

A Phase I, dose escalation trial of Recombinant Modified Vaccinia Ankara (MVA)-based Vaccine encoding Epstein-Barr virus target antigens.

Background:

Epstein-Barr virus (EBV) is a B-lymphotropic virus that is present as a persistent asymptomatic infection in most adults. Primary infection is typically acquired in childhood, at which time it presents as a nondescript febrile illness. Delayed primary infection in adolescence or adulthood is commonly associated with a benign self-limiting lymphoproliferative syndrome termed infectious mononucleosis. EBV has the property of transforming newly infected B cells into permanently proliferating B lymphoblastoid cell lines (LCL) *in vitro*. In such cells, EBV expresses a limited range of latent antigens including EBV nuclear antigens (EBNA) 1, 2, 3A, 3B, 3C and LP, and latent membrane proteins (LMP) 1 and 2 (1). EBV infection of B cells *in vivo* also leads to B cell activation and growth with full virus latent gene expression; however these cells are either removed by the immune response or go on to differentiate and down-regulate transformation-associated antigens. This results in the presence, in healthy EBV carriers, of circulating non-proliferating, non-activated EBV-positive B-cells with a post-germinal centre phenotype with little or no viral antigen expression. EBV+ malignant cells can be targeted and killed by virus-specific T lymphocytes *in vivo* with therapeutic effect. Infused donor-origin EBV-specific T lymphocytes have been shown to home to tumour sites and bring about complete regression of Lymphoproliferative disease (LPD) lesions. EBV+ LPD occur in a wide range of clinical settings in which patients are immunocompromised, including solid organ transplant recipients, patients with HIV and patients receiving chronic immunosuppression for autoimmune diseases.

The MVA-EBNA1/LMP2 vaccine is designed to amplify CD8+ cytolytic and CD4+ helper T lymphocyte responses directed against the tumour-associated viral antigens EBNA 1 and LMP 2 in individuals with EBV-positive malignancies. Both helper and killer T lymphocytes are able to mediate anti-tumour effects *in vivo*.

Aims:

Primary Objectives:

- To determine safety and to characterise the toxicity profile of MVA-EBNA1/LMP2 vaccine.
- To describe changes in the frequency of functional T-cell responses to MHC class I and II-restricted epitopes within EBNA1 and LMP2 in peripheral blood at sequential time-points before, during and up to nine months after the vaccination course.

Secondary Objectives:

- To assess changes in levels of EBV genome levels in plasma.

Entry Criteria:

Inclusion Criteria:

1. Histologically confirmed malignancy in which the presence of EBV within the malignant cells has been demonstrated by EBER (EBV early RNA) in situ hybridisation in more than 50% of the malignant cells. **2.** Patients in remission from disease, ie complete response (CR) or unconfirmed complete response (Cru). **3.** Aged ≥ 18 yrs. **4.** WHO performance status 0-1. **5.** Life expectancy of at least 4 months. **6.** Completion of standard therapy for malignancy at least 12 weeks before trial entry. **7.** *Hb > 10.0 g/dl. Lymphocytes $\geq 1.0 \times 10^9$ /L. Neutrophils $\geq 1.5 \times 10^9$ /L. Platelets $\geq 100 \times 10^9$ /L. Serum Bilirubin $\leq 1.5 \times \text{ULN}$. Serum ALP, ALT and/or AST $\leq 1.5 \times \text{ULN}$. Calculated creatinine clearance ≥ 50 ml/min (uncorrected value) or isotope clearance measurement ≥ 50 ml/min. **8.** Female patients of child-bearing potential are eligible, provided they have a negative serum pregnancy test prior to enrolment and agree to use appropriate medically approved contraception during the study up to six months after the last vaccination. **9.** Male patients must agree to use appropriate medically approved contraception during the study up to six months after the last vaccination.
***Within 8 days prior to going on study.**

Exclusion Criteria:

1. Receiving current chemotherapy or radiotherapy, or received within 12 weeks of trial entry. **2.** Known chronic active infection with Hepatitis B, Hepatitis C or Human Immunodeficiency Virus (HIV). **3.** Current active autoimmune disease. **4.** Current active skin diseases requiring therapy (psoriasis, eczema etc). **5.** Ongoing active infection. **6.** History of anaphylaxis or severe allergy to vaccination. **7.** Allergy to eggs or egg products. **8.** Previous myeloblastic therapy followed by an autologous or allogeneic haematopoietic stem cell transplant. **9.** Patients who have had a splenectomy or splenic irradiation, or with known splenic dysfunction. **10.** Receiving current immunosuppressive medication, including corticosteroids. **11.** Pregnant and lactating women. **12.** Ongoing toxic manifestations of previous treatment. Exceptions to this are alopecia or certain Grade 1 toxicities, which in the opinion of the Investigator and Cancer Research UK should not exclude the patient. **13.** Major thoracic and/or abdominal surgery in the preceding four weeks from which the patient has not yet recovered. **14.** Patients with any other condition which in the Investigator's opinion would not make the patient a good candidate for the clinical trial. **15.** Concurrent congestive heart failure or prior history of class III/ IV cardiac disease (New York Heart Association [NYHA]).

Points to Note:

-40ml blood samples for Viral Immunity (cellular & humoral immunity) will be taken at Screening (2 samples to be taken at least 1 week apart), on day 1 & 8 of each cycle, off-treatment visit (wk 10), and & wk 11, wk 14, Month 6 & Month 12 follow-up visits. Samples will be sent to Dr Graham Taylor, Institute for Cancer Studies.

-EBV genome levels 4ml sample will be taken on Day 1 of Cycle 1, Cycle 2 and Cycle 3. Samples will be batched and the assay performed after the patient has

completed the study. Samples will be sent to Mr Steve Wilson at Heartlands Hospital.

- An interval of 21 days (+/- 7 days) should occur between any two vaccinations. To proceed to the next vaccination the previous vaccination(s) must have been well tolerated. The second and third vaccinations will be given over alternate deltoid muscles or the outer thigh surface.
- Patients will be asked to remove the dressing the day after their injection, and keep the dressing in a sealed bag for return at the next clinic visit.
- Patients will be followed up at week 11 & Week 14 (4 & 7 weeks after the last vaccination), and at Month 6 & Month 12 following the first vaccination.
- For female patients of childbearing potential, a urine pregnancy test will be performed on Day 1 of Cycle 2 & Cycle 3 prior to the vaccination; the results must be reviewed prior to the vaccination.

Recruitment Number: 15 patients

Screening: Physical exam, FBC, Chemistry + (ALT). Urea & Creatinine. ECG. Chest x-ray. Pregnancy test (serum HCG). Urinalysis (pH, protein, glucose, ketones, blood). Assessment of EBV status of the tumour. HLA typing. Blood samples (viral immunity for cellular & humoral immunity & EBV genome levels – **(2 samples to be taken at least 1 week apart)**). Assessment of disease status – clinical assessment, and if appropriate a CT scan of areas previously involved in the disease (within 4 weeks prior to study day 1).

Treatment Plan:

Patients will receive three vaccinations of MVA-EBNA1/LMP2 at three-week intervals. The starting dose will be 5×10^7 plaque forming units (pfu) given by intradermal vaccination over the deltoid muscle on the arm, or on the outer thigh surface. Cohorts of three patients will receive escalating doses of the vaccine (100%, 100%, 67% and 50%). The dose escalation scheme is 5×10^7 pfu, 1×10^8 pfu, 2×10^8 pfu, 3.3×10^8 pfu, 5×10^8 pfu. This will be dependant on toxicity. Approximately 15 patients in remission having had a histologically confirmed EBV+ malignancy will be entered into this study. The final number will depend on the number of dose escalations required to reach the study endpoints.

Side Effects:

'Flu-like' symptoms, pain, malaise, chills, headache.

Follow-up: 30 day

Research Nurse: Sr Ceri Davies & Sr Libby Hewitt.

Trial Co-ordinator: Manjit Tanday

Gene therapy Pharmacist: Julie Simpson

Review date: Sept 04

Schedule of Events:

	Screen -ing	Cycle 1		Cycle 2		Cycle 3		off Rx	Follow-Up			
	Wk -1 to -4	Wk 1	Wk 2	Wk 4	Wk 5	Wk 7	Wk 8	Wk 10 ^f	Wk 11 ^g	Wk 14 ^g	Mth 6	Mth 12
	day -1 to -28	day 1	day 8	day 1	day 8	day 1	day 8	3wks post last vac	4wks post last vac	7wks post last vac	6mths post 1 st vacc.	12mths post 1 st vacc.
Informed consent	◆											
Medical history	◆											
Prior Diagnosis & Treatment	◆											
Physical examination	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
Vital signs	◆	◆ ^d	◆	◆ ^d	◆	◆ ^d	◆	◆	◆	◆	◆	◆
Weight	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
ECG	◆								◆			
Chest X-Ray	◆								◆			
Pregnancy test (if applic.)*	◆ ^b			◆ [*]		◆ [*]			◆			
Laboratory tests (haem & biochem) ^a	◆ ^b	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆	◆
EBV status of tumour **	◆											
HLA typing**	◆											
Urinalysis	◆								◆		◆	◆

Intradermal vaccination		◆		◆		◆						
Review of injection site		◆	◆	◆	◆	◆	◆					
Blood sample: viral immunity (& antibody responses when applicable) ^h	◆ ◆ ^{c, h} (2 samples at least 1 week apart)	◆	◆	◆	◆	◆	◆	◆	◆ ^h	◆	◆	◆
Blood sample: EBV levels ^e	◆	◆		◆ ^e		◆ ^e		◆	◆		◆ ^e	◆ ^e
Adverse Events		← monitored throughout treatment period & up to Wk 11 visit → (4 weeks post last vaccination)							← drug-related AEs →			
Concom diseases & Treatment	◆	← monitored throughout treatment period & up to Wk 11 visit → (4 weeks post last vaccination)										

* Pregnancy Test (if female of child-bearing potential) – results to be reviewed prior to the vaccination.
 ** Assessment of EBV status of tumour and HLA typing - only if not performed previously.
^a Haematology:- Hb, WBC & differentials, platelets.
 Biochemistry:- Na⁺, K⁺, Ca²⁺, total protein, urea, creatinine, bilirubin, ALT, alkaline phosphatase.
^b Samples to be taken < 8 days prior to Study Day 1.
^c Viral Immunity:- two samples during Screening taken at least 1 week apart.
^d Vital Signs on Day 1 of each cycle:- pre-vaccination, and 30, 60, 120 and 180 minutes post-vaccination.
^e EBV levels:- samples to be taken at all time-points and stored for later analysis. Aliquot of Screening & Week 11 sample to be sent for analysis. If detectable EBV levels in these aliquots, complete set of aliquots from all time-points to be sent for quantitation.
^f Off-Treatment Visit (Wk 10):- if discontinue treatment prematurely to be performed 3 weeks after last vaccination.
^g Follow-Up visits:- at Wk 11 & Wk 14 (4 & 7 weeks after last vaccination), and at Month 6 & 12 after 1st vaccination.
^h Blood sample viral immunity:- plasma from this sample at Screening & Wk 11 stored for analysis of antibody response.

Note: Allowed visit window of +/- 7 days during treatment period, and +/- 2 weeks during the Follow-Up period.