UNIVERSITY OF Southampton

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Leukaemia & Lymphoma Research
BEATING BLOOD CANCERS

ECMC

Imperial College Healthcare NHS
NHS Trust

WIN

WT1 Immunity via DNA fusion Gene Vaccination in Haematological Malignancies by intramuscular injection followed by intramuscular electroporation

Protocol version 6, 31-07-2012

SPONSOR: University Hospital Southampton NHS Foundation Trust

COORDINATING CENTRE: University of Southampton Clinical Trials Unit

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Protocol Information

This protocol describes the WIN trial and provides information about procedures for entering patients. The protocol should not be used as a guide for the treatment of patients who are not eligible for the WIN Trial; every care was taken in writing this protocol but corrections or amendments may be necessary as the trial progresses. These will be circulated to all investigators participating in the trial. It is the responsibility of the Investigator to ensure that the most updated approved version of the protocol is used throughout the conduct of the study. Sites entering patients for the first time may contact the University of Southampton Clinical Trials Unit to confirm they have the most recent version.

Compliance

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and all other regulatory requirements, as appropriate.

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List of Abbreviations

ABPI	Association of the British Pharmaceutical Industry
AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AML	Acute myeloid leukaemia
APC	Antigen presenting cell
AR	Adverse Reaction
AST	Aspartate aminotransferase
BP	Blood Pressure
CCyR	Complete Cytogenetic Response
CEA	Carcinoembryonic Antigen
CIRB	Central Institutional Review Board
CK	Creatine Kinase
CML	Chronic myeloid leukaemia
CML-CP	Chronic myeloid leukaemia in chronic phase
CMR	Complete Molecular response
CMV	Cytomegalovirus
CR	Complete Response
CRF	Case Report Form
CT	Computerised tomography
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T cell/(s)
CXR	Chest X ray
DMEC	Data Monitoring and Ethics Committee
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DTH	Delayed-Type Hypersensitivity
ECG	Electro-cardiogram
EDLI	Educated donor lymphocyte infusion
EP	Electroporation
ESR	Erythrocyte Sedimentation Rate
FBC	Full Blood Count
FDA	Food and Drug Administration
FrC	Fragment C from tetanus toxin
FU	Follow up
GMCSF	Granulocyte/Macrophage Colony Stimulating Factor
GTAC	Gene Therapy Advisory Committee
HLA A2	Human Leukocyte Antigen A2
IB	Investigator’s Brochure
ICH GCP	International Conference on Harmonisation of Good Clinical Practice
IFNγ	Interferon gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISS	Immune Stimulating sequence
MDS	Myelodysplastic syndrome
MHC	Major Histocompatability Complex
MHRA	Medicines and Healthcare products Regulatory Authority
MRD	Minimal Residual Disease

MRNA	Messenger Riboneuclic acid
NCI	National Cancer Institute
NK	Natural Killer Cell
NYHA	New York Heart Association
PCR	Polymerase chain reaction
PD	Progressive Disease
p.DOM	Domain 1 from Fragment C of Tetanus Toxin (FrC), used in the vaccine construct as an immune alert signal
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
scFv	Single chain fragment of variable regions
SD	Stable Disease
SLE	Systemic Lupus Erythematosus
SOP	Standard Operating Procedure
UHS	Southampton University Hospitals Trust
SUSAR	Suspected Unexpected Serious Adverse Reaction
TH1/2	T Helper 1/2 cells
TKI	Tyrosine Kinase Inhibitor
TMG	Trial Management Group
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
ULN	Upper limit of normal
UoSCTU	University of Southampton Clinical Trials Unit
WBC	White Blood Cells
WHO	World Health Organisation
WT1	Wilms Tumour gene 1

TRIAL SYNOPSIS

Title:	WT1 Immunity via DNA fusion Gene Vaccination in Haematological Malignancies by intramuscular injection followed by intramuscular electroporation.
Sponsor:	University Hospital Southampton NHS Foundation Trust
Sponsor Ref Number:	RHMCAN0700
Funder:	Leukaemia & Lymphoma Research, and the Efficacy and Mechanism Evaluation (EME) programme.
Trial Phase:	II
Indication:	Chronic Myeloid Leukaemia (CML) and Acute Myeloid Leukaemia (AML)
Primary Objective:	CML: Molecular response of BCR-ABL. AML: Time to disease progression.
Secondary Objective:	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, overall survival
Trial Design:	Open label, single dose level, Phase II study in two patient groups (CML and AML) using genetic randomisation. Consented and eligible HLA A2+ve patients will be vaccinated with two DNA vaccines. HLA A2 –ve patients will be followed up on trial without vaccination.
Sample size : **(split by treatment group)**	Vaccination Arm (HLA A2+ve patients): 32 patients with data to at least 8 weeks will be recruited into the CML treatment group. 39 patients with data to at least 8 weeks will be recruited into the AML treatment group. Control Arm: this arm will consist of all eligible and consenting patients who are HLA A2 –ve.
Main inclusion Criteria:	CML: Philadelphia chromosome positive CML in chronic phase, in complete cytogenetic response (CCyR) but with detectable BCR-ABL transcripts and maintained the CCyR on tyrosine kinase inhibitor (TKI) monotherapy for a minimum of 24 months. AML: $WT1^+$ AML in CR or morphologic CR with incomplete blood count recovery (CRi).
Main exclusion Criteria:	CML: accelerated phase or blast crisis or having achieved CMR at any point during tyrosine kinase inhibitor therapy. Tyrosine kinase inhibitor dose modification in the previous year or interruption for > 15 days in the previous 6 months prior to recruitment.
Investigational Products:	p.DOM-WT1-37 DNA Vaccine p.DOM-WT1-126 DNA Vaccine
Dosage Regimen / Duration of Treatment:	p.DOM-WT1-37: 1mg/dose/vaccine and p.DOM-WT1-126: 1mg/dose/vaccine The DNA vaccine will be administered 12 times. In the first 6 months patients will receive the vaccine at 4 weekly intervals into separate sites. After this vaccinations will be administered every 3 months to a maximum of 24 months. Vaccines will be injected intramuscularly and followed by intramuscular electroporation.
Concomitant Therapy:	Steroids or other drugs with a likely effect on immune competence are not permitted during the course of the trial. Concomitant medication may be given as medically indicated. Patients with CML-CP will continue on tyrosine kinase inhibitor therapy.

REFERENCE DIAGRAM

- **Both patient groups (AML & CML) will follow the same schedule**

Consent

↓

Register patient with UoSCTU

↓

Blood: HLA A2 analysis

↓

Notify UoSCTU of HLA A2 status

HLA A2 –ve

↓

Follow up only

HLA A2 +ve

↓

Vaccination

Vaccine administered 6 times, at 4 weekly intervals initially and then every 3 months to a maximum of 24 months

SCHEDULE OF OBSERVATIONS AND PROCEDURES FOR HLA A2 POSITIVE PARTICIPANTS

	baseline	w0	w2	w4	w8	W10	w12	w16	w20	w22	w24-w28	w32	w34	m11 d0	m14 d0	m17 d0	m17 d14	m20 d0	m23 d0	m24 d0	m27	m30	m33	m36 EOT
Visit number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Informed consent	*																							
HLA status Expression of WT1 in tumour cells	*																							
Demographic Data	*																							
Medical history/ Malignancy	*																							
Prior Treatment Malignancy	*																							
Height & Weight	*	*	*	*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Physical examination	*	*	*	*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
WHO Performance Status	*	*	*	*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Vital signs	*	*	*	*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Assessment of pain/ discomfort and distress	*	*		*	*		*	*	*			*		*	*	*		*	*					
ECG Echocardiogram	* Echo at baseline only unless clinically indicated	*(1)		*	*		*	*	*			*		*	*	*		*	*					
FBC and differential blood count, ESR	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Clotting	*																							*
Biochemistry including CK Urine test for proteinuria	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Syphilis, Hep B, Hep C, HIV, EBV, CMV	*																							
Immunological monitoring (2)	* 80ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml
qPCR for BCR-ABL/WT	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

	baseline	w0	w2	w4	w8	W10	w12	w16	w20	w22	w24-w28	w32	w34	m11 d0	m14 d0	m17 d0	m17 d14	m20 d0	m23 d0	m24 d0	m27	m30	m33	m36 EOT
Visit number	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Chest X-ray	*																							
Vaccination		*		*	*		*	*	*			*		*	*	*		*	*					
Bone Marrow, **Clinical assessment** (3)	*										*													
Leukapheresis for immunological studies	*										*													
Autoimmune profile (4)	*							*			*						*							*
DTH reaction to peptide (5)							*				*													
Concomitant Diseases and Treatment	*																							
Adverse Events	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

(1) Before and after vaccination

(2) Includes anti-tetanus antibodies, Tetanus-reactive helper T cells, etc… 70ml of anticoagulated blood will be collected per timepoint for these immunological studies

(3) AML : morphology, cytogenetics and immunological studies. CML patients immunological studies only

(4) Autoimmune profile includes: anti-muscle antibodies, antinuclear antibodies, anti-DNA antibodies, rheumatoid factors and serum Ig electrophoresis.

(5) Skin biopsy for immunological evaluation where DTH reaction observed (to be carried out if wherever feasible)

SCHEDULE OF OBSERVATIONS AND PROCEDURES FOR HLA A2 NEGATIVE PARTICIPANTS

	baseline	w0	w2	w4	w8	W10	w12	w16	w20	w22	w24-w28	w32	w34 (6)	m11 d0	m14 d0	m17 d0	m17 d14 (6)	m20 d0	m23 d0	m24 d0	m27	m30	m33	m36 EOT
Visit number	0	1	2^	3	4	5^	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Informed consent	*																							
HLA status Expression of WT1 in tumour cells	*																							
Demographic Data	*																							
Medical history/ Malignancy	*																							
Prior Treatment Malignancy	*																							
Height & Weight	*	*		*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Physical examination	*	*		*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
WHO Performance Status	*	*		*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Vital signs	*	*		*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
FBC and differential blood count, ESR	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Clotting	*																							*
Biochemistry & Urine test for proteinuria	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
qPCR for BCR-ABL/WT	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Bone Marrow, **Clinical assessment** (1&7))	*																							
Autoimmune profile (3)	*																							
Concomitant Diseases and Treatment	*																							
Adverse Events	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

^Not done for HLA A2 negative patients

(1) AML : morphology, cytogenetics and immunological studies. CML patients immunological studies only

(2) Not to be performed in CML patients who are HLA A2 negative

(3) Autoimmune profile includes: anti-muscle antibodies, antinuclear antibodies, anti-DNA antibodies, rheumatoid factors and serum Ig electrophoresis.

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 HAEMATOLOGICAL MALIGNANCIES

In the UK 7,279 patients were diagnosed with leukaemia in 2005 (http://info.cancerresearchuk.org/cancerstats) but despite continuing advances in diagnosis and treatment the majority of these individuals will eventually die from their disease (1).

1.1.2 CML

Chronic myeloid leukaemia (CML) is a clonal disease of the haematopoietic stem cell in which a reciprocal translocation, t(9;22)(q34;q11), known as the Philadelphia chromosome, results in a fusion gene, BCR-ABL, which in turn expresses an activated tyrosine kinase and is regarded as the initiating lesion of CML (2, 3). Until quite recently the only treatment to offer the possibility of long-term disease free survival was allogeneic stem cell transplantation (allo-SCT), the 'curative' effect of which is mediated in large part through the allo-immune graft-versus-leukaemia effect (4). However, allo-SCT carries a substantial risk of mortality and is only available to a minority of patients. Because of their lower toxicity and impressive efficacy, tyrosine kinase inhibitors, notably imatinib, have replaced allo-SCT as first-line therapy for CML. Although over 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 q-PCR for BCR-ABL transcripts and almost all will relapse following imatinib withdrawal (5, 6). Functional leukaemic CD34+ progenitor cells have been identified in such patients in CCyR, suggesting the presence of a reservoir of leukaemic cells resistant to the TKI (7). Furthermore the durability of these responses has not yet been established. In contrast long-term survivors of allo-SCT very rarely have any detectable molecular disease, indicating that all leukaemic cells must be susceptible to immune destruction (graft versus leukaemia [GVL] effect). Therefore novel strategies to eradicate quiescent CML stem cells are required, especially because these cells provide a reservoir for disease relapse.

The immunlogical effect of allo-SCT and donor lymphocyte infusions (DLI) 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 WT1 using DNA vaccination is an attractive choice for delivering this immune attack. The validity of WT1 as a target for immunotherapy in CML was recently shown in work published by Yong, Rezvani and colleagues in John Barrett's group at the National Institutes of Health (USA) (8). This group studied the expression of leukaemia-associated antigens including WT1 within the CD34+ primitive stem and committed progenitor cell pools in CML patients. WT1 is significantly overexpressed in all CD34+ subpopulations in CML encompassing the most primitive HSC to the most mature cells (8), which escape control by imatinib. Taken in the context of Dr. Rezvani's clinical data and that from other groups, which show that even suboptimal vaccination with peptide can have clinical effects (9-14) (discussed below) these data strongly suggest that active immunotherapy other than allo-transplantation holds significant promise by the induction of tumour antigen specific CD8+ T cells without adding toxicity.

Clearly it is critical to choose the best clinical setting in which to vaccinate. The data show that the effect of TKI as a drug class on the immune system is variable (15), and can be either suppressive or stimulatory. For imatinib specifically, in vivo data show that it can be immunostimulatory, supporting our proposed study, both in murine (16, 17) as well as human studies (18-20). Furthermore, Wang et al 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 (16). In patients, low frequency CD8+ T-cell responses to 4 leukaemia-associated antigens (LAAs), Abl kinase, Proteinase 3, Telomerase, and WT 1, were detected in CML patients on imatinib (21) and show the immune system's ability to respond to LAAs in the presence of imatinib. It is therefore unsurprising that two vaccine studies using BCR-ABL peptides in patients with CML treated with imatinib (22, 23) clearly demonstrated the successful induction of CD8+ and CD4+ T-cell against the vaccine, even with a suboptimal peptide vaccine approach. Bocchia et al found that anti-leukaemia T-cell responses could be stimulated after vaccination in 9 of 14 patients (22). In the Epic study 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 (23). Dr. Rezvani's group recently performed a prospective analysis of immune responses to vaccination against influenza virus (Flu) and Pneumococcus in 50 CP-CML patients treated with imatinib, dasatinib or nilotinib and 15 healthy controls. Significant CD8+ and CD4+ T cells responses against Flu were induced in patients with CML-CP on TKI 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.

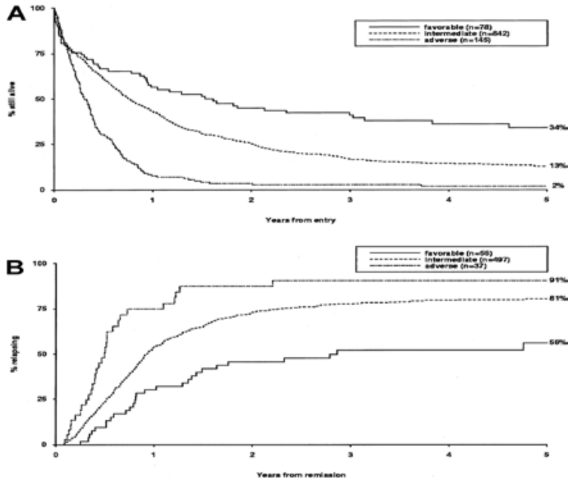
1.1.3 AML

AML is a disease of older adults with a median age of 68 years (24) and an incidence of 8-12/100,000. Advances in our 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 between ages 18–60 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 (Figure 1) (25). Treatment also causes significant morbidity and mortality. Allo-SCT 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 commonest cause of treatment failure following transplant. At this point a minority of patients respond to chemotherapy and donor lymphocyte infusions (DLI), but remission rates are around 15% with only a fraction being durable (19, 20). 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) (26). Additionally WT1 gene expression has been suggested to carry adverse prognostic implications in AML based on data from a number of studies (27, 28). A recent study by the European Leukemianet defined and standardised a WT1 real-time quantitative PCR assay as a marker for MRD monitoring and risk stratification in AML (Cilloni et al JCO 2009). We intend to exploit this for the proposed study of WT1 vaccination. As in CML, peptide vaccination has been tested with some success (10, 13, 29-32) and the data support that active immunotherapy other than allo-transplantation holds significant promise by the induction of tumour antigen specific CD8+ T cells without added toxicity.

We propose to build on our established programme of DNA fusion gene vaccination delivered by intramuscular injection and exploiting our 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. The aim of the trial is to evaluate an identical vaccine strategy in two parallel settings with the purpose of identifying the most promising context for eventual phase III testing. We intend to test the hypothesis 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.

Studying two patient groups will maximise the knowledge gained from this vaccine trial: Patients with CML will allow a direct and objective assessment of the anti-leukaemia 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. We can assess the anti-leukaemia effect of vaccination objectively by measuring WT1 gene expression levels. More importantly, we can gain data on the clinically highly relevant question of whether vaccination will prevent relapse in this patient group. Based on the MRC AML 11 trial data, it is anticipated that 60-75% of patients enrolled in this trial will relapse in 2 years (Figure 1) (25).
For both CML and AML the HLA A2 negative patients will be prospectively followed as control groups.



Grimwade, D. et al. Blood 2001;98:1312-1320

Figure 1. Survival and risk of relapse in AML

Overall survival (A) and relapse risk (B) in MRC AML11 trial are shown by hierarchical risk group. The risk groups were as follows. Favourable group: t(15;17), t(8;21), or inv(16), whether alone or in conjunction with other abnormalities. Intermediate group: normal karyotype, all other noncomplex abnormalities. Adverse group: complex karyotype (5 or more unrelated abnormalities), excluding cases with t(15;17), t(8;21), and inv(16).

1.2 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 cohorts to be studied here have therefore been chosen to reflect this conclusion.

1.3 IMMUNOTHERAPY IN HAEMATOLOGICAL MALIGNACIES TARGETING WT1

DNA fusion vaccines were initially developed by our group to treat B-cell malignancies (33). We showed that fusion of the microbial sequence, Fragment C (FrC) from tetanus toxin to idiotypic tumour antigen provided the T cell help required to induce humoral (34) and CD4+ T cell responses in pre-clinical models (35). Early clinical testing was undertaken in a phase I/II dose escalation study (LIFTT trial; GTAC 029A), with individual idiotypic DNA fusion vaccines to treat patients with follicular lymphoma. The vaccine was safe, and 14/18 patients showed an antibody and/or CD4+ T-cell responses against the FrC portion of the fusion gene. Encouragingly, 6/16 showed responses to the tumour-specific idiotypic antigen (manuscript in preparation). Between doses ranging from 500-2500µg/dose there was no evidence of a dose/response (36). Overall however, the levels of response were relatively low and improvements were sought.

An important development has been electroporation (EP), which dramatically increased DNA vaccine performance in mice (37) and rhesus macaques (38) and this has been included in our clinical trial in patients with prostate cancer. We find 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 fragment C (FrC) sequence to a single domain (p.DOM). This decreased the potential for peptide competition but retained the MHC class II-restricted peptide p30 (40). An epitope-specific sequence was then inserted at the C terminus of FrC to aid processing/presentation. In multiple models (36), this p.DOM-epitope design (**Figure 2A**) was able to induce high levels of epitope-specific CD8+ T cells.

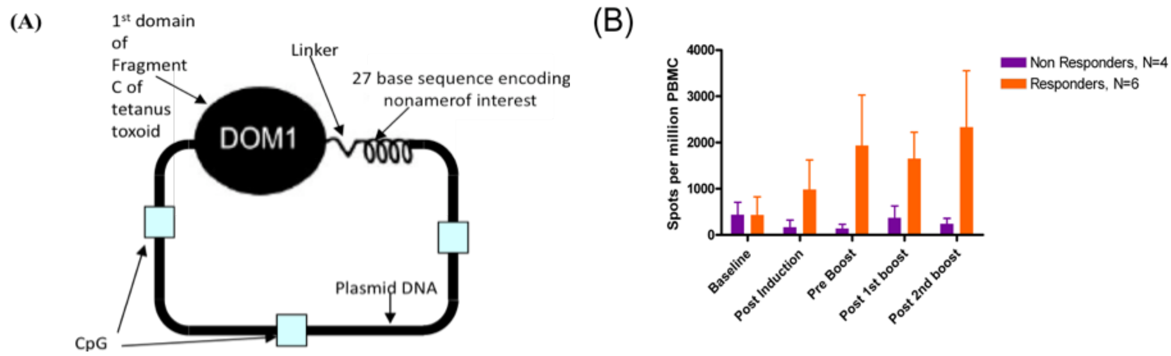


Figure 2. Vaccination of patients with the p.DOM.epitope vaccine.

(A)The p.DOM epitope vaccine consists of a DNA plasmid backbone incorporating CpGsites. The first domain of tetanus toxin (DOM; TT865-1120) provides T cell help, when linked to a tumour associated nucleotide sequence, encoding the HLAI binding epitope of interest. This format allows the appropriate processing and presentation of the peptide.

(B)HLA A2+ patients with biochemical failure of prostate cancer were treated in a Phase I/II, two arm, dose escalation study. Patients were eligible if their tumor expressed PSMA. 3 monthly doses of DNA (p.DOM.PSMA27) were delivered either by intramuscular (i.m.) injection (800, 1600, 3200µg) or i.m. electroporation (EP) (400, 800, 1600µg) with 5 patients at each dose level. Booster 1 was given at 6, boost 2 at 12 months. 30 patients have been recruited. Immunological monitoring is being undertaken by ELISPOT assays, validated to GCLP. The figure shows data from the first dose cohort, analyzed in a cultured ELISPOT. 6/10 patients responded to vaccination with a significant increase in the spots/million PBMCs producing IFNg compared with base line levels, measured at week 0.

Importantly, provision of high levels of T-cell help enables induction of immune responses in tolerant settings (36, 40).

We are also able to show that the preclinical data appear to predict for responses in humans (41). For patients with relapsed prostate cancer, a p.DOM-epitope design incorporating a peptide sequence from PSMA (GTAC 089) has induced high levels of epitope-specific IFN-γ, producing CD8+ T cell responses in 66% (10/15) patients (42) (data from the 10 patients in the lowest dose levels of DNA and DNA/EP are shown in **Figure 2B**. This was the first ever study to exploit delivery of DNA by electroporation, and we found this approach to be safe and readily accepted by our patients (41). Responses are robust and persistent over many months to the end of follow up on trial at 18 months (**Figure 2B**).
Figure 3 (A)-(D) illustrates the CD8 analyses in more detail. In **panel (A)** and **(B)** two non-responders are shown, one of which **(B)** had pre-existing levels of PSMA27 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 1st booster injection at 6 months. Further data are required to allow interpretation of this observation. In **Panel (C)** and **(D)** two of the 6 responders at dose level 1 are shown. The patient in **panel (C)** was treated with DNA alone followed by DNA delivered by electroporation, the patient in **panel (D)** with DNA/Electroporation on 5 occasions.

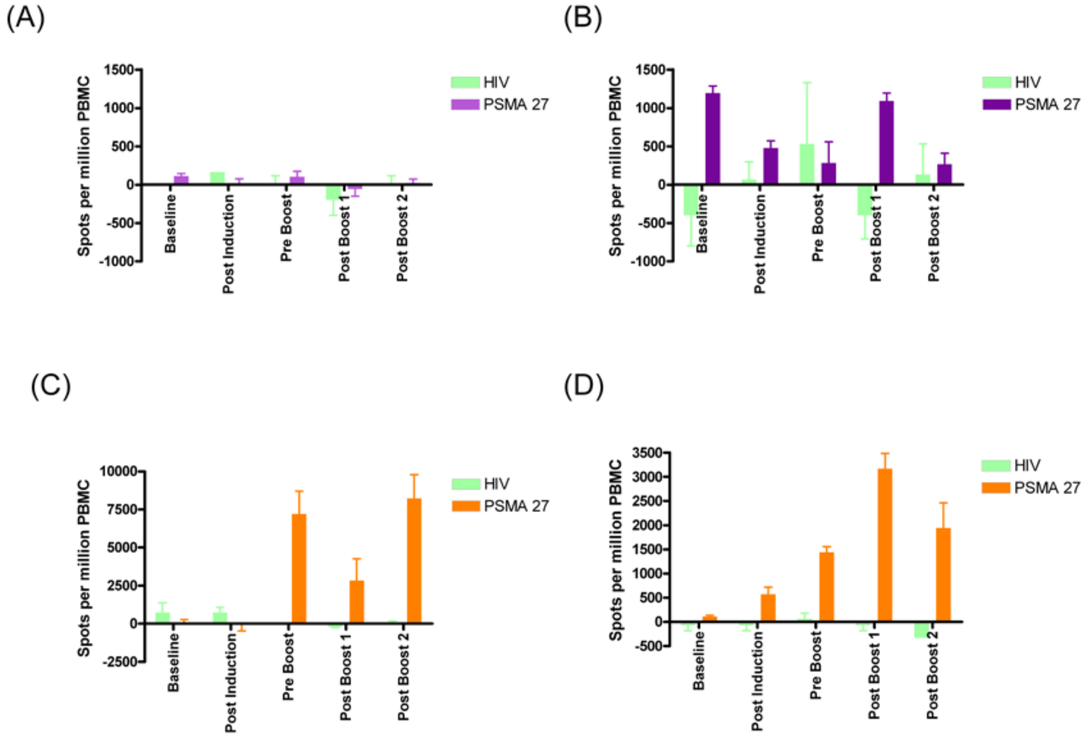


Figure 3. CD8 responses to DNA vaccination analyzed over time by ELISPOT.

(A) and (B) shows data on 2/4 non responders, of which the patient in (B) shows a low-level CD8 response to the PSMA27 at baseline. As there is no significant increase in levels of IFNg producing PBMC above the base line, this is patient has been classified as a non-responder.

(C) and (D) show examples of patients that have significantly increased levels of IFNg producing PBMCs compared to the baseline levels and to the HIV negative control. (N=6 in first dose cohort).

Based on these clinical results, we now wish to explore the effectiveness of the p.DOM-epitope design for the treatment of myeloid malignancies. Wilms' Tumour gene 1 (WT1) has emerged as one of the most promising targets for immunotherapy of haematological malignancies including CML, AML and MDS (10-12, 14). Additionally it is also a potential target for the treatment of solid tumours (14, 43-45). 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 (46-49) where expression is significantly lower (10-100 fold) (46). This could raise a concern about autoimmunity but reassuringly the available data document selectivity of attack against tumour cells, sparing the CD34+ cells (50, 51) and without any evidence of renal or other autoimmune-toxicity in murine models (52-54) or patients (10-12, 14).

We (9, 10) and others have tested WT1 peptide vaccines both in preclinical models (9, 50, 51) and in clinical trials (10-14). 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 in poor persistence of the detected CD8 responses. In contrast we find that the p.DOM-epitope fusion vaccines appear to be able to deliver CD8 responses, which show long term persistence (Figure 2B, Figure 3 (C) and (D)).

Recently we have evaluated three DOM-epitope vaccines, each encoding a different, previously described, WT1-derived, HLA A2-restricted peptide (9). All were able to induce CD8+ T cell responses in "humanized", and presumably tolerized, mice expressing HLA A2 and these killed 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 (9). 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. WT1.37 and WT1.126 peptides were selected for current studies. We have already documented clinically the ability of p.DOM-epitope vaccines to induce CTL 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 study we wish to continue using electroporation as a delivery strategy.

The aim of the study proposed here is to bring together our substantial preclinical and clinical expertise to exploit the advantages of DNA fusion vaccines to form the basis for larger, randomized studies.

1.4 ELECTROPORATION TO AMPLIFY THE RESPONSE TO NAKED DNA VACCINATION

Electroporation (EP) is the delivery of electrical pulses to destabilize the cell membrane and make it permeable for macromolecules such as DNA. Electroporation has been used to introduce DNA into different cell types in vitro, and has recently also shown success in in vivo applications. Gene transfer by EP has been obtained in skin (55), corneal endothelium (56), tumours (57-59), brain (60), liver (61, 62) and muscle (63, 64) of experimental animals. Electroporation is used in clinical treatments of tumours to enhance uptake of water soluble cytostatica (65).

DNA immunization has shown to be potent in small animals but on its own may be less efficient in larger animals. A limiting factor is the uptake of DNA. Low levels of uptake will result in low expression levels and the antigen may then be expressed at levels below the limit needed to induce an immune response. Electroporation enhances the antigen expression and the immune response is increased significantly. Small animal studies that show compared to naked DNA alone, DNA in combination with electroporation can be given at much reduced levels and still induce similar or better humoral and cellular immune responses.(66-68)

Electroporation is thought to enhance immunization in several ways. Antigen expression may be improved and the resulting high concentration of vaccine derived protein may be important for reaching a threshold for the immune response to the vaccine. Secondly transient and reversible muscle cell damage will occur; this damage is likely to have effects similar to those of adjuvant and will provide a “danger signal” that attracts antigen presenting cells (APC) to the site (69). It is also possible that other cell types beyond muscle cells may be transfected in the muscle tissue when electrical stimulation is used. (70)

An interesting effect of electroporation mediated DNA immunization is an IgG subclass shift. By electroporation the IgG2 and hence the cellular response is much more pronounced than without EP (Tollefsen et al., 2002). Ulmer reported enhanced cellular immune responses in non-human primates at the DNA vaccine meeting in Edinburgh 2002. Rath et al have shown that using gene gun, an efficient IgG1 response is obtained. However, using electroporation, both IgG1 and IgG2a responses were enhanced (66).

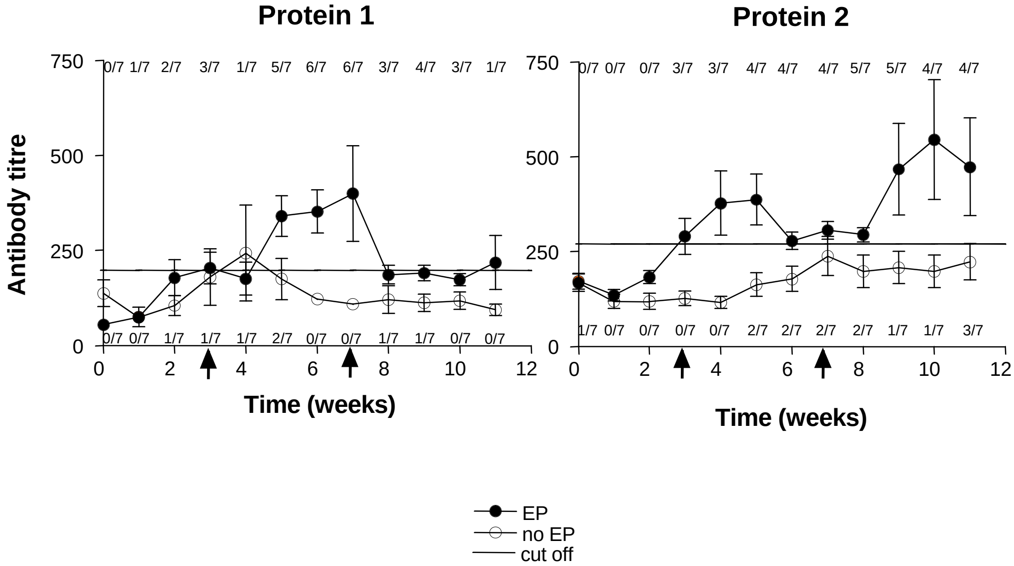


Figure 4: Humoral immune response following DNA vaccination with two different H. contortus Ag in sheep. *Ab titre in serum of each animal was determined and the group average was calculated. The line across the graph represents the average of the negative controls. DNA vaccination with EP is depicted as black symbols while the non-EP group is shown as white symbols. SEM are depicted for each point, The ratios represent the number of animals out of the total with titres above the control values for the EP group (top) and the non-EP group (bottom). The arrow represents DNA boosts with EP*

In collaboration with Inovio AS, we have shown that electroporation based gene delivery results in a stronger immune response, both cellular and humoral, compared to plasmid injections without EP (37). While in our hands 50µg in 50µl of naked DNA appears to deliver the optimal immune response in mice, suboptimal concentrations and volumes could be injected while still retain a strong response if the EP was used. This may be a considerable advantage in larger animals and humans were it is impossible to scale up the dose and volume proportionally to the body mass.

It is possible that we will find that the immune response is weak in patients and we may have to explore heterologous methods of boosting this immune response. However our current murine data show that our vaccine can deliver a very strong and consistent CTL response when we use p.DOM as an immune alert signal in the construct. It appears likely that electroporation will improve the antigen delivery to muscle cells and to local APC and enhance the amount of antigen available for stimulation of an immune response. For this reason we feel that a trial with the proposed construct is warranted without further heterologous boosting strategies.

1.5 INVESTIGATIONAL PRODUCT

The analyses of the immune response to FrC-encoding DNA vaccines has revealed the promotion of antibody and CD4+ T cell responses. If the aim however is to induce cytotoxic T cells, the MHC I motifs encoded in FrC might compete with the target sequence for immunodominance. Removal of the second, C-terminal domain of FrC results in a DNA sequence, which is a potent stimulator for CTL responses against peptide sequences, linked 3' to the N terminal domain of FrC.(71)

We have so far used DNA vaccination to induce immunity against tumour derived idiotypic protein for patients with B-cell malignancies. These vaccines are patient specific and contain the tumour-derived immunoglobulin variable region genes, linked to an immune alert signal, which is the non-toxic fragment C (FrC) from tetanus toxin.

In vitro DNA idiotype vaccine against the tumour (35) unexpectedly but reproducibly protected animals against myeloma. The response was not antibody driven because there was no surface Ig on the tumour cells. Moreover, there is no candidate CTL motif in the scFv of the murine model myeloma, 5T33. It is likely that CD4+ T cells were the mediators of protection, suggesting that DNA vaccination favours the Th_1 response needed for protection.

The bacterial DNA which forms the plasmid backbone of the vaccine has potent adjuvant or immunostimulatory properties. These can be exploited to generate highly effective CTL and Th_1 responses.(72) These immunostimulatory effects are determined by the presence of specific unmethylated CG-containing sequences. DNA containing these sequences stimulates the innate immune system, leading to the production of IFNγ by NK cells and IFNα, IFNγβ, IL-12 and IL-18 by macrophages. The cytokine milieu created by the immunostimulatory sequences (ISS) biases the immune response to simultaneously delivered antigen, favouring the differentiation of naïve T helper cells to the Th_1 phenotype. Secretion of IFNγ by Th_1 cells favours the activation of CTLs.

Several studies are currently ongoing or have completed. They enrol patients with follicular lymphoma (LIFTT), multiple myeloma after autograft (MMIFTT), prostate cancer and CEA expressing malignancies. The latter two studies use vaccines with the p.DOM-epitope design.

All studies to date focus on immunological readouts. We find a reproducible and durable induction of immunity (cellular and humoral) against FrC and DOM in all studies. More importantly we find strong evidence for induction of immunity against the tumour associated antigens, both in the FrC based vaccines (LIFTT and MIFFTT studies) and in the solid tumour studies using the p.DOM-epitope design. In the prostate study, strong and durable anti-epitope specific responses to the HLA A2 restricted epitope encoded in the vaccine were observed in over 2/3 of the patients analyzed to date. (73)

Based on our preclinical data using WT1 constructs (9) we now propose to test these in patients with haematological malignancies and using disease response as the primary endpoint.

1.5.1 STRUCTURE OF VACCINE

The vaccine is a circular plasmid DNA (pUMCV). The insert of DNA unique to the vaccine consists of p.DOM linked to the WT1 epitope sequence. The expression of the insert p.DOM-WT1 is regulated by a CMV promoter. The sequence of the DNA insert is:

pcUMCV – 4030 nucleotides
italics: domain 1 (p.DOM) from Fragment C
* WT1 epitope.

Two vaccines will be evaluated in this study:

p.DOM-WT1-37 with the sequence:

VLDFAPPGA (aa37-45) *GTGCTGGACTTTGCGCCCCCGGGCGCT*

p.DOM-WT1-126 with the sequence :

RMFPNAPYL (aa126-134) AGGATGTTTCCTAACGCGCCCTACCTG

TAA is a stop codon for translation

AGCTTGCCGCCACCATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACC
TTGATTGTTGGGTCGACAACGAAGAAGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAAC
AACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCTGTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCA
ACGGCAAAGCTATCCACCTGGTTAACAACGAATCTTCTGAAGTTATCGTGCACAAGGCCATGGACATCGAATACAAC
GACATGTTCAACAACTTCACCGTTAGCTTCTGGCTGCGCGTTCCGAAAGTTTCTGCTTCCCACCTGGAACAGTACGGC
ACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTGAAG
GGTAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTCAGATCACTTTCCGCGACCTGCCGGACAA
GTTCAACGCGTACCTGGCTAACAAATGGGTTTTCATCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATC
AACGGCGTTCTGATGGGCTCCGCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCT
GGACCGTTGCAACAACAACAACCAGTACGTATCCATCGACAAGTTCCGTATCTTCTGCAAAGCACTGAACCCGAAAG
*AGATCGAAAAACTGTATACCAGCTACCTGTCTATCACC*WT1 epitope*TAA*

1.5.2 MECHANISM OF ACTION OF VACCINE

The DNA vaccines are designed to induce protein expression of the p.DOM-WT1-37 and p.DOM-WT1-126 fusion proteins, respectively, in muscle cells and/or local antigen presenting cells.
Presentation of the protein by antigen presenting cells will stimulate anti p.DOM CD4 helper cells, which induce linked T cell help for the induction of CD8+ T cells against WT1-37 and WT1-126.

1.6 PRE-CLINICAL ANTI-TUMOUR ACTIVITY

Preclinical data from mice expressing the human HLA A2 molecule (HHD mice) have been obtained with the proposed DNA vaccine, encoding WT1 epitopes and p.DOM from FrC. We see strong induction of peptide specific CD8+ T cells which are able to kill peptide loaded T2 cells as well as human tumour cells, expressing WT1. No toxicity was observed in these animals after vaccination (9).

1.7 ANIMAL AND HUMAN TOXICOLOGY OF DNA VACCINATION

No adverse events have been observed in any of the animal cohorts studied with any of our DNA vaccine constructs. There is now a large body of data from human subjects, who have been vaccinated in our own clinical trials; the main side effects have been flu-like symptoms (WHO1) and tiredness (WHO1).

Other groups confirm the conclusions that DNA vaccination is safe in patients in a variety of clinical settings (74, 75).

In preparation for this clinical trial we examined, whether there could be bone marrow toxicity associated WT1 vaccination. In our preclinical model in HLA A2 transgenic mice we found no evidence for bone marrow toxicity. Colony formation from bone marrow cells was unchanged, even if high levels of WT1 specific T cells were induced in the animals (9).

1.8 ANIMAL AND HUMAN TOXICOLOGY OF ELECTROPORATION

Electroporation is a physical method which requires the delivery of energy in the form of electrical current to the target tissue. The current is delivered as defined pulses of controlled magnitude, polarity and duration. Too much current, too high current density or too long pulses can be harmful and cause permanent tissue damage. If however the right amount of current is delivered, a transient permeability change is induced in the cell membrane. The collaboration with Inovio allows us access to this group's considerable expertise in this field and we are therefore able to safely incorporate this strategy into our own DNA vaccination approach.

1.8.1 SMALL ANIMALS

Based on experience from small animal studies some muscle tissue damage will occur and is desired. It is however important to minimize the amount of tissue damaged since only cells that recover from transient damage are able to recover and to express the vaccine derived antigen. Skeletal muscle cells regenerate from satellite cells in the vicinity of the damaged muscle cells. This process begins rapidly after injury has occurred. Within 2-3 weeks the regenerated muscle cells appear normal. There is evidence that this process may be prolonged when antigens are expressed by the muscle cells. (Erik Grønnevik et. al unpublished data) and this may be linked to the immune response against the expressed antigens.

1.8.2 LARGE ANIMALS

A limited number of studies have been performed on larger animals in pigs.(76, 77) These studies were performed both with and without anaesthesia. The treatment was well tolerated without anaesthesia. Only limited muscle cell damage was seen, it is however difficult to find the exact same location of the electroporation since the treatment would influence about 1 x 0.5 x 1.5 cm of the muscle mass per injection/electroporation.

1.8.3 HUMANS

Recently, a clinical trial was performed at Ullevål University Hospital, (Oslo, Norway) to investigate the toxicology and side effects of electroporation alone. 6 healthy volunteers were approached and consented after local ethical approval of the study had been obtained. Only saline was injected and up to 4 applications per volunteer were given over 30 minutes, each into a separate site in the thigh muscles. Pain was assessed by a graded questionnaire and with a visual analogue scale.

First, a pulse (20 ms) at 20 V was given, a second pulse was given at <70 V/<250 mA. Then six pulses of 20 ms were delivered within a period of 1.4 seconds. Delivery of 6 unipolar pulses led to strong contractions of the muscle as well to significant discomfort (CTC $\leq$3) and moderate pain (CTC 2-3). The volunteers where then asked if they would accept a fourth pulse sequence of 6 pulses but now with a bipolar pattern (the polarity was switched during the pulse, 10 ms + 70 V/ 10 ms -70 V). 4/6 volunteers accepted the bipolar treatment. The bipolar treatment caused higher levels of pain and discomfort and may require sedation but quantitative differences was not assessable in this study. The treatment was very short (a total of 1.4 sec) only localized to the muscle and it was the conclusion of the patients that this method of vaccine delivery was tolerable. Acute pain and discomfort resolved immediately after application. The short term side effect was ache at the site of treatment which lasted for 2-3 days, much like pain after physical exercise. No skin reactions were observed. The first post treatment blood samples taken (full blood count, full biochemistry including CK and LDH) showed an increase of creatine kinase level 16-19 h

after the treatment. However, these levels were back to normal at next blood sampling (5 days after the treatment). By this time all subjective sensations related to the EP had resolved.
Our own dataset is now of 150 applications of EP to patients. In the study in prostate cancer no significant toxicity was detected in either biochemical, haematological or autoimmune parameters after delivery of DNA with EP (41). While painful, the pain and discomfort in our patients resolved within minutes of vaccination (41) and prophylactic or symptomatic administration of pain medication was not required (41).

1.9 RATIONALE FOR THE PROPOSED STUDY

The information of DNA vaccination in humans has closely resembled the murine data so far. DNA vaccination in humans appears to be safe (73, 78). The data from our own experience is now based on >80 patients in the mentioned studies. No vaccine related serious adverse events have been observed; the major side effects were ache at the site of injection, mild flu-like symptoms and tiredness.

In trials other than our own, DNA vaccines given to patients, including immunocompromised individuals have had only minor ill effects, comparable to what is seen with conventional vaccines. DNA vaccines against malaria have now been safely administered to normal volunteers (75, 78, 80). Monitoring of safety on this study will follow our previous protocols.

We plan to study the effect of DNA vaccination with two DNA vaccines, p.DOM-WT1-37 and p.DOM-WT1-126 in HLA A2+ patients with CML-CP and AML. Patients will be tested for HIV, HepB, C and Syphilis to protect the laboratory personnel and because these infections may have a significant impact on the immunocompetence of the patient.

2 TRIAL OBJECTIVES

The objectives are to evaluate:

1) Molecular response in patients with CML (BCR-ABL, WT1) and AML (WT1).
2) Time to disease progression, 2 year survival rate (patients with AML)
3) Correlation of molecular responses with immunological responses.

2.1 PRIMARY ENDPOINT

2.1.1 CML:

Molecular response of BCR-ABL (major or minor response or CMR, as defined in section 9.6).

2.1.2 AML:

Time to disease progression. Disease progression in AML is defined as disease relapse.

2.2 SECONDARY ENDPOINTS

2.2.1 CML:

1) Molecular response of BCR-ABL (major response or CMR, as defined in Section 9.6).
2) Molecular response of WT1 (major or minor response or CMR, as defined in Section 9.6).
3) 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 (81):
   - WBC < 10 x 10^9/L
   - Basophils < 5%
   - No myelocytes, promyelocytes, myeloblasts in the differential
   - Platelet count < 450 x 10^9/L

- Spleen nonpalpable

4) 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.
5) Duration of molecular response:
    a. Measured from the beginning of tyrosine kinase inhibitor treatment.
    b. Measured from the time of obtaining informed consent
6) Overall survival.
7) Toxicity assessed according to NCI CTCAE v4.0.
8) Pain assessment immediately after vaccination
9) Pain assessment at 48 hrs post vaccination

2.2.2 AML:

1) 2 year overall survival
2) 2 year progression free survival.
3) Overall survival.
4) Molecular response of WT1 (major or minor response or CMR, as defined in section 9.6).
5) Toxicity assessed according to NCI CTCAE v4.0
6) Pain assessment immediately after vaccination
7) Pain assessment at 48 hrs post vaccination

2.2.3 HLA A2 positive patients only (CML and AML):

1) Immune response to WT1-epitope specific T cells in blood and/or bone marrow, using validated assays by ELISPOT and/or ICS and tetramer staining. A positive response using the validated assays by ELISPOT is defined in Section 9.6.
2) Number of WT1 specific T-cells after peptide challenge to the skin (wherever assessment is feasible).
3) Immune response to DOM, using validated assays by ELISA.
4) Immune response to DOM specific T cells, using validated assays by ELISPOT.
5) Number of humoral responses (B-cells) to the vaccine components, by ELISA.

3 TRIAL DESIGN

This is a non-randomised open label, single dose level Phase II study in two patient groups (CML and AML) based on HLA A2 genotype. Consented and eligible HLA A2+ patients will be vaccinated with two DNA vaccines, p.DOM-WT1-37 (epitope sequence: VLDFAPPGA) and p.DOM-WT1-126 (epitope sequence: RMFPNAPYL). Patients with HLA A2-ve genotype will therefore form the unvaccinated control group. The sample size for the HLA A2+ group has been determined using a A'Hern's single stage study design.

Intervention group of HLA A2+ patients:
32 HLA A2 +ve patients with data to at least 16 weeks will be recruited into the CML treatment group.
39 HLA A2 +ve patients with data to at least 16 weeks will be recruited into the AML treatment group.

Control groups of HLA A2-negative but otherwise eligible, consenting patients:
CML: Control group: all eligible and consenting patients who are HLA A2 negative
AML: Control group: all eligible and consenting patients who are HLA A2 negative

3.1 PATIENT EVALUABILITY AND REPLACEMENT

Where patients do not fulfil the necessary criteria to be eligible for analysis (as set out in Section 10.2) additional patients may be recruited in their place.

4 SELECTION AND WITHDRAWAL OF PATIENTS

4.1 INCLUSION CRITERIA

CML patients:

Philadelphia chromosome positive CML in chronic phase, in complete cytogenetic response (CCyR) but with detectable BCR-ABL transcripts and maintained the CCyR on tyrosine kinase inhibitor monotherapy for a minimum of 24 months

AML patients:

Patients with WT1+ AML complete remission (CR) post chemotherapy or AML in morphologic CR with incomplete blood count recovery (CRi) defined as patients who fulfil all of the criteria for CR except for residual neutropenia (<1,000/µL) or thrombocytopenia (<100,000/ µL).

As the vast majority of AML express WT and evaluation in CR or CRi is technically not feasible, formal demonstration of WT1 expression in AML cells is not required. Where historical or relapsed samples become available, WT1 expression status will be evaluated post hoc.

All patients:

- ≥ 18 years of age,
- Written informed consent
- WHO Performance status of 0 or 1.
- For vaccination groups: HLA-A0201 positive in at least one allele
- For control groups: HLA A2 negative in both alleles
- Renal function and liver function (Creatinine <1.5 x upper limit of normal, liver function tests < 1.5 x upper limit of normal); Lymphocyte count $\geq$ 1.0 x10^9/l*; normal clotting
- HB>100 g/l
- Adequate venous access for repeated blood sampling according to protocol schedule.
- If sexually active and possibly fertile, patients must agree to use appropriate contraceptive methods during the trial and for six months afterwards.

* If the lymphocyte count is below 1.0 at the time of entry into the trial but has been over 1.0 in the last 6 months and has also not declined rapidly in the days and weeks preceding entry, then the patient is eligible.

4.2 EXCLUSION CRITERIA

CML patients:

- CML in accelerated phase or blast crisis or having achieved CMR at any point during tyrosine kinase inhibitor therapy
- Tyrosine kinase inhibitor change or dose modification in the previous year, therapy interruption for more than 15 days in the previous 6 months to enrolment
- Prior interferon-α therapy
- Hypocellular bone marrow (<20%) (indicated by blood counts and most recent bone marrow (where available)
- Complete molecular response (CMR)

AML patients:

- AML in haematological relapse or eligible for allogeneic SCT.
- Hypocellular bone marrow (<20%)
- AML patients with the "good-risk" abnormalities comprised 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 are forbidden during the trial. The predictable need of their use will preclude the patient from trial entry. Inhaled steroids are allowed.
- Major surgery in the preceding three to four weeks from which the patient has not yet recovered.
- Patients who are 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 (NYHA) class III/ 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 have undergone potentially curative therapy for a prior malignancy, have no evidence of that disease for five years and are deemed at low risk for recurrence, are eligible for the study.
- Patients who are serologically positive for or are known to suffer from Hepatitis B, C, Syphilis or HIV. Counselling will be offered to all patients prior to testing.

4.3 REGISTRATION PROCEDURE

All registration and baseline assessments should be completed prior to registration.
Only patients fulfilling the eligibility criteria should be registered, any queries should be discussed with the University of Southampton Clinical Trials Unit before registration

All patients must be registered via the University of Southampton Clinical Trials Unit on:

Tel: +44 (0) 23 8079 4507

The patient's eligibility will be checked during the registration process. Eligible patients will be allocated a unique patient trial ID by the University of Southampton Clinical Trials Unit.

4.4 WITHDRAWAL CRITERIA

Patients who are removed from the study treatment due to adverse experiences (clinical or laboratory) will be treated and followed according to accepted medical practice. All pertinent information concerning the outcome of such treatment must be recorded in the CRF.

Patients who withdraw their consent from participating in the trial will be withdrawn from protocol procedures. Data that has been collected on these patients to this timepoint will be included in the analyses.

The discontinuation of study treatment will occur under the following circumstances

- Unacceptable toxicity

- Any patient who experiences a WHO CTC grade 3 adverse reaction (AR), possibly or likely to be related to vaccination will discontinue further vaccination.

- Severe local toxicity (CTC 3 or greater) at the site of injection, development of clinically relevant anti-DNA antibodies or rheumatoid factors, clinical or biochemical evidence of muscle destruction (CTC 3 or greater) or other unexpected toxicity will lead to termination of the vaccination of that patient.

- Unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable

- SAE requiring discontinuation of treatment

- The patient withdraws consent - where a patient is not evaluable, additional patients will be recruited to replace them (See Section 3.1)

- Serious violation of the study protocol (including persistent patient attendance failure and persistent non-compliance)
- Evidence of disease progression

- In patients with CML requirement to discontinue or change the dose of tyrosine kinase inhibitor therapy

4.5 DISCONTINUATION OF THE CLINICAL STUDY

The entire trial will be stopped if:

- Life-threatening vaccination-related toxicity is observed in more than one patient, termination of the trial will be discussed among all collaborators and the trial sponsor.
- The vaccine and/or its application is considered too toxic to continue treatment prior to meeting the recruitment target.

If the trial is discontinued patients should be treated according to accepted medical practice.

5 TREATMENTS

HLA A2-ve patients will receive standard care in accordance with accepted medical practice.

HLA A2+ patients will receive the treatment described in Sections 5.1 to 5.6:

5.1 VACCINE DOSE

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

5.2 VACCINE SCHEDULE

The DNA vaccine will be administered 6 times every 4 weeks followed by a further 6 vaccinations every 3 months to maximum of 24 months.

5.3 VACCINE ROUTE AND SITE

The vaccines will be injected by deep intramuscular injection (IM) followed by electroporation (EP) by a trained health care professional. The vaccines will not be mixed but will be administered into separate sites.
Refer to Section 6.4 for vaccine administration details.

5.4 DOSE MODIFICATIONS, REDUCTIONS AND DOSE DELAYS

There will be no dose reductions or dose modifications.

Haematological toxicity – If patients experience CTC toxicity >2, vaccination will not be administered. Further vaccination may be offered after normalization if appropriate in the opinion of the treating clinician and only after documented discussion with the UoSCTU and lead investigators.

Severe local toxicity (CTC 3 or greater) at the site of injection, development of clinically relevant anti-DNA antibodies or rheumatoid factors, clinical or biochemical evidence of muscle destruction (CTC 3 or greater) or other unexpected toxicity will lead to termination of the vaccination of that patient.

5.5 DOSE ESCALATION

There will be no dose escalation

5.6 CONCURRENT MEDICATION AND REPLACEMENT

Systemic steroids or other drugs with a likely effect on immune competence are forbidden during the trial. The predictable need of their use will preclude the patient from trial entry. Inhaled steroids are allowed. Concomitant medication may be given as medically indicated.

Patients with CML-CP will continue on tyrosine kinase inhibitor therapy and the dose of tyrosine kinase inhibitor therapy will remain unchanged throughout the course of the study. Patients will continue on the same dose of tyrosine kinase inhibitor therapy as prior to enrolment. Details of the concomitant medication given must be recorded in the Case Report Form (CRF).

The patient must not receive other anti-cancer therapy or investigational drugs while on study.

6 PHARMACEUTICAL INFORMATION

6.1 SUPPLY OF VACCINE

The DNA vaccine construct has been prepared and sequenced in the Department of Molecular Immunology, Cancer Sciences Division, University of Southampton.

The bulk preparation and sterile fill will be performed in accordance with Good Manufacturing Practice (GMP) at the MHRA approved laboratory at the Clinical Biotechnology Centre, Bristol. This facility has also received approval for vaccine preparation by the FDA and conforms to GMP standards according to the new European guidelines. The vaccine will comply with stringent Quality Control (QC) criteria, as for our previous trials. Vaccine batches will be controlled for sterility, purity, endotoxin level and by restriction enzyme digest and nucleotide sequencing before release for clinical use.
A complete certificate of analysis will be provided with each batch of vaccine and will be retained in the trial Investigator's File/ Pharmacy File.

For information on the vaccine contact either the Coordinating Investigator or:

Paul Lloyd Evans
Clinical Biotechnology Centre
Bristol Institute for Transfusion Science
Work address
Churchill Bldg, Langford House, Lower Langford
Bristol BS40 5DU
Telephone (0117) 928-9388
E-mail address paul.lloyd-evans@nbs.nhs.uk

A copy of the drug shipment form will be sent to the MHRA by the supplier.

6.2 PHARMACEUTICAL DATA

6.2.1 STUDY AGENT FORMULATION

The concentration of DNA will be 1mg/dose for p.DOM-WT1-37 and 1mg/dose for p.DOM-WT1-126 (*at a final concentration of 1 mg/0.8mL*). The vaccine is supplied in standard Phosphate Buffer Solution (PBS). The DNA for injection will be divided into aliquots for storage at -70^{O}C in sterile glass vials and aliquots for sterility and stability testing. The testing will be based on the guidelines for injectables described in the European Pharmacopoeia. The most likely contaminant is protein, this is expected to be <1%. The material will be confirmed as pyrogen free by using a limulus test (Bio Whittaker (UK) Ltd). After delivery to the Hospital Pharmacy the vaccine will be stored at -70^{o}C.

6.2.2 STORAGE CONDITIONS & STABILITY OF UN-RECONSTITUTED INVESTIGATIONAL DRUG FORMULATION

The vaccine must be stored -70°C or below. Before thawing, the vaccine is stable for at least 2 years @ -70°C, based on our previous experience with DNA fusion vaccine plasmids produced in Bristol.

6.2.3 METHOD OF RECONSTITUTION

The vaccine should be thawed for approximately 5 minutes before injection at room temperature, and is stable for 24hrs. Shaking is not necessary.

6.2.4 STABILITY AFTER RECONSTITUTION AND LABELLING

The thawed vaccine must be used within 24 hours.

<u>Labelling requirements for the vaccine</u>
Amount and Name of Vaccine
Total volume
Concentration
Date of preparation
Expiry date and time

6.3 ELECTROPORATION DEVICE

The Elgen1000 is an electroporation 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 which temporarily increases cellular membrane permeability allowing the plasmid DNA to enter the cells.

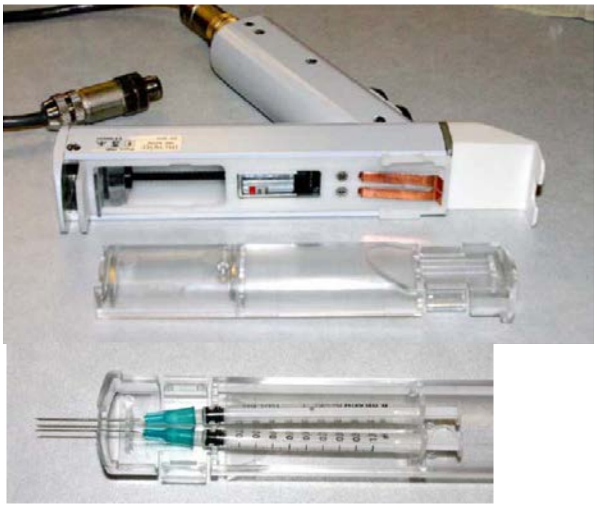
The Elgen1000 consists of an electrical pulse generator (Control Unit) and an automated injector unit for delivery of drug (DNA) and electrical pulses. Upon user activation the disposable needles mounted in the Injector Unit advances into the muscle at the same time as the DNA is injected. Upon user activation, when the insertion/injection comes to an end, the Control Unit to deliver a sequence of electric pulses to the needle electrodes on the Injector Unit. The resulting electrical field at the treatment site produces the required environment to enhance cell membrane permeability allowing DNA to efficiently enter the cell interior.

The Control Unit is a durable medical electronic apparatus that provides series of short duration, moderate voltage electric pulses. Treatment protocols defining electrical pulsing patterns as well as injection volumes and electrode insertion depth can be pre-programmed on an onboard flash card. Patient data and treatment records can also be stored in the flash card memory. For the proposed trial a pulse sequence of 2 pulses of 60ms pulses at 400 mA will be used. Studies in rhesus macaques shows that these parameters give an electrical field of approximately 125V/cm using two parallel needle electrodes 4mm apart and inserted approximately 15 mm into the tissue.

The unit connects to line voltage and consists of a medical grade power supply unit (PSU), high-voltage power circuit and a low-voltage digital control circuit. The PSU provides patient / user electrical isolation and converts local line power to the voltage required by the instrument. The control circuit consists of a standard microprocessor-based controller board for the user interface, and a separate and insulated embedded microprocessor for the real time hardware interface. The high voltage is generated by a series of DC-DC converters on the power circuit board and the voltage is discharged and regulated through a power transistor. An array of power transistors is used to route the sequence of pulses to the needles, as well as to control the polarity of pulses.

The Injector Unit is an automated two motor device; one electrical motor drives a movable carriage where a sterile lid containing two disposable syringes with needles are mounted. The second motor operates the pistons of the syringes for the injection of DNA. Needle insertion depth (0-20 mm), and injection volume (20-600 ul) are programmed in the control unit. After the treatment, the lid containing syringes and needles will be disposed of along with a single use injector tip. The needles are connected to the Control Unit through insulated wires, which run through the Injector Unit handle and terminates in an integrated connector. An insulated cable, reaching 6.5 feet, connects Injector and Control Units. The connector attaches to the front panel of the Elgen 1000 Control Unit.

The DNA filled syringes with needles are positioned in a disposable lid and mounted on the top of the Injector Unit. The lids are manufactured to accommodate 22 Gauge needles. The distance between the needles electrodes are 4 mm.



Control Unit Injection Unit with lid and syringes containing the vaccine

The Elgen1000 electroporation device

6.3.1 TRAINING AND EXPERIENCE REQUIRED

The investigation will be performed under the supervision of a trained and experienced physician. The operator will be competent in the use of all parts of the device and be able to identify the proper area to be injected. The operator will have undergone formal training and be familiar with:
The instructions for use
Handling of the injection device (mounting the standard syringes and needles)
Operation of the electroporator

Training will be provided by INOVIO BC, San Diego and/or by the clinical trials team from Southampton.

6.4 VACCINE ADMINISTRATION

6.4.1 DNA VACCINATION WITH ELECTROPORATION

The two syringes with two parallel needles, 4 mm apart, will be filled with DNA and mounted in the Injection unit. The front of the Injector will be placed against the skin of the patient and insertion of the needles initiated. At approximately 5mm tissue depth, injection of the DNA starts automatically during further insertion (15-20 mm). A total of 400 ul DNA will be injected. Immediately after injection and insertion has stopped, electrical pulses are delivered to the tissue using the injection needles as electrodes. When the stimulation has ended, the needles are automatically withdrawn. For each treatment 2 unipolar pulses of 60 ms at 200 ms interval using a current of 400mA (approximately 125V/cm) will be applied. The procedure from needle insertion to needle withdrawal will take approximately 5 seconds.

Painkillers (eg. paracetamol) will be provided. Of note, this was not necessary for any patient in the study GTAC 089.

6.4.2 EXAMINATION OF THE INJECTION SITE

At all visits the physical examination will include a careful examination of the injection site including measurements of the circumference of the extremity where appropriate if there is clinical evidence of a local reaction. Patients will be monitored throughout the study period for anti-DNA antibodies, rheumatoid factors and evidence of muscle destruction. Levels of anti-DNA antibodies and rheumatoid factors will be measured according to standard local ranges. If these tests should become significantly positive, after previously being absent or normal or other clinically significant signs of autoimmunity appear, vaccination will be terminated and rheumatology consultation will be sought.

6.4.3 ASSESSMENT OF PAIN AFTER INJECTION

Acute pain immediately after injection and delayed pain will be assessed by questionnaire for all patients (see Appendix 1 and 2).

6.4.4 ACCIDENTAL SPILLAGES

Accidental spillages should be dealt with according to hospital policy. The product is non-toxic and non-infectious. Spillage will be treated with 1000ppm hypochloride solution. It can then be wiped up and the tissue disposed of in autoclavable bags/containers. Syringes will be disposed of in autoclavable containers.

6.5 DRUG ACCOUNTABILITY

Accurate records of all drug shipments, vaccine dispensed, and all vaccine returned must be maintained. This inventory record must be available for inspection by UHS or the UoSCTU at any time. Drug supplies are to be used only in accordance with this protocol and under the supervision of the Investigator.

Empty vials of vaccine will be shipped back to, the Quality Assurance Manager, Cancer Research Building, Southampton General Hospital, Tremona Road, Southampton SO16 6YD by first class post using appropriate packaging. Alternatively they will be collected by a member of the UoSCTU staff at a scheduled monitoring visit.

The Investigator undertakes not to destroy any unused drug unless directed to by UHS or the UoSCTU. Any destroyed study drug must be destroyed according to hospital procedures and properly accounted for. At the conclusion of the study the overall numbers of the drug shipped to the centre, and the number destroyed or returned will be provided by the pharmacy, and an account given of any discrepancy. Any vaccine remaining will be returned to the Chief Investigator.

7 ASSESSMENT OF SAFETY

The recent reporting of trials using DNA vaccination in normal volunteers and in HIV infected individuals confirms our own findings that the procedure is safe and extends the safe dose range up to 2,500µg. Thus, although the potential toxicities are discussed below and will be looked for, the procedure appears very safe.

7.1 SAFETY CONSIDERATIONS OF DNA VACCINATION +/- ELECTROPORATION

7.1.1 SAFETY OF THE ELGEN PULSE GENERATOR AND THE TWIN INJECTOR

The Elgen pulse generator and a syringe based electrode system has been tested and approved according to Electromagnetic Compatibility (EN 60601-1-2:1993, EN 61000-3-2:2000, EN 61000-3-3:1995 + A1:2001). It has also been tested according to: IEC 60601-2-10, 1st ed. 1987 + A1: 2001, EN 60601-1-10:2000+A1:2001, Medical equipment, part 2: Particular requirements for the safety of nerve muscle stimulators. (The system failed to pass the limitations requirements for output parameters for nerve muscle stimulators, this is because the currents and voltages needed to perform EP is larger than what is necessary to perform standard nerve-muscle stimulation).

7.1.2 LOCAL TOXICITY AT THE INJECTION SITE

There exists the possibility of pain, tenderness and inflammation at the site of intramuscular injection of the DNA construct, caused by the DNA itself, impurities in the DNA, or by the protein encoded by the DNA construct. Additionally electroporation has so far only been tested in a cohort of healthy volunteers and in 32 patients with prostate cancer. While no local toxicity of grade WHO >2 has been found, careful monitoring of the injection site is therefore crucial:

- Assessment of local toxicity will be according to CTC criteria.
- Any visible local injection sites will be documented electronically by digital photography
- CK and anti-skeletal muscle antibodies will be measured to assess auto-immune muscle damage. If there is evidence of muscle damage myoglobin will be measured using local standards.
- Persistent or rising levels of CK or anti-skeletal muscle antibodies will be taken to indicate muscle damage.
  This is unlikely based on animal experiment but if it occurs the vaccination will be stopped and full rheumatological assessment will be arranged.

7.1.3 ASSESSMENT OF PAIN AND DISCOMFORT POST INJECTION

For all patients pain or discomfort and level of distress will be assessed 1 hr after injection (or after recovery from sedation) and at 48 hrs. The information will be collected, using a questionnaire (see Appendix 1 & 2).

7.1.4 PAIN MANAGEMENT AFTER VACCINATION:

The following are suggested for pain control after evaluation of the pain using the pain evaluation tools in Appendix 1 & 2:

Mild pain/discomfort - 1-4	No intervention
Moderate pain - 5-6	Paracetamol 1 g qds
Severe Pain - 7-10	Co-codamol 30/500 2 tabs qds +/- Ibuprofen 400 mg qds

Patients will be offered paracetamol to take home and will be reviewed early if severe pain occurs.

7.1.5 IMMEDIATE HYPERSENSITIVITY

Immediate hypersensitivity to injected DNA has not previously been observed. Nevertheless, the possibility of anaphylactic, febrile or other systemic reactions exists, either to the DNA itself or to impurities present in the vaccine preparation.

- Vital signs will be monitored closely after administration of the DNA. If anaphylaxis occurs, the patient should be treated immediately, initially with adrenaline, hydrocortisone and chlorpheniramine.

7.1.6 ANTI-DNA ANTIBODIES

Anti-nuclear and anti-DNA antibodies are characteristic of systemic lupus erythematosus (SLE), an autoimmune disease with diverse clinical manifestations. It is not clear whether the anti-DNA antibodies are important in the pathogenesis of this disorder, although the antibody titre does carry prognostic significance. It is conceivable that repeated inoculations with unmodified plasmid DNA could give rise to anti-DNA antibody responses leading to the development of an SLE-like syndrome. However, experimental evidence from animal and our own human studies with ca 100 patients and close to 1000 DNA vaccine applications suggests that this is a very unlikely possibility.

- Anti DNA antibodies will be measured sequentially.
- In the unlikely event that such antibodies should appear, the treatment will be stopped and the rheumatologists will be consulted.

7.1.7 GERM LINE GENE TRANSFER

Gene integration is expected to take place after IM injections at a very low frequency. When EP is applied the probability of integration will increase. Since the amount of DNA entering each cell is likely to increase between 100-1000 fold, a corresponding increase in integration probability will take place. In addition we do not know to which extent the electrical currents will influence the structure of the plasmid or nuclear DNA and if that could increase the integration rate. If there is an enhanced probability of integration, this is likely to be effective only at site of injection and these cells will be eliminated as a result of the immune response.

Plasmid DNA administered by the intramuscular route may gain access to the blood circulation through the walls of small blood vessels in the vicinity of the inoculum. Damage to these blood vessels may occur at the time of DNA inoculation. There is therefore a theoretical possibility that circulating plasmid DNA could localise in gonadal tissues, enter the nuclei and integrate into the chromosomes of germ cells or their precursors. Such genetic modification of germ line cells is undesirable and intentional modification of the human germ line is not permitted in any country.

- Any sexually active and fertile patients will be instructed to use appropriate contraceptive measures in line with the advice given to patients undergoing treatment for potentially mutagenic drugs (eg. cytotoxic chemotherapy). They will be asked to take such precautions at study entry, during the period of vaccination and for six months afterwards.

7.1.8 OTHER SYSTEMIC TOXICITY

Haematological toxicity: It is possible that vaccination with WT1 may cause cytopaenias in patients who have a hypocellular low marrow reserve (hypocellular bone marrow). This is because WT1 is also expressed at low levels in normal CD34+ stem cells. To minimise the possibility of this, we will exclude patients with low marrow reserve (bone marrow cellularity <20%). Patients who develop cytopaenia will be treated with 5 days of intravenous methylprednisone (1-2 mg/kg). This steroid treatment will not start for 2-3 weeks after the start of cytopaenia to allow time to determine if cell counts rebound. They will also be given supportive care including blood products, growth factors and antibiotics as clinically indicated until the counts recover. The development of other systemic toxicity appears unlikely. FBC, biochemistry, in particular renal function and clinical AE will be assessed by CTC criteria.

- If CTC toxicity >2 occurs in a particular patient, vaccination will be paused. Further vaccination may be offered after normalization if appropriate in the opinion of the treating clinician and only after documented discussion with the UoSCTU and lead investigators.

Cardiac or renal toxicity: It is possible but from the available dataset in the literature unlikely, that the patients may develop immune related toxicity that affects the kidney or cardiac function. Patients will therefore have regular monitoring of their renal function, including urine sampling for proteinuria, and an ECG before any vaccination dose is given. Additional tests will be undertaken as clinically indicated, should signs of renal or cardiac dysfunction occur.

8 PHARMACOVIGILANCE

8.1 DEFINITIONS

The Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, provides the following definitions relating to adverse events in trials with an investigational medicinal product:

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered.
All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure (IB) for an unapproved investigational product*).*
When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the IB which occur in a more severe form than anticipated are also considered to be unexpected.

Serious Adverse Event (SAE) or **Serious Adverse Reaction (SAR):** any untoward medical occurrence or effect that at any dose:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

8.2 CAUSALITY

Most adverse events and adverse drug reactions that occur in this trial, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this trial. The assignment of the causality should be made by the investigator responsible for the care of the subject using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the University of Southampton Clinical Trials Unit (UoSCTU) who will notify the Chief Investigator. Pharmaceutical companies and/or other clinicians may be asked for advice in these cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the subject's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the subject's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

8.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the UoSCTU in the first instance. A flowchart will be provided to aid in the reporting procedures.

8.3.1 PRE-EXISTING CONDITIONS

A pre-existing condition should not be reported as an AE unless the condition worsens by at least one CTCAE grade during the trial. The condition, however, must be reported in the pre-treatment section of the CRF, if symptomatic at the time of entry, or under concurrent medical conditions if asymptomatic.

8.3.2 NON SERIOUS AR/AES

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the UoSCTU within one month of the form being due.

8.3.3 SERIOUS ADVERSE EVENTS AND REACTIONS

Fatal or life threatening SAEs and SUSARs should be reported within 24 hours of the local site becoming aware of the event. The SAE/SUSAR form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should assign the causality and expectedness of the event with reference to the current IMP IB and use the event terms and grades given in the NCI CTCAE v4.0. Additional information should be provided as soon as possible if the event/reaction has not resolved at the time of reporting.

Relapse and death due to CML or AML, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

8.3.4 REPORTING DETAILS

An SAE/SUSAR form should be completed for all SAEs and SUSARS and faxed to the UoSCTU within 24 hours.

Complete the SAE/SUSAR form & fax or email a scanned copy of the form with as many details as possible to the UoSCTU together with anonymised relevant treatment forms and investigation reports.

Or

Contact the UoSCTU by phone for advice and then fax or email a scanned copy of the completed SAE/SUSAR form.

SAE/SUSAR REPORTING CONTACT DETAILS

Please fax or email SAE/SUSAR forms to:

Fax: 08447 740621 or Email: ctu@soton.ac.uk

FAO: Quality & Regulatory Team

Tel: 023 8079 5154 (Mon to Fri 09.00 – 17.00)

The UoSCTU will notify the necessary competent authorities and main REC of all SUSARs occurring during the trial according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the trial.

Local investigators should report any SUSARs and /or SAEs as required by their Local Research Ethics Committee and/or Research & Development Office.

8.3.5 FOLLOW UP AND POST-STUDY SAES

The reporting requirement for SAEs affecting subjects applies for all events occurring up to 4 weeks after the last administration of study drugs. All unresolved adverse events should be followed by the investigator until resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's general practitioner, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

8.3.6 SARS NOT REQUIRING IMMEDIATE REPORTING

The most recent Investigator Brochure (IB) for the IMPs being used in this trial will be circulated to participating centres by the UoSCTU and is to be stored in the local site file.

Side effects and toxicities that are expected are listed in the IB do not require immediate reporting in this trial, unless they are of an unexpected severity.
Death as a result of disease progression and other events that are primary or secondary outcome measures are also not considered to be SAEs.
SAEs of the above types should be recorded on the SAE/SUSAR form provided and this should be forwarded to the UoSCTU in a timely manner.

8.3.7 PREGNANCY

If a subject or his/her partner becomes pregnant whilst taking part in the trial or during a stage where the foetus could have been exposed to an IMP, the investigator must ensure that the subject and the subject's healthcare professional are aware that follow up information is required on the outcome of the pregnancy. If the subject leaves the area, their new healthcare professional should also be informed.

9 ASSESSMENT AND FOLLOW-UP OF PATIENTS

It is intended that all patients will be followed on study for up to 36 months from date of informed consent. Date and type of subsequent treatments and survival data for all patients will be collected prospectively.

9.1 PRE-TREATMENT EVALUATIONS

The following investigations should be performed within 1 month of starting treatment. (It is anticipated that these investigations will be performed as standard clinical care; if they are not, consent must be obtained prior to any study related procedure/s):

- Bone Marrow aspirate *for assessment of bone marrow cellularity and immunological evaluation (all patients except HLA A2 negative CML patients) and for disease evaluation (in AML)*
  If a bone marrow sample is available within 1 month prior to recruitment in the study, a second bone marrow examination is not performed, unless clinically indicated.
- Chest X-Ray (HLA A2 positive patients only)
- ECG (HLA A2 positive patients only)
- Echocardiogram (HLA A2 positive patients only)

The following investigations/evaluations should be performed after consent and registration, and prior to starting treatment:

- HLA Status
- Demographic data
- Medical history
- Prior treatment malignancy
- Height and weight
- Physical examination
- WHO Performance status
- Vital Signs
- FBC and differential blood count, ESR
- Clotting
- Biochemistry
- CK test (HLA A2 positive patients only)
- Urine analysis (HLA A2 positive patients only)
- Syphilis, Hep B, Hep C, HIV
- Blood for immunological assays (80 ml of anticoagulated blood)
- qPCR for BCR-ABL and WT1 in CML, for WT1 in AML
- Leukapheresis for immunological studies (HLA A2 positive patients only)

- Autoimmune profile
- Concomitant diseases/treatments
- Adverse Events

Please also refer to the tabulated Schedule of Observations and Procedures (on pages 13-16).

9.2 VACCINE SHIPMENT TO THE PARTICIPATING TRIAL CENTRES

Vaccine will be shipped to sites around the time of activation. Stock levels should be monitored by sites and drug orders should be placed through the UoSCTU. It is advised that at least 7 days notice should be given.

The UoSCTU will place the drug order with Dr. Paul Lloyd-Evans, in Bristol, or his designated deputee who will then authorize and organise shipment of vaccines p.DOM-WT1-37 and p.DOM-WT1-126 on dry ice to the participating centre.

9.3 EVALUATIONS DURING AND AT THE END OF THE STUDY

HLA Status: Registration
Visit 0: Baseline (once HLA status confirmed)

Visit 1: week 0	within 7 days of baseline visit
Visit 2: week 2	14 days after v1 +/- 3 days (Not done for HLA A2 negative patients)
Visit 3: week 4	14 days after v2 +/- 7 days
Visit 4: week 8	28 days after v3 +/- 7 days
Visit 5: week 10	14 days after v4 +/- 3 days (Not done for HLA A2 negative patients)
Visit 6: week 12	14 days after v5 +/- 7 days
Visit 7: week 16	28 days after v6 +/- 7 days
Visit 8: week 20	28 days after v7 +/- 7 days
Visit 9: week 22	14 days after v8 +/- 3 days
Visit 10: week 24-28	14-42 days after v9 +/- 14 days
Visit 11: week 32	56-28 days after v10 (depending on date of v10) +/- 14 days
Visit 12: week 34	14 days after v11 +/- 3 days
Visit 13:	11 months after baseline +/- 14 days
Visit 14:	14 months after baseline +/- 14 days
Visit 15:	17 months after baseline +/- 14 days
Visit 16:	14 days after visit 15 +/- 3 days
Visit 17:	20 months after baseline +/- 14 days
Visit 18:	23 months after baseline +/- 14 days
Visit 19:	24 months after baseline +/- 14 days
Visit 20:	27 months after baseline +/- 14 days
Visit 21:	30 months after baseline +/- 14 days
Visit 22:	33 months after baseline +/- 14 days
Visit 23:	36 months after baseline +/- 14 days

After Visit 23, month 36, follow-up outcomes should continue to be recorded.

If delays occur on vaccination visits, all subsequent visits will be slipped to keep the interval between vaccinations at 1 and 3 months, respectively. The day 14 post vaccination visits (v2, v5, v9 and v12) should be kept as close to 14 days as possible, allowed +/- 3 days.

<u>Investigations required at each visit:</u>

- FBC and differential blood count, ESR
- Biochemistry
- CK test (HLA A2 positive patients only)
- Urine analysis (HLA A2 positive patients only)

- Immunological monitoring*; *65 ml of anticoagulated blood (Lithium Heparin Tubes) and 5 mL clotted blood for serum will be taken for this purpose. (Blood should be taken prior to vaccine being administered).*

Investigations required at visits 1, 2, 3, 4, 6, 7, 8, 11, 13, 14, 15, 17 and 18:
- Height and weight
- Physical examination
- WHO performance status
- Vital signs

HLA A2 positive patients only - Additional investigations required after each vaccination*(Visits 1, 3, 4, 6, 7, 8, 11, 13, 14, 15, 17 and 18):
- ECG
- Echocardiogram (if clinically indicated)
- Assessment of pain and discomfort of vaccination sites
- Additionally, patients will be observed for 2 hours post each vaccination at the trial site. If the absence of adverse events supports this, the TMG may decide to waive this requirement after 12 patients have completed the first 6 doses of vaccination.

**The first HLA A2 positive patient recruited at each site will be evaluated at 48 hours after her/his first vaccination, before additional doses can be given or before additional patients can be vaccinated at that site.*

HLA A2 positive patients only - Additional investigation required Visit 6
- DTH assessment (to be carried out if wherever feasible)

HLA A2 positive patients only - Additional investigations required at Visit 7
- Autoimmune profile

HLA A2 positive patients only - Additional investigations required at Visit 10
- Bone marrow for immunological (CML and AML) and disease (AML) evaluation
- Leukapheresis
- Autoimmune profile
- DTH assessment (to be carried out if wherever feasible)

HLA A2 positive patients only - Additional investigations required at Visit 16
- Autoimmune profile

HLA A2 positive patients only - Additional investigations required at Visit 23
- Autoimmune profile

Please also refer to the tabulated Schedule of Observations and Procedures (on pages 13-16).

9.4 TRANSFER OF SAMPLES

Local laboratories will be used for the analyses of FBC, biochemistry analysis, viral serologies and autoimmune profiles.

Central laboratories

Hammersmith (qPCR)

pPCR for BCR-ABL and pPCR for WT1 (Hammersmith) – all patients, all time points. For shipment from Southampton and Exeter this shipment will be taken by next day delivery post in Royal Mail safeboxes. Samples will be processed in the molecular haematology laboratory, Hammersmith Hospital, Du Cane Road, London.

Royal Mail safeboxes will be provided by the UoSCTU to investigators at participating sites. If further supplies are required, please contact the WIN Clinical Trial Coordinator at UoSCTU.

Southampton (Immunological)
Immunological analyses for vaccine responses, including leukapheresis samples and bone marrow samples, will be processed and frozen locally according to agreed SOP and stored in liquid nitrogen. Sample transport to the Cancer Sciences Division, Southampton, in dry ice and using a temperature logger will be undertaken once a sufficient number or samples have been collected locally (Hammersmith, Exeter).

9.5 ASSESSMENT OF EFFICACY

In CML the primary endpoint of the study will be molecular responses of BCR-ABL transcripts by qPCR which will be done centrally at the Hammersmith (Prof. Letizia Foroni) in a Clinical Pathology Accredited (CPA) accredited MRD laboratory. This is an accepted and routine test for monitoring of this disease. We intend to monitor this at every visit to the end of the study and we know already that fluctuations on stable tyrosine kinase inhibitors, notably imatinib, are very small (less than 0.5 log over 1 year). WT1 qPCR is to be undertaken in these patients in parallel also at every time point from the same blood sample using the standardised WT1 qPCR assay recently reported by Cilloni et al (JCO 2009).

We will measure only WT1 transcripts in patients with AML (vaccination group, control group) at every follow up visit.

The molecular monitoring will allow us to assess efficacy of the vaccine over time and also give us effectiveness data on the size of the biological effect.

Immunological efficacy and mechanism evaluation of the vaccine is intended to the completion of study for each patient by measuring the increase in WT1 specific CD8 T cells over time, compared to baseline. For this we will use ELISPOT and/or tetramer analysis for WT1 in conjunction with T cell memory, activation and differentiation markers (such as but not limited to CD3, CD4, CD8, CD45RA, CCR7 + 'dump channel'; with appropriate controls).

Additional immunological analyses (research tests) that will be undertaken, depending on availability of material:
CD4 and CD8 responses to WT1 and control antigens by ELISPOT or intracellular cytokine/tetramer analysis by flow cytometry, cytotoxicity studies of patient derived T cells against either CML/AML cells or appropriate surrogate targets, phenotyping of blood B cell and NK/NKT cell numbers and phenotype. It is likely that evaluation will use other tumour derived and non-tumour derived antigen as controls antigens. Examples for this are CMV, Flu, HIV, or PRAME, PASD-1 ecc.

Given the funding envelope the study is begun under it is expected that the duration of the immunological evaluation of the study will extend beyond the evaluation of the primary endpoints and secondary endpoints. Immunological evaluation is also a rapidly changing field in which the tools are improved and newly developed quickly. To reflect this, in the consent form patients will be asked to permit this later use of material for additional immunological evaluations beyond the submission of the end of study report to the regulatory bodies.

9.6 CRITERIA FOR IMMUNOLOGICAL/MOLECULAR RESPONSE

9.6.1 MOLECULAR RESPONSE:

Definition of BCR-ABL Response

1) For patients with a baseline BCR-ABL transcript level less than 11:
   CMR: 0 BCR-ABL transcript level with ABL control copy greater than or equal to 32,000; in TWO CONSECUTIVE tests.
   These patients cannot be assessed for a major or minor response as defined below.

2) For patients with a baseline BCR-ABL transcript level greater than or equal to 11:
   a. CMR: 0 BCR-ABL transcript level with ABL control copy greater than or equal to 32,000; in TWO CONSECUTIVE tests.
   b. Major response: a fall greater than 1 log in the BCR-ABL transcript level ratio.*
   c. Minor response: a fall greater than 0.5 log in the BCR-ABL transcript level ratio.*

   *Confirmed in an ABL control copy greater than or equal to 32,000 in two consecutive samples at any time during follow up.

Definition of WT1 Response

1) For patients with a baseline WT1/GUS ratio less than 0.1%:
   CMR: 0% WT1/GUS ratio with GUS control copy greater than or equal to 32,000; in TWO CONSECUTIVE tests.
   These patients cannot be assessed for a major or minor response as defined below.

2) For patients with a baseline WT1/GUS ratio greater than or equal to 0.1%:
   a. CMR: 0% WT1/GUS ratio with GUS control copy greater than or equal to 32,000; in TWO CONSECUTIVE tests.
   b. Major response: a fall greater than 1 log in the WT1/GUS ratio.*
   c. Minor response: a fall greater than 0.5 log in the WT1/GUS ratio.*

   *Confirmed in a GUS control copy greater than or equal to 32,000 in two consecutive samples at any time during follow up.

9.6.2 IMMUNOLOGICAL RESPONSE:

Definition of validated assay by ELISPOT response:

A positive response using the validated assays by ELISPOT will be determined by having all of the following criteria:

- More than 25 spots per million Peripheral Blood Mononuclear Cell (PBMC) assay;
- (increase in spot number exceeds spot number at baseline + 2 x standard deviation at baseline;
- p-value from the t-test (post-baseline time point vs baseline time point) < 0.05.

Immunological response may also be assessed by ICS and/or tetramer staining. Only results produced by validated assays will be used in the analysis.

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

10 STATISTICS AND DATA ANALYSIS

10.1 SAMPLE SIZE CALCULATION

10.1.1 SAMPLE SIZE CALCULATION FOR CML

The sample size calculation is based on A'Hern's Single-Stage Design and the primary outcome measure of molecular response (BCR-ABL or WT1).

A molecular response (BCR-ABL or WT1) rate of 20% would imply the vaccine clearly warrants further investigation. A molecular response rate of 5% or less 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 study a treatment that has a true PFS rate 30%) is set at 10%. The probability of a false negative result, β (i.e. incorrectly rejecting for further study a treatment with a true PFS rate of 50%) is set at 10%. Using these parameters, α = 0.1, β=0.1, p0=5%, p1=20%, 32 HLA A2+ve patients need to be recruited to the study. The drop-out rate is expected to be less than 10%, hence a total of 36 patients will be recruited.

This trial needs to observe a minimum of 4 out of 32 patients who are molecular responders in order to provide evidence that the vaccine warrants further investigation. Patients will be followed up for 2 years to assess molecular response.

10.1.2 SAMPLE SIZE CALCULATION FOR AML

The sample size calculation is based on A'Hern's Single-Stage Design and the primary outcome measure of progression free survival (PFS) at 2 years.

A progression free survival rate of 50% at 2 years would imply the vaccine clearly warrants further investigation. A PFS rate of 30% or less at 2 years 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 study a treatment that has a true PFS rate 30%) is set at 10%. The probability of a false negative result, β (i.e. incorrectly rejecting for further study a treatment with a true PFS rate of 50%) is set at 10%. Using these parameters, α = 0.1, β=0.1, p0=30%, p1=50%, 39 HLA A2+ve patients need to be recruited to the study. The drop-out rate is expected to be less than 10% (see section 3.1 for details of patient replacement).

This trial needs 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.

10.2 ANALYSIS PLAN

Molecular Analysis
The analyses on molecular response will be performed on all patients with molecular data at a minimum of 2 post-baseline time points (HLA A2 positive patients must also have received at least 1 dose of the vaccine).

Immunological Analysis
To be evaluable for immunological response, positive HLA A2 patients must receive at least 1 dose of the vaccine and the immunological testing must be available until at least week 8 post first dose.

Safety Analysis
For safety analyses, all patients with positive HLA A2 status who received at least one study drug administration will be evaluable for toxicity. All controls will be included in the safety analyses, where relevant.

Other Analysis
For all other analyses, an intention to treat principle will be used.

10.2.1 ANALYSIS FOR CML PATIENTS ONLY:

Primary:
The primary endpoint is molecular response of BCR-ABL (major or minor response or CMR). For the purpose of analysis, response (as defined by section 9.6) will be measured at weeks 4, 8, 12, 16, 20, 32 and 11, 17 and 23 months. Fisher's exact test will be used to compare differences in proportions of molecular response in CML patients versus controls.

Secondary:
The primary analysis will be repeated for major response or CMR of BCR-ABL.

The analysis methods used for BCR-ABL will be repeated for WT1.

Progression free survival, duration of response and overall survival (as defined in Section 2.2) will be estimated using the method of Kaplan and Meier for each group. Survival estimates will be presented with 90% confidence intervals. These endpoints will also be compared between the CML patients and controls using log-rank tests.

Progression free survival for CML patients is defined in Section 2.2 and is measured as the time from date of consent to the study to date of progression or death from any cause. Alive patients who are progression free will be censored at their date of last follow up. Similar analysis will be performed for overall survival and duration of response.

Toxicity will be collected and evaluated according to the NCI CTC toxicity scale (v4.0) and compared between the vaccinated and control groups by Fisher's exact test.

10.2.2 ANALYSIS FOR AML PATIENTS ONLY:

Primary:
The primary endpoint is time to disease progression measured as the time from date of consent to the study to date of progression or death from any cause (progression is defined as disease relapse). Alive patients who are progression free will be censored at their date of last follow up. Progression free survival will be estimated using the method of Kaplan and Meier for each group. Survival estimates will be presented with 90% confidence intervals. A log rank test will be computed to compare AML patients and their controls.

Secondary:
The primary analysis will be repeated for survival time.

A secondary endpoint is molecular response of WT1 (major or minor response or CMR). For the purpose of analysis, response (as defined in section 9.6) will be measured at weeks 4, 8, 12, 16, 20, 32 and 11, 17 and 23 months. Proportions of responders in the vaccinated group will be compared with the control group using Fisher's exact test for the CML and AML groups. This analysis will be repeated for major molecular response or CMR of WT1.

Toxicity will be collected and evaluated according to the NCI CTC toxicity scale (v4.0) and compared between the vaccinated and control groups by Fisher's exact test.

10.2.3 ANALYSIS FOR VACCINATED PATIENTS ONLY – CML AND AML

Another secondary endpoint is immune response (as defined in Section 9.6) of WT1-epitope specific T cells in blood and/or bone marrow, using validated assays by ELISPOT and/or ICS and tetramer staining. The proportion of responders in the CML group will be compared with the AML group using Fisher's exact test. This analysis will be repeated for the immune response to DOM and the immune response to DOM specific T cells.

The number of WT1 specific T-cells after peptide challenge to the skin and the number of humoral responses (B-cells) to the vaccine components will be summarised using descriptive statistics (wherever assessments have been feasible). Data on kinetics, duration and level of WT1 CD8 T cell responses will also be collected.

11 REGULATORY ISSUES

11.1 CLINICAL TRIAL AUTHORISATION

This trial has a Clinical Trial Authorisation from the UK Competent Authority the MHRA.

11.2 ETHICS APPROVAL

The trial protocol has received the favourable opinion of the Gene Therapy Advisory Committee.

The trial will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 as revised and recognised by governing laws and EU Directives. Each subject's consent to participate in the trial should be obtained after a full explanation has been given of treatment options, including the conventional and generally accepted methods of treatment. The right of the subject to refuse to participate in the trial without giving reasons must be respected.

After the subject has entered the trial, the clinician may give alternative treatment to that specified in the protocol, at any stage, if they feel it to be in the best interest of the subject. However, reasons for doing so should be recorded and the subject will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the subject remains free to withdraw at any time from protocol treatment and trial follow-up without giving reasons and without prejudicing their further treatment.

The investigator must ensure that subject's anonymity will be maintained and that their identities are protected from unauthorised parties. On CRFs subjects will not be identified by their names, but by an identification code. The investigator should keep a subject enrolment log showing codes, names and addresses.

11.3 ETHICAL CONSIDERATIONS

Amendments to the protocol may only be made with the approval of the Chief Investigator, and will be subject to review by GTAC and the MHRA. Written documentation of the approval must be received before the amendment can be implemented.

11.4 CONSENT

Consent to enter the trial must be sought from each subject only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed subject consent should be obtained. The right of the subject to refuse to participate without giving reasons must be respected.

After the subject has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the subject's best interest, but the reasons for doing so should be recorded. In these cases the subjects remain within the trial for the purposes of follow-up and data analysis. All subjects are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

The Investigator must also ensure the following points are made:

- The patient must be informed that the study drug is new and that the exact degree of activity is at present unknown, but that treating him will contribute to further knowledge.
- A copy of the written informed consent form will be retained by the patient and the original filed in the Investigator's Site File (unless otherwise agreed that the original will be stored in the patients notes and the copies kept in the ISF)
- An explanation of whom to contact for answers to pertinent questions about the research and research subject's rights, and who to contact in the event of a research-related injury to the subject must be given.

11.5 CONFIDENTIALITY

Subjects' identification data will be required for the registration process. The UoSCTU will preserve the confidentiality of subjects taking part in the trial.

11.6 INDEMNITY

The sponsor of the trial is University Hospital Southampton NHS Foundation Trust. For NHS sponsored research HSG (96) 48 reference no.2 refers. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

11.7 SPONSOR

University Hospital Southampton NHS Foundation Trust, Southampton is acting as the main sponsor for this trial. The UoSCTU has been delegated duties by the Sponsor relating to submissions to regulatory authorities, ICH GCP and phamacovigilance. Other delegated duties will be assigned to the NHS Trusts or others taking part in this trial by means of the site clinical trial agreement.

11.8 FUNDING

Leukaemia and Lymphoma Research Fund, and the Efficacy and Mechanisms Evaluation board of the MRC/DOH are funding this trial.

11.9 AUDITS AND INSPECTIONS

The trial may be subject to inspection and audit by University Hospital Southampton NHS Foundation Trust, Southampton, under their remit as sponsor, the UoSCTU as the Sponsor's delegate and other regulatory bodies to ensure adherence to ICH GCP, Research Governance Framework for Health and Social Care, applicable contracts/agreements and national regulations.

12 TRIAL MANAGEMENT

12.1 TRIAL MANAGEMENT GROUP (TMG) AND DATA MONITORING ETHICS COMMITTEE (DMEC)

TMG

Trial Management Group (TMG) is responsible for overseeing progress of the trial. The day-to-day management of the trial will be co-ordinated through the UoSCTU and oversight will be maintained by the Trial Steering Committee and the Data Monitoring and Ethics Committee.

Listings of AR, AE, SAE and SUSARs will be reviewed every 2 to 3 months jointly by the study team (Trial Management Group to include members of UoSCTU and the clinical and laboratory investigators).

DMEC

A DMEC will be convened on behalf of and with input from the Trial Management Group at regular intervals to review the safety listings and recruitment. AR frequency and type will be collected and presented to the Trial Management Group and the independent DMEC. It will then fall to the DMEC to decide whether there is sufficient reason to suspend or terminate the study.

Additionally the DMEC will evaluate on an ongoing basis whether there is any evidence that the vaccination might disadvantage patients in either cohort of the study and in this case might recommend discontinuation.

Life-threatening vaccination-related toxicity is observed in more than one patient, termination of the trial will be discussed among all collaborators and the trial sponsor.

Unplanned DMEC meetings will be called if required during the study.

12.2 COMPLETION OF THE CRF

Data will be collected and retained in accordance with the Data Protection Act (1988). CRFs will be used to collect the data. The Investigator is responsible for ensuring the accuracy, completeness, legibility and timelines of the data reported in the CRFs. Study documents will be retained in a secure location during and after the trial has finished.

The CRFs must be completed in black ink. Only the Investigator and those personnel authorised by them should enter or change data the CRFs. All laboratory data and Investigator observations must be transcribed into the CRF. ECG, MRI and CT scans must be reported in summary in the CRF. The original reports, traces and films must be retained by the Investigators for future reference.

Vital signs may be collected directly into the CRF.

Corrections can be made only by striking out any errors, with a single stroke, and not by using correction fluid. The correct entry must be entered by the side. The incorrect figure must remain visible and the correction should be initialled and dated by the person authorised by the Investigator to make the correction.

After all the queries have been resolved at the end of the study, the Investigator will confirm this by signing off the CRFs. The original CRFs will subsequently be submitted to the UoSCTU for archiving. The Investigator will receive copies of the CRFs and Data Clarification Forms.

12.3 STUDY PERFORMANCE AND MONITORING

Before the study can be initiated, the prerequisites for conducting the study must be clarified and the organisational preparations made with the trial centre. The suitability of the Investigator’s co-workers, technical facilities and availability of eligible patients at the trial centre must be ensured as well as the supply and the storage of the drug. When making the appointment for the initiation visit, it will be pointed out that all those who are involved in the study at the trial centre should be present. This visit involves a detailed presentation of the study documents and discussion of unanswered questions. The Investigator must ensure that the entire study information is passed on continuously to all those who are involved. The UoSCTU must be informed immediately of any change in the persons involved in the conduct of the study.

The study will be monitored and audited in accordance with UoSCTU procedures. All trial related documents will be made available on request for monitoring and audit by UoSCTU staff, UHS, REC and for inspection by the MHRA or other relevant bodies. Prior to the study start, the Investigator will be advised of the anticipated frequency of the monitoring visits. The Investigator will receive reasonable notification prior to each monitoring visit.

It is the duty of the Clinical Trial Coordinator (CTC) to review study records and compare them with source documents, discuss the conduct of the study and the emerging problems with the Investigator, check that the drug storage, dispensing and retrieval are reliable and appropriate and verify that the available facilities remain acceptable.

At the final close-down visit, the UoSCTU has to clarify any open questions, verify that all data requested and corrections have been entered correctly on the CRFs and collect the study material that is no longer required. All the unused drug supplied will be returned to the co-ordinating investigator.

12.4 SOURCE DOCUMENT VERIFICATION

The Investigator will allow the UoSCTU direct access to relevant source documentation for verification of data entered onto the CRFs taking into account data protection regulations. Entries in the CRF will be compared with patients' medical records and the results will be documented in the monitoring report form. Access should also be given to trial staff and departments (i.e. pharmacy).

The patients' medical records, and other relevant data, may also be reviewed by appropriate qualified personnel independent from the UoSCTU appointed to audit the study, and by regulatory authorities. Details will remain confidential and patients' names will not be recorded outside the hospital.

12.5 STUDY REPORT

At appropriate intervals, interim data listings will be prepared to give the Investigator the possibility to review the data and check the completeness of information collected. All clinical data will be presented at the end of the study on final data listings. A study report will be prepared based on the final data listings. The report will be submitted to the Investigator for review and confirmation it accurately represents the data collected during the course of the study.

12.6 RECORD RETENTION

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure the Investigator will maintain all source documents, study related documents and copies of the CRFs and Data Clarification Forms. The UoSCTU, Cancer Sciences Division, University Hospital Southampton NHS Foundation Trust /University of Southampton will maintain sponsor specific study related documents and the original CRFs. The CI will maintain specific study related documents and the original CRFs. All source documents will be retained for a period of fifteen (15) years following the end of the study.

13 PUBLICATION POLICY

All publications and presentations relating to the trial will be authorised by the Trial Management Group. Named authors will include at least the trial's Chief Investigators, Statistician and a member of UoSCTU staff. Members of the TMG and the Data Monitoring Committee may be listed and contributors will be cited by name if published in a journal where this is in accordance with the journal's policy. Any proposed publication will be submitted to UoSCTU at least 28 days in advance of being submitted for publication to allow time for UoSCTU to schedule a review and resolve any outstanding issues. Abstracts and press releases must be submitted to UoSCTU at least 14 days in advance of being made released.

14 REFERENCES

1. Goldstone, A. H., A. K. Burnett, K. Wheatley, A. G. Smith, R. M. Hutchinson, and R. E. Clark. 2001. Attempts to improve treatment outcomes in acute myeloid leukaemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. *Blood* 98:1302-1311.
2. Lugo, T. G., A. M. Pendergast, A. J. Muller, and O. N. Witte. 1990. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 247:1079-1082.
3. Daley, G. Q., R. A. Van Etten, and D. Baltimore. 1990. Induction of chronic myelogenous leukaemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 247:824-830.
4. Kolb, H. J., C. Schmid, A. J. Barrett, and D. J. Schendel. 2004. Graft-versus-leukaemia reactions in allogeneic chimeras. *Blood* 103:767-776.
5. Druker, B. J., F. Guilhot, S. G. O'Brien, I. Gathmann, H. Kantarjian, N. Gattermann, M. W. Deininger, R. T. Silver, J. M. Goldman, R. M. Stone, F. Cervantes, A. Hochhaus, B. L. Powell, J. L. Gabrilove, P. Rousselot, J. Reiffers, J. J. Cornelissen, T. Hughes, H. Agis, T. Fischer, G. Verhoef, J. Shepherd, G. Saglio, A. Gratwohl, J. L. Nielsen, J. P. Radich, B. Simonsson, K. Taylor, M. Baccarani, C. So, L. Letvak, and R. A. Larson. 2006. Five-year follow-up of patients receiving imatinib for chronic myeloid leukaemia. *N Engl J Med* 355:2408-2417.
6. Hughes, T., and S. Branford. 2003. Molecular monitoring of chronic myeloid leukaemia. *Semin Hematol* 40:62-68.
7. Bhatia, R., M. Holtz, N. Niu, R. Gray, D. S. Snyder, C. L. Sawyers, D. A. Arber, M. L. Slovak, and S. J. Forman. 2003. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukaemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 101:4701-4707.
8. Yong, A. S., K. Keyvanfar, R. Eniafe, B. N. Savani, K. Rezvani, E. M. Sloand, J. M. Goldman, and A. J. Barrett. 2008. Hematopoietic stem cells and progenitors of chronic myeloid leukaemia express leukaemia-associated antigens: implications for the graft-versus-leukaemia effect and peptide vaccine-based immunotherapy. *Leukaemia*.
9. Chaise, C., S. L. Buchan, J. Rice, J. Marquet, H. Rouard, M. Kuentz, G. E. Vittes, V. Molinier-Frenkel, J. P. Farcet, H. J. Stauss, M. H. Delfau-Larue, and F. K. Stevenson. 2008. DNA vaccination induces WT1-specific T-cell responses with potential clinical relevance. *Blood* 112:2956-2964.
10. Rezvani, K., A. S. Yong, S. Mielke, B. N. Savani, L. Musse, J. Superata, B. Jafarpour, C. Boss, and A. J. Barrett. 2008. Leukaemia-associated antigen-specific T-cell responses following combined PR1 and WT1 peptide vaccination in patients with myeloid malignancies. *Blood* 111:236-242.
11. Kawakami, M., Y. Oka, A. Tsuboi, Y. Harada, O. A. Elisseeva, Y. Furukawa, M. Tsukaguchi, T. Shirakata, S. Nishida, H. Nakajima, S. Morita, J. Sakamoto, I. Kawase, Y. Oji, and H. Sugiyama. 2007. Clinical and immunologic responses to very low-dose vaccination with WT1 peptide (5 microg/body) in a patient with chronic myelomonocytic leukaemia. *Int J Hematol* 85:426-429.
12. Oka, Y., A. Tsuboi, M. Murakami, M. Hirai, N. Tominaga, H. Nakajima, O. A. Elisseeva, T. Masuda, A. Nakano, M. Kawakami, Y. Oji, K. Ikegame, N. Hosen, K. Udaka, M. Yasukawa, H. Ogawa, I. Kawase, and H. Sugiyama. 2003. Wilms tumor gene peptide-based immunotherapy for patients with overt leukaemia from myelodysplastic syndrome (MDS) or MDS with myelofibrosis. *Int J Hematol* 78:56-61.
13. Oka, Y., A. Tsuboi, T. Taguchi, T. Osaki, T. Kyo, H. Nakajima, O. A. Elisseeva, Y. Oji, M. Kawakami, K. Ikegame, N. Hosen, S. Yoshihara, F. Wu, F. Fujiki, M. Murakami, T. Masuda, S. Nishida, T. Shirakata, S. Nakatsuka, A. Sasaki, K. Udaka, H. Dohy, K. Aozasa, S. Noguchi, I. Kawase, and H. Sugiyama. 2004. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci U S A* 101:13885-13890.
14. Tsuboi, A., Y. Oka, K. Udaka, M. Murakami, T. Masuda, A. Nakano, H. Nakajima, M. Yasukawa, A. Hiraki, Y. Oji, M. Kawakami, N. Hosen, T. Fujioka, F. Wu, Y. Taniguchi, S. Nishida, M. Asada, H. Ogawa, I. Kawase, and H. Sugiyama. 2002. Enhanced induction of human WT1-specific cytotoxic T lymphocytes with a 9-mer WT1 peptide modified at HLA-A*2402-binding residues. *Cancer Immunol Immunother* 51:614-620.
15. Hipp, M. M., N. Hilf, S. Walter, D. Werth, K. M. Brauer, M. P. Radsak, T. Weinschenk, H. Singh-Jasuja, and P. Brossart. 2008. Sorafenib, but not sunitinib, affects function of dendritic cells and induction of primary immune responses. *Blood* 111:5610-5620.
16. Wang, H., F. Cheng, A. Cuenca, P. Horna, Z. Zheng, K. Bhalla, and E. M. Sotomayor. 2005. Imatinib mesylate (STI-571) enhances antigen-presenting cell function and overcomes tumor-induced CD4+ T-cell tolerance. *Blood* 105:1135-1143.

17. Mumprecht, S., M. Matter, V. Pavelic, and A. F. Ochsenbein. 2006. Imatinib mesylate selectively impairs expansion of memory cytotoxic T cells without affecting the control of primary viral infections. *Blood* 108:3406-3413.
18. Larmonier, N., N. Janikashvili, C. J. LaCasse, C. B. Larmonier, J. Cantrell, E. Situ, T. Lundeen, B. Bonnotte, and E. Katsanis. 2008. Imatinib mesylate inhibits CD4+ CD25+ regulatory T cell activity and enhances active immunotherapy against BCR-ABL- tumors. *J Immunol* 181:6955-6963.
19. Collins, R. H., Jr., O. Shpilberg, W. R. Drobyski, D. L. Porter, S. Giralt, R. Champlin, S. A. Goodman, S. N. Wolff, W. Hu, C. Verfaillie, A. List, W. Dalton, N. Ognoskie, A. Chetrit, J. H. Antin, and J. Nemunaitis. 1997. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 15:433-444.
20. Vettenranta, K., L. Hovi, and U. M. Saarinen-Pihkala. 2003. Adoptive immunotherapy as consolidation of remission in pediatric AML relapsing post-transplant. *Pediatric transplantation* 7:446-449.
21. Gannage, M., M. Abel, A. S. Michallet, S. Delluc, M. Lambert, S. Giraudier, R. Kratzer, G. Niedermann, L. Saveanu, F. Guilhot, L. Camoin, B. Varet, A. Buzyn, and S. Caillat-Zucman. 2005. Ex vivo characterization of multiepitopic tumor-specific CD8 T cells in patients with chronic myeloid leukaemia: implications for vaccine development and adoptive cellular immunotherapy. *J Immunol* 174:8210-8218.
22. Bocchia, M., S. Gentili, E. Abruzzese, A. Fanelli, F. Iuliano, A. Tabilio, M. Amabile, F. Forconi, A. Gozzetti, D. Raspadori, S. Amadori, and F. Lauria. 2005. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet* 365:657-662.
23. Rojas, J. M., K. Knight, L. Wang, and R. E. Clark. 2007. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. *Leukaemia* 21:2287-2295.
24. Jemal, A., L. X. Clegg, E. Ward, L. A. Ries, X. Wu, P. M. Jamison, P. A. Wingo, H. L. Howe, R. N. Anderson, and B. K. Edwards. 2004. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. *Cancer* 101:3-27.
25. Grimwade, D., H. Walker, G. Harrison, F. Oliver, S. Chatters, C. J. Harrison, K. Wheatley, A. K. Burnett, and A. H. Goldstone. 2001. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukaemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood* 98:1312-1320.
26. Brieger, J., E. Weidmann, U. Maurer, D. Hoelzer, P. S. Mitrou, and L. Bergmann. 1995. The Wilms' tumor gene is frequently expressed in acute myeloblastic leukemias and may provide a marker for residual blast cells detectable by PCR. *Ann Oncol* 6:811-816.
27. Karakas, T., C. C. Miething, U. Maurer, E. Weidmann, H. Ackermann, D. Hoelzer, and L. Bergmann. 2002. The coexpression of the apoptosis-related genes bcl-2 and wt1 in predicting survival in adult acute myeloid leukaemia. *Leukaemia* 16:846-854.
28. Bergmann, L., C. Miething, U. Maurer, J. Brieger, T. Karakas, E. Weidmann, and D. Hoelzer. 1997. High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid leukemias are associated with a worse long-term outcome. *Blood* 90:1217-1225.
29. Mailander, V., C. Scheibenbogen, E. Thiel, A. Letsch, I. W. Blau, and U. Keilholz. 2004. Complete remission in a patient with recurrent acute myeloid leukaemia induced by vaccination with WT1 peptide in the absence of hematological or renal toxicity. *Leukaemia* 18:165-166.
30. Oka, Y., A. Tsuboi, O. A. Elisseeva, H. Nakajima, F. Fujiki, M. Kawakami, T. Shirakata, S. Nishida, N. Hosen, Y. Oji, I. Kawase, and H. Sugiyama. 2007. WT1 peptide cancer vaccine for patients with hematopoietic malignancies and solid cancers. *TheScientificWorldJournal* 7:649-665.
31. Molldrem, J., S. Dermime, K. Parker, Y. Z. Jiang, D. Mavroudis, N. Hensel, P. Fukushima, and A. J. Barrett. 1996. Targeted T-cell therapy for human leukaemia: cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukaemia cells. *Blood* 88:2450-2457.
32. Keilholz, U., A. Letsch, A. Aseminssen, W. K. Hofmann, L. Uharek, W. Blau, E. Thiel, and C. Scheibenbogen. 2006. Clinical and immune responses of WT1-peptide vaccination in patients with acute myeloid leukaemia. *J.Clin.Oncol., 2006 Asco Annual Meeting Proceedings 24, 2511*.
33. Stevenson, F. K., D. Zhu, C. A. King, L. J. Ashworth, S. Kumar, and R. E. Hawkins. 1995. Idiotypic DNA vaccines against B-cell lymphoma. *Immunol Rev*:211-228.
34. Spellerberg, M. B., D. Zhu, A. Thompsett, C. A. King, T. J. Hamblin, and F. K. Stevenson. 1997. DNA vaccines against lymphoma: promotion of anti-idiotypic antibody responses induced by single chain Fv genes by fusion to tetanus toxin fragment C. *J Immunol* 159:1885-1892.
35. King, C. A., M. B. Spellerberg, D. Zhu, J. Rice, S. S. Sahota, A. R. Thompsett, T. J. Hamblin, J. Radl, and F. K. Stevenson. 1998. DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma *Nat Med* 4:1281-1286.

36. Rice, J., C. H. Ottensmeier, and F. K. Stevenson. 2008. DNA vaccines: precision tools for activating effective immunity against cancer. *Nat Rev Cancer* 8:108-120.
37. Buchan, S., E. Gronevik, I. Mathiesen, C. A. King, F. K. Stevenson, and J. Rice. 2005. Electroporation as a "prime/boost" strategy for naked DNA vaccination against a tumor antigen. *J Immunol* 174:6292-6298.
38. Otten, G., M. Schaefer, B. Doe, H. Liu, I. Srivastava, J. zur Megede, D. O'Hagan, J. Donnelly, G. Widera, D. Rabussay, M. G. Lewis, S. Barnett, and J. B. Ulmer. 2004. Enhancement of DNA vaccine potency in rhesus macaques by electroporation. *Vaccine* 22:2489-2493.
39. Ottensmeier, C., L. L, A. Mander, A. Williams, T. Tjelle, J. Campos-Perez, C. Heath, D. P. Dearnaley, I. Mathiesen, and F. Stevenson. 2008. DNA fusion gene vaccination, delivered with or without in vivo electroporation - a potent and safe strategy for inducing anti-tumor immune responses in prostate cancer. . *American Association for Cancer Research Annual Meeting. 2008*:abstract 300.
40. Rice, J., S. Buchan, H. Dewchand, E. Simpson, and F. K. Stevenson. 2004. DNA fusion vaccines induce targeted epitope-specific CTLs against minor histocompatibility antigens from a normal or tolerized repertoire. *J Immunol* 173:4492-4499.
41. Low, L., A. Mander, K. McCann, D. Dearnaley, T. Tjelle, I. Mathiesen, F. Stevenson, and C. H. Ottensmeier. 2009. DNA Vaccination with Electroporation Induces Increased Antibody Responses in Patients with Prostate Cancer. *Hum Gene Ther*.
42. Ottensmeier, C. H. H., L. Low, A. Mander, A. Williams, T. Tjelle, J. Campos-Perez, C. Heath, D. P. Dearnaley, I. Mathiesen, and F. Stevenson. 2008. DNA fusion gene vaccination, delivered with or without in vivo electroporation - a potent and safe strategy for inducing anti-tumor immune responses in prostate cancer. . *American Association for Cancer Research Annual Meeting. 2008*:abstract 300.
43. Oji, Y., M. Yano, Y. Nakano, S. Abeno, S. Nakatsuka, A. Ikeba, T. Yasuda, Y. Fujiwara, S. Takiguchi, H. Yamamoto, S. Fujita, K. Kanato, K. Ito, T. Jomgeow, M. Kawakami, A. Tsuboi, T. Shirakata, S. Nishida, N. Hosen, Y. Oka, K. Aozasa, M. Monden, and H. Sugiyama. 2004. Overexpression of the Wilms' tumor gene WT1 in esophageal cancer. *Anticancer Res* 24:3103-3108.
44. Iiyama, T., K. Udaka, S. Takeda, T. Takeuchi, Y. C. Adachi, Y. Ohtsuki, A. Tsuboi, S. Nakatsuka, O. A. Elisseeva, Y. Oji, M. Kawakami, H. Nakajima, S. Nishida, T. Shirakata, Y. Oka, T. Shuin, and H. Sugiyama. 2007. WT1 (Wilms' tumor 1) peptide immunotherapy for renal cell carcinoma. *Microbiol Immunol* 51:519-530.
45. Hosen, N., T. Shirakata, S. Nishida, M. Yanagihara, A. Tsuboi, M. Kawakami, Y. Oji, Y. Oka, M. Okabe, B. Tan, H. Sugiyama, and I. L. Weissman. 2007. The Wilms' tumor gene WT1-GFP knock-in mouse reveals the dynamic regulation of WT1 expression in normal and leukemic hematopoiesis. *Leukaemia* 21:1783-1791.
46. Inoue, K., H. Ogawa, Y. Sonoda, T. Kimura, H. Sakabe, Y. Oka, S. Miyake, H. Tamaki, Y. Oji, T. Yamagami, T. Tatekawa, T. Soma, T. Kishimoto, and H. Sugiyama. 1997. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukaemia. *Blood* 89:1405-1412.
47. Lee, S. B., and D. A. Haber. 2001. Wilms tumor and the WT1 gene. *Exp Cell Res* 264:74-99.
48. Rauscher, F. J., 3rd. 1993. The WT1 Wilms tumor gene product: a developmentally regulated transcription factor in the kidney that functions as a tumor suppressor. *Faseb J* 7:896-903.
49. Hosen, N., Y. Sonoda, Y. Oji, T. Kimura, H. Minamiguchi, H. Tamaki, M. Kawakami, M. Asada, K. Kanato, M. Motomura, M. Murakami, T. Fujioka, T. Masuda, E. H. Kim, A. Tsuboi, Y. Oka, T. Soma, H. Ogawa, and H. Sugiyama. 2002. Very low frequencies of human normal CD34+ haematopoietic progenitor cells express the Wilms' tumour gene WT1 at levels similar to those in leukaemia cells. *Br J Haematol* 116:409-420.
50. Gao, L., I. Bellantuono, A. Elsasser, S. B. Marley, M. Y. Gordon, J. M. Goldman, and H. J. Stauss. 2000. Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* 95:2198-2203.
51. Oka, Y., O. A. Elisseeva, A. Tsuboi, H. Ogawa, H. Tamaki, H. Li, Y. Oji, E. H. Kim, T. Soma, M. Asada, K. Ueda, E. Maruya, H. Saji, T. Kishimoto, K. Udaka, and H. Sugiyama. 2000. Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type Wilms' tumor gene (WT1) product. *Immunogenetics* 51:99-107.
52. Gaiger, A., V. Reese, M. L. Disis, and M. A. Cheever. 2000. Immunity to WT1 in the animal model and in patients with acute myeloid leukaemia. *Blood* 96:1480-1489.
53. Gao, L., S. A. Xue, R. Hasserjian, F. Cotter, J. Kaeda, J. M. Goldman, F. Dazzi, and H. J. Stauss. 2003. Human cytotoxic T lymphocytes specific for Wilms' tumor antigen-1 inhibit engraftment of leukaemia-initiating stem cells in non-obese diabetic-severe combined immunodeficient recipients. *Transplantation* 75:1429-1436.
54. Ohminami, H., M. Yasukawa, and S. Fujita. 2000. HLA class I-restricted lysis of leukaemia cells by a CD8(+) cytotoxic T-lymphocyte clone specific for WT1 peptide. *Blood* 95:286-293.
55. Titomirov, A. V., S. Sukharev, and E. Kistanova. 1991. In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochim Biophys Acta* 1088:131-134.
56. Oshima, Y., T. Sakamoto, T. Hisatomi, C. Tsutsumi, Y. Sassa, T. Ishibashi, and H. Inomata. 2002. Targeted gene transfer to corneal stroma in vivo by electric pulses. *Exp Eye Res* 74:191-198.

57. Rols, M. P., and J. Teissie. 1998. Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. *Biophys J* 75:1415-1423.
58. Bettan, M., M. A. Ivanov, L. M. Mir, F. Boissiere, P. Delaere, and D. Scherman. 2000. Efficient DNA electrotransfer into tumors. *Bioelectrochemistry* 52:83-90.
59. Goto, T., T. Nishi, T. Tamura, S. B. Dev, H. Takeshima, M. Kochi, K. Yoshizato, J. Kuratsu, T. Sakata, G. A. Hofmann, and Y. Ushio. 2000. Highly efficient electro-gene therapy of solid tumor by using an expression plasmid for the herpes simplex virus thymidine kinase gene. *Proc Natl Acad Sci U S A* 97:354-359.
60. Saito, T., and N. Nakatsuji. 2001. Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. *Dev Biol* 240:237-246.
61. Suzuki, T., B. C. Shin, K. Fujikura, T. Matsuzaki, and K. Takata. 1998. Direct gene transfer into rat liver cells by in vivo electroporation. *FEBS Lett* 425:436-440.
62. Heller, R., M. Jaroszeski, A. Atkin, D. Moradpour, R. Gilbert, J. Wands, and C. Nicolau. 1996. In vivo gene electroinjection and expression in rat liver. *FEBS Lett* 389:225-228.
63. Mir, L. M., M. F. Bureau, R. Rangara, B. Schwartz, and D. Scherman. 1998. Long-term, high level in vivo gene expression after electric pulse-mediated gene transfer into skeletal muscle. *C R Acad Sci III* 321:893-899.
64. Aihara, H., and J. Miyazaki. 1998. Gene transfer into muscle by electroporation in vivo. *Nat Biotechnol* 16:867-870.
65. Gothelf, A., L. M. Mir, and J. Gehl. 2003. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat Rev* 29:371-387.
66. Rath, A., D. Batra, R. Kaur, S. Vrati, and S. K. Gupta. 2003. Characterization of immune response in mice to plasmid DNA encoding dog zona pellucida glycoprotein-3. *Vaccine* 21:1913-1923.
67. Zucchelli, S., S. Capone, E. Fattori, A. Folgori, A. Di Marco, D. Casimiro, A. J. Simon, R. Laufer, N. La Monica, R. Cortese, and A. Nicosia. 2000. Enhancing B- and T-cell immune response to a hepatitis C virus E2 DNA vaccine by intramuscular electrical gene transfer. *J Virol* 74:11598-11607.
68. Widera, G., M. Austin, D. Rabussay, C. Goldbeck, S. W. Barnett, M. Chen, L. Leung, G. R. Otten, K. Thudium, M. J. Selby, and J. B. Ulmer. 2000. Increased DNA vaccine delivery and immunogenicity by electroporation in vivo. *J Immunol* 164:4635-4640.
69. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 5:1249-1255.
70. Gronevik, E., S. Tollefsen, L. I. Sikkeland, T. Haug, T. E. Tjelle, and I. Mathiesen. 2003. DNA transfection of mononuclear cells in muscle tissue. *J Gene Med* 5:909-917.
71. Rice, J., T. Elliott, S. Buchan, and F. K. Stevenson. 2001. DNA fusion vaccine designed to induce cytotoxic T cell responses against defined peptide motifs: implications for cancer vaccines. *J Immunol* 167:1558-1565.
72. Weiner, G. J., H. M. Liu, J. E. Wooldridge, C. E. Dahle, and A. M. Krieg. 1997. Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in tumor antigen immunization. *Proc Natl Acad Sci U S A* 94:10833-10837.
73. Ottensmeier, C. H. H., L. Low, A. Mander, A. Williams, T. Tjelle, J. Campos-Perez, C. Heath, D. P. Dearnaley, I. Mathiesen, and F. Stevenson. 2008. DNA fusion gene vaccination, delivered with or without in vivo electroporation - a potent and safe strategy for inducing anti-tumor immune responses in prostate cancer. In *American Society of Gene Therapy, 11th Annual Meeting*, Boston, MA, USA. Abstract 450987.
74. Moorthy, V. S., S. McConkey, M. Roberts, P. Gothard, N. Arulanantham, P. Degano, J. Schneider, C. Hannan, M. Roy, S. C. Gilbert, T. E. Peto, and A. V. Hill. 2003. Safety of DNA and modified vaccinia virus Ankara vaccines against liver-stage P. falciparum malaria in non-immune volunteers. *Vaccine* 21:2004-2011.
75. McConkey, S. J., W. H. Reece, V. S. Moorthy, D. Webster, S. Dunachie, G. Butcher, J. M. Vuola, T. J. Blanchard, P. Gothard, K. Watkins, C. M. Hannan, S. Everaere, K. Brown, K. E. Kester, J. Cummings, J. Williams, D. G. Heppner, A. Pathan, K. Flanagan, N. Arulanantham, M. T. Roberts, M. Roy, G. L. Smith, J. Schneider, T. Peto, R. E. Sinden, S. C. Gilbert, and A. V. Hill. 2003. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat Med* 9:729-735.
76. Babiuk, S., M. E. Baca-Estrada, M. Foldvari, M. Storms, D. Rabussay, G. Widera, and L. A. Babiuk. 2002. Electroporation improves the efficacy of DNA vaccines in large animals. *Vaccine* 20:3399-3408.
77. Tollefsen, S., M. Vordermeier, I. Olsen, A. K. Storset, L. J. Reitan, D. Clifford, D. B. Lowrie, H. G. Wiker, K. Huygen, G. Hewinson, I. Mathiesen, and T. E. Tjelle. 2003. DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. *Scand J Immunol* 57:229-238.
78. Wang, R., D. L. Doolan, T. P. Le, R. C. Hedstrom, K. M. Coonan, Y. Charoenvit, T. R. Jones, P. Hobart, M. Margalith, J. Ng, W. R. Weiss, M. Sedegah, C. de Taisne, J. A. Norman, and S. L. Hoffman. 1998. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. *Science* 282:476-480.
79. Hassett, D. E., M. K. Slifka, J. Zhang, and J. L. Whitton. 2000. Direct ex vivo kinetic and phenotypic analyses of CD8(+) T-cell responses induced by DNA immunization. *J Virol* 74:8286-8291.

80. Tacket, C. O., M. J. Roy, G. Widera, W. F. Swain, S. Broome, and R. Edelman. 1999. Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine* 17:2826-2829.
81. Baccarani, M. Cortes, J. Pane, F. *et al*. 2009 Chronic Myeloid Leukemia: An Update of Concepts and Management Recommendations of European LeukemiaNet. *J Clin Oncol*. 27:6041-6051.
82. Simon, R. 1989. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials* 10:1-10.
83. Simon, R. M., S. M. Steinberg, M. Hamilton, A. Hildesheim, S. Khleif, L. W. Kwak, C. L. Mackall, J. Schlom, S. L. Topalian, and J. A. Berzofsky. 2001. Clinical trial designs for the early clinical development of therapeutic cancer vaccines. *J Clin Oncol* 19:1848-1854.

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 AT 48 HRS POST 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*