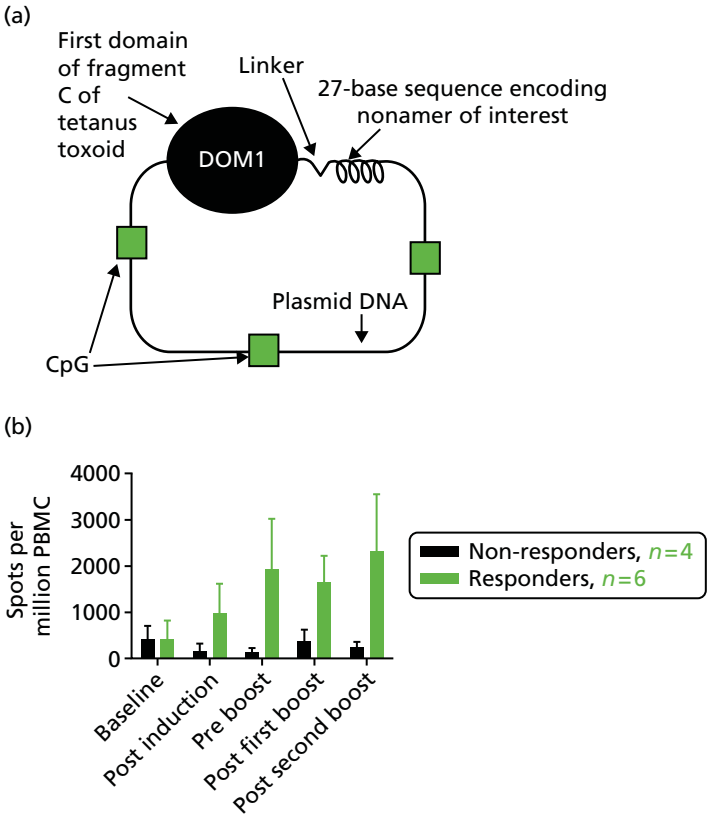

FIGURE 1 Vaccination of patients with the p.DOM epitope vaccine. (a) The p.DOM epitope vaccine consists of a DNA plasmid backbone incorporating cytosine–phosphate–guanine sites. The first domain of the tetanus toxin (DOM; TT865–1120) provides T-cell help when linked to a tumour-associated nucleotide sequence encoding the human leucocyte antigen class I binding epitope of interest. This format allows the appropriate processing and presentation of the peptide. (b) Spots per million peripheral blood mononuclear cell producing IFN-γ in human leucocyte antigen A2-positive patients treated with three monthly doses of DNA (p.DOM.PSMA27). The figure shows data from the first dose cohort, analysed in a cultured.

Figure 2 illustrates the CD8+ T-cell analyses in more detail. In *Figure 2a* and *2b* two non-responders are shown, one of whom (*Figure 2b*) had pre-existing levels of PSMA peptide 27-specific T cells at baseline. It is interesting to note that these cells appear to leave the circulation post vaccination and become visible again after the first booster injection at 6 months. Further data are required to allow interpretation of this observation. In *Figure 2c* and *2d*, two of the six responders at dose level 1 are shown. The patient in *Figure 2c* was treated with DNA alone followed by DNA delivered by electroporation, whereas the patient in *Figure 2d* was treated with DNA/electroporation on five occasions.

The effectiveness of the p.DOM epitope design for the treatment of myeloid malignancies has been explored based on these clinical results. *WT1* has emerged as one of the most promising targets for immunotherapy of haematological malignancies including CML, AML and myelodysplastic syndromes.[29,41–43] It is also a potential target for the treatment of solid tumours.[43–46] 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,[47–50] where expression is significantly lower (10- to 100-fold).[47] This could raise a concern about autoimmunity, but, reassuringly, the available data document selectivity of attack against tumour cells, sparing the CD34+ cells[51,52] and without any evidence of renal or other autoimmune toxicity in murine models[53–55] or patients.[29,41–43]

The WT1 peptide vaccines have been tested both in preclinical models[51,52,56] and in clinical trials.[12,41–43] The data from clinical trials 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

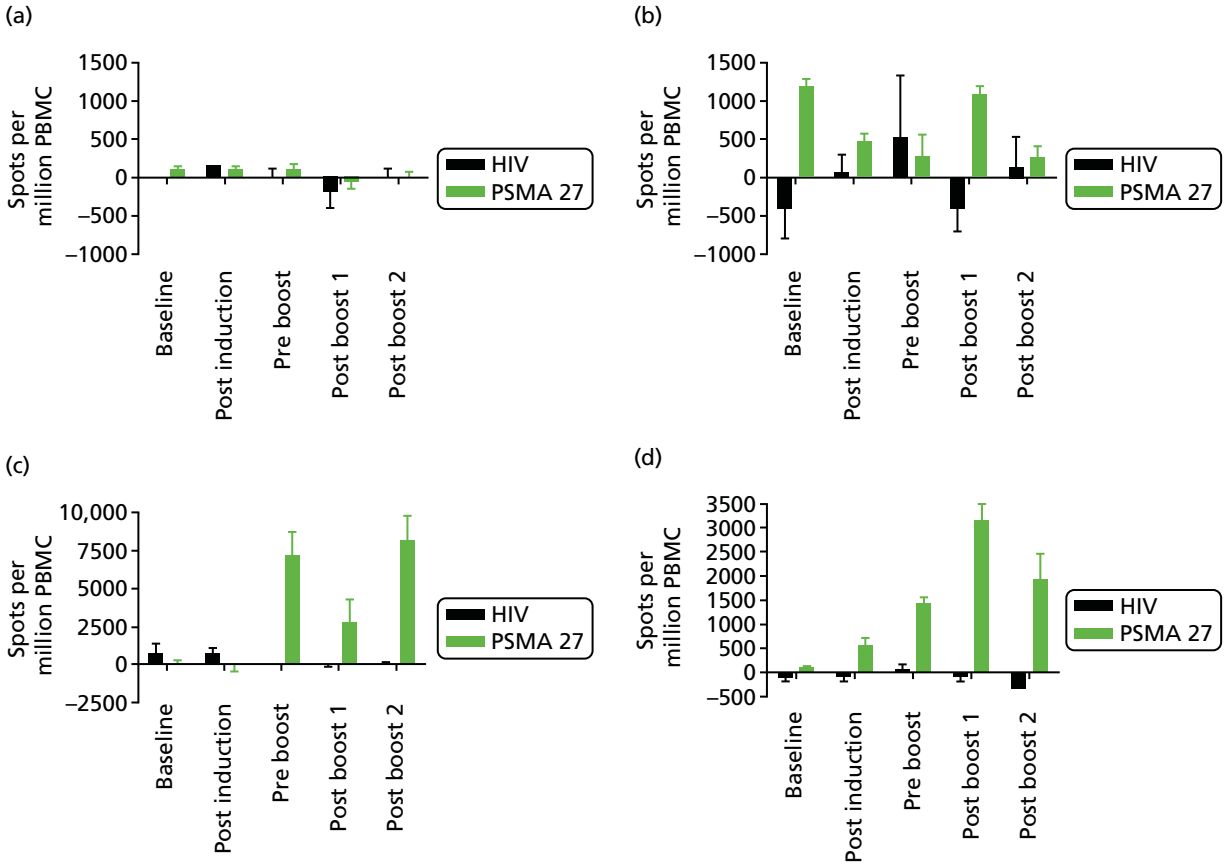

FIGURE 2 The CD8+ T-cell responses to DNA vaccination analysed over time by enzyme-linked immunospot. (a) and (b) show data on two out of four non-responders, of which the patient in (b) shows a low-level CD8+ T-cell response to the PSMA peptide 27 at baseline. As there is no significant increase in levels of PMBCs producing IFN-γ above the baseline, this patient has been classified as a non-responder; (c) and (d) show examples of patients that have significantly increased levels of PMBCs producing IFN-γ compared with baseline levels and to the human immunodeficiency virus-negative control. ($n = 6$ in the first dose cohort.) HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell.

inability of this approach to provide linked CD4+ T-cell help, which is crucial for the maintenance of tumour antigen-specific CD8+ T-cell populations. In the clinic, this is visible in poor persistence of the detected CD8+ T-cell responses. In contrast, it was that the p.DOM epitope fusion vaccines appeared to be able to deliver CD8+ T-cell responses, which show long-term persistence (see *Figures 1b* and *2c* and *2d*).

Recently, 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.[56] 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.[56] In parallel, we showed that low numbers of human WT1 peptide-specific T cells could be expanded in vitro to kill WT1+ HLA A2+ leukaemia cells. The WT1 peptide 37 (WT1-37) and WT1 peptide 126 (WT1-126) peptides were selected for current studies. We have already documented clinically the ability of p.DOM epitope vaccines to induce cytotoxic T lymphocytes (CTLs) and anticipate that dual attack against more than one epitope will provide added clinical benefit.[9,10] 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.[9,10,56]

Chapter 2 Aims

Recently, three DOM epitope vaccines were developed, each encoding a different, previously described, WT1-derived, HLA A2-restricted peptide.[56] All vaccines were able to induce CD8+ T-cell responses in 'humanised', and presumably tolerised, mice expressing HLA A2, and these T cells were capable of killing human 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.[56] In parallel, we showed that low numbers of human *WT1* peptide-specific T cells could be expanded in vitro to kill HLA A2+ WT1+ leukaemia cells.[56] Peptides WT1-37 (amino acid sequence: VLDFAPPGA) and WT1-126 (amino acid sequence: RMFPNAPYL) were selected for current studies.

The ability of p.DOM epitope vaccines to induce CTLs has been documented clinically and we 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 chosen as the vaccine delivery strategy.[10]

The aim of this 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 the:

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

Chapter 3 Trial design and 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 (HLA A2–) genotype were not vaccinated and formed the control group. Patients were tested for the presence of the human immunodeficiency virus (HIV), hepatitis B, hepatitis C and syphilis to protect the laboratory personnel, and because these infections may have a significant impact on the immunocompetence of the patient.

Significant change to trial design

The original trial design used Simon's optimal Phase II trial design.[57,58] This allowed us to undertake a 'start/stop' evaluation once 12 patients had been enrolled into each vaccination group of the study and had been evaluated to 6 months (molecular monitoring). An interim analysis of these CML patients' molecular data (*BCR–ABL* and *WT1*) was to be carried out including up to 6 months of data. If a molecular response (change in transcript level of *BCR–ABL* or *WT1*) was observed the CML arm may recruit an additional 25 participants. It was designed that the AML arm would also be opened to recruitment at this point.

It became evident early on in the trial that recruitment of the CML patients was significantly behind target. At the same time, new data emerged which illustrated that immune responses detected in the blood appear to evolve by 6 months post vaccination in most patients.[56] These data were not available when the WT1 Immunity via DNA (WIN) trial protocol was developed. Clinical responses (reduction in *BCR–ABL* transcript levels) are therefore also expected to happen late which would necessitate a halt in recruitment to 'observe' outcomes in the first stage prior to initiating the second stage.

This new information manifested to protocol amendment six. The following changes were included in this amendment to increase the available eligible CML patient population and help speed up recruitment:

- The AML arm was to be opened, independent of the CML result, as a result of the new data that had emerged illustrating that immune responses detected in the blood appear to evolve by 6 months in most patients.
- Patients on any TKI became eligible (rather than just those on imatinib).
- The trial design was changed from Simon's two-stage design[58] to A'Hern's single-stage design.[59]
- Amendment to the sample size for the trial to observe a minimum of 4 out of 32 CML patients who are molecular responders in order to provide evidence that the vaccine warrants further investigation. This is an increase proportionally from 10.8% (4/37) to 12.5% (4/32) and allowed all patients to receive all 12 vaccinations rather than only receive an additional six based on response being observed.

Early closure of the trial

The CML arm of the trial was terminated early by the funders because of poor recruitment. This meant that recruitment of the AML arm could not be implemented until an assessment was done to secure funding to continue with this arm of the trial. The outcome of this assessment was to close the trial. The Research Ethics Committee and the Medicines and Healthcare products Regulatory Agency (MHRA) were notified on the 3 April 2014.

The study met its primary decision-making target with one major molecular response in *BCR–ABL* transcript levels and, in parallel, new data emerged which illustrated that immune responses detected in the blood appear to evolve by 6 months post vaccination in most patients.[56] The early onset of the major molecular response suggests that vaccination has achieved this molecular event. In parallel, a *WT1* molecular response was observed.

A significant amount of time and effort was utilised in an attempt to overcome multiple hurdles that prevented successful recruitment, but despite this the study did not complete recruitment. The failure to achieve the recruitment target was exclusively driven by the lack of recruitment of CML patients.

Some of the major hurdles which contributed to the failure of the studies are as follows:

- The agreement and enthusiasm of the principal investigator (PI) for the centre at Hammersmith to participate in the study was not matched by a main member of the team to recruit to the study, leading to a lack of recruitment from the centre where the largest number of CML patients was expected.
- At the feasibility stage, the expert haemato-oncologist (PI) at Hammersmith assessed the cohort size of eligible patients to be 500 patients with CML who would fulfil the entry criteria for the study. This meant that 70% of the recruitment would come from the cohort size at this centre. Unfortunately, neither the cohort size nor recruitment materialised and this was key to the success of the study.
- Apparent lack of clinical efficacy by the vaccination and, therefore, less interest in the study.
- The unexpected loss of the PI to a US centre, leaving the study unsupervised in the key centre.

The following key reasons contributed to the failure to achieve the recruitment target:

- Extensive attempts were made by the chief investigator via the National Institute for Health Research (NIHR) clinical studies group and by visiting national key centres, which mange CML, to recruit new centres to the study. Although there was significant academic interest for this study, it competed directly with an ongoing study in CML, precluding their participation.
- As highlighted above, the NIHR Efficacy and Mechanism Evaluation (EME) programme did not support recruitment of AML patients. Therefore, recruitment of this study arm was stopped and no patients were vaccinated; it was deemed unethical to recruit one or two patients to the AML arm and then stop the trial.

This was 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.

Ethical approval and research governance

Ethical approval for the trial was given by the GTAC, National Research Ethics Service National Patient Safety Agency (24 September 2010, reference number GTAC 173). The trial was registered with the International Standard Randomised Controlled Trial Register (ISRCTN) under the reference number ISRCTN 62678383.

Changes to original protocol

A summary of the changes to the original protocol is given in *Table 1*.

Trial setting and sample

Three hospitals (Southampton General Hospital, University Hospital Southampton NHS Foundation Trust; Hammersmith Hospital, Imperial College Healthcare NHS Trust; and Royal Devon and Exeter Hospital, The Royal Devon and Exeter NHS Foundation Trust) were selected to undertake the trial based on their interest, clinical experience and the number of patients with AML and CML patients who may be suitable to participate in the trial. It was anticipated that the Hammersmith Hospital would contribute the majority

TABLE 1 Summary of changes to the original protocol

Change in protocol	REC/MHRA approvals
Amendment 1	
Protocol version 1 (20 June 2010): MHRA non-acceptance	28 September 2010
Protocol version 1 (7 July 2010): conditional approval only	
Protocol version 2 (9 September 2010): conditions addressed and protocol approved	
Addition of electrocardiography at baseline; logistical changes made for supply and return of IMP; and clarification on monitoring	
Amendment 2	
Protocol version 3 (7 October 2010): used to open sites	1 November 2010
Update in the IMPD included in the protocol	
Amendment 3	
Protocol version 4 (29 June 2011): clarifications	12 August 2011
Clarification of patient pathway throughout the trial from consent; clarification on the schedule of observations and procedure for HLA A2– participants; clarification on inclusion criteria for AML patients with regards to *WT1* status; clarification determination of bone status for trial inclusion; clarification on resupply of IMP to sites; clarification of local and central laboratory responsibilities and shipment of samples; amendment of pain assessment case report form to remove patient identifiers; and addition of delayed-type hypersensitivity reaction to be carried out wherever feasible	
Amendment 4	
Protocol version 5 (18 October 2011): eligibility broadened	16 January 2012
Eligibility criteria amended to allow patients with a 6-month history of lymphocyte counts of just below 1 to be included in the trial	
Amendment 5	
Protocol version 5 (18 October 2011): changes to the IMPD; no changes to the protocol	17 May 2012
Stability data for p.DOM–WT1 DNA vaccines to support the proposed expiry date extension plan at the predetermined 18-month time point	
Amendment 6	
Protocol version 6 (31 July 2012): eligibility broadened and trial design changed	8 November 2012
Eligibility criteria widened to include all TKIs to increase recruitment; change in trial design from a two-stage design to a single-stage design; sample size adjusted to 32 CML patients and 37 AML patients; all HLA A2+ patients to receive all 12 vaccinations instead of receiving the second six only if a response is observed	
Amendment 8	
Protocol version 6 (31 July 2012): no change to the protocol; temporary halt to the trial	11 April 2013
CML arm of the trial stopped and temporary halt on trial for AML arm	
Amendment 10	
Protocol version 7 (1 October 2013): reduction in follow-up visits	7 November 2013
Patients last follow-up 12 months post final vaccination. The enzyme-linked immunospot (ELISPOT) assay removed from end-point analysis (replaced by tetramer staining). The 36-month follow-up visit removed	
End of trial notification	3 April 2014

IMP, investigational medicinal product; IMPD, investigational medicinal product dossier; REC, Research Ethics Committee.

of the CML patients based on the cohort of about 500 patients treated with imatinib with stable disease. Smaller numbers were expected from Exeter and Southampton.

Inclusion criteria

Chronic lymphocytic leukaemia patients

Those CML patients:

- who have Philadelphia chromosome-positive CML in chronic phase
- who are in CCyR but with detectable *BCR–ABL* transcripts and maintain the CCyR on TKI monotherapy for a minimum of 24 months.

Acute myeloid leukaemia patients

Those AML patients who have either:

- WT1-positive 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).

All patients

- Aged ≥ 18 years.
- Written informed consent.
- World Health Organization (WHO) performance status of 0 or 1.
- For vaccination groups: HLA A2+ in at least one allele.
- For control groups: HLA A2– in both alleles.
- Renal function and liver function (creatinine < 1.5 × upper limit of normal, liver function tests < 1.5 × upper limit of normal); lymphocyte count ≥ 1.0×10^9/l (if the lymphocyte count was < 1.0×10^9/l at the time of entry into the trial but had been > 1.0×10^9/l in the last 6 months and had also not declined rapidly in the days and weeks preceding entry, then the patient was eligible); and normal clotting.
- Haemoglobin level of > 100 g/l.
- Adequate venous access for repeated blood sampling according to the protocol schedule.
- If sexually active and possibly fertile, patients must have agreed to use appropriate contraceptive methods during the trial and for 6 months afterwards.

Eligibility criteria were widened to include all TKIs (previously imatinib only) to increase recruitment (amendment 6, 8 November 2012; see *Table 1*).

Exclusion criteria

Chronic lymphocytic leukaemia patients

- Chronic lymphocytic leukaemia in accelerated phase or blast crisis or having achieved complete molecular response (CMR) at any point during TKI therapy.
- A TKI change or dose modification in the previous year, therapy interruption for > 15 days in the 6 months prior to enrolment.
- Prior interferon alpha therapy.
- Hypocellular bone marrow (< 20%; indicated by blood counts and most recent bone marrow, where available).
- A CMR.

Acute myeloid leukaemia patients

- Acute myeloid leukaemia in haematological relapse or eligible for alloSCT.
- Hypocellular bone marrow (< 20%).
- Acute myeloid leukaemia patients with the 'good-risk' abnormalities comprising by the core-binding factor leukaemias [i.e. AML with the translocation (8;21) and inversion of chromosome 16, and acute promyelocytic leukaemia with the translocation (15;17)].

All patients

- Systemic steroids or other drugs with a likely effect on immune competence were forbidden during the trial. The predictable need of their use precluded the patient from trial entry. Inhaled steroids were allowed.
- Major surgery in the preceding 3–4 weeks from which the patient had not yet recovered.
- Patients who were of high medical risk because of non-malignant systemic disease, as well as those with active uncontrolled infection.
- Patients with any other condition which, in the investigator's opinion, would not make the patient a good candidate for the clinical trial, such as concurrent congestive heart failure or prior history of New York Heart Association class III or IV cardiac disease.
- Current malignancies at other sites, with the exception of adequately treated basal or squamous cell carcinoma of the skin. Cancer survivors, who had undergone potentially curative therapy for a prior malignancy, had no evidence of that disease for 5 years and were deemed at low risk of recurrence, were eligible for the trial.
- Patients who are serologically positive for, or are known to suffer from, hepatitis B or C, syphilis or HIV. Counselling was offered to all patients prior to testing.

Trial interventions

The trial was an open-label trial with two groups for both the CML and AML arms:

- intervention group: all eligible and consenting patients who were HLA A2+
- control group: all eligible and consenting patients who were HLA A2–.

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

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

The original protocol planned that patients would be given vaccines six times, at 4-weekly intervals, and only if a response was observed would they go on to receive the remaining six vaccinations at 3-monthly intervals up to a maximum of 24 months. New data emerged which illustrated that immune responses detected in the blood appear to evolve by 6 months in most patients;[58] therefore, the protocol was amended to allow all HLA A2+ patients to receive all 12 vaccinations instead of receiving the second six only if a response was observed.

The vaccine was manufactured at the MHRA-approved Clinical Biotechnology Centre at the Bristol Institute for Transfusion Science (Bristol, UK) in accordance with good manufacturing practice.

The amount of DNA used was 1 mg/dose for p.DOM–WT1-37 and 1 mg/dose for p.DOM–WT1-126 (at a final concentration of 1 mg/0.8 ml). The vaccine was supplied in standard phosphate-buffered saline. The DNA for injection was divided into aliquots for storage at –70 °C in sterile glass vials, and aliquots for

sterility and stability testing. The testing was based on the guidelines for injectables described in the European Pharmacopoeia (http://edpm.eu). The most likely contaminant is protein, which was expected to be < 1%. The material was confirmed as pyrogen free by using a *Limulus* test (BioWhittaker UK Ltd, Wokingham, UK). After delivery to the hospital pharmacy, the vaccine was stored at –70 °C.

The vaccine was thawed for approximately 5 minutes so that it was at room temperature before administration. The vaccines were injected by deep intramuscular injection into separate sites followed by electroporation.

The electroporation device (Elgen1000, Inovio Biomedical corporation, San Diego, CA, USA) was a system specifically designed for the delivery of electrical pulses to selected tissues, including muscle, to facilitate the intracellular uptake of plasmid DNA. The device locally applies controlled, short-duration electric pulses to target tissues to create an electric field that temporarily increases cellular membrane permeability allowing the plasmid DNA to enter the cells.

Operators underwent formal training before being considered competent in the use of the device. Training was provided by Inovio Pharmaceuticals, Inc. (San Diego, CA, USA).

Vaccination schedule

The DNA vaccine was administered 12 times. Patients received the vaccine at 4-weekly intervals into separate sites for the first 6 months, followed by vaccinations every 3 months up to a maximum of 24 months. Vaccines were injected intramuscularly and followed by intramuscular electroporation. Pain assessments were conducted immediately after vaccination and at 48 hours post vaccination.

The first HLA A2+ patient recruited at each site was evaluated 48 hours after administration of the first vaccination, before additional doses were given or before additional patients were vaccinated at that site.

Trial procedures

Recruitment and informed consent

The PIs identified potential eligible patients from their existing patient population either during routine consultation or from a database search. Patients identified from their database were approached for the trial at their next clinic appointment. 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.

Registration

After written informed consent was obtained from the patient and before screening commenced, sites registered the patient with the Southampton Clinical Trials Unit (SCTU) to obtain the unique patient identification number. The patient's eligibility was checked during the registration process to ensure that only patients fulfilling the eligibility criteria were registered. Subsequently, the patient identification number was assigned.

Data collection and management

Sites entered trial-specific data, as specified in the protocol, onto paper case report forms (pCRFs). Completed pCRFs were sent to the SCTU, which was responsible for the data management of the trial. Data were transcribed from pCRFs into an InForm database (InForm version 5.0, ORACLE) at the SCTU. A range of data validation checks were carried out within both InForm and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) or above to minimise incorrect or missing data.

Molecular samples were sent to Haematology department at Hammersmith Hospital (a clinical pathology-accredited MRD laboratory) for molecular monitoring (qPCR for *BCR–ABL*/*WT1* in CML and *WT1* in AML). This is an accepted and routine test for monitoring of this disease. Results from the analysed samples were regularly sent back to SCTU for statistical analysis.

Immunological analyses for vaccine responses, including leukapheresis samples and bone marrow samples, were processed and frozen locally according to an agreed standard operating procedure and stored in liquid nitrogen. Samples were transported to the Cancer Sciences Division (Southampton General Hospital, Southampton, UK) in dry ice and using a temperature logger once a sufficient number or samples had been collected locally (Hammersmith Hospital, Exeter, UK).

Source data verification was undertaken during site monitoring visits, in accordance with the SCTU's trial monitoring plan. Sites were visited at least once during the trial. At least one monitoring visit was undertaken for each participating site. A total of three planned monitoring visits and one triggered monitoring visit were carried out.

Baseline

The baseline investigations/evaluations that were performed on patients before vaccination included bone marrow aspiration, electrocardiogram (ECG), chest X-ray, HLA A2 status verification, vital signs, WHO performance status, urinalysis, full blood counts (FBCs), blood clotting test, biochemistry (levels of sodium, potassium, calcium, phosphorus, urea and creatinine; total protein, albumin, bilirubin, alkaline phosphate, alanine transaminase, aspartate transaminase mad gamma-glutamyl transferase levels), the creatine kinase (CK) test (for HLA A2+ patients), tests for the presence of syphilis, hepatitis B, hepatitis C and HIV, qPCR (for *BCR–ABL* and *WT1* in CML patients and for *WT1* in AML patients), leukapheresis for immunological studies (HLA A2+ patients only), autoimmune profiling and checks for concomitant diseases/treatments.

Follow-up

Patients were followed up as outpatients from the start of treatment at the time points outlined below. Each visit was done from baseline ± 14 days.

- Visit 1: week 0, within 7 days of baseline.
- Visit 2: week 2.
- Visit 3: week 4.
- Visit 4: week 8.
- Visit 5: week 10.
- Visit 6: week 12.
- Visit 7: week 16.
- Visit 8: week 20.
- Visit 9: week 22.
- Visit 10: week 24.
- Visit 11: week 32.
- Visit 12: week 34.
- From visit 13 onwards, patients were seen at the following time points from baseline ± 14 days: months 11, 14, 17, 17 + 14 days, 20, 23, 24, 27, 30, 33 and 36.

Patients were observed in hospital for 2 hours post vaccination for adverse events (AEs). It was planned that if no AEs were observed after 12 patients completed six doses of vaccination, the 2-hour hospital stay may be discontinued; however, because of the early termination of the study, this was not implemented.

Time points were adjusted if visits were delayed to keep visits as close as possible to the 14-day post-vaccination visits. Many of the baseline investigations were repeated throughout the follow-up period at varying time points. The repeated tests included FBCs and differential blood counts; biochemistry including the CK test and urinalysis; immunological monitoring [65 ml of anticoagulated blood (in lithium heparin tubes) and 5 ml of clotted blood for serum would be taken for immunological monitoring]; ECG; echocardiogram (ECHO), if clinically indicated; bone marrow for immunological (CML and AML) and disease (AML) evaluation; leukapheresis; and molecular analysis of *BCR–ABL* and *WT1* transcripts in CML patients and *WT1* transcripts in AML patients (20 ml of anticoagulated blood was taken for qPCR).

All patients were asked to consent to information about their health status being held and maintained by the Health and Social Care Information Centre and the NHS Central Register, to enable long-term follow-up.

Outcome measures

Molecular response

Definition of the *BCR–ABL* response

For patients with a baseline *BCR–ABL* transcript level of > 11 transcripts/mg of ribonucleic acid (RNA):

- Complete molecular response: a *BCR–ABL* transcript level of 0 transcripts/mg of RNA with an Abelson murine leukaemia viral oncogene homologue 1 (*ABL*) control level of ≥ 32,000 transcripts/mg of RNA in two consecutive tests. These patients cannot be assessed for a major or minor response as defined below.

For patients with a baseline *BCR–ABL* transcript level of ≥ 11 transcripts/mg of RNA:

- Complete molecular response: a *BCR–ABL* transcript level of 0 transcripts/mg of RNA, with an *ABL* control level of ≥ 32,000 transcripts/mg of RNA in two consecutive tests.
- Major response: a fall of > 1-log in the breakpoint cluster region (*BCR*) to *ABL* transcript level ratio. Confirmed in an *ABL* control level of ≥ 32,000 transcripts/mg of RNA in two consecutive samples at any time during follow-up.
- Minor response: a fall of > 0.5-log in the *BCR* to *ABL* transcript level ratio. Confirmed in an *ABL* control copy transcript level of ≥ 32,000 transcripts/mg of RNA in two consecutive samples at any time during follow-up.

Definition of *WT1* response

For patients with a baseline *WT1* to β-glucuronidase (*GUS*) transcript level ratio of < 0.1%:

- Complete molecular response: 0% *WT1* to *GUS* transcript level ratio, with a *GUS* control level of ≥ 32,000 transcripts/mg of RNA; in two consecutive tests these patients cannot be assessed for a major or minor response as defined below.

For patients with a baseline *WT1* to *GUS* transcript level ratio of ≥ 0.1%:

- Complete molecular response: 0% *WT1* to *GUS* transcript level ratio, with a *GUS* control level ≥ 32,000 transcripts/mg of RNA; in two consecutive tests.
- Major response: a fall of > 1-log in the *WT1* to *GUS* transcript level ratio. Confirmed in a *GUS* control level of ≥ 32,000 transcripts/mg of RNA in two consecutive samples at any time during follow-up.
- Minor response: a fall of > 0.5-log in the *WT1* to *GUS* transcript level ratio. Confirmed in a GUS control level of ≥ 32,000 transcripts/mg of RNA in two consecutive samples at any time during follow-up.

Immunological response

Definition of validated assay by tetramer

A positive response using the validated assays by tetramer will be determined by the following criteria:

- tetramer staining at the post-vaccination time point is over the cut-off value for the specific tetramer when the pre-vaccination baseline time point (visit number 00 or 01) is below this cut-off value or
- tetramer staining at the post-vaccination time point is more than twofold above the baseline time point when the baseline time point measures over the cut-off value for the specific tetramer
- tetramer staining is reviewed and confirmed by at least two independent, flow cytometric-experienced scientists.

Immunological responses may also be assessed by other research assays such as intracellular cytokine staining and enzyme-linked immunospot. Only results produced by validated end-point assays will be reported on.

All patients who are removed from the study for reasons other than progressive disease will be re-evaluated at the time of treatment discontinuation.

Primary outcome

Chronic myeloid leukaemia

The primary outcome for the CML treatment group was a molecular *BCR–ABL* response (major or minor response or CMR), as defined in *Definition of* BCR–ABL *response*.

Acute myeloid leukaemia

The primary outcome for the AML treatment group was time to disease progression. Disease progression in AML is defined as disease relapse.

Secondary outcomes

Chronic myeloid leukaemia

The secondary outcomes for the CML treatment group are as follows:.

- A *BCR–ABL* molecular response (major response or CMR), as defined in *Definition of BCR–ABL response*.
- A *WT1* molecular response of (major or minor response or CMR), as defined in *Definition of* WT1 *response.*
- Time to disease progression. Disease progression for CML patients is defined as a loss in complete haematological response, where at least one factor falls out of the following ranges:[60]
    - a white blood cell count of $< 10 \times 10^9$/l
    - basophils levels of $< 5\%$
    - no myelocytes, promyelocytes or myeloblasts in the differential blood count
    - a platelet count of $< 450 \times 10^9$/l
    - a non-palpable spleen.
- Time to next treatment. A next treatment is defined as the first drug taken during the course of the study with an indication to treat CML.
- Time to molecular response (for both *BCR–ABL* and *WT1*):
    - measured from the beginning of TKI treatment
    - measured from the time of obtaining informed consent.

- Overall survival.
- Toxicity assessed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.[1]
- Pain assessment immediately after vaccination (see *Appendix 1*).
- Pain assessment at 48 hours post vaccination (see *Appendix 2*).

Acute myeloid leukaemia

The secondary outcomes for the AML treatment group are as follows:

- 2-year overall survival.
- 2-year progression-free survival (PFS).
- Overall survival.
- A *WT1* molecular response (major or minor response or CMR), as defined in *Definition of* WT1 *response*.
- Toxicity assessed according to the National Cancer Institute's CTCAE, version 4.0.
- Pain assessment immediately after vaccination.
- Pain assessment at 48 hours post vaccination.

Human leucocyte antigen A2-positive patients only (chronic myeloid leukaemia and acute myeloid leukaemia)

The secondary outcomes for only the HLA A2+ patients are as follows:

- Immune response to WT1 epitope-specific T cells in blood and/or bone marrow, using validated assays by tetramer staining. A positive response using the validated assays by tetramer staining is defined in *Definition of validated assay by tetramer*.
- Number of WT1-specific T-cells after peptide challenge to the skin (wherever assessment is feasible).
- Immune response to FrC, evaluated using validated assays by enzyme-linked immunosorbent assay (ELISA).
- Number of humoral responses (B lymphocytes) to the vaccine components evaluated using ELISA.

Sample size

Original sample size

For sample size calculation, we used Simon's optimal Phase-II trial design for clinical development of therapeutic cancer vaccines.[60,61] This would allow us to undertake a 'start/stop' evaluation once 12 patients had been enrolled into each vaccination group of the study and had been evaluated to 6 months (molecular monitoring).

If one or more responders were observed in the 12 initial patients, this particular vaccination group would be extended to 37 patients. This would allow the study to distinguish between $p_0 = 5\%$ (standard response) and $p_1 = 20\%$ (expected response) with $\alpha = 0.1\%$ (two-sided) and $\beta = 0.10$, where p_0 is the probability of a clinically uninteresting true response rate and p_1 is the probability of a sufficiently promising true response rate. This gives a less than 10% chance of rejecting a useful vaccine, with error probability limits of α 0.10 and $\beta < 0.10$, even if the true response rate were to be $p_1 = 20\%$.

If no molecular responses were seen in the first 12 CML or AML patients, recruitment would have ceased for this patient group, as there will not be sufficient clinical interest to pursue the trial further. This optimal design had an expected sample size under H_0 (the null hypothesis) of 24 and a maximum sample size of 37 in each patient group (AML and CML).

Revised sample size

The sample size was revised in line with the change in trial design from Simon's two-stage[60] to A'Hern's one-stage trial design.[59] This was approved in version 6 of the protocol on 8 November 2012 (see *Table 1*).

Chronic myeloid leukaemia

The sample size for the HLA A2+ group had been determined using A'Hern's single-stage trial design[59] and the primary outcome measure of molecular response (*BCR–ABL* or *WT1* transcript level) for CML patients.

A molecular response (*BCR–ABL* or *WT1* transcript level) rate of 20% would imply that the vaccine clearly warrants further investigation. A molecular response rate of $\leq 5\%$ would be unacceptable and would indicate that the vaccine does not warrant further investigation. The probability of obtaining a false-positive result, α (i.e. incorrectly accepting for further trial a treatment that has a true response rate of 5%), was set at 10%. The probability of a false-negative result, β (i.e. incorrectly rejecting for further trial a treatment with a true response rate of 20%), was set at 10%. Using these parameters ($\alpha = 0.1$, $\beta = 0.1$, $p_0 = 5\%$, $p_1 = 20\%$), 32 HLA A2+ patients needed to be recruited to the trial. The drop-out rate was expected to be $< 10\%$ and hence a total of 36 patients would be recruited. The control arm consisted of all eligible and consenting patients who were HLA A2–.

This trial needed to observe a minimum of four molecular responders out of 32 patients in order to provide evidence that the vaccine warrants further investigation.

Acute myeloid leukaemia

The sample size calculation for the AML treatment group was based on A'Hern's single-stage design[59] and the primary outcome measure of PFS at 2 years post vaccination.

A PFS rate of 50% at 2 years post vaccination would indicate that the vaccine warrants further investigation. A PFS rate of $\geq 30\%$ at 2 years post vaccination would be unacceptable and would indicate the vaccine does not warrant further investigation. The probability of obtaining a false-positive result, α (i.e. incorrectly accepting for further trial a treatment that has a true PFS rate of 30%), was set at 10%. The probability of a false-negative result, β (i.e. incorrectly rejecting for further trial a treatment with a true PFS rate of 50%) was set at 10%. Using these parameters, $\alpha = 0.1$, $\beta = 0.1$, $p_0 = 30\%$, $p_1 = 50\%$, 39 HLA A2+ patients needed to be recruited to the trial. The dropout rate was expected to be $> 10\%$. The control arm consisted of all eligible and consenting patients who were HLA A2–.

This trial needed to observe a minimum of 16 out of 39 patients who are progression free and alive at 2 years in order to provide evidence that the vaccine warrants further investigation.

Interim analyses and stopping guidelines

Following the original trial design of Simon's optimal phase II trial design,[60] there was a 'start/stop' evaluation once 12 patients had been enrolled into the trial (*Figure 3*). The molecular data for these patients will be evaluated (molecular monitoring). If no molecular responses are seen in the first 12 vaccinated patients with CML, recruitment will cease for CML and will not begin for AML, as there will not be sufficient clinical interest to pursue further. After the change in trial design to A'Hern's one-stage design,[59] this stopping evaluation was no longer in required.

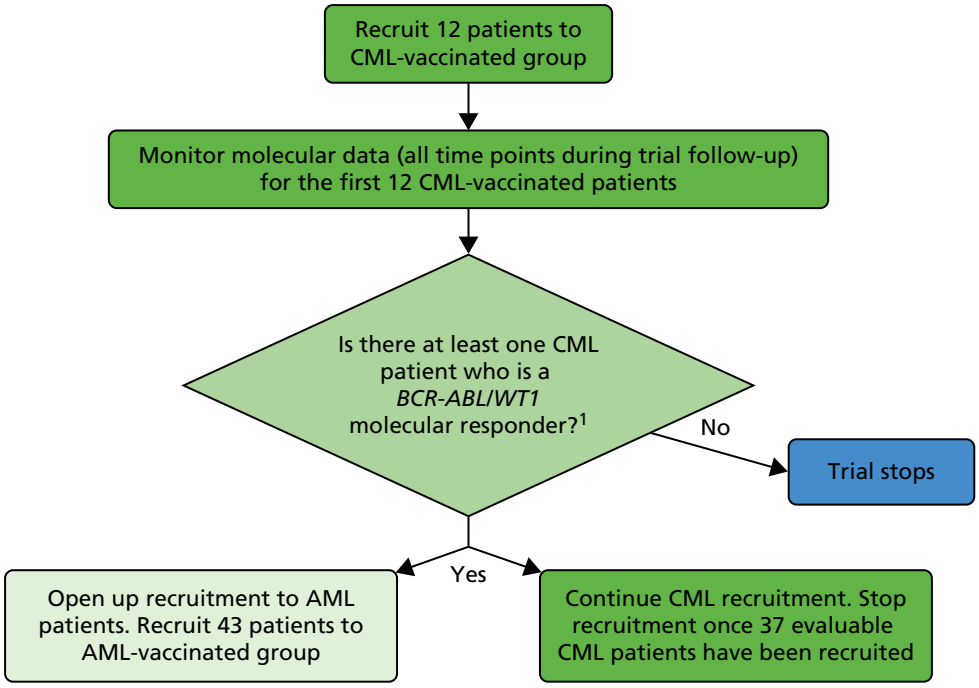

FIGURE 3 Flow diagram showing the original two-stage design for the CML patient group.

A Data Monitoring and Ethics Committee (DMEC) was convened on behalf of, and with input from, the Trial Management Group. The committee's remit was to meet at regular intervals to review the safety listings and recruitment and to make a recommendation on whether or not to continue beyond 12 patients in the CML and AML cohorts. Unplanned DMEC meetings were to be called if required during the study.

In addition, the DMEC would review any cases of CTCAE grade 3 adverse reactions, possibly or likely to be related to vaccination to assess if there were sufficient reason to suspend or terminate the study.

Statistical analysis

All trial analyses and reporting were carried out following the Consolidated Standards of Reporting Trials (CONSORT) guidelines. Statistical analysis was carried out in SAS, version 9.3, following a predefined statistical analysis plan.

Analysis populations

Molecular analysis

The analyses of the molecular responses were performed on all patients with molecular data at a minimum of two post-baseline time points (HLA A2+ patients must also have received at least one dose of the vaccine).

Immunological analysis

To be evaluable for an immunological response, HLA A2+ patients must have received at least one dose of the vaccine and the immunological testing must have been available until at least week 8 post first dose.

Safety analysis

For safety analyses, all HLA A2+ patients who received at least one trial drug administration were evaluable for toxicity. All controls were included in the safety analyses, where relevant.

Other analysis (intention-to-treat population)

For all other analyses, an intention-to-treat (ITT) principle (i.e. the planned treatment regimen) was used, which included all registered patients who obtained HLA A2+ status.

Preliminary analyses

Disposition of patients and follow-up information on the ITT population were analysed separately for HLA A2+ and HLA A2– patients (see *Figure 4* and *Table 2*). Major protocol deviations were also listed for the ITT population (see *Box 1*).

Patient characteristics recorded at baseline, including the patient's age, gender, ECHO result and WHO performance status, were summarised by HLA A2 status for the ITT population (see *Table 3*). In addition, baseline *BCR–ABL* and *WT1* molecular data were analysed separately for the molecular analysis population and summarised by HLA A2 status (see *Tables 4* and *5*).

Dosing information on pre-trial imatinib treatment was summarised by HLA A2 status for the ITT population (see *Table 6*).

Treatment analyses

Vaccination administration and electroporation failure summarises were presented for the ITT population (HLA A2+ patients only), together with details of any reasons for premature withdrawals from treatment (see *Tables 7–9*).

Safety analyses

Serious adverse events (SAEs), including a SAE summary and listing of SAEs by system organ class and CTCAE term, were presented by HLA A2 status for the safety analysis population (*Tables 10* and *11*). In addition, a complete SAE listing was provided for the safety analysis population (see *Table 12*).

Information on all AEs was also presented by HLA A2 status for the safety analysis population, which comprised a summary by worst CTCAE grade recorded across the following type of AEs:

- all AEs (see *Table 13*)
- cardio-related AEs (see *Table 14*)
- renal-related AEs (see *Table 15*)
- bone marrow-related AEs (see *Table 16*)
- other-related AEs (see *Table 17*).

Fisher's exact test was used to compare differences in rates of toxicity in CML patients with controls for the safety population. No adjustment was made for multiple testing and, therefore, borderline significance should not be overinterpreted.

A list of all AEs was also presented for the safety analysis population (see *Table 18*). In addition, any concomitant medications taken because of pain within 48 hours following vaccination were summarised for HLA A2+ patients only in the safety analysis population (see *Table 19*).

Primary analyses

The primary outcome is a *BCR–ABL* molecular response (major or minor response or CMR), which was summarised for the molecular analysis population by HLA A2 status (see *Table 20*).

Secondary analyses

Secondary outcomes carried out on the molecular analysis population included:

- *BCR–ABL* (major response or CMR), summarised by HLA A2 status (see *Table 21*)
- *WT1* (major or minor response or CMR), summarised by HLA A2 status (see *Table 22*).

In addition, the following time-to-event secondary outcomes were also analysed by HLA A2 status for the ITT population:

- time to last follow-up (see *Table 23* and *Figure 5*)
- time to progression or death (see *Table 24* and *Figure 6*)
- time to death (see *Table 25* and *Figure 7*)
- time to next treatment (see *Table 26* and *Figure 8*).

In each case, the median time to event was presented along with time to event listings, a log-rank test, results from an unadjusted Cox proportional hazards model with alpha set at the 10% two-sided level and a Kaplan–Meier plot.

Time to a *BCR–ABL* response (major or minor or CMR) and time to a *WT1* response (major or minor or CMR) from the beginning of inhibitor treatment and also from the date of informed consent were also analysed using a similar approach for the molecular analysis population, together with the duration of *BCR–ABL* and *WT1* responses (see *Table 27* and *Figure 9*, and *Table 28* and *Figure 10*, respectively).

Pain assessment information recorded immediately after vaccination and recorded 48 hours post vaccination were summarised in terms of the median pain score recorded and the worst pain score recorded for HLA A2+ patients in the ITT population (see *Tables 29* and *30*, respectively).

Immunological analyses

The immunological analyses carried out on vaccinated HLA A2+ patients included:

- anti-FrC antibody in serum, as evaluated by a validated ELISA
- WT1-specific T-cell responses in blood and bone marrow, as evaluated by a validated WT1 tetramer assay.

The ELISA acceptance criteria included a post-vaccination time point level > twofold increase, as well as significantly greater levels of anti-FrC antibody compared with pre-vaccination baseline ($p < 0.05$, Dunnett's multiple comparisons test).

The WT1 tetramer acceptance criteria included a post-vaccination time point level > twofold over baseline and confirmed by three independent flow cytometric experts.

Chapter 4 Trial results

The intention to recruit 39 AML patients was not realised because of the early termination of the trial. The information included in this section only relates to patients in the CML treatment group.

Recruitment

Below is a summary of the recruitment milestones in the WIN trial:

- Southampton (first site) opened: 1 February 2011.
- Hammersmith opened: 9 May 2011.
- Exeter opened: 21 November 2011.
- First patient registered: 1 June 2011.
- Chronic myeloid leukaemia arm closed: 26 February 3013.
- Last patient, last visit: 31 July 2014.
- Trial termination: 25 March 2014.

Flow of participants through the trial

A total of 91 CML patients were assessed for eligibility for entry into the WIN trial and 23 CML patients were registered. Of these, 13 patients were HLA A2+ and 12 out of 13 began vaccination with a control group of nine HLA A2– patients.

Twelve HLA A2+ patients were vaccinated and 12 out of 12 as well as nine out of nine control patients were included in both the safety and molecular analyses. Four vaccinated and two control patients completed the study protocol treatment. The other patients either withdrew consent ($n = 11$) or underwent modification of their TKI treatment, thus requiring study termination.

Only one AML patient was assessed for eligibility for entry into the WIN trial, but decided not to enter into the trial.

Figure 4 shows the CONSORT diagram for the CML group only.

Major protocol deviations

There were no major protocol deviations. *Box 1* summarises the protocol deviations that occurred during the trial.

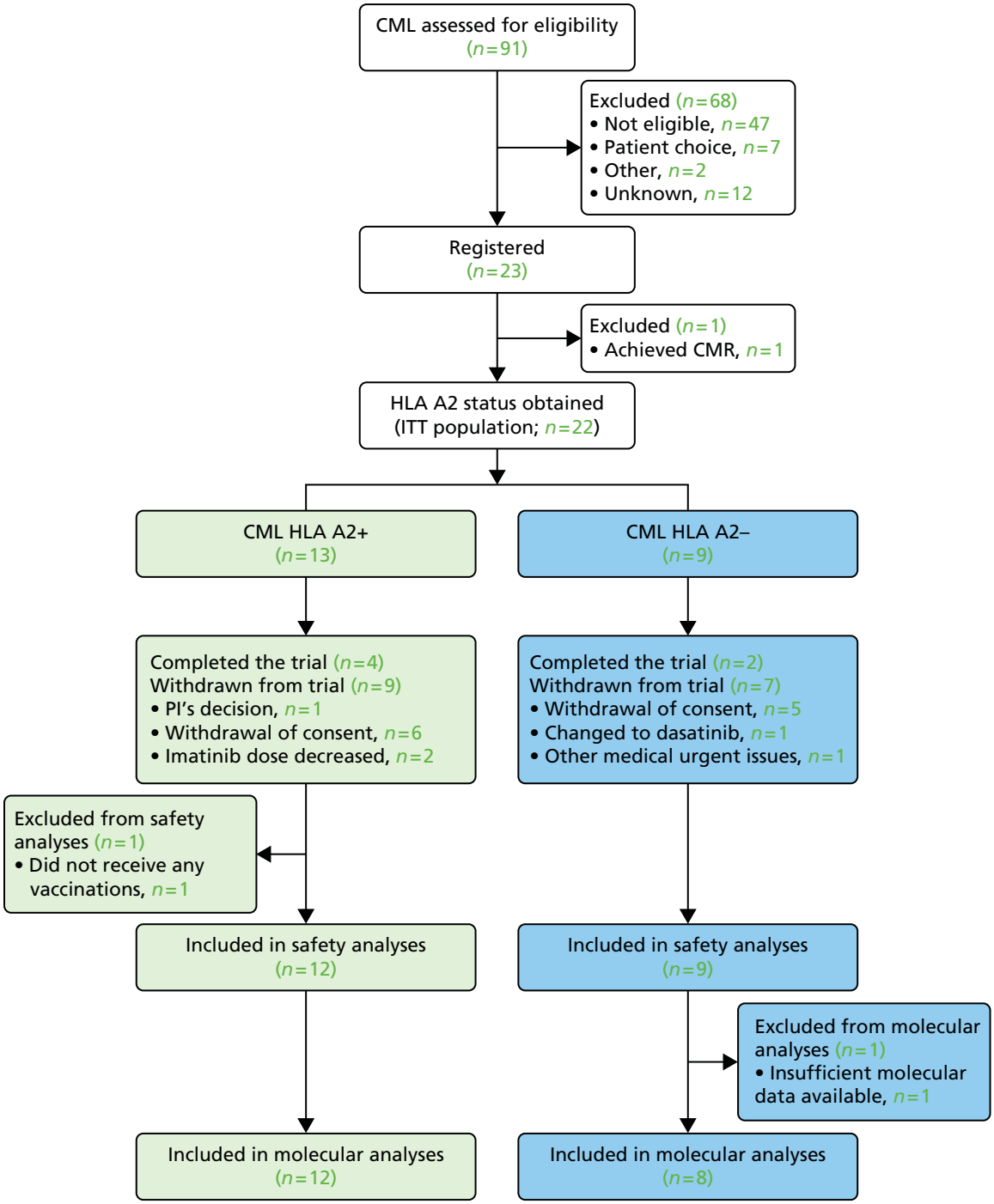

FIGURE 4 Consolidated Standards of Reporting Trials flow chart (CML group only).

TABLE 2 Withdrawals from trial: ITT population

Characteristic	HLA A2+ (*n* = 13), *n* (%)	HLA A2– (*n* = 9), *n* (%)	Total (*n* = 22), *n* (%)
Number who completed the trial	4 (30.8)	2 (22.2)	6 (27.3)
Number who discontinued the trial	9 (69.2)	7 (77.8)	16 (72.7)
PI's decision[a]	1 (11.1)	0	1 (6.3)
Withdrawal of consent[a]	6 (66.7)	5 (71.4)	11 (68.8)
Difficulty in complying with study visits because of work commitments and because patient found vaccinations distressing[b]	1 (16.7)	0	1 (9.1)
As there are going to be no more vaccinations[b]	1 (16.7)	0	1 (9.1)
Good response, not keen on the number of visits[b]	1 (16.7)	0	1 (9.1)
Patient choice[b]	3 (50.0)	5 (100)	8 (72.7)
Other[a]	2 (22.2)	2 (28.6)	4 (25.0)
Changed to dasatinib[c]	0	1 (50.0)	1 (25.0)
Imatinib dose decreased to 300 mg o.d.[c]	1 (50.0)	0	1 (25.0)
Imatinib dose reduced to 400 mg o.d.[c]	1 (50.0)	0	1 (25.0)
Other medical urgent issues[c]	0	1 (50.0)	1 (25.0)
Time from consent date to end of study (months)			
n	9	7	16
Median (IQR)	18.27 (14.23–26.68)	14.95 (14.46–18.69)	14.98 (14.34–23.00)
Range	5.42–33.48	3.94–23.00	3.94–33.48

IQR, interquartile range; o.d., once daily.
a Denominator is the number of patients in the ITT population who discontinued the trial.
b Denominator is the number of patients in the ITT population who discontinued the trial owing to a withdrawal of consent.
c Denominator is the number of patients in the ITT population who discontinued the trial owing to another reason.

BOX 1 Summary of protocol deviations

Protocol deviation

Missed patient visits:

- 2-C-008: visit 7 and visit 8.
- 2-C-015: visit 3 and visit 4.
- 2-C-016: visit 1 and visit 2.
- 2-C-020: visit 6.

Immunological blood sample volumes below the protocol requirements.

1-C-023: baseline molecular sample taken, but not sent to central laboratory.

Characteristics of the trial sample

Tables 3–5 detail the baseline characteristics of the trial sample. The distribution of patient baseline characteristics was fully consistent with the expected distribution. The arms appear well balanced, within the limits of a small sample size.

As expected from the entry criteria of the trial, the *BCR–ABL* transcript levels were low. *WT1* transcripts were detected in all but one patient, in line with published data sets.

TABLE 3 Baseline characteristics: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Gender,* n *(%)[a]			
Male	6 (46.2)	4 (50.0)	10 (47.6)
Female	7 (53.8)	4 (50.0)	11 (52.4)
Missing	0 (0)	1 (11.1)[b]	1 (4.5)
WHO performance status,* n *(%)[a]			
0: Asymptomatic	13 (100)	8 (100%)	21 (100)
1: Symptomatic but completely ambulatory	0 (0)	0 (0)	0 (0)
Missing	0 (0)	1 (11.1)[b]	1 (4.5)
ECHO result,* n *(%)[a]			
Normal	10 (76.9)	NA[c]	10 (76.9)
Abnormal	0 (0)	NA[c]	0 (0)
Not done	3 (23.1)	NA[c]	3 (23.1)
Age at baseline (years)			
n	13	9	22
Median (IQR)	52 (42–60)	56 (51–63)	53 (46–63)
Range	23–66	42–73	23–73

IQR, interquartile range; NA, not applicable.
a Percentages are based on the number with non-missing data for each characteristic.
b No baseline information was received for patient 1-C-003, who withdrew from the trial early owing to 'other urgent medical issues'.
c ECHO result recorded for HLA A2+ patients only.

TABLE 4 Baseline *BCR–ABL* molecular data: molecular analysis population

Characteristic	HLA A2+ (*n* = 12)	HLA A2– (*n* = 8)	Total (*n* = 20)
Transcript type,* n *(%)			
e13/e14a2	0 (0)	1 (12.5)	1 (5.0)
e13a2	2 (16.7)	3 (37.5)	5 (25.0)
e13a2/e14a2	0 (0)	1 (12.5)	1 (5.0)
e14a2	7 (58.3)	2 (25.0)	9 (45.0)
Unknown	3 (25.0)	1 (12.5)	4 (20.0)
Result,* n *(%)			
Positive	12 (100)	8 (100)	20 (100)
Undetectable	0 (0)	0 (0)	0 (0)
Failed	0 (0)	0 (0)	0 (0)
Transcript level (transcripts/mg of RNA)			
n	12	8	20
Median (IQR)	6.5 (2.5–12.5)	16.5 (7.0–29.5)	7.0 (4.0–23.5)
Range	2–42	1–68	1–68
ABL *control (transcripts/mg of RNA)*			
n	12	8	20
Median (IQR)	28,100 (15,900–46,100)	30,950 (20,450–69,300)	29,550 (18,150–47,450)
Range	14,300–61,800	14,300–97,900	14,300–97,900
BCR *to* ABL *transcript level ratio*			
n	12	8	20
Median (IQR)	0.021 (0.013–0.056)	0.036 (0.023–0.079)	0.027 (0.015–0.061)
Range	0.003–0.098	0.005–0.117	0.003–0.117

IQR, interquartile range.

TABLE 5 Baseline *WT1* molecular data: molecular analysis population

Characteristic	HLA A2+ (*n* = 12)	HLA A2– (*n* = 8)	Total (*n* = 20)
Result,* n *(%)			
Positive	12 (100)	7 (87.5)	19 (95.0)
Undetectable	0 (0)	1 (12.5)	1 (5.0)
Failed	0 (0)	0 (0)	0 (0)
Transcript level (transcripts/mg of RNA)			
n	12	8	20
Median (IQR)	5.9 (2.0–9.5)	3.0 (1.5–7.5)	4.5 (2.0–9.5)
Range	1–83	0–74	0–83
GUS *control (transcripts/mg of RNA)*			
n	12	8	20
Median (IQR)	40,027.2 (23,402.5–59,431.5)	45,856.9 (36,828.5–59,618.8)	41,656.9 (31,270.0–59,431.5)
Range	16,828.8–99,100.0	22,644.4–12,3530.8	16,828.8–123,530.8
WT1 *to* GUS *transcript level ratio*			
n	12	8	20
Median (IQR)	0.011 (0.006–0.048)	0.004 (0.003–0.019)	0.010 (0.003–0.022)
Range	0.002–0.294	0.000–0.025	0.000–0.294

IQR, interquartile range.

Treatment adherence

Pre-trial information is described in *Table 6* and treatment adherence is described in *Tables 7–9*.

Maintenance imatinib treatment was given throughout the trial (see *Table 6*). *Table 6* also describes the duration of pre-trial treatment with imatinib. A course of six pairs of vaccinations (with p.DOM–WT1-37 and p.DOM–WT1-126, respectively) were planned for weeks 0, 4, 8, 12, 16 and 20, followed by maintenance vaccinations. Median vaccination was the expected 12 doses, with a range of 3–6 pairs of vaccinations delivered. Only one patient went on to receive one further set of maintenance vaccinations.

There were two technical failures of electroporation delivery in the same patient at one vaccination time point (*Table 8*). Ten out of 12 patients completed all six pairs of vaccination to week 20. Two patients withdrew consent for further injections after three doses of the vaccine (see *Table 9*).

TABLE 6 Pre-trial imatinib dosing information: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Received pre-trial imatinib, [a] **n *(%)***	***12 (92.3)***	***8 (88.9)***	***20 (90.9)***
Maximum imatinib dose received [b]			
300 mg o.d.	1 (8.3)	0	1 (5.0)
400 mg o.d.	6 (50.0)	4 (50.0)	10 (50.0)
500 mg o.d.	0	1 (12.5)	1 (5.0)
600 mg o.d.	4 (33.3)	1 (12.5)	5 (25.0)
800 mg o.d.	1 (8.3)	2 (25.0)	3 (15.0)
Imatinib duration (months) [c]			
n	12	8	20
Median (IQR)	89.1 (69.8–101.7)	79.1 (67.9–98.0)	85.0 (69.8–100.8)
Range	28.1–110.1	27.8–105.6	27.8–110.1
Imatinib duration (years) [d]			
n	12	8	20
Median (IQR)	7.4 (5.8–8.5)	6.6 (5.7–8.2)	7.1 (5.8–8.4)
Range	2.3–9.2	2.3–8.8	2.3–9.2

IQR, interquartile range; o.d., once daily.
a Percentages are based on the number with non-missing treatment data.
b Percentages are based on the number receiving pre-trial imatinib treatment.
c Imatinib duration defined as time from the earliest start date of imatinib pre-trial to the stop date of imatinib pre-trial (in months).
d Imatinib duration defined as time from the earliest start date of imatinib pre-trial to the stop date of imatinib pre-trial (in years).

TABLE 7 Vaccination treatment summary: ITT population – HLA A2+ patients only

Characteristic	HLA A2+ ($n = 13$)
Received treatment,[a] n (%) *Initial p.DOM–WT1-37 or p.DOM–WT1-126 vaccination administration*	***12 (92.3)***
No vaccines administered[b]	0 (0)
At least one vaccine administered[b]	12 (100)
At least one p.DOM–WT1-37 vaccination administered where no p.DOM–WT1-126 vaccination administered (or vice versa)[b]	0 (0)
Number of initial p.DOM–WT1-37 or p.DOM–WT1-126 vaccinations received	
n	12
Median (IQR)	6 (6–6)
Range	3–6
Proceeded onto additional vaccinations,[a] n (%) *Additional p.DOM–WT1-37 or p.DOM–WT1-126 vaccinations*	***1 (7.7)***
No vaccines administered[c]	0 (0)
At least one vaccine administered[c]	1 (100)
At least one p.DOM–WT1-37 vaccination administered where no p.DOM–WT1-126 vaccination administered (or vice versa)[c]	0 (0)
Number of additional p.DOM–WT1-37 or p.DOM–WT1-126 vaccinations received	
n	1
Median (IQR)	1 (1–1)
Range	1–1
Received treatment,[a] n (%) *Any (initial or additional) p.DOM–WT1-37 or p.DOM–WT1-126 vaccinations*	***12 (92.3)***
No vaccines administered[b]	0 (0)
At least one vaccine administered[b]	12 (100)
At least one p.DOM–WT1-37 vaccination administered where no p.DOM–WT1-126 vaccination administered (or vice versa)[b]	0 (0)
Number of any (initial or additional) p.DOM–WT1-37 or p.DOM–WT1-126 vaccinations received	
n	12
Median (IQR)	6 (6–6)
Range	3–7

IQR, interquartile range.
a Percentages are based on the number with non-missing treatment data.
b Percentages are based on the number receiving initial treatment.
c Percentages are based on the number proceeding onto additional treatment.
Notes
Initial vaccinations = vaccinations administered on weeks 0, 4, 8, 12, 16 or 20.
Additional vaccinations = vaccinations administered on week 32, or month 11 day 0, month 14 day 0, month 17 day 0, month 20 day 0 or month 23 day 0.

TABLE 8 Electroporation treatment summary: ITT population – HLA A2+ patients only

Characteristic	HLA A2+ ($n = 13$)
Received treatment,[a] n (%) *Initial p.DOM–WT1-37 or WT1-126 electroporation failures*	***12 (92.3)***
No electroporation failures	10 (83.3)
At least one electroporation failure	2 (16.7)
Electroporation failure details	
Electroporation failure in initial p.DOM–WT1-37 vaccination[b]	1 (50.0)
Electroporation failure in initial p.DOM–WT1-126 vaccination[c]	1 (50.0)
Initial p.DOM–WT1-37 or WT1-126 electroporation failures	
n	12
Median (IQR)	0 (0–0)
Range	0–1
Proceeded onto additional vaccinations,[d] n (%) *Additional p.DOM–WT1-37 or p.DOM–WT1-126 electroporation failure*	***1 (7.7)***
No electroporation failures	1 (100)
At least one electroporation failure	0 (0)
Additional p.DOM–WT1-37 or 126 electroporation failures	
n	0
Median (IQR)	0 (0–0)
Range	0–0
Received treatment,[e] n (%)	***12 (92.3)***
Any (initial or additional) p.DOM–WT1-37 or WT1-126 electroporation failures	
No electroporation failures	10 (83.3)
At least one electroporation failure	2 (16.7)
Electroporation failure details:	
Electroporation failure in initial p.DOM–WT1-37 vaccination[b]	1 (50.0)
Electroporation failure in initial p.DOM–WT1-126 vaccination[c]	1 (50.0)
Any (initial or additional) p.DOM–WT1-37 or WT1-126 electroporation failures	
n	12
Median (IQR)	0 (0–0)
Range	0–1

IQR, Interquartile range.
a Percentages are based on the number receiving initial treatment.
b Initial p.DOM–WT1-37 vaccination location was the same as the previous vaccination in the right thigh. Initial 126 vaccination location was the same as the previous vaccination in the left thigh.
c Initial p.DOM–WT1-37 vaccination location was the same as the previous vaccination in the right arm. Initial p.DOM–WT1-126 vaccination location was the same as the previous vaccination in the left arm.
d Percentages are based on the number proceeding onto additional treatment.
e Percentages are based on the number with non-missing treatment data.

Notes
Initial electroporation: electroporation administered on weeks 0, 4, 8, 12, 16 or 20.
Additional electroporation: electroporation administered on week 32, or month 11 day 0, month 14 day 0, month 17 day 0, month 20 day 0 or month 23 day 0.

TABLE 9 Premature withdrawals from treatment: ITT population – HLA A2+ patients only

Characteristic	HLA A2+ ($n = 13$), n (%)
Received treatment[a]	12 (92.3)
Patients who received all six initial vaccinations[b]	10 (83.3)
Patients who did not receive all six initial vaccinations[b]	2 (16.7)
Withdrew consent (from treatment only)[c]	2 (100)
Patients who proceeded to receive additional vaccinations[b]	1 (7.7)
Patients who received all six additional vaccinations[d]	0 (0)
Patients who did not receive all six additional vaccinations	1 (100)
Withdrew consent (from trial)[e]	1 (100)

a Percentages are based on the number with non-missing treatment data.
b Percentages are based on the number receiving treatment.
c Percentages are based on the number who did not receive all six initial vaccinations.
d Percentages are based on the number proceeding onto additional treatment (see *Chapter 3*, *Trial interventions*).
e Percentages are based on the number who did not receive all six additional vaccinations.
Notes
Initial vaccinations: vaccinations administered on weeks 0, 4, 8, 12, 16 or 20.
Additional vaccinations: vaccinations administered on week 32, or month 11 day 0, month 14 day 0, month 17 day 0, month 20 day 0 or month 23 day 0.

Safety

Serious adverse events

Details of SAEs are provided in *Tables 10–12*. No vaccine-related SAEs were observed. Three SAEs were observed in the control group and are not vaccine related.

Adverse events

In the vaccine group, 11 grade 1–3 AEs were observed, compared with nine grade 1–3 AEs in the control group (*Table 13*). Of these AEs, palpitations in one vaccinated patient were of special interest (*Table 14*). In one patient, 12 grade 1–3 renal AEs were documented (pre-existing grade 1 renal dysfunction), with no such AEs observed in the control group (*Table 15*). No haematological toxicities were observed (*Table 16*).

A range of mild to moderate AEs was observed (*Table 17*). No detectable significance was observed between the vaccination and observation arms.

Table 18 provides a complete list of all AEs for the safety analysis population.

Concomitant medications taken because of pain within 48 hours of vaccination

Two patients used painkillers after vaccination/electroporation, with good symptomatic relief (*Table 19*). No new clinical insights beyond the pre-trial data on electroporation were observed.

TABLE 10 Serious adverse event summary: safety analysis population

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)
Total number of SAEs, *n* (%)	0 (0)	3	3
Number of patients experiencing at least one SAE,[a] *n* (%)	0 (0)	1 (11.1)	1 (4.8)
PI assessment,[b] n (%)			
SUSAR	0 (0)	0 (0)	0 (0)
SAR	0 (0)	0 (0)	0 (0)
SAE	0 (0)	3 (100)	3 (100)
Clinical reviewer assessment,[b] n (%)			
SUSAR	0 (0)	0 (0)	0 (0)
SAR	0 (0)	0 (0)	0 (0)
SAE	0 (0)	3 (100)	3 (100)
Grade,[b] n (%)			
Mild	0 (0)	0 (0)	0 (0)
Moderate	0 (0)	2 (66.7)	2 (66.7)
Severe	0 (0)	1 (33.3)	1 (33.3)
Life-threatening	0 (0)	0 (0)	0 (0)
Death related to AE	0 (0)	0 (0)	0 (0)
Why was the event serious?,[b] n (%)			
Resulted in death	0 (0)	0 (0)	0 (0)
Life-threatening	0 (0)	0 (0)	0 (0)
Required hospitalisation or prolongation of existing hospitalisation	0 (0)	3 (100)	3 (100)
Persistent or significant disability/incapacity	0 (0)	0 (0)	0 (0)
Congenital anomaly/birth defect	0 (0)	0 (0)	0 (0)

SAR, serious adverse reaction; SUSAR; suspected unexpected serious adverse reaction.
a Percentages are based on the number of patients in the safety analysis population.
b Percentages are based on the total number of SAEs.

TABLE 11 Summary of SAEs by system organ class and CTCAE term: safety analysis population

CTCAE, version 4, system organ class/term[a]	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)
Gastrointestinal disorders,[b] *n* (%)	0 (0)	3 (100)	3 (100)
Abdominal pain,[c] *n* (%)	0 (0)	3 (100)	3 (100)

a Percentages are based on the total number of SAEs.
b CTCAE, version 4, system organ class.
c CTCAE, version 4, term.

TABLE 12 Listing of all SAEs: safety analysis population

HLA A2 status	PI assessment	Clinical reviewer assessment	CTCAE version 4 system organ class	CTCAE version 4 term	Main symptom	Patient ID	Date of informed consent	Date of onset of SAE	Grade	Why was event serious?	Was the vaccine given?[a]	Last administration of vaccine
Negative	SAE	SAE	Gastrointestinal disorders	Abdominal pain	Abdominal pain (cause unknown)	1-C-003	3 August 2011	30 November 2011[b]	3 = severe	3 = hospitalisation or prolongation of hospitalisation	No/no	NA
Negative	SAE	SAE	Gastrointestinal disorders	Abdominal pain	Abdominal pain	1-C-003	3 August 2011	09 December 2011	2 = moderate	3 = hospitalisation or prolongation of hospitalisation	No/no	NA
Negative	SAE	SAE	Gastrointestinal disorders	Abdominal pain	Abdominal pain	1-C-003	3 August 2011	23 December 2011	2 = moderate	3 = hospitalisation or prolongation of hospitalisation	No/no	NA

ID, identification number; NA, not applicable; SUSAR, suspected unexpected serious adverse reaction.

a There are two responses as two vaccines were administered.

b On reconciliation with AEs it was noted that the site reported the date of onset on the SAE/SUSAR form as 30 November 2011 but on the AE form as 1 December 2011. The event started at home on 30 November 2011, but the patient was not admitted until 1 December 2011 hence why the dates are different. The stop date, outcome and grade of the event are the same on both forms.

TABLE 13 Overall worst CTCAE grade across all AEs: safety analysis population[a,b]

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)
Worst CTCAE grade experienced,* n *(%)			
None	1 (8.3)	0 (0)	1 (4.8)
Grade 1: mild	6 (50.0)	4 (44.4)	10 (47.6)
Grade 2: moderate	5 (41.7)	4 (44.4)	9 (42.9)
Grade 3: severe	0 (0)	1 (11.1)	1 (4.8)
Grade 4: life-threatening	0 (0)	0 (0)	0 (0)
Grade 5: death related to an AE	0 (0)	0 (0)	0 (0)
At least one severe (CTCAE grade 3 or above) AE	0 (0)	1 (11.1)	1 (4.8)

a If a patient experienced more than one AE with different CTCAE grades, then the worst CTCAE grade is counted in this table.
b Multiple events per patient are counted once.

TABLE 14 Worst CTCAE grade for cardio-related AEs: safety analysis population[a,b,c]

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)	p-value[d]
Worst CTCAE grade for cardio-related AEs experienced,* n *(%)				
None	11 (91.7)	9 (100)	20 (95.2)	–
Grade 1: mild	0 (0)	0 (0)	0 (0)	–
Grade 2: moderate	1 (8.3)	0 (0)	1 (4.8)	–
Grade 3: severe	0 (0)	0 (0)	0 (0)	–
Grade 4: life-threatening	0 (0)	0 (0)	0 (0)	–
Grade 5: death related to AE	0 (0)	0 (0)	0 (0)	–
At least one severe (CTCAE grade 3 or above) cardio-related AE	0 (0)	0 (0)	0 (0)	–
CTCAE, version 4, term,* n *(%)				
Palpitations	1 (8.3)	0	1 (4.8)	> 0.999

a If a patient experienced more than one cardio-related AE with different CTCAE grades, then the worst CTCAE grade is counted in this table.
b If a patient experienced more than one cardio-related AE with the same worst CTCAE grade, then they are counted once in the first section and once under each nature of AE.
c Multiple events per patient are counted once.
d Fisher's exact tests were calculated to compare differences between HLA A2+ and HLA A2– groups for each type of CTCAE term.

TABLE 15 Worst CTCAE grade for renal-related AEs: safety analysis population[a,b,c]

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)	p-value[d]
Worst CTCAE grade for renal-related AEs experienced,* n *(%)				
None	8 (66.7)	9 (100)	17 (81.0)	–
Grade 1: mild	3 (25.0)	0 (0)	3 (14.3)	–
Grade 2: moderate	1 (8.3)	0 (0)	1 (4.8)	–
Grade 3: severe	0 (0)	0 (0)	0 (0)	–
Grade 4: life-threatening	0 (0)	0 (0)	0 (0)	–
Grade 5: death related to AE	0 (0)	0 (0)	0 (0)	–
At least one severe (CTCAE grade 3 or above) renal-related AE	0 (0)	0 (0)	0 (0)	–
CTCAE, version 4, term,* n *(%)				
CK increased	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Renal and urinary disorders, other	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Renal calculi	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Urinary tract pain	1 (8.3)	0 (0)	1 (4.8)	> 0.999

a If a patient experienced more than one renal-related AE with different CTCAE grades, then the worst CTCAE grade is counted in this table.
b If a patient experienced more than one renal-related AE with the same worst CTCAE grade, then they are counted once in the first section and once under each nature of AE.
c Multiple events per patient are counted once.
d Fisher's exact tests were calculated to compare differences between HLA A2+ and HLA A2– groups for each type of CTCAE term.

TABLE 16 Worst CTCAE grade for bone marrow-related AEs: safety analysis population[a,b,c]

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)
Worst CTCAE grade for bone marrow-related AEs experienced,* n *(%)			
None	12 (100)	9 (100)	21 (100)
Grade 1: mild	0 (0)	0 (0)	0 (0)
Grade 2: moderate	0 (0)	0 (0)	0 (0)
Grade 3: severe	0 (0)	0 (0)	0 (0)
Grade 4: life-threatening	0 (0)	0 (0)	0 (0)
Grade 5: death related to AE	0 (0)	0 (0)	0 (0)
At least one severe (CTCAE grade 3 or above) bone marrow-related AE	0 (0)	0 (0)	0 (0)

a If a patient experienced more than one bone marrow-related AE with different CTCAE grades, then the worst CTCAE grade is counted in this table.
b If a patient experienced more than one bone marrow-related AE with the same worst CTCAE grade, then they are counted once in the first section and once under each nature of AE.
c Multiple events per patient are counted once.

TABLE 17 Worst CTCAE grade for other-related AEs: safety analysis population[a,b,c,d]

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)	p-value[e]
***Worst CTCAE grade for other-related AEs experienced*, n (%)**				
None	1 (8.3)	0	1 (4.8)	–
Grade 1: mild	7 (58.3)	4 (44.4)	11 (52.4)	–
Grade 2: moderate	4 (33.3)	4 (44.4)	8 (38.1)	–
Grade 3: severe	0 (0)	1 (11.1)	1 (4.8)	–
Grade 4: life-threatening	0 (0)	0 (0)	0 (0)	–
Grade 5: death related to AE	0 (0)	0 (0)	0 (0)	–
At least one severe (CTCAE grade 3 or above) other-related AE	0 (0)	1 (11.1)	1 (4.8)	–
***CTCAE, version 4, term*, n (%)**				
Abdominal pain	0 (0)	1 (11.1)	1 (4.8)	0.429
Anorexia	0 (0)	1 (11.1)	1 (4.8)	0.429
Bloating	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Bone pain	0 (0)	1 (11.1)	1 (4.8)	0.429
Bruising	2 (16.7)	0 (0)	2 (9.5)	0.486
Cough	1 (8.3)	2 (22.2)	3 (14.3)	0.553
Depression	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Diarrhoea	1 (8.3)	1 (11.1)	2 (9.5)	> 0.999
Dyspepsia	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Ear and labyrinth disorders, other	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Oedema limbs	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Epistaxis	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Eye disorders, other	2 (16.7)	1 (11.1)	3 (14.3)	> 0.999
Eye infection	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Fatigue	4 (33.3)	0 (0)	4 (19.0)	0.104
Headache	0 (0)	2 (22.2)	2 (9.5)	0.171
Hypotension	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Injury, poisoning and procedural complications, other	2 (16.7)	0 (0)	2 (9.5)	0.486
Musculoskeletal and connective tissue disorders, other	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Myalgia	3 (25.0)	0 (0)	3 (14.3)	0.229
Nail infection	0 (0)	1 (11.1)	1 (4.8)	0.429
Nausea	1 (8.3)	0 (0)	1 (4.8)	> 0.999

continued

TABLE 17 Worst CTCAE grade for other-related AEs: safety analysis population[a,b,c,d] (*continued*)

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 9$)	Total ($n = 21$)	p-value[e]
Nervous system disorders, other	1 (8.3)	2 (22.2)	3 (14.3)	0.553
Pain in extremity	1 (8.3)	0 (0)	1 (4.8)	> 0.999
Respiratory, thoracic and mediastinal disorders, other	0 (0)	1 (11.1)	1 (4.8)	0.429
Skin and subcutaneous tissue disorders, other	2 (16.7)	1 (11.1)	3 (14.3)	> 0.999
Skin ulceration	0 (0)	1 (11.1)	1 (4.8)	0.429
Tooth infection	0 (0)	1 (11.1)	1 (4.8)	0.429
Upper respiratory infection	2 (16.7)	5 (55.6)	7 (33.3)	0.159
Vomiting	1 (8.3)	1 (11.1)	2 (9.5)	> 0.999
Watering eyes	0 (0)	1 (11.1)	1 (4.8)	0.429

a Other AEs correspond to AEs that are not cardio, renal or bone marrow related.
b If a patient experienced more than one other-related AE with different CTCAE grades, then the worst CTCAE grade is counted in this table.
c If a patient experienced more than one other-related AE with the same worst CTCAE grade, then they are counted once in the first section and once under each nature of AE.
d Multiple events per patient are counted once.
e Fisher's exact tests were calculated to compare differences between HLA A2 groups for each type of CTCAE term.

TABLE 18 Listing of AEs: safety analysis population

AE category	CTCAE version 4 system organ class	CTCAE version 4 term	Grade	HLA A2 status	Patient ID	Was the event serious?	Relationship	Outcome	AE start date	AE end date
Cardio	Cardiac disorders	Palpitations	Moderate	Positive	2-C-004	No	Unrelated	Ongoing	March 2013	Ongoing
Cardio	Cardiac disorders	Palpitations	Mild	Positive	2-C-004	No	Unrelated	Resolved	September 2012	September 2012
Other	Ear and labyrinth disorders	Ear and labyrinth disorders, other	Mild	Positive	3-C-017	No	Unlikely to be related	Unknown	12 September 2012	Unknown
Other	Eye disorders	Eye disorders, other	Mild	Negative	2-C-016	No	Unrelated	Resolved	2 August 2012	20 August 2012
Other	Eye disorders	Eye disorders, other	Mild	Positive	1-C-005	No	Unrelated	Resolved	27 September 2011	13 October 2011
Other	Eye disorders	Eye disorders, other	Mild	Positive	2-C-013	No	Unrelated	Ongoing	4 June 2012	Ongoing
Other	Eye disorders	Watering eyes	Mild	Negative	3-C-011	No	Unrelated	Unknown	14 March 2012	Unknown
Other	Gastrointestinal disorders	Abdominal pain	Severe	Negative	1-C-003	Yes	Unrelated	Resolved	1 December 2011	6 December 2011
Other	Gastrointestinal disorders	Abdominal pain	Moderate	Negative	1-C-003	No	Unrelated	Resolved	22 November 2011	22 November 2011
Other	Gastrointestinal disorders	Abdominal pain	Moderate	Negative	1-C-003	Yes	Unrelated	Resolved	9 December 2011	21 December 2011
Other	Gastrointestinal disorders	Abdominal pain	Moderate	Negative	1-C-003	Yes	Unrelated	Resolved	23 December 2011	29 December 2011
Other	Gastrointestinal disorders	Bloating	Moderate	Positive	3-C-017	No	Unlikely to be related	Unknown	12 September 2012	Unknown
Other	Gastrointestinal disorders	Diarrhoea	Mild	Negative	3-C-018	No	Unrelated	Unknown	8 February 2013	Unknown
Other	Gastrointestinal disorders	Diarrhoea	Mild	Positive	3-C-017	No	Unlikely to be related	Unknown	12 September 2012	Unknown
Other	Gastrointestinal disorders	Dyspepsia	Mild	Positive	3-C-017	No	Unlikely to be related	Unknown	12 September 2012	Unknown
Other	Gastrointestinal disorders	Nausea	Moderate	Positive	2-C-012	No	Unrelated	Resolved	16 August 2012	18 August 2012
Other	Gastrointestinal disorders	Vomiting	Moderate	Positive	2-C-012	No	Unrelated	Resolved	16 August 2012	18 August 2012
Other	Gastrointestinal disorders	Vomiting	Mild	Negative	3-C-011	No	Unrelated	Resolved	12 March 2012	14 March 2012
Other	General disorders and administration site conditions	Oedema limbs	Mild	Positive	1-C-005	No	Unrelated	Resolved	5 May 2012	8 May 2012

continued

TABLE 18 Listing of AEs: safety analysis population (*continued*)

AE category	CTCAE version 4 system organ class	CTCAE version 4 term	Grade	HLA A2 status	Patient ID	Was the event serious?	Relationship	Outcome	AE start date	AE end date
Other	General disorders and administration site conditions	Fatigue	Mild	Positive	1-C-005	No	Unrelated	Unknown	20 June 2012	Unknown
Other	General disorders and administration site conditions	Fatigue	Mild	Positive	1-C-023	No	Unlikely to be related	Resolved	31 January 2013	31 January 2013
Other	General disorders and administration site conditions	Fatigue	Mild	Positive	2-C-014	No	Unlikely to be related	Resolved	11 July 2012	18 July 2012
Other	General disorders and administration site conditions	Fatigue	Mild	Positive	2-C-014	No	Unlikely to be related	Resolved	11 July 2012	18 July 2012
Other	General disorders and administration site conditions	Fatigue	Mild	Positive	3-C-017	No	Possibly related	Resolved	12 June 2012	27 June 2012
Other	Infections and infestations	Eye infection	Mild	Positive	2-C-004	No	Unrelated	Resolved	14 October 2012	19 October 2012
Other	Infections and infestations	Nail infection	Moderate	Negative	3-C-011	No	Unrelated	Resolved	November 2013	12 March 2014
Other	Infections and infestations	Tooth infection	Moderate	Negative	2-C-009	No	Unrelated	Ongoing	18 August 2012	Ongoing
Other	Infections and infestations	Upper respiratory infection	Moderate	Negative	2-C-008	No	Unrelated	Resolved	10 December 2011	9 January 2012
Other	Infections and infestations	Upper respiratory infection	Moderate	Negative	2-C-009	No	Unrelated	Resolved	12 April 2012	May 2012
Other	Infections and infestations	Upper respiratory infection	Mild	Negative	2-C-006	No	Unrelated	Resolved	23 December 2011	25 December 2011
Other	Infections and infestations	Upper respiratory infection	Mild	Negative	2-C-008	No	Unrelated	Resolved	14 November 2011	18 November 2011
Other	Infections and infestations	Upper respiratory infection	Mild	Negative	2-C-020	No	Unrelated	Ongoing	7 October 2012	Ongoing
Other	Infections and infestations	Upper respiratory infection	Mild	Negative	3-C-011	No	Unrelated	Resolved	6 March 2013	20 March 2013

AE category	CTCAE version 4 system organ class	CTCAE version 4 term	Grade	HLA A2 status	Patient ID	Was the event serious?	Relationship	Outcome	AE start date	AE end date
Other	Infections and infestations	Upper respiratory infection	Mild	Positive	2-C-010	No	Unrelated	Resolved	8 February 2012	18 February 2012
Other	Infections and infestations	Upper respiratory infection	Mild	Positive	2-C-013	No	Unrelated	Ongoing	21 May 2012	Ongoing
Other	Injury, poisoning and procedural complications	Bruising	Mild	Positive	1-C-022	No	Unrelated	Ongoing	2013	Ongoing
Other	Injury, poisoning and procedural complications	Bruising	Mild	Positive	2-C-002	No	Possibly related	Resolved	24 June 2011	25 June 2011
Other	Injury, poisoning and procedural complications	Injury, poisoning and procedural complications, other	Moderate	Positive	1-C-005	No	Unrelated	Resolved	21 September 2011	21 September 2011
Other	Injury, poisoning and procedural complications	Injury, poisoning and procedural complications, other	Mild	Positive	2-C-010	No	Unrelated	Resolved	January 2012	February 2012
Renal	Investigations	CK increased	Mild	Positive	3-C-017	No	Possibly related	Resolved	30 May 2012	26 June 2012
Other	Metabolism and nutrition disorders	Anorexia	Mild	Negative	3-C-011	No	Unrelated	Unknown	28 April 2012	Unknown
Other	Musculoskeletal and connective tissue disorders	Bone pain	Mild	Negative	2-C-008	No	Unrelated	Resolved	6 December 2011	12 December 2011
Other	Musculoskeletal and connective tissue disorders	Bone pain	Mild	Negative	2-C-008	No	Unrelated	Ongoing	20 June 2012	Ongoing
Other	Musculoskeletal and connective tissue disorders	Musculoskeletal and connective tissue disorders, other	Mild	Positive	1-C-005	No	Probably related	Resolved	28 September 2011	29 September 2011
Other	Musculoskeletal and connective tissue disorders	Myalgia	Moderate	Positive	2-C-013	No	Unrelated	Resolved	1 June 2012	1 June 2012
Other	Musculoskeletal and connective tissue disorders	Myalgia	Mild	Positive	2-C-007	No	Unrelated	Ongoing	21 November 2011	Ongoing

continued

TABLE 18 Listing of AEs: safety analysis population (*continued*)

AE category	CTCAE version 4 system organ class	CTCAE version 4 term	Grade	HLA A2 status	Patient ID	Was the event serious?	Relationship	Outcome	AE start date	AE end date
Other	Musculoskeletal and connective tissue disorders	Myalgia	Mild	Positive	2-C-013	No	Unrelated	Resolved	1 June 2011	1 June 2012
Other	Musculoskeletal and connective tissue disorders	Myalgia	Mild	Positive	2-C-013	No	Unrelated	Ongoing	12 August 2012	Ongoing
Other	Musculoskeletal and connective tissue disorders	Myalgia	Mild	Positive	2-C-013	No	Unrelated	Resolved	15 June 2012	15 June 2012
Other	Musculoskeletal and connective tissue disorders	Myalgia	Mild	Positive	3-C-017	No	Possibly related	Resolved	12 June 2012	27 June 2012
Other	Musculoskeletal and connective tissue disorders	Pain in extremity	Mild	Positive	2-C-010	No	Unrelated	Resolved	19 April 2012	20 April 2012
Other	Nervous system disorders	Headache	Moderate	Negative	2-C-008	No	Unrelated	Ongoing	14 September 2012	Ongoing
Other	Nervous system disorders	Headache	Moderate	Negative	2-C-015	No	Unrelated	Resolved	9 June 2012	9 June 2012
Other	Nervous system disorders	Headache	Mild	Negative	2-C-008	No	Unrelated	Ongoing	6 April 2012	Ongoing
Other	Nervous system disorders	Headache	Mild	Negative	2-C-015	No	Unrelated	Resolved	7 October 2012	11 October 2012
Other	Nervous system disorders	Nervous system disorders, other	Moderate	Negative	2-C-015	No	Unrelated	Resolved	July 2012	September 2012
Other	Nervous system disorders	Nervous system disorders, other	Mild	Negative	3-C-011	No	Unrelated	Resolved	2011	12 September 2012
Other	Nervous system disorders	Nervous system disorders, other	Mild	Positive	2-C-002	No	Unrelated	Ongoing	June 2013	Ongoing
Other	Psychiatric disorders	Depression	Mild	Positive	3-C-017	No	Possibly related	Unknown	12 September 2012	Unknown
Renal	Renal and urinary disorders	Renal and urinary disorders, other	Mild	Positive	2-C-013	No	Unrelated	Ongoing	16 May 2012	Ongoing
Renal	Renal and urinary disorders	Renal calculi	Mild	Positive	2-C-010	No	Unrelated	Ongoing	October 2013	Ongoing
Renal	Renal and urinary disorders	Urinary tract pain	Moderate	Positive	2-C-012	No	Unrelated	Resolved	April 2013	April 2013

AE category	CTCAE version 4 system organ class	CTCAE version 4 term	Grade	HLA A2 status	Patient ID	Was the event serious?	Relationship	Outcome	AE start date	AE end date
Other	Respiratory, thoracic and mediastinal disorders	Cough	Moderate	Negative	2-C-009	No	Unrelated	Resolved	12 April 2012	May 2012
Other	Respiratory, thoracic and mediastinal disorders	Cough	Mild	Negative	2-C-020	No	Unrelated	Ongoing	7 October 2012	Ongoing
Other	Respiratory, thoracic and mediastinal disorders	Cough	Mild	Positive	2-C-010	No	Unrelated	Resolved	8 February 2012	18 February 2012
Other	Respiratory, thoracic and mediastinal disorders	Epistaxis	Mild	Positive	1-C-005	No	Unrelated	Resolved with sequelae	28 September 2011	28 September 2011
Other	Respiratory, thoracic and mediastinal disorders	Respiratory, thoracic and mediastinal disorders, other	Mild	Negative	3-C-011	No	Unrelated	Unknown	28 April 2012	12 March 2014
Other	Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders, other	Mild	Negative	2-C-009	No	Unrelated	Ongoing	12 April 2012	Ongoing
Other	Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders, other	Mild	Positive	1-C-005	No	Unrelated	Resolved	5 May 2012	8 May 2012
Other	Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders, other	Mild	Positive	3-C-017	No	Possibly related	Unknown	12 September 2012	Unknown
Other	Skin and subcutaneous tissue disorders	Skin ulceration	Moderate	Negative	2-C-009	No	Unrelated	Ongoing	15 June 2012	Ongoing
Other	Vascular disorders	Hypotension	Mild	Positive	1-C-005	No	Unrelated	Resolved	24 November 2011	24 November 2011

ID, identification number.

TABLE 19 Summary of pain relief taken following vaccination: safety analysis population – HLA A2+ patients only

Characteristic	HLA A2+ (*N* = 12), *n* (%)
Did the patient receive any concomitant medications due to pain within 48 hours of vaccinations?	
Yes[a]	2 (18.2)
No[a]	9 (81.8)
Missing	1 (8.3)
Types of concomitant medications taken for pain within 48 hours of vaccinations[b]	
Paracetamol	1 (14.3)
Paracetamol and ibuprofen	2 (28.6)
Tramadol for arthritis	4 (57.1)
Total	7 (100)
Within 48 hours of vaccination, how much relief have pain treatments or medications provided?[c] *Median score*[d]	
n	2
Median (IQR)	80% (80–80%)
Range	80–80%
Worst score[e]	
n	2
Median (IQR)	25% (10–40%)
Range	10–40%

IQR, interquartile range.
a Percentages are based on non-missing data.
b All types of medications taken because of pain within 48 hours of vaccination. Percentages are based on the total number of concomitant medications taken.
c How much relief: range of 0% to 100%, where 0% means no relief and 100% means complete relief.
d Median score calculated for each patient across all vaccination visits.
e Worst score calculated for each patient across all vaccination visits.

Primary outcome

The primary outcome was *BCR–ABL* response (CMR or minor or major response) observed during the trial. There were two *BCR–ABL* responses, both of which were defined as a major response, one in each HLA A2 group. Hence, there were no differences observed between the groups (*Table 20*).

TABLE 20 The *BCR–ABL* response: CMR or minor or major – molecular analysis population[a]

Characteristic	HLA A2+	HLA A2–	Total
BCR–ABL *response (CMR or minor or major),* n *(%)*			
No	11 (91.7)	7 (87.5)	18 (90.0)
Yes	1 (8.3)	1 (12.5)	2 (10.0)
Total	12 (100)	8 (100)	20 (100)
Fisher's exact test *p*-value[b] ≥ 0.999			

a Only responses measured at weeks 4, 8, 12, 16, 20, 32 and months 11, 17 and 23 were considered in the analysis, as predefined in the protocol.
b Fisher's exact tests were calculated to compare differences between HLA A2+ and HLA A2– by *BCR–ABL* response.

Secondary outcomes

Breakpoint cluster region–Abelson response (major or complete molecular response)

The *BCR–ABL* major and CMR responses were evaluated, with no change in results to those seen in the primary end point (*Table 21*).

WT1 *response*

There were three *WT1* responses observed, all of which were defined as CMR. Two responses were observed in the HLA A2+ group and one in the HLA A2– group. There were no differences observed between the groups (*Table 22*).

Follow-up

Median follow-up time was 18 months for HLA A2+ patients and 15 months for HLA A2– patients. No significant differences between the study arms were detected (*Table 23*).

A Kaplan–Meier plot for time to last follow-up in the ITT population is presented in *Figure 5*.

TABLE 21 The *BCR–ABL* response: CMR or major – molecular analysis population[a]

Characteristic	HLA A2+	HLA A2–	Total
BCR–ABL *response (CMR or minor or major),* n *(%)*			
No	11 (91.7)	7 (87.5)	18 (90.0)
Yes	1 (8.3)	1 (12.5)	2 (10.0)
Total	12 (100)	8 (100)	20 (100)
Fisher's exact test *p*-value[b]	≥ 0.999		

a Only responses measured at weeks 4, 8, 12, 16, 20, 32 and months 11, 17 and 23 were considered in the analysis as predefined in the protocol.
b Fisher's exact tests were calculated to compare differences between HLA A2+ and HLA A2– by *BCR–ABL* response.

TABLE 22 The *WT1* response: CMR or minor or major – molecular analysis population[a]

Characteristic	HLA A2+	HLA A2–	Total
WT1 *response (CMR or minor or major),* n *(%)*			
No	10 (83.3)	7 (87.5)	17 (85.0)
Yes	2 (16.7)	1 (12.5)	3 (15.0)
Total	12 (100)	8 (100)	20 (100)
Fisher's exact test *p*-value[b]	≥ 0.999		

a Only responses measured at weeks 4, 8, 12, 16, 20, 32 and months 11, 17 and 23 were considered in the analysis as predefined in the protocol.
b Fisher's exact tests were calculated to compare differences between HLA A2+ and HLA A2– by *BCR–ABL* response.

TABLE 23 Follow-up information: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Time to last follow-up from date of consent (months)[a]			
Median (90% CI)	18.3 (14.6 to 26.7)	15.0 (14.5 to 23.0)	18.1 (14.6 to 23.0)
Follow-up at 6 months, % (90% CI)	92.3 (66.0 to 98.5)	88.9 (54.3 to 97.8)	90.9 (73.7 to 97.1)
Follow-up at 12 months, % (90% CI)	84.6 (58.6 to 94.9)	88.9 (54.3 to 97.8)	86.4 (68.4 to 94.5)
Follow-up at 18 months, % (90% CI)	53.8 (29.4 to 73.1)	44.4 (17.8 to 68.3)	50.0 (31.7 to 65.8)
Follow-up at 24 months, % (90% CI)	30.8 (12.2 to 51.7)	22.2 (5.1 to 46.7)	27.3 (13.3 to 43.3)
Log-rank test			
Number of events observed, *n* (%)	13 (100)	9 (100)	22 (100)
p-value	0.463		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	0.721 (0.343 to 1.516)		
p-value	0.469		

CI, confidence interval.
a Follow-up is defined as time from date of consent to date of last follow-up. Patients who die are censored at the date of death.
b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio of > 1 represents a favourable outcome for HLA A2– patients; and a hazard ratio of < 1 represents a favourable outcome for HLA A2+ patients.

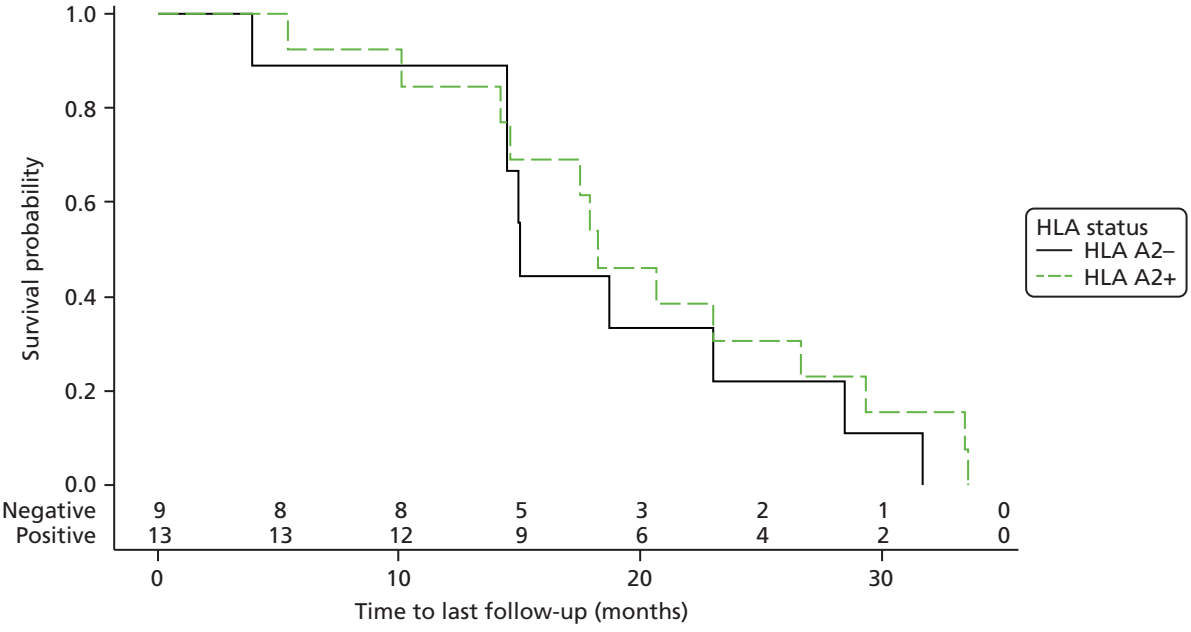

FIGURE 5 Kaplan–Meier for time to last follow-up: ITT population.

Progression-free survival

There were no disease progression or deaths observed in this trial (*Table 24*).

A Kaplan–Meier plot for time to disease progression or death in the ITT population is presented in *Figure 6*.

TABLE 24 Progression-free survival: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Time to progression or death (PFS) from date of consent (months)[a]			
Median (90% CI)	NR (NR to NR)	NR (NR to NR)	NR (NR to NR)
PFS at 6 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
PFS at 12 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
PFS at 18 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
PFS at 24 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Log-rank test			
Number of events observed	0	0	0
p-value	NA		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	NA		
p-value	NA		

CI, confidence interval; NA, not applicable; NR, not reached.
a Progression-free survival defined as time from date of consent to date of progression, last follow-up or death (whichever occurs first). Patients who die and do not progress are censored at the date of death/last follow-up.
b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio of > 1 represents a favourable outcome for HLA A2– patients; and a hazard ratio of < 1 represents a favourable outcome for HLA A2+ patients.

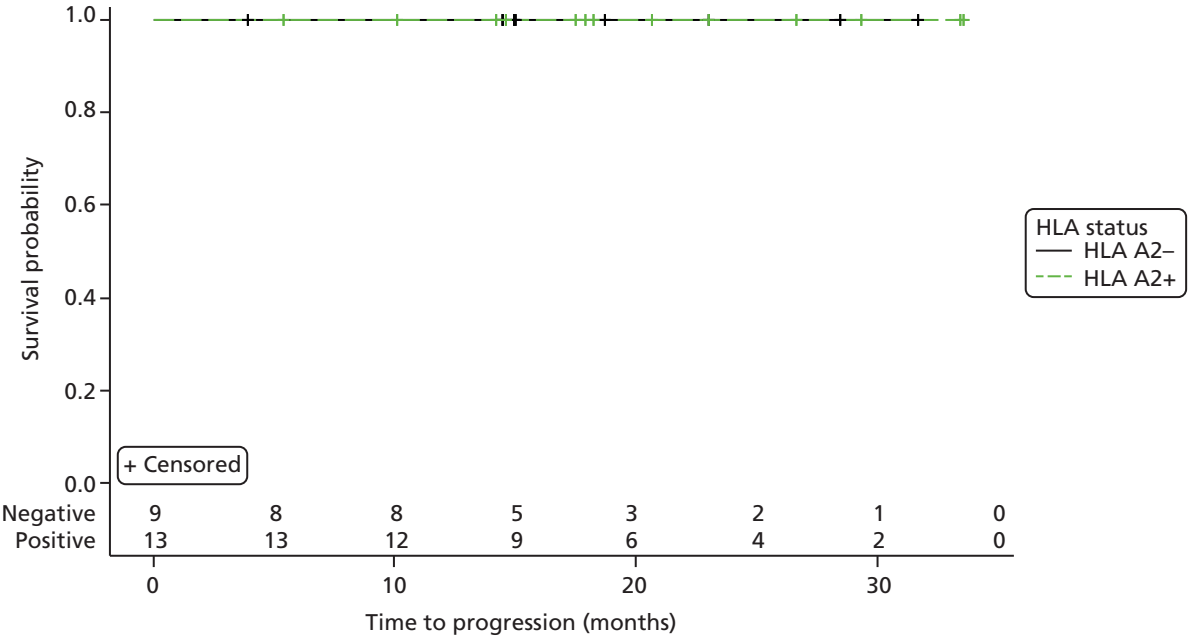

FIGURE 6 Kaplan–Meier for time to disease progression or death: ITT population.

Overall survival

There were no deaths observed in this trial (*Table 25*).

A Kaplan–Meier plot for time to death in the ITT population is presented in *Figure 7*.

TABLE 25 Overall survival: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Time to death (OS) from date of consent (months)[a]			
Median (90% CI)	NR (NR to NR)	NR (NR to NR)	NR (NR to NR)
OS at 6 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
OS at 12 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
OS at 18 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
OS at 24 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Log-rank test			
Number of events observed	0	0	0
p-value	NA		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	NA (NA)		
p-value	NA		

CI, confidence interval; NA, not applicable; NR, not reached; OS, overall survival.
a OS defined as time from date of consent to date of last follow-up or death (whichever occurs first). Patients who do not die are censored at the date of last follow-up.
b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio of > 1 represents a favourable outcome for HLA A2– patients; and a hazard ratio of < 1 represents a favourable outcome for HLA A2+ patients.

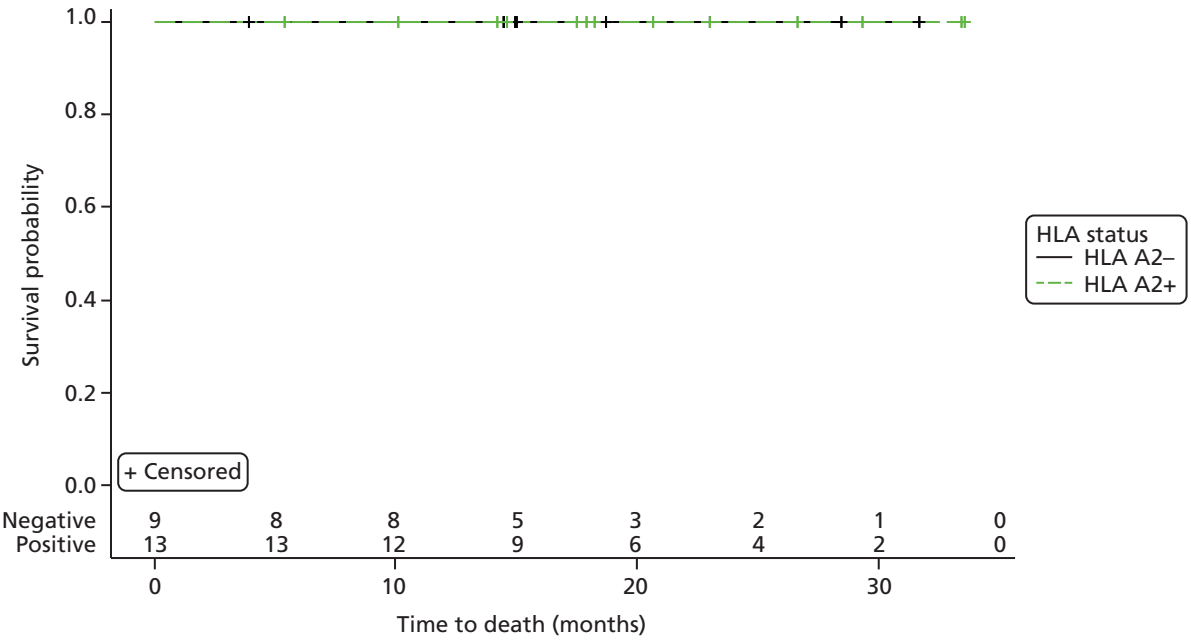

FIGURE 7 Kaplan–Meier for time to death: ITT population.

Time to next treatment

Time to next treatment is defined as time from date of consent to the date of next CML treatment or date of last follow-up (whichever occurs first). No additional CML treatment was administered to any patients within this trial (*Table 26*).

A Kaplan–Meier plot for time to next treatment in the ITT population is presented in *Figure 8*.

TABLE 26 Time to next treatment: ITT population

Characteristic	HLA A2+ ($n = 13$)	HLA A2– ($n = 9$)	Total ($n = 22$)
Time to next treatment from date of consent (months)[a]			
Median (90% CI)	NR (NR to NR)	NR (NR to NR)	NR (NR to NR)
Time to next treatment at 6 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Time to next treatment at 12 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Time to next treatment at 18 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Time to next treatment at 24 months, % (90% CI)	100 (NA)	100 (NA)	100 (NA)
Log-rank test			
Number of events observed	0	0	0
p-value	NA		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	NA (NA)		
p-value	NA		

NA, not applicable; NR, not reached.

a Time to next treatment defined as time from date of consent to date of last follow up or next CML treatment (whichever occurs first). Patients who die or do not have a next treatment are censored at the date of death/last follow-up.

b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio of > 1 represents a favourable outcome for HLA A2– patients; and a hazard ratio of < 1 represents a favourable outcome for HLA A2+ patients.

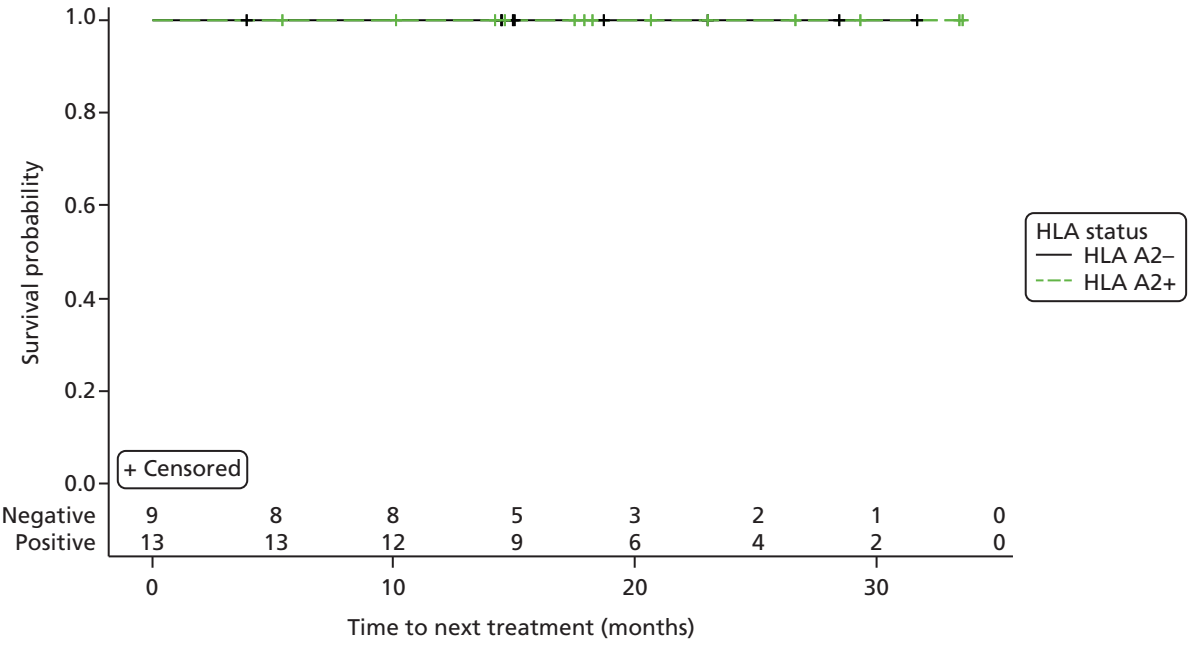

FIGURE 8 Kaplan–Meier for time to next treatment: ITT population.

Time to response

Two *BCR–ABL* responses were observed. One patient in the HLA A2+ group had a response at week 8 and one patient in the HLA A2– group had a response at month 23. The data suggest a vaccine response in the HLA A2+ patient and a late imatinib response in the HLA A2– patient (*Table 27*).

A Kaplan–Meier plot for time to for time to *BCR–ABL* molecular response in the molecular analysis population is presented in *Figure 9*.

TABLE 27 Time to, and duration of, *BCR–ABL* response: major or minor or CMR – molecular analysis population

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 8$)	Total ($n = 20$)
***Time to* BCR–ABL *response from beginning of inhibitor treatment (years)*[a]**			
Median time (90% CI)	NR (NR to NR)	NR (NR to NR)	NR (NR to NR)
Log-rank test			
Number of events observed, *n* (%)	1 (8.3)	1 (12.5)	2 (10.0)
p-value	0.768		–
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	0.661 (0.064 to 6.782)		–
p-value	0.770		–
***Time to* BCR–ABL *response from date of informed consent (months)*[c]**			
Median time (90% CI)	NR (NR to NR)	NR (22.8 to NR)	NR (22.8 to NR)
Log-rank test			
Number of events observed, *n* (%)	1 (8.3)	1 (12.5)	2 (10.0)
p-value	0.695		–
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	0.577 (0.056 to 5.947)		–
p-value	0.699		–
***Duration of* BCR–ABL *response (weeks)*[d]**			
n	1[e]	1	2
Median (IQR)	23.1 (23.1–23.1)	4.1 (4.1–4.1)	13.6 (4.1–23.1)
Range	23.1–23.1	4.1–4.1	4.1–23.1

CI, confidence interval; IQR, interquartile range; NR, not reached.

a Time to *BCR–ABL* response in years is defined as time from date of beginning TKI treatment to date of last *BCR–ABL* sample or the first date of *BCR–ABL* response (whichever occurs first). Patients who did not respond were censored at the date of last *BCR–ABL* response.

b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio of > 1 represents a favourable outcome for HLA A2– patients; a hazard ratio of < 1 represents a favourable outcome for HLA A2+ patients.

c Time to *BCR–ABL* response in months is defined as time from date of consent to date of last *BCR–ABL* sample or the first date of *BCR–ABL* response (whichever occurs first). Patients who did not respond were censored at the date of last *BCR–ABL* response.

d Duration of *BCR–ABL* response is calculated by date of sample where *BCR–ABL* molecular response definition no longer satisfied minus first date *BCR–ABL* response confirmed.

e *BCR–ABL* response definition was still satisfied at their last sample in the trial.

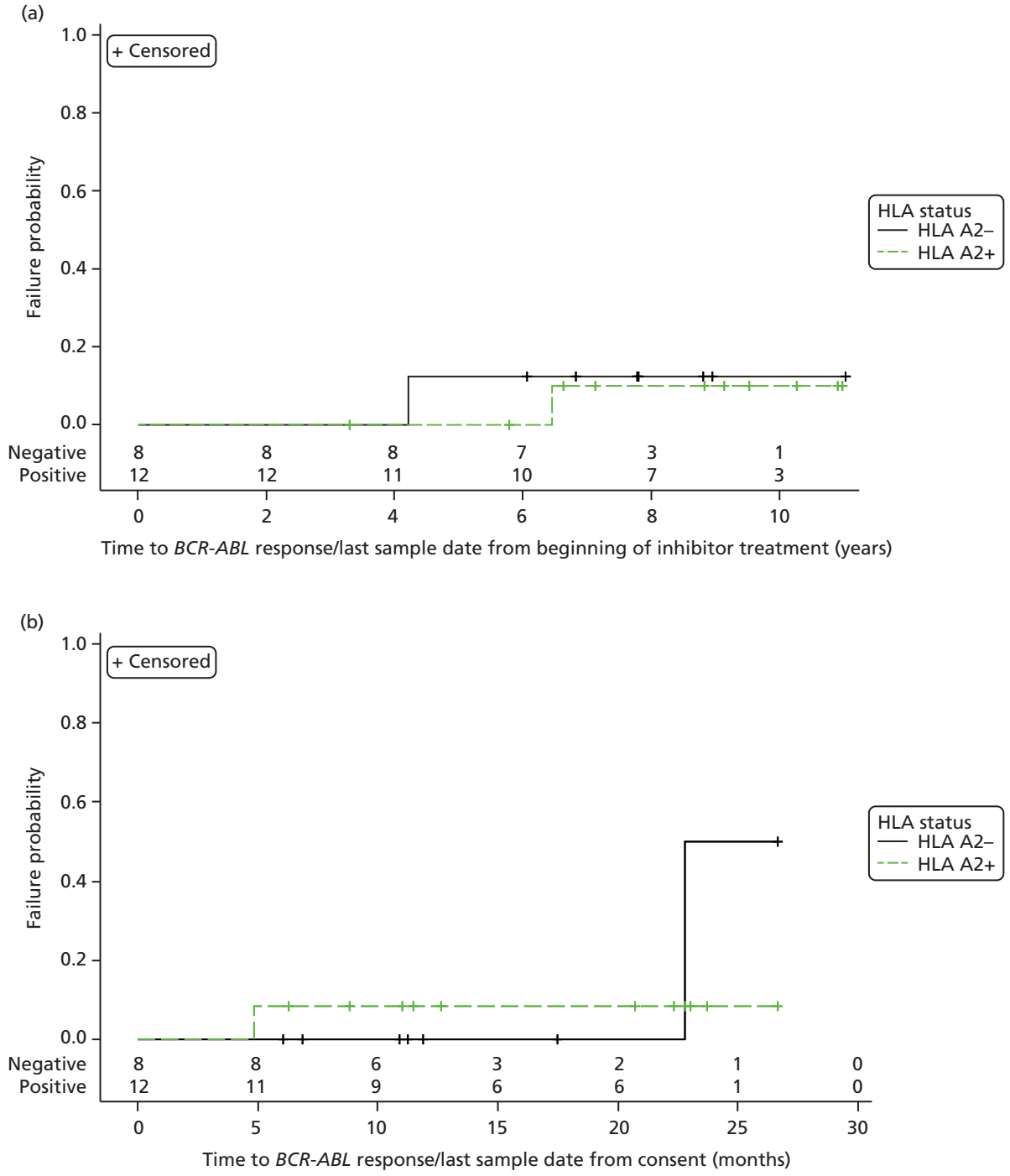

FIGURE 9 Kaplan–Meier for time to *BCR–ABL* molecular response: molecular analysis population. (a) From beginning of TKI treatment (in years); and (b) from informed consent (in months).

There were three *WT1* responses observed. HLA A2+ patients experienced responses at week 20 and week 32. The patient in the HLA A2– group had a response at week 8 (*Table 28*).

A Kaplan–Meier plot for time to for time to *WT1* molecular response in the molecular analysis population is provided in *Figure 10*.

TABLE 28 Time to, and duration of, *WT1* response: major or minor or CMR – molecular analysis population

Characteristic	HLA A2+ ($n = 12$)	HLA A2– ($n = 8$)	Total ($n = 20$)
***Time to* WT1 *response from beginning of inhibitor treatment (years)*[a]**			
Median time (90% CI)	NR (8.0 to NR)	NR (8.7 to NR)	NR (8.7 to NR)
Log-rank test			
Number of events observed, *n* (%)	2 (16.7)	1 (12.5)	3 (15.0)
p-value	0.764		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	0.688 (0.088 to 5.397)		
p-value	0.765		
***Time to* WT1 *response from date of informed consent (months)*[c]**			
Median time (90% CI)	NR (NR to NR)	NR (NR to NR)	NR (NR to NR)
Log-rank test			
Number of events observed, *n* (%)	2 (16.7)	1 (12.5)	3 (15.0)
p-value	0.879		
Cox's proportional hazards model			
Hazard ratio (90% CI)[b]	1.205 (0.161 to 9.050)		
p-value	0.879		
***Duration of* WT1 *response (weeks)*[d]**			
n	2	1	3
Median (IQR)	2.0 (1.7– 2.3)	5.0 (5.0–5.0)	2.3 (1.7–5.0)
Range	1.7–2.3	5.0–5.0	1.7–5.0

CI, confidence interval; IQR, interquartile range; NR, not reached.

a Time to *WT1* response in years is defined as time from date of beginning TKI treatment to date of last *WT1* sample or the first date of *WT1* response (whichever occurs first). Patients who did not respond were censored at the date of last *WT1* response.

b The reference category for the hazard ratio is HLA A2– patients, i.e. a hazard ratio > 1 represents a favourable outcome for HLA A2– patients; a hazard ratio < 1 represents a favourable outcome for HLA A2+ patients.

c Time to *WT1* response in months is defined as time from date of consent to date of last *BCR–ABL* sample or the first date of *WT1* response (whichever occurs first). Patients who did not respond were censored at the date of last *WT1* response.

d Duration of *BCR–ABL* response is calculated by date of sample where *WT1* molecular response definition no longer satisfied – first date *WT1* response confirmed.

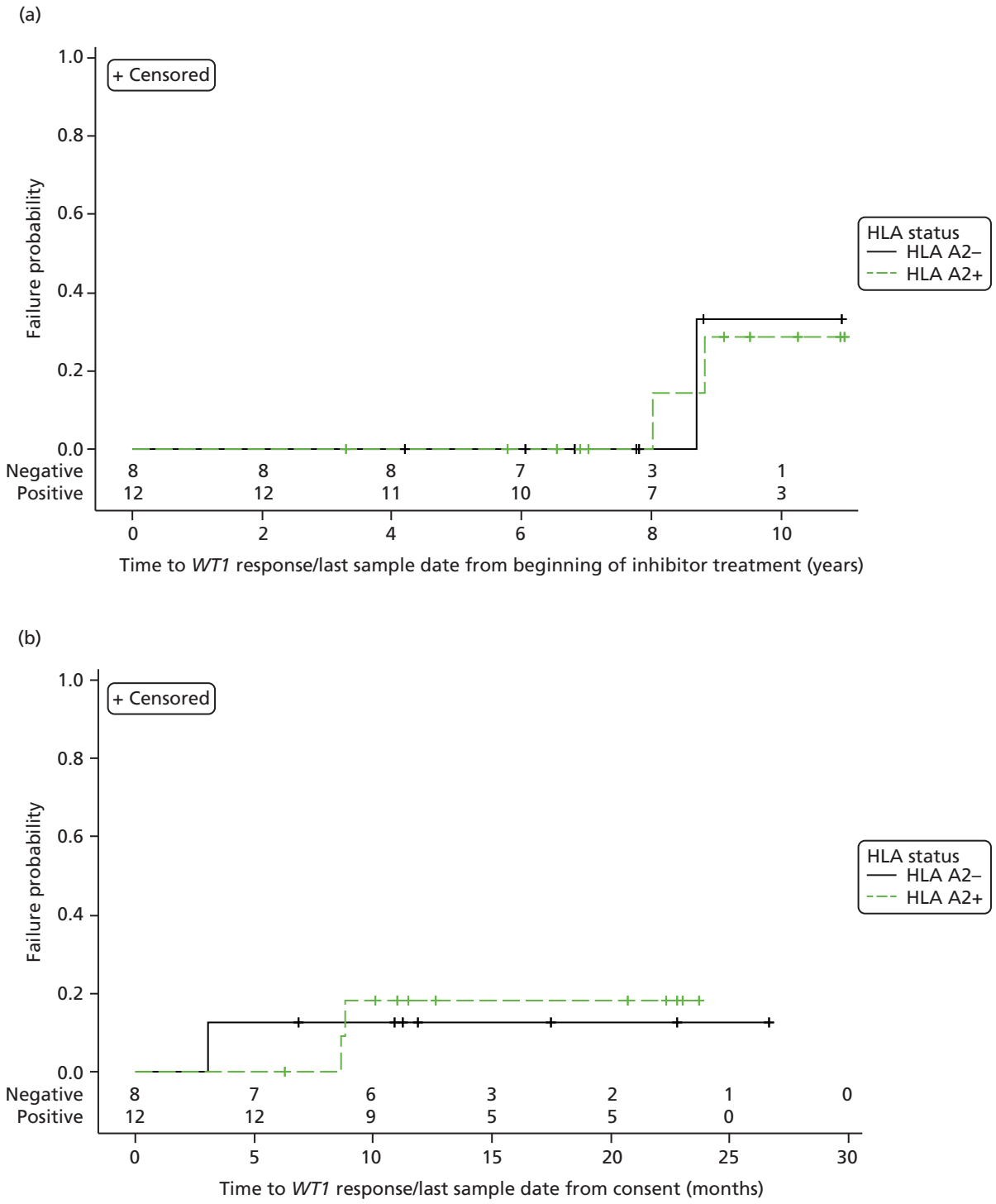

FIGURE 10 Kaplan–Meier for time to *WT1* molecular response: molecular analysis population. (a) From beginning of inhibitor treatment (in years); and (b) from informed consent (in months).

Pain assessment immediately after vaccination

Patient 2-C-021 did not receive any treatment, as outlined by the missing pain assessment data values described in *Table 29*.

Pain assessment 48 hours post vaccination

Pain and discomfort data were measured on a visual analogue scale ranging from 0 (no pain/discomfort) to 10 (worst ever pain/discomfort). Electroporation was well tolerated, with short-lived clinical effects on the patient. No new safety information emerged on electroporation.

Patient 2-C-021 did not receive any treatment, as outlined by the missing pain assessment data values described in *Table 30*.

TABLE 29 Pain assessment[a] immediately after vaccination: ITT population – HLA A2+ patients only

Characteristic	HLA A2+ (*n* = 13)
Median pain score recorded[b] *How severe is your pain or discomfort now?*	
n	12
Median (IQR)	1.0 (1.0–2.3)
Range	0.0–5.0
How severe was your pain or discomfort during and immediately after the injection?	
n	12
Median (IQR)	3.0 (2.0–5.0)
Range	1.0 to 9.0
How distressing is your pain or discomfort now?	
n	12
Median (IQR)	1.0 (0.3–1.0)
Range	0.0–3.0
How distressing was your pain or discomfort during and immediately after the injection?	
n	12
Median (IQR)	2.3 (1.0–4.0)
Range	0.0–8.5

TABLE 29 Pain assessment[a] immediately after vaccination: ITT population – HLA A2+ patients only (*continued*)

Characteristic	HLA A2+ ($n = 13$)
Worst pain score recorded[c] *How severe is your pain or discomfort now?*	
n	12
Median (IQR)	3 (1.50–5.0)
Range	1.0–6.0
How severe was your pain or discomfort during and immediately after the injection?	
n	12
Median (IQR)	5.0 (3.5–7.0)
Range	1.0–9.0
How distressing is your pain or discomfort now?	
n	12
Median (IQR)	2.0 (1.0–2.0)
Range	1.0–5.0
How distressing was your pain or discomfort during and immediately after the injection?	
n	12
Median (IQR)	4.0 (2.0–7.0)
Range	1.0–9.0

IQR, interquartile range.
a Pain assessment scoring based on a scale of 0 to 10, where 0 represents no pain and 10 represents worst pain imaginable.
b Median score for each pain assessment question obtained by calculating the summary statistics using the median score for the corresponding pain assessment question recorded for each patient across all vaccination visits.
c Worst pain score for each pain assessment question obtained by calculating the summary statistics using the maximum (worst) score for the corresponding pain assessment question recorded for each patient across all vaccination visits.

TABLE 30 Pain assessment[a] 48 hours post vaccination: ITT population – HLA A2+ patients only

Characteristic	HLA A2+ ($n = 13$)
Median pain score recorded[b] *Pain at its worst in the last 48 hours?*	
n	12
Median (IQR)	0.5 (0.0–1.0)
Range	0.0–4.0
Pain at its least in the last 48 hours?	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
Pain at its average in the last 48 hours?	
n	12
Median (IQR)	0.3 (0.0–1.0)
Range	0.0–1.0

continued

TABLE 30 Pain assessment[a] 48 hours post vaccination: ITT population – HLA A2+ patients only (*continued*)

Characteristic	HLA A2+ (*n* = 13)
How much pain do you have right now?	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **general activity**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **mood**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **walking ability**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–0.0
*During the past 48 hours, has pain interfered with your **normal work**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **relations with other people**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–0.0
*During the past 48 hours, has pain interfered with your **sleep**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–2.5
*During the past 48 hours, has pain interfered with your **enjoyment of life**?*	
n	12
Median (IQR)	0.0 (0.0–0.5)
Range	0.0–1.5
Worst pain score recorded[c] *Pain at its worst in the last 48 hours?*	
n	12
Median (IQR)	1.0 (1.0–2.0)
Range	0.0–8.0
Pain at its least in the last 48 hours?	
n	12
Median (IQR)	0.0 (0.0–1.0)
Range	0.0–1.0

TABLE 30 Pain assessment[a] 48 hours post vaccination: ITT population – HLA A2+ patients only (*continued*)

Characteristic	HLA A2+ ($n = 13$)
Pain at its average in the last 48 hours?	
n	12
Median (IQR)	1.0 (0.5–1.0)
Range	0.0–4.0
How much pain do you have right now?	
n	12
Median (IQR)	0.0 (0.0–0.5)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **general activity**?*	
n	12
Median (IQR)	0.0 (0.0–0.5)
Range	0.0–3.0
*During the past 48 hours, has pain interfered with your **mood**?*	
n	12
Median (IQR)	0.0 (0.0–1.0)
Range	0.0–6.0
*During the past 48 hours, has pain interfered with your **walking ability**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–0.0
*During the past 48 hours, has pain interfered with your **normal work**?*	
n	12
Median (IQR)	0.0 (0.0–0.5)
Range	0.0–2.0
*During the past 48 hours, has pain interfered with your **relations with other people**?*	
n	12
Median (IQR)	0.0 (0.0–0.0)
Range	0.0–1.0
*During the past 48 hours, has pain interfered with your **sleep**?*	
n	12
Median (IQR)	0.0 (0.0–0.5)
Range	0.0–4.0
*During the past 48 hours, has pain interfered with your **enjoyment of life**?*	
n	12
Median (IQR)	0.0 (0.0–1.0)
Range	0.0–4.0

IQR, interquartile range.

a Pain assessment scoring based on a scale of 0 to 10, where 0 represents no pain and 10 represents worst pain imaginable.

b Median score for each pain assessment question obtained by calculating the summary statistics using the median score for the corresponding pain assessment question recorded for each patient across all vaccination visits.

c Worst pain score for each pain assessment question obtained by calculating the summary statistics using the maximum (worst) score for the corresponding pain assessment question recorded for each patient across all vaccination visits.

Immunological analysis

Immunological humoral (antibody) and cellular responses to the vaccine were assessed, using validated assays, in blood and/or bone marrow of 10 out of 12 evaluable vaccinated patients. Results are reported for 10 out of 12 vaccinated patients. Samples for patient 3-C-019 were lost in a freezer thaw at a site prior to the analysis, as were baseline and multiple later time points for serum and cellular samples for patient 3-C-017. Patients 3-C-019 and 3-C-017 were therefore not immunologically evaluable. Immunological analyses demonstrated that the p.DOM–WT1 vaccine can stimulate measurable immune responses against both the DOM and the *WT1* components in CML patients in chronic phase on imatinib.

Positive responses to the tetanus-derived component of the p.DOM–WT1 vaccine were assessed by measuring levels of anti-FrC antibody in the patient serum by validated ELISAs. A positive response was assigned for a post-vaccination time point with a greater than twofold increase as well as significantly greater levels of anti-FrC antibody compared with pre-vaccination baseline ($p < 0.05$, Dunnett's multiple comparisons test). Positive FrC-antibody responses were detected in a total of 10 out of 10 (100%) evaluable vaccinated patients, providing confirmation of successful vaccine delivery (see *Appendix 3*).

Immunological responses to the antigen-specific (WT1) component of the vaccine were measured using the validated WT1 tetramer assay. A positive response was assigned when tetramer staining for a time point was greater than twofold over baseline and confirmed by three out of three members of an independent flow cytometry expert panel. WT1-specific T cells were detected for total of 7 out of 10 (70%) evaluable vaccinated patients (see *Appendix 4*). This included responses to the WT1-37 peptide in 6 out of 10 (60%) and to WT1-126 peptide in 2 out of 10 (20%) evaluable patients.

Chapter 5 Discussion and summary of findings

The study met its primary decision-making target with one major molecular response in *BCR–ABL* transcript levels. This was the decision-making point for opening the study to the full cohort as well as the AML part of the trial (funded by Bloodwise). The early onset of the major molecular response suggests that vaccination has achieved this molecular event. Two *WT1* molecular responses were observed and one *WT1* response was seen in the HLA A2– group. One of the control patients developed a *BCR–ABL* response at 23 weeks.

The vaccine and electroporation appeared safe, with no new toxicities observed or noted. The evaluation of patients for AEs of special interest did not reveal safety concerns. One patient with pre-existing compensated renal failure had fluctuating renal function, but no significant change from baseline was observed. No significant cardiac toxicity or bone marrow toxicities were observed. One event of palpitation was most likely related to anxiety pre-vaccination/pre-electroporation.

No significant differences in frequency of AEs between the HLA A2+ or HLA A2– groups were seen. AEs related to vaccination site reaction were numerically more frequent in the HLA A2+ group; this did not reach significance – a reflection of small sample size.

At an immunological level the vaccine performed as expected. Positive responses to the tetanus-derived component of the p.DOM–WT1 vaccine were assessed by measuring levels of anti-FrC antibody in the patient serum by validated ELISA. A positive response was assigned for a post-vaccination time point with greater than twofold increase as well as significantly greater levels of anti-FrC antibody compared with pre-vaccination baseline ($p < 0.05$, Dunnett's multiple comparisons test). Positive FrC-antibody responses were detected in a total of 10 out of 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. A positive response was assigned when tetramer staining for a time point was greater than twofold over baseline and confirmed by three independent flow cytometry experts. Overall, WT1-specific T cells were detected for total of 7 out of 10 (70%) evaluable vaccinated patients. This included responses to WT1-37 peptide in 6 out of 10 (60%) and to WT1-126 peptide in 2 out of 10 (20%) evaluable patients.

The immunological analyses for the WIN trial provide evidence to show that the p.DOM–WT1 vaccine can stimulate measurable immune responses against both the DOM and the WT1 components in chronic-phase CML patients on imatinib.

Recruitment could not be completed for multiple reasons. The key study centre had access to only a fraction of the predicted clinical cohort, severely limiting recruitment of CML patients. Unexpectedly, there was no access to AML patients in this study centre. The principal clinical investigator moved out of the country, and this resulted in loss of oversight and recruitment in the key centre. Attempts to recruit other centres were hampered by directly competing studies and, therefore, the CML extension cohort and the AML cohorts could not be pursued.

This is particularly 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.

The observation that only 1 out of 12 patients exhibited amolecular response suggests that the rate of clinical benefit in patients on imatinib in chronic-phase CML is low, although if it were possible to withdraw imatinib in 10% of patients this would be clinically significant. Although immunogenicity supports 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 in AML remains attractive clinically, but 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 currently being developed.

Acknowledgements

Funding

Chronic myeloid leukaemia arm – NIHR EME programme.

Acute myeloid leukaemia arm – Bloodwise.

Sponsor

University Hospital Southampton NHS Foundation Trust.

Clinical Trials Unit

Joshua Caddy, Mary Ellis, Alex Allen, Nicole Vaughan-Spickers, Emily Moore, Elizabeth Dixon, Jo Musgrove and Kirsty Rogers.

Data Monitoring and Ethics Committee

Professor Alan Melcher, Dr Christy Ralph and Dr Emma Hall.

Trial Steering Committee

Dr Stephen Falk, Dr Andrew Protheroe, Professor Sally Stenning and Professor Diana Eccles.

Investigational medicinal product manufacturer

Bristol Institute of Transfusion Sciences Clinical Biotechnology Centre (BITS), University of Bristol.

Inovio

Provision of the Electroporation Device and training of site staff.

Laboratory

Experimental Cancer Medicine Centre (ECMC) Imperial College Healthcare NHS Trust.

National Cancer Research Network

National Cancer Research Network (NCRN).

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Data sharing statement

The available data can be obtained from the SCTU. The results will also be published on the European Clinical Trials Database (EudraCT).

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Appendix 1 Pain assessment tool: immediately after vaccination

Anti-WT1 DNA vaccination – assessment immediately post vaccination

Participant Trial ID ☐ / ☐ / ☐☐☐ Participant Initials ☐☐☐

Please mark the circle (as appropriate) below to show how intense your pain is.
A zero (0) means no pain and ten (10) means extreme pain.

How **severe** is your pain or discomfort **now**?

0 1 2 3 4 5 6 7 8 9 10
No pain Extreme pain

How severe was your pain or discomfort during and immediately after the injection?

0 1 2 3 4 5 6 7 8 9 10
No pain Extreme pain

Now please use the same method to describe how **distressing** your pain or discomfort is.

0 1 2 3 4 5 6 7 8 9 10
No pain Extreme pain

How **distressing** is your pain or discomfort **now**?

0 1 2 3 4 5 6 7 8 9 10
No pain Extreme pain

How distressing was your pain or discomfort during and immediately after the injection?

0 1 2 3 4 5 6 7 8 9 10
No pain Extreme pain

Appendix 2 Pain assessment tool: 48 hours after vaccination

Anti-WT1 DNA vaccination – Assessment at 48 hrs post vaccination

Participant Trial ID [ ] / [ ] / [ ][ ][ ] Participant Initials [ ][ ][ ]

Injection type (please circle) DNA alone / DNA+Electroporation

Week [ ][ ]

Vaccination dose [ ][ ][ ].[ ]

Vaccination date [d][d] / [m][m] / [y][y]

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

Yes ☐ No ☐

2. Please mark an X next to the areas where you feel pain.

Injection site		
Left arm		
Right arm		
Left leg		
Right leg		
Trunk		
Other (please specify)		Specify:

3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 48 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain Worst pain imaginable

4. Please rate your pain by circling the one number that best describes your pain at its least in the last 48 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain Worst pain imaginable

5. Please rate your pain by circling the one number that best describes your pain at its average in the last 48 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain Worst pain imaginable

6. Please rate your pain by circling the one number that best describes how much pain you have right NOW.

0 1 2 3 4 5 6 7 8 9 10
No pain Worst pain imaginable

7. What treatments have you had for your pain in the last 48 hours?

_____

_____

8. In the last 48 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
No relief complete relief

9. Circle the one number that describes how, during the past 48 hours, pain has interfered with you:

General Activity
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Mood
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Walking Ability
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Normal Work (includes both work outside the home and housework)
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Relations with other people
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Sleep
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely interferes

Enjoyment of life
0 1 2 3 4 5 6 7 8 9 10
Does not interfere at all Completely Interferes

Appendix 3 Anti-fragment C from tetanus toxin antibody enzyme-linked immunosorbent assay results

Anti-FrC antibody ELISA results are shown as mean relative antibody units at each time point (0–23 weeks). Positive responses (shown highlighted in green) represent a greater than twofold, and significantly greater than pre-vaccination, baseline ($p < 0.05$, Dunnett's multiple comparisons test). Blue shading represents no sample. The final column shows overall response, with a maximum fold increase relative to baseline.

APPENDIX 3

	Time point (weeks)										
FrC	00	01	02	03	04	05	06	07	08	09	10
1-C-005	0.4215	0.4151	0.6618	0.6491	0.8572	0.8491	0.7884	0.7357	0.8395	0.8159	0.8159
1-C-022	0.0517	0.0624	0.0899	0.4052	1.1441		1.4248	1.6481	1.7005	1.7878	1.7795
1-C-023	0.6330	0.5645	1.0376	1.0156	1.2872	1.2920	1.2381	1.8903	1.6056	1.7529	
2-C-002	1.5938	2.2106	4.4236	2.9914	3.6068	4.7370	3.1848	3.2868	2.8729	5.1475	4.5656
2-C-004	0.1043	0.1389	0.1340	0.0573	0.1130	0.2688	0.2195	0.2440	0.2212	0.4068	0.3736
2-C-007	0.0854	0.1092	0.1098	0.1417	0.3030	0.4984	0.6170	0.7719	0.4890	0.8262	0.7443
2-C-010	0.4321	0.5565	1.0132	1.2200	0.9896	1.4916	1.4232	1.4452	1.3271	1.3318	1.2191
2-C-012	0.6950	0.7023	1.2529	1.1530	1.1180	1.0267	1.1176	1.3059	1.6607	1.6134	1.5593
2-C-013	0.1304	0.1458	0.3887	0.3314	0.3412	0.4077	0.4102	0.4713	0.4341	0.4839	
2-C-014	0.3324	0.3583	1.0392	1.0211	1.1308	1.0485	1.0133	1.2261	1.2952	1.2738	
3-C-017	NA										0.5324
Response frequency	0	0	4	4	8	8	6	8	8	9	6

NA, not applicable.

11	12	13	14	15	16	17	18	19	20	23	Overall result
	0.7603		0.7135	0.5409	0.5194	0.4560		0.6307	0.5164	0.5923	2.0
1.8024		1.2027	0.9147							0.7652	34.9
1.4973	1.4630	1.3148	1.0102							0.9034	3.0
	2.3297	4.5745	3.0819				3.1852	2.7010	3.3740		3.2
0.1449		0.1633	0.0702			0.0691					3.9
0.3447											9.7
0.8340	0.7065			0.6568	0.6074	0.7267					3.5
1.2250	1.8651	1.0415									2.7
			0.2557								3.7
			0.5880								3.9
0.4440	0.4829	0.3703									NA
3	2	3	1	0	0	0	0	0	1	1	10/11

Appendix 4 Tetramer analysis results for WT1-37 and WT1-126 peptides

Tetramer analysis results for WT1-37 and WT1-126 peptides are shown as percentage of tetramer-positive cells within the CD8+ T-cell population. Positive responses (shown highlighted in green) represent a post-vaccination time point greater than twofold increase over baseline and confirmed as real by three independent flow cytometrists. Blue shading represents no sample. The final two columns show overall response, with a maximum fold increase relative to baseline.

Peptide	Time point (weeks)										
	00	01	02	03	04	05	06	07	08	09	10
WT1-37											
1-C-005	0.0152	0.0152	0.0210	0.0120	0.0117	0.0088	0.0120	0.0183	0.0093	0.0125	0.0135
1-C-022	0.0025	0.0013	0.0036	0.0039	0.0016	0.0024	0.0012	0.0021	0.0022	0.0004	0.0035
1-C-023	0.0074	0.0115	0.0110	0.0186	0.0243	0.0267	0.0277	0.0119	0.0276	0.0162	
2-C-002	0.0378	0.0455	0.0667	0.0547	0.0844	0.0760	0.0663	0.0794	0.0775	0.0612	0.0631
2-C-004	0.0026	0.0008	0.0046	0.0034	0.0122	0.0042	0.0063	0.0023	0.0040	0.0057	0.0054
2-C-007	0.0032	0.0027	0.0074	0.0031	0.0079	0.0036	0.0038	0.0035	0.0074	0.0037	0.0020
2-C-010	0.0031	0.0016	0.0037	0.0047	0.0037	0.0037	0.0036	0.0052	0.0021	0.0031	0.0047
2-C-012	0.0028	0.0041	0.0027	0.0158	0.0274	0.0227	0.0182	0.0147	0.0256	0.0162	0.0093
2-C-013	0.0136	0.0117	0.0097	0.0175	0.0148	0.0117	0.0147	0.0132	0.0091	0.0117	
2-C-014	0.0056	0.0041	0.0521	0.0096	0.0126	0.0144	0.0093	0.0128	0.0184	0.0148	
3-C-017	0.0121										0.0184
Response frequency	0	0	2	2	5	4	2	3	5	3	0
WT1-126											
1-C-005	0.0040	0.0040	0.0035	0.0055	0.0082	0.0090	0.0085	0.0085	0.0067	0.0043	0.0062
1-C-022	0.0005	0.0008	0.0016	0.0004	0.0008	0.0008	0.0013	0.0026	0.0009	0.0004	0.0018
1-C-023	0.0050	0.0055	0.0060	0.0070	0.0068	0.0064	0.0085	0.0076	0.0140	0.0112	
2-C-002	0.0021	0.0028	0.0031	0.0035	0.0018	0.0028	0.0036	0.0030	0.0027	0.0022	0.0021
2-C-004	0.0007	0.0007	0.0008	0.0000	0.0032	0.0010	0.0011	0.0006	0.0017	0.0025	0.0024
2-C-007	0.0012	0.0035	0.0023	0.0040	0.0037	0.0026	0.0019	0.0013	0.0006	0.0029	0.0013
2-C-010	0.0021	0.0021	0.0000	0.0010	0.0005	0.0010	0.0031	0.0026	0.0016	0.0021	0.0032
2-C-012	0.0020	0.0015	0.0006	0.0005	0.0015	0.0015	0.0010	0.0000	0.0019	0.0017	0.0023
2-C-013	0.0015	0.0026	0.0000	0.0015	0.0015	0.0017	0.0036	0.0036	0.0035	0.0046	
2-C-014	0.0023	0.0000	0.0009	0.0045	0.0038	0.0013	0.0012	0.0031	0.0021	0.0036	
3-C-017	0.0020										0.0048
Response frequency	0	0	0	0	0	1	1	1	1	0	0

11	12	13	14	15	16	17	18	19	20	23	Overall result
	0.0160		0.0155	0.0090	0.0074	0.0075		0.0086	0.0149	0.0085	
0.0017	0.0012	0.0017	0.0009							0.0015	
0.0181	0.0230	0.0223	0.0225							0.0099	3.7
	0.0696	0.0490	0.0483				0.0633	0.0648	0.0592		2.3
0.0051		0.0045	0.0069			0.0060					4.7
0.0024											2.3
	0.0036			0.0026	0.0042	0.0030					
0.0143	0.0149	0.0120									9.8
			0.0077								
			0.0116								9.3
0.0195	0.0176	0.0128									
2	2	1	2	0	0	1	0	0	0	1	6/11
	0.0048		0.0065	0.0030	0.0050	0.0045		0.0050	0.0045	0.0065	2.2
0.0004	0.0009	0.0008	0.0005							0.0010	
0.0120	0.0065	0.0123	0.0120							0.0097	2.8
	0.0023	0.0035	0.0093				0.0102	0.0035	0.0064		
0.0007		0.0020	0.0000			0.0007					
0.0006											
	0.0042			0.0047	0.0031	0.0029					
0.0005	0.0020	0.0000									
			0.0025								
			0.0022								
0.0010	0.0073	0.0035									
1	0	1	1	0	0	0	0	0	0	1	2/11

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