AdUP

Investigators:
Mr Prashant Patel, Professor Nicholas James, Dr Emilio Porfiri, Mr Richard Viney, Dr Vivien Mautner, Dr Peter Searle

Sponsors: University of Birmingham

Coordinating Centre

The trial is being conducted under the auspices of the Cancer Research UK Clinical Trials Unit (CRCTU), University of Birmingham according to their local procedures.

Background and Rationale:

Locally relapsed prostate cancer presents an attractive target for gene therapy because the tumour remains localised to the prostate or to the surrounding tissue for a long phase of the disease. Also the delivery of gene therapy vectors to the prostate is straightforward using either ultrasound-guided, trans-rectal injection or adapting methods developed for brachytherapy, to deliver multiple, template-guided trans-perineal injections to achieve saturation coverage of the prostate. Several gene therapy and gene-immunotherapy strategies have been tested in patients with locally relapsed or metastatic prostate cancer, including Virus Directed Enzyme Prodrug Therapy (VDEPT), Conditionally Replicating Adenoviruses (CRAds) and GMCSF based immune stimulation[2]. These treatments were well tolerated and showed some evidence of anti-tumour activity[3].

The nitroreductase/CB1954 VDEPT system

The nitroreductase/CB1954 system employs *E. coli* **nitroreductase (NTR)** as the prodrug activating enzyme, and **CB1954** as the prodrug to be activated. To exploit this system we have previously generated an E1-E3 deleted, replication-defective human adenovirus (Ad5) designated CTL102 which contains the NTR gene under the control of the cytomegalovirus (CMV) promoter. CTL102 was able to infect cancer cells and to induce NTR expression *in vitro*, in addition NTR expressing cells are sensitised to CB1954. Experiments carried out in animal models demonstrated that NTR expression could be detected in prostate cancer xenografts injected with CTL102 and that growth of the xenografts was inhibited by intratumoural injection of CTL102 followed 2 days later by intra peritoneal administration of CB1954[4].

CB1954 prodrug conversion and clinical development

CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] is a weak alkylating agent that is reduced by the rat nitroreductase enzyme DT diaphorase to a potent, bifunctional alkylating agent (5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide) that cross-links DNA and is able to kill both dividing and non-dividing cells[5]. The human and murine forms of DT diaphorase are deficient in this reaction[6]. A nitroreductase enzyme encoded by the *nfsB* gene of *E. coli* was found to perform the same reduction 60 to 100-fold faster than the rat DT diaphorase[7], and this is the enzyme (NTR) encoded in CTL102 and AdNRGM.

A phase I pharmacokinetic trial of CB1954 defined its maximum tolerated dose (MTD), dose-limiting toxicities and plasma concentration-time profile. Ten cohorts of three patients each were treated with CB1954 at dose levels of 3mg/m^2 to 37.5 mg/m^2 [8]. No marrow suppression, nephrotoxicity or alopecia was observed. Dose limiting toxicities (one grade 4 diarrhoea and two grade 2 hepatic toxicity) were seen at 37.5 mg/m^2; other toxicities included nausea, vomiting, anorexia and fatigue. Side effects recorded at 24 mg/m^2 were grade 3 nausea and fatigue, therefore this dose was recommended as the standard dose for future clinical trials[8]. A clouding of the lens of the eye, also known as cataract, occurred in a few animals given the CB1954 drug. We believe this change is highly unlikely to occur in humans and none of the patients who were treated with this drug in previous clinical trials has suffered from this complication.

Prostate cancer trial

A phase I/II clinical trial of CTL102/CB1954 was completed in men with prostate cancer[11]. In the first stage of the trial 20 patients who were scheduled for radical prostatectomy received CTL102 alone, via intraprostatic injection, prior to surgery. Objectives of

this part of the study were safety, tolerability and level of NTR expression. Immunohistochemistry demonstrated NTR staining of the glandular epithelium of the tumour and of the normal prostate tissue at all dose levels which were used ($1x10^{10}$ to $1x10^{12}$ virus particles). Expression of NTR was found in 30%-50% of the prostate specimen slides and appeared to increase when using higher vector doses or multiple injections, however there was no statistically significant relationship between virus dose and extent of NTR expression. Gene therapy was well tolerated, although one patient who received $5x10^{10}$ vp had a DLT (transient bilirubin increase). The cohort was therefore expanded but no other DLT was observed and dose escalation could be resumed. (The DLT was subsequently attributed to a post-operative myocardial infarction.) Other adverse events were transient grade 3 lymphopaenia (3 patients) and grade 2 hepatic enzymes increase (4 patients). There were no treatment-related serious adverse events.

Trial Design:

Open-label, phase I, non-randomised, single centre clinical trial.

Sample Size:

It is planned to treat 15 patients in this Phase I study, however the actual number of patients will depend on whether treatment-related dose-limiting toxicity (DLT) is encountered, in which case cohort expansion (which could lead to a maximum of 30 patients) or early study termination will be necessary.

Primary Objectives:

To determine safety and tolerability of escalating doses of AdNRGM administered by multiple template-guided intraprostatic injections, followed by intravenous administration of CB1954

Secondary Objectives:

To investigate changes in PSA levels and kinetics following treatment with AdNRGM and CB1954

Inclusion Criteria

- Patients who present with biopsy-proven local recurrence of prostate cancer following radical radiotherapy and a rising PSA while on androgen suppression with anti-androgens or LHRH agonist/antagonist therapy or after bilateral orchidectomy. A rising PSA is defined as 2 consecutive increases over 3 or 4 readings over a minimum period of 6 weeks, with time-points separated by at least 2 weeks. If the patient is on anti-androgens or LHRH agonist/antagonist therapy, this therapy should be continued.
- PSA value ≥ 2 and ≤ 100 ng/ml at study entry.
- Adequate hepatic, renal and haematological function.
- Patients must agree not to father a child within 12 months following AdNRGM administration, and must use at least two methods of contraception, one of which is barrier, starting from the time of AdNRGM administration for at least 12 months.
- No known immuno-incompetence.

Main Exclusion Criteria

- Patients with a prostate or tumour which is deemed clinically unsuitable for trans-perineal template-guided injection.
- Patients who have previously been treated with prostate brachytherapy.
- Patients who have previously been treated with AdNRGM, CB1954 or any other gene therapy trial involving an adenovirus vector.
- Patients who have received chemotherapy, radiotherapy or immunotherapy within 28 days of study entry.
- Acute active infection (viral, bacterial, or fungal) which requires specific therapy.
- Known hepatitis B or C infection, HIV positive patients. (Patients will be tested for HBV/HCV, but not HIV).
- Tumours of other organs or tissues still active or treated radically less than 3 years before (except that successfully treated, non-metastatic skin cancers are not an exclusion criterion).
- Concurrent corticosteroids, or any medication known to have significant immunosuppressive action.
- Evidence of adenovirus infection and/or shedding at baseline.

For further details please see AdUP_Protocol_vn5.0_vd18_Dec_2013