



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



Protocol version 4.0, 29-06-2011

SPONSOR: Southampton University Hospitals NHS Trust

COORDINATING CENTRE: University of Southampton Clinical Trials Unit



EudraCT reference no: 2009-017340-14

Gene Therapy Advisory Committee no: 173

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This trial is funded by Leukaemia & Lymphoma Research, and the Efficacy and Mechanism Evaluation (EME) programme.

Protocol Information

This protocol describes the WIN trial and provides information about procedures for entering subjects. The protocol should not be used as a guide for the treatment of other subjects; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the trial, but sites entering subjects for the first time are advised to 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-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
ECG	Electro-cardiogram
EDLI	Educated donor lymphocyte infusion
ER	Electroporation
ESR	Erythrocyte Sedimentation Rate
FBC	Full Blood Count
FDA	Food and Drug Administration
FrC	Fragment C from tetanus toxin
FU	Follow up
GM-CSF	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
IFN γ	Interferon gamma
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISS	Immune Stimulating sequence
MDS	Myelodysplastic syndrome
MHC	Major Histocompatibility Complex
MHRA	Medicines and Healthcare products Regulatory Authority
MRNA	Messenger Ribonucleic acid
NCI	National Cancer Institute
NK	Natural Killer Cell
NYHA	New York Heart Association

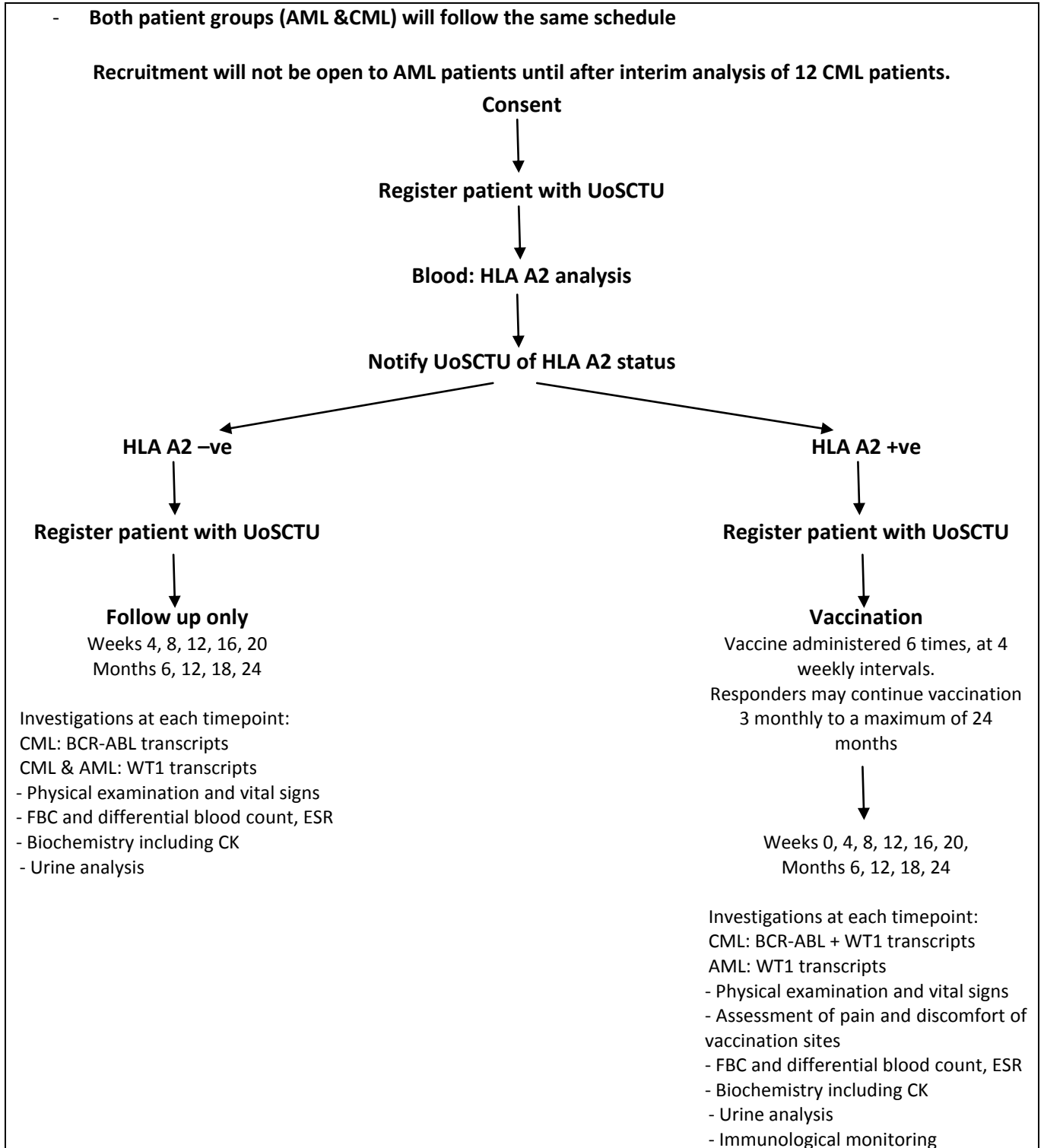
PCR	Polymerase chain reaction
PD	Progressive Disease
p.DOM	Domain 1 from Fragment C of Tetanus Toxin (FrC), used in the vaccine construct as an immune alert signal
PI	Principal Investigator
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
scFv	Single chain fragment of variable regions
SD	Stable Disease
SLE	Systemic Lupus Erythematosus
SOP	Standard Operating Procedure
SUHT	Southampton University Hospitals Trust
SUSAR	Suspected Unexpected Serious Adverse Reaction
TH1/2	T Helper 1/2 cells
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:	Southampton University Hospitals NHS Trust
Sponsor Ref Number:	RHMCAN0700
Funder:	Leukaemia & Lymphoma Research, and the Efficacy and Mechanism Evaluation (EME) programme.
Trial Phase:	II
Indication:	Chronic Myeloid Leukaemia and Acute Myeloid Leukaemia
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 and HLA A2 –ve patients will be followed up with molecular monitoring only.
Sample size : (split by treatment group)	Vaccination Arm (HLA A2+ve patients): 37 patients will be recruited in each treatment group. CML patients will be recruited first and an interim analysis will occur after the first 12 patients; if 1 or more molecular responder is observed an additional 25 patients will be recruited in this group, together with 12 in the AML group. If molecular responses (to WT1) are detected in 1 or more patients in the 1st 12 AML patients recruitment to the full cohort of 37 patients can proceed. Follow-up Arm (HLA A2 –ve patients): 50-55 patients are anticipated in each treatment group, based on the distribution of HLA A2 in the study population.
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 Imatinib 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 imatinib therapy. Imatinib dose modification in the previous year or interruption for > 15 days in the previous 6months 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 6 times at 4 weekly intervals into separate sites. Responders (immunological and/or clinical) may continue vaccination 3 monthly to maximum of 24 months. Vaccines will be injected intramuscularly 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 Imatinib.

REFERENCE DIAGRAM



SCHEDULE OF OBSERVATIONS AND PROCEDURES FOR HLA A2 POSITIVE 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	*	*	*	*	*		*	*	*			*		*	*	*		*	*		*	*	*	*
Assessment of pain/ discomfort and distress	*	*		*	*		*	*	*			* (6)		* (6)	* (6)	* (6)		* (6)	* (6)					
ECG Echocardiogram	* Echo at baseline only unless clinically indicated	*(1)		*	*		*	*	*			* (6)		* (6)	* (6)	* (6)		* (6)	* (6)					
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	* (6) 70ml	* 70ml	* (6) 70ml	* (6) 70ml	* (6) 70ml	* 70ml	* (6) 70ml	* (6) 70ml	* 70ml	* 70ml	* 70ml	* 70ml	* 70ml

	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
Immunological monitoring (2)	*	*	*	*	*	*	*	*	*	*	*	*(6)	*	*(6)	*(6)	*(6)	*	*(6)	*(6)	*	*	*	*	*
qPCR for BCR-ABL/WT	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Chest X-ray	*																							
Vaccination		*		*	*		*	*	*			*(6)		*(6)	*(6)	*(6)		*(6)	*(6)					
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... 70 ML 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)

(6) Not to be performed in patients who have not obtained response to the initial 6 vaccinations

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
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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	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

(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

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 (CP) CML 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 immunological 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 (Cillonni 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.

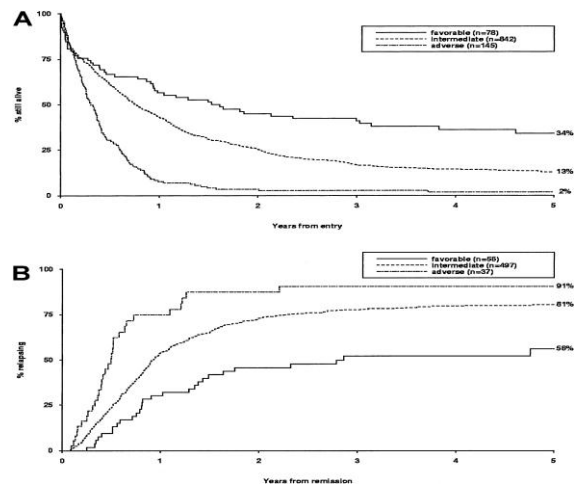
We propose to build on our established programme of DNA fusion gene vaccination delivered by intramuscular injection and exploiting our unique experience with electroporation, to induce durable immune responses with the aim of controlling disease by precision attack of the tumour by CD8+ T cells. The 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.

Figure 1. Survival and risk of relapse in AML



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

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 cohort to be studied here have therefore been chosen to reflect this conclusion:

In this study we propose to:

1. Assess the frequency of clinical responses by evaluation of
 - a. quantitative measurements of BCR-ABL transcripts (CML) and of WT1 transcripts in the blood (patients with CML and AML) at weeks 4, 8, 12, 16, 20 and months 6, 12, 18 and 24.
 - b. time to disease progression and 2 year survival rate (patients with AML)
2. Evaluate the level and kinetics of CD8 responses induced to both epitopes encoded in the vaccines in the different clinical contexts and to assess the correlation to clinical effects

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.

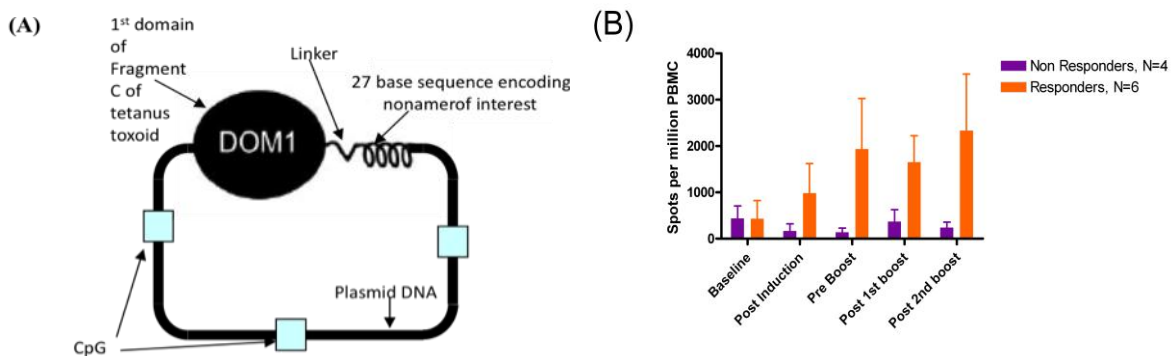


Figure 2. Vaccination of patients with the p.DOM.epitope vaccine.

(A) The p.DOM epitope vaccine consists of a DNA plasmid backbone incorporating CpG sites. The first domain of tetanus toxin (DOM; TT865-1120) provides T cell help, when linked to a tumour associated nucleotide sequence, encoding the HLA I 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 IFNγ 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.

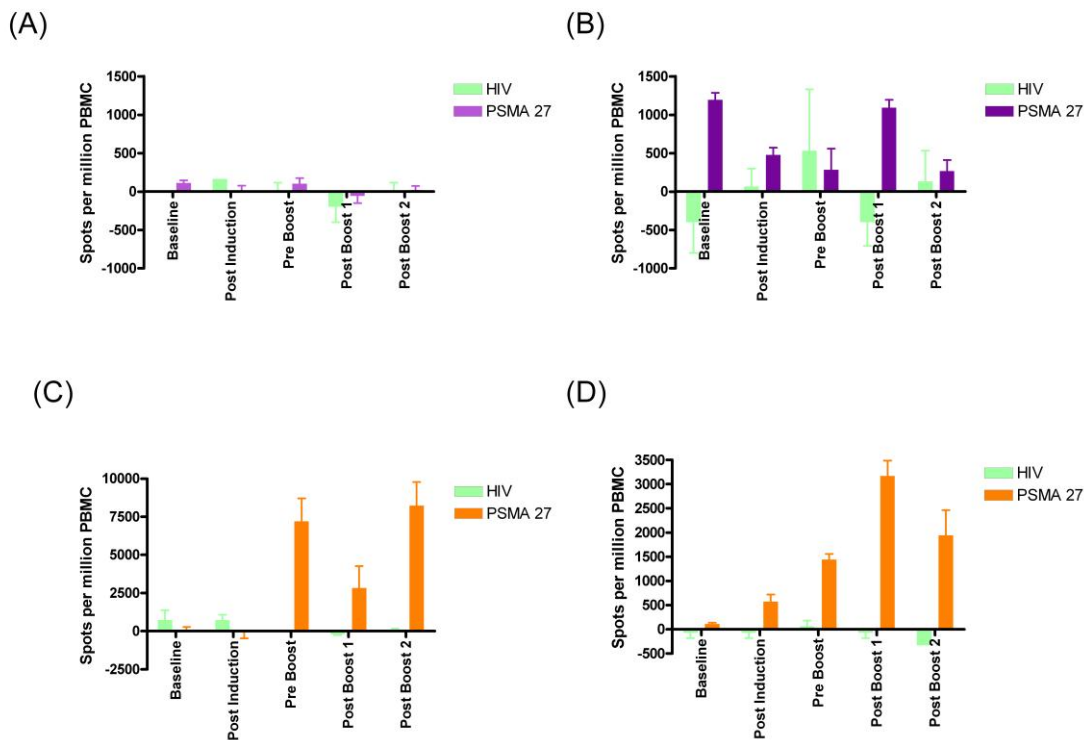


Figure 3. CD8 responses to DNA vaccination analyzed over time by ELISPOT.

(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 IFN γ producing PBMC above the base line, this patient has been classified as a non-responder.

(C) and (D) show examples of patients that have significantly increased levels of IFN γ 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).

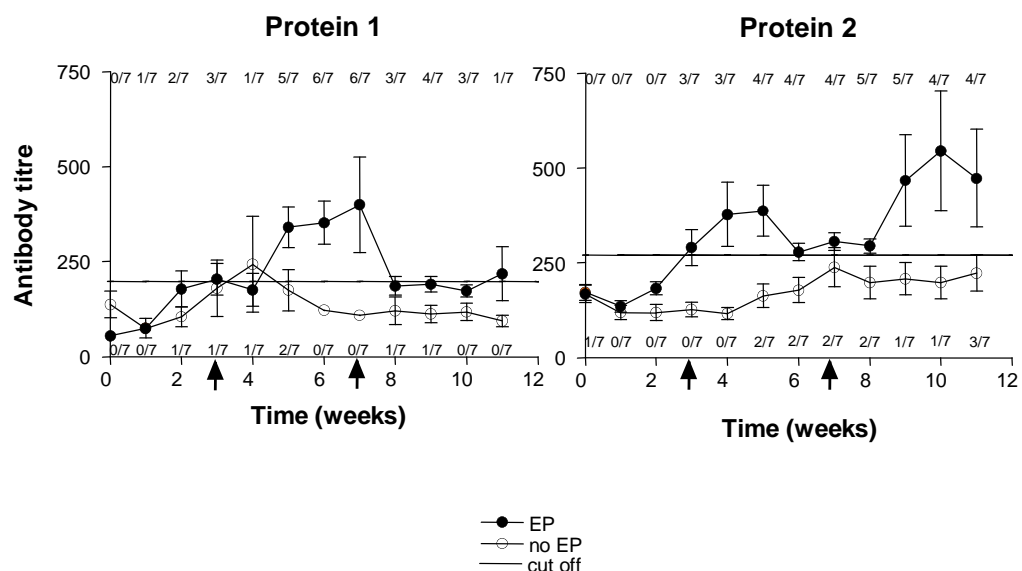


Figure 4: Humoral immune response following DNA vaccination with two different *H. contortus* Ag in sheep. Ab titre in serum of each animal was determined and the group average was calculated. The line across the graph represents the average of the negative controls. DNA vaccination with EP is depicted 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 idiotype 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 idioype 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 Th₁ 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 Th₁ 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 IFN γ by NK cells and IFN α , IFN γ β , 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 Th₁ phenotype. Secretion of IFN γ by Th₁ 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 MIFTT 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) *GTGCTGGACTTTGCGCCCCGGGCGCT*

p.DOM-WT1-126 with the sequence :

RMFPNAPYL (aa126-134) *AGGATGTTTCCTAACGCGCCCTACCTG*

TAA is a stop codon for translation

*AGCTTGCCGCCACCATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACC
TTGATTGTTGGGTGCGACAACGAAGAAGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAAC
AACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCTGTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCA
ACGGCAAAGCTATCCACCTGGTTAAACAACGAATCTTCTGAAGTTATCGTGCACAAGGCCATGGACATCGAATACAAC
GACATGTTCAACAACCTTACCCTTAGCTTCTGGCTGCGGTTCCGAAAGTTTCTGCTTCCACCTGGAACAGTACGGC
ACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTGAAG
GGTAAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTAGATCACTTCCGCGACCTGCCGGACAA
GTTCAACGCGTACCTGGCTAACAAATGGGTTTTCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATC
AACGGCGTTCTGATGGGCTCCGCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCT
GGACCGTTGCAACAACAACAACAGTACGTATCCATCGACAAGTTCGTATCTTCTGCAAAGCACTGAACCCGAAAG
AGATCGAAAACTGTATACCAGCTACCTGTCTATCACC*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 which 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 with 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 were 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 subjects 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

CLINICAL STUDY OBJECTIVES

The objectives are to evaluate:

- 1) Molecular response following p.DOM-epitope DNA vaccination in patients with CML (BCR-ABL, WT1) and AML (WT1) at weeks 4, 8, 12, 16, 20 and at months 6, 12, 18 and 24.
- 2) Time to disease progression, 2 year survival rate (patients with AML)
- 3) Correlation of molecular responses with immunological responses.

2.1 PRIMARY ENDPOINT

CML: Molecular response of BCR-ABL transcripts (minor + major)

Minor response: a fall greater than 0.5 log in the BCR-ABL transcript level, at any time during follow up.

Major response: a fall > 1 log in the BCR-ABL transcript level, at any time during follow up.

A responder will have decrease in transcript levels at two time-points during/following vaccination

AML: time to disease progression.

2.2 SECONDARY ENDPOINTS

Molecular response of WT1 transcripts for both CML and AML, criteria as above

Toxicity assessed according to the NCI CTC toxicity scale v4.0

CML: Time to disease progression and next treatment, survival

AML: 2 year survival, overall survival

Vaccinated patients only:

Frequency of WT1-epitope specific T cells by 6 months in blood and bone marrow. A responder will have ≥ 2 fold increase in WT1 specific CD8 T cells, using validated assays by ELISPOT and/or ICS/tetramer staining.

WT1 specific T-cells after peptide challenge to the skin.

Immune responses to DOM

Humoral responses to the vaccine components.

3. TRIAL DESIGN

This is an 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 be followed up with molecular monitoring only. Evaluation will be on an intention to treat basis (from time of consent). Each vaccination group will therefore have an unvaccinated control group.

It is intended the allocation of vaccination or follow-up only, based on A2 genotype will reduce the selection bias as there is no evidence for an effect of the presence of the HLA A2 gene on outcome in either CML or AML.

12 CML patients will initially be recruited into the vaccine arm followed by interim analysis. If a response is observed, an additional 25 CML patients will be recruited into the treatment arm.

Following the interim analysis in the 12 CML patients, recruitment to the AML arm of the study will be permitted. After 12 AML patients an interim analysis of molecular responses will be undertaken. If a response is observed, 25 additional AML patients will be recruited into the treatment arm.

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

It is anticipated that 50-55 patients will be recruited to each of the AML & CML groups, based on the distribution of HLA A2 in the study population. CML and AML patients are monitored closely at 4-6 weekly intervals as part of their standard of care.

3.1 STUDY TIMELINES AND ACCRUAL RATE

Patients will be recruited from three haematology centres: Hammersmith Hospital, Imperial NHS trust; Southampton University Hospitals Trust, and Royal Devon & Exeter Healthcare NHS Trust. No recruitment is intended outside of the UK.

The Hammersmith is currently following a cohort of about 500 patients with CML whose disease is stable on Imatinib, which are reviewed 6 weekly in the clinical department. Southampton and Exeter will be able to contribute a smaller number of patients to the CML group. We anticipate being able to recruit the maximum number of patients within 12 months therefore. Retrospective evaluation supports that the three centers see about 100 new patients with AML per annum between them. These patients are also monitored closely in the first 2 years post treatment and we anticipate being able to recruit the maximum number of patients in 18 months.

3.2 DURATION OF THE CLINICAL TRIAL

It is expected that the study will commence recruitment in September 2010. It is expected that the accrual rate will be 2-3/month and it is therefore expected that recruitment will be complete in 18 months.

3.3 PATIENT EVALUABILITY AND REPLACEMENT

All patients receiving at least one study drug administration will be evaluable for toxicity. To be evaluable for molecular and immunological response, patients must receive at least 1 dose of the vaccine and the immunological testing must be available until at least week 6 post first dose. Patients who go off study before week 16 for any reason may be replaced by recruiting additional patients to the appropriate study arm.

4. SELECTION AND ENROLEMENT OF SUBJECTS

4.1 PATIENT SELECTION

1. CML-CP if they have previously received ≥ 400 mg/day of imatinib, achieved CCyR but not CMR, and have been treated with imatinib for a minimum of 24 months and who are on a stable dose of imatinib. Patients will continue to receive imatinib (at the same dose that was being given prior to study entry) throughout the vaccine study.

2. 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 ($<1,000/\mu\text{L}$) or thrombocytopenia ($<100,000/\mu\text{L}$). Patients must be unsuitable for allo-SCT (comorbidity, age over sixty or unavailability of a fully matched donor) or have made an informed decision not to undergo transplant procedure. 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) will be excluded).

Patients that fulfil the entry criteria and who consent will be recruited into the study and followed clinically and by molecular means. HLA A2+ patients will be vaccinated; HLA A2 negative patients will followed clinically and by molecular means.

4.2 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 imatinib monotherapy for a minimum of 24 months

AML patients:

WT1⁺ AML in CR or morphologic CR with incomplete blood count recovery (CRi);

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
- 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 $> 1.0 \times 10^9/\text{l}$; 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.

4.3 EXCLUSION CRITERIA

CML patients:

- CML in accelerated phase or blast crisis or having achieved CMR at any point during imatinib therapy.
- Imatinib dose modification in the previous year, Imatinib 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
- 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.

4.4 REGISTRATION PROCEDURE

Before registering the patient, the Investigator or designated representative should determine the eligibility of the patient. Where there is doubt as to their eligibility, the Investigator should consult with the University of Southampton Clinical Trials Unit or the Chief Investigator. If the agreed opinion is that the patient is eligible, the patient may be registered.

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 and actual laboratory measurements will be requested. If eligible for the study, the patient will be allocated a patient trial ID by the University of Southampton Clinical Trials Unit.

4.7 WITHDRAWAL CRITERIA

Any patient who experiences a WHO CTC grade 3 adverse reaction (AR), possibly or likely to be related to vaccination will discontinue further vaccination. While with the current knowledge it is unlikely that such vaccine related events will be observed, 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.

The Investigator will make every reasonable effort to keep each patient on study. However, if the Investigator removes a patient from the study or if the patient declines further participation, prior to any therapeutic intervention, final assessments will be performed, if possible. These include a physical examination, laboratory samples, an assessment of AE's and molecular/clinical response, recording

concomitant medication and drug accountability. All the results of the evaluations and observations, together with a description of the reasons for study withdrawal, must be recorded in the CRF.

Patients who are removed from the study 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.

The following are justifiable reasons for the Investigator to withdraw a patient from study:

- unacceptable toxicity
- unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable
- SAE requiring discontinuation of treatment
- withdrawal of consent - where a patient is not evaluable, additional patients will be recruited to replace them
- serious violation of the study protocol (including persistent patient attendance failure and persistent non-compliance)
- withdrawal by the Investigator for clinical reasons not related to the study drug treatment
- evidence of disease progression
- In patients with CML requirement to discontinue or change the dose of imatinib

4.8 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/its application is considered too toxic to continue treatment prior to the required number of patients being recruited
- The stated number of patients to be recruited is reached.

Regardless of the reason for termination, all data available for the patient at the time of discontinuation of follow-up must be recorded in the CRF. All reasons for discontinuation of treatment must be documented.

In terminating the study, the Investigators will ensure that adequate consideration is given to the protection of the patient's interest.

5. TREATMENTS

HLA A2+ patients, will receive the following treatment:

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 at 4 weekly intervals. Responders (Immunological but without molecular progression) may continue vaccination 3 monthly to maximum of 24 months. The vaccines will be injected intramuscularly (im) followed by electroporation (EP) into separate locations.

5.3 VACCINE ROUTE AND SITE

The vaccines will be injected by deep intramuscular injection (im) followed by delivery im +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, independent of the clinical and immunological endpoints.

Haematological toxicity - 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 document 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.

If serious 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. Pain at CTC grade 4 will be considered as an SAE.

All patients are free to remove themselves from the trial at any time.

5.5 DOSE ESCALATION:

There will be no dose escalation

5.6 CONCURRENT MEDICATION AND REPLACEMENT

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 imatinib and the dose of imatinib will remain unchanged throughout the course of the study. Patients will continue on the same dose of imatinib 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.

6.0 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 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 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°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°C.

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

The vaccine must be stored -70C 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.

Labelling requirements for the vaccine

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.

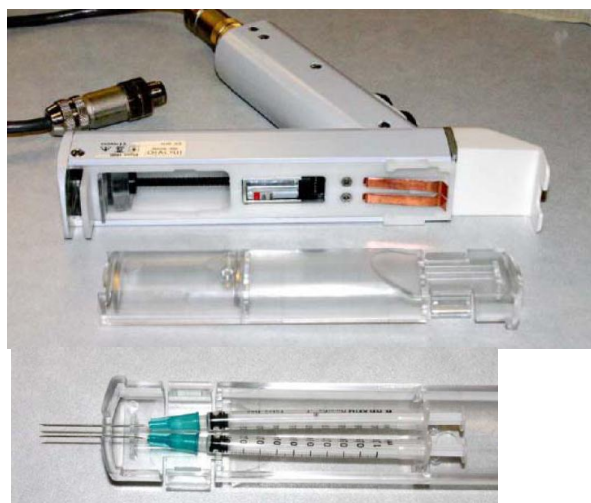
The unit connects to line voltage and consists of a medical grade power supply unit (PSU), high-voltage power circuit and a low-voltage digital control circuit. The PSU provides patient / user electrical isolation and converts local line power to the voltage required by the instrument. The control circuit 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 needle 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 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 SUHT 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 SUHT 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.

7.0 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.

Additionally a visual analogue scale will be used to assess the level of discomfort/pain in all patients in the study.

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 with the 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 and should anaphylaxis occur, it would 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 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 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 document 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: 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.



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 SUBJECTS

It is intended that all patients will be followed on study for up to 24 months. 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 investigation/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 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
- ECG
- baseline echocardiogram

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

- HLA Status
- qPCR for BCR-ABL and WT1 in CML, for WT1 in AML
- Blood for immunological assays (80 ml of anticoagulated blood)- Syphilis, Hep B, Hep C, HIV
- Autoimmune profile

- Leukapheresis for immunological studies
- Height and weight
- WHO Performance status
- Vital Signs
- Demographic data
- History and physical examination
- FBC and differential blood count, ESR
- Clotting
- Biochemistry including CK
- urine analysis

-

The following evaluation should be performed immediately after vaccination:

- Pain/discomfort and distress assessment

The following evaluation should be performed 48 hours after vaccination:

- Pain/discomfort and distress assessment

Please also refer to the tabulated Schedule of Observations and Procedures.

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 and drug orders should be placed through the UoSCTU and it is advised that at least 7 days notice is given.

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

For patients, eligible for the vaccinations at 6, 9, 12, 18 and 24 months the clinical trials centres will contact the WIN trial coordinator at UoSCTU to request shipment of additional vaccine vials. UoSCTU will then contact Dr. Paul Lloyd-Evans or his designated depute to request release and shipment.

9.3 EVALUATIONS DURING AND AT THE END OF THE STUDY

Visit 0: Baseline (registration)

Visit 1: week 0 within 7 days of baseline visit

Visit 2: week 2 14 days after v1 +/- 3 days

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

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

From v13 onwards, patients will be seen at the following timepoints from baseline +/- 14 days.

Months 11, 14, 17, 17day14, 20, 23, 24, 27, 30, 33, 36.

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.

Investigations required at each visit:- Physical examination and vital signs

- FBC and differential blood count, ESR
- Biochemistry including CK
- Urine analysis - Immunological monitoring; 65 ml of anticoagulated blood (Lithium Heparin Tubes) and 5 mL clotted blood for serum will be taken for this purpose

Additional investigations required after each vaccination:

- ECG, Echocardiogram (if clinically indicated), assessment of pain and discomfort of vaccination sites
- the first patient recruited at each site will be evaluated at 48 hrs after her/his first vaccination, before additional doses can be given or before additional patients can be vaccinated at that site.
- 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.

Additional investigation required visit 6

- DTH assessment (to be carried out if wherever feasible)

Additional investigations required at visit 10

- Bone marrow for immunological (CML and AML) and disease (AML) evaluation
- Leukapheresis
- DTH assessment (to be carried out if wherever feasible)

Additional investigations required for vaccine patients:

- Molecular (20ml anticoagulated blood for qPCR for BCR-ABL and WT1 in CML, for WT1 in AML) and blood sampling for immunological assays.
- Blood should be taken prior to vaccine being administered.

Additional investigations required for non vaccinated patients in follow-up group:

- Molecular only (20ml anticoagulated blood for qPCR for BCR-ABL and WT1 in CML, for WT1 in AML)

Please also refer to the tabulated Schedule of Observations and Procedures.

9.4 TRANSFER OF SAMPLES

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

Central laboratories are being used for the analysis of:

1) pPCR for BCR-ABL and pPCR for WT1 (Hammersmith) – all patients, all timepoints. 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.

2) the immunological analyses for vaccine responses, including leukapheresis samples and bone marrow samples. These 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 of 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 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 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 timepoint 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.

Time to disease progression will be calculated for each patient from time of consent in an intention to treat analysis.

Additionally the vaccinated and unvaccinated groups will be compared using a log-rank test in terms of molecular responses, time to disease progression and immunological events.

Immunological efficacy and mechanism evaluation of the vaccine is intended to the completion of the first 6 months on study for each patient by measuring the increase in WT1 specific CD8 T cells over time, compared to baseline. For this we will use high and low affinity tetramer analysis for WT1-126 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 (for example CMV/EBV as positive and HIV as negative control). We are currently making and validating the WT1-37 tetramer. ELISPOT assays are fully validated also for clinical trials use and do not require further work for either WT1-126 or WT1-37 epitopes. A WT1 immune-responder will have >2 fold increase in WT1 specific CD8 T cells, using validated assays.

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 CLINICAL/MOLECULAR RESPONSE

- Molecular response:
 - Minor response is defined as a fall greater than 0.5 log in the levels of BCR-ABL (in CML patients) or WT1 (in patients with AML) transcripts , at any two timepoints during follow up.

- Major response is defined as a fall greater than 1 log in the levels of BCR-ABL (CML) or WT1 (in patients with AML) transcripts, at any two timepoints during follow up.
- an immune response to the WT1-126 or WT1-37 vaccines will be defined as the emergence of detectable T cell frequencies against WT1-126 or WT1-37, at any time during the follow up, when the pre-study analysis found no response or a twofold increase in T cell frequency to WT1-126 or WT1-37 following vaccination .

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

9.7 RECORDING OF RESPONSE IN THE CRF

An overall response will be recorded for each visit that includes disease assessment. The applicable overall response category for each visit will be recorded, even though the criteria for determination of CR or PR by the protocol must be confirmed after two consecutive observations, no less than four weeks apart.

9.8 OTHER DEFINITIONS OF RESPONSE

Early Progression: Progression within the first course of treatment.

Early death: Death during the first course of treatment without severe toxicity.

Toxic death: Any death to which drug toxicity is thought to have a major contribution.

10. STATISTICS AND DATA ANALYSIS

All analyses will be performed on an intention to treat basis.

CML: the primary outcome is molecular response (major + minor; decrease in transcript levels at two time-points during/following vaccination) to BCR-ABL measured at baseline (recruitment) and follow-up at 6, 12, 18 and 24 months. Fisher's exact test will be used to compare differences in proportions of molecular response in CML patients versus controls.

AML: the primary outcome is time to disease progression from date of consent to end of study participation for eligible patients. Median survival times and a Log-rank test will be computed to AML patients and their controls.

The proportion surviving at 2 years will be compared between AML patient group and their controls using the Fisher's exact test.

CML & AML: the secondary molecular response outcome measures and frequency of WT1-epitope specific T cells will be compared using Fisher's exact test.

CML& AML: Descriptive statistics will be used to describe the immunological response rate in HLA A2+ patients from baseline to 6 months in % responders of vaccinated individuals with an increase of >2 fold in WT1 specific CD8 T cells compared to baseline. Data on kinetics, duration and level of WT1 CD8 T cell responses will also be collected.

Comparison between groups using a log-rank test will be undertaken.

Time to progression, time to next treatment(s), response and duration of remission and overall survival will be evaluated.

Toxicity will be collected and evaluated according to the NCI CTC toxicity scale (v 4) and compared between the vaccinated and control groups by Fisher's exact test.

10.1 STATISTICAL PLAN INCLUDING INTERIM ANALYSIS

For sample size calculation we have used Simon's optimal phase II trial design for clinical development of therapeutic cancer vaccines (81, 82). This allows us to undertake a 'start/stop' evaluation once 12 patients have been enrolled into each vaccination group of the study and have been evaluated to 6 months (molecular monitoring).

If 1 or more than 1 molecular responder is observed in the 12 initial patients, this particular vaccination group will be extended to 37 patients. This will allow the study to distinguish between $p_0=5\%$ (standard response) from $p_1=20\%$ (expected response) with $\alpha=10\%$ and $1-\beta=90\%$. This gives a less than 10% chance of rejecting a useful vaccine with error probability limits of $\alpha < 0.10$ and $\beta < 0.10$, even if the true response rate were to be $p_1=20\%$.

If no molecular responses are seen in the first 12 patients with either CML or AML, recruitment will cease for this patient group, as there will not be sufficient clinical interest to pursue further. This optimal design has an expected sample size under H_0 of 24 and a maximum sample size of 37 in each patient group (AML and CML).

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 whom 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 Southampton University Hospitals NHS 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

Southampton University Hospitals NHS 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, GCP and pharmacovigilance. 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 Southampton University Hospitals NHS 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 AND DATA MONITORING ETHICS COMMITTEE

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). A DMEC will be convened on behalf of and with input from the Trial Management Group at regular intervals to review the safety listings, recruitment and to make a recommendation on whether to continue beyond 12 patients in the CML and AML cohorts, respectively. 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 him 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, SUHT, 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 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 by 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, Southampton University Hospitals/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.

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APPENDIX 1: VISUAL ANALOGUE SCALE FOR ASSESSMENT OF INJECTION RELATED PAIN

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

Brief Pain Inventory

Participant Trial ID / /

Participant Initials

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

Week

Vaccination dose .

Vaccination date dd / mm / yy

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: <input type="text"/>

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

