Abbreviated Title: EPOCH-R + vaccine for MCL
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PILOT STUDY OF IDIOTYPE VACCINE AND EPOCH-RITUXIMAB CHEMOTHERAPY IN UNTREATED MANTLE CELL LYMPHOMA

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Investigational Agents:

Drug Name:	Id-KLH Vaccine / GM-CSF (Sargramostim)
IND Numbers:	IND#: 5427 / BB IND#: 2632
Sponsor:	CTEP
Manufacturer:	TSI Washington / Immunex

Commercial Agents:

EPOCH-R = etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab

PRÉCIS

Background:

- Mantle cell lymphoma presents a particular clinical challenge because it is aggressive and incurable with chemotherapy. Thus, novel treatment approaches are needed.
- In follicular center cell lymphomas, another incurable disease, recent evidence suggests that molecular complete remissions may be achieved following idiotype vaccination in patients who have achieved minimal residual disease with combination chemotherapy.
- These results suggest that idiotype vaccines may be able to induce a clinically significant immune response against lymphoma.

Objectives:

- To assess if EPOCH-R/idiotype vaccination is associated with a median progression-free survival consistent with 36 months;
- To assess if rituximab affects generation of T-cell immunity against the idiotype.
- To compare T-cell immunity using two different methods of isolating the idiotype protein.

Eligibility:

- Tissue diagnosis of mantle cell lymphoma
- Age ≥ 18 years.
- Previously untreated with cytotoxic chemotherapy. All stages of disease.
- Lymph node of ≥ 2 cm accessible for biopsy/harvest or > 1000/μl of circulating tumor cells in the blood.
- ECOG performance status ≤3

Design:

- In the present study, we propose to investigate the efficacy of idiotype vaccine treatment in previously untreated patients with mantle cell lymphomas. In order to achieve minimal residual disease, patients will receive 6 cycles EPOCH chemotherapy and rituximab (EPOCH-R) followed by 5 idiotype vaccine injections.
- This study has completed accrual of 26 patients and is only open for follow-up.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

- To assess if EPOCH-R followed by idiotype vaccination is associated with a median progression-free survival (PFS) consistent with 36 months
- To assess if idiotype vaccine will generate a tumor specific T cell response

1.1.2 Secondary

- To assess the response rate and toxicity of EPOCH-R in previously untreated mantle cell lymphoma
- To assess the use of molecular markers of mantle cell lymphoma, including PCR-based methodologies for detection of bcl-1 and IgH rearrangements
- To obtain cDNA microarray profiling of untreated mantle cell lymphomas
- To determine overall and progression-free survival

1.1.3 Exploratory

- To explore molecular and genomic studies that may predict response and outcomes

1.2 BACKGROUND AND RATIONALE

1.2.1 Mantle cell lymphoma

In the 1980's, Weisenburger and Palutke described atypical small lymphoid cells in the mantle surrounding the benign germinal center that they named mantle zone lymphoma (MCL)(1). The cytology of MCL consists of monotonous population of atypical small to medium-size lymphoid cells with indented nuclei, and scant cytoplasm. Histologically, the cells may range from those with predominantly round nuclei or slight nuclear irregularity to those with markedly angulated and cleaved nuclei. In 20 % of cases, MCL can present as "anaplastic centrocyte" or blastic variant (2). The MCL phenotype corresponds to that of a monoclonal B cell, CD19, CD20, CD22, CD 24, IgM, IgD positive, with co-expression of IgG in 20% and monoclonal lambda light chain in 60% of cases. Classically, the cells are CD5 positive and CD10 negative. The blastic variant is less likely to express CD5, IgD and CD43 and may express CD10. (1-2). MCL has a characteristic cytogenetic abnormality t(11;14)(q13; q32). The molecular counterpart involves a translocation of the oncogene bcl-1 breakpoint into the proximity of the enhancer region of the heavy chain gene. The putative gene has been named CCND1 and encodes for cyclin D1 that is overexpressed in most of cases of MCL (3). The t(11; 14) can be used to evaluate minimal residual disease by either PCR or FISH. The presence of a complex karyotype, and hyperdyploidy has been associated with an aggressive clinical behavior.

MCL comprises 2.5% to 4% of all NHL in USA (1-2, 4). The clinical presentation of MCL frequently includes an elevated LDH, lymphadenopathy, B-symptoms (40%), advance stage III or IV (90%), splenomegaly, positive BM involvement and the GI tract is involved in 20% of cases. The clinical behavior of MCL tends to be aggressive but may vary from relatively indolent to very aggressive. Median survival is 3 to 4 years and patients are rarely if ever cured. The

response rate varies by series, ranging from 17% to 68 % CR and 28% to 52% PR (Table 1). Studies have shown event-free survivals ranging from 8 to 19 months, and disease free survival ranging from 19 to 25 months, depending on the series (Table 1). Specifically, Teodorovic et al treated 29 patients with mantle cell lymphoma using an aggressive doxorubicin-containing regimen (5). Overall 52% achieved a complete remission and the median disease free survival was 20 months. This was not significantly different from their PFS of 19 months.

Series	n	PR (%)	CR (%)	Median DFS (months)	Median PFS (months)	Median Survival (months)
G.A. Velders[6]	41	---	32	25	---	24
W.Hiddenmann[7]	45	52	17	---	8	28
H. Somaha[8]	121	28	68	---	---	38
I. Teodorovic[5]	65	---	52	20	19	45
E. Zucca[9]	26	---	50	19	---	33
J. Armitage[10]	83	---	---	---	---	32

Khouri et al tested an aggressive regimen of hyper-CVAD and high-dose methotrexate/cytarabine followed by stem cell transplantation in patients with relapsed or previously untreated mantle cell lymphoma (11). Among the 25 previously untreated patients, the overall survival (OS) and event-free survival (EFS) at 3 years were 92% and 72% respectively. In contrast, the EFS at 3 years was 17% in the previously treated patients. Although the results are impressive in the untreated group, the results from the previously treated group suggest that this combination approach can salvage few if any patients and is therefore unlikely to be curative in the untreated group. Furthermore, other studies have shown little evidence that high-dose treatment with transplant is potentially curative. Freedman et al treated 28 MCL patients with high-dose chemo-radiotherapy and anti-B-cell monoclonal antibody purged autologous bone marrow transplantation (12). Twenty patients had received prior regimens before transplant, and 8 were in first CR/PR following CHOP. Nineteen (68%) patients relapsed at a median of 21 months, and of 8 patients in first CR/PR, 5 had relapsed. With a median follow-up of 24 months, DFS and OS were estimated to be 31% and 62%, respectively, at 4 years, indicating that transplant cures few if any patients.

Preliminary results with rituximab and CHOP chemotherapy suggest that, although rituximab may increase the response rate, there is yet no evidence for prolonged response. Howard et al reported their results of CHOP and rituximab in 40 newly diagnosed patients with mantle cell lymphoma (13). Overall, 95% of patients responded, with 19 (48%) CR/CRu, but the median progression-free survival was 16.2 (1.7-26.8+) months). There results show that many of the responding patients have continued to relapse.

Taken together, these results suggest that no approach, including aggressive chemotherapy, monoclonal antibody/toxin and transplant, has produced a significant incidence of long-term PFS in mantle cell lymphoma, and indicate that patients are rarely if ever cured. Thus, novel therapeutic approaches are needed

1.2.2 EPOCH-R treatment

EPOCH-Rituximab (EPOCH-R) is a novel combination of chemotherapy and the CD-20 monoclonal antibody, rituximab. In a phase II trial of EPOCH alone in relapsed and refractory non-Hodgkin's lymphomas, EPOCH produced a 74% response rate, and a 57% response rate in patients with refractory disease (Gutierrez et al, submitted) (14). Additionally, dose-adjusted EPOCH has produced a 73% progression-free survival at 38 months in patients with previously untreated aggressive B-cell lymphomas (unpublished data), and a 92% complete remission rate (15).

Recent evidence suggests that rituximab may increase the efficacy of chemotherapy. Czuczman et al treated 40 patients with rituximab and CHOP and obtained an overall response rate of 95% of which 55% were complete (16). Of 8 patients in this trial who achieved CR and had pretreatment evidence of a BCL-2 translocation by PCR, 7 had no evidence post-treatment of the BCL-2 clone by PCR, a result that is rarely achieved with CHOP alone. Furthermore, in 30 patients with aggressive B-cell lymphomas treated with rituximab and CHOP, 93% responded and 63% were complete. Recently, we have treated 6 patients with relapsed and refractory NHL with EPOCH-R and have observed a high rate of response (data too early). There is also preclinical evidence that rituximab and chemotherapy are synergistic. Based on these findings, we project that EPOCH-R can achieve at least 80% CR/CRu in previously untreated patients with mantle cell lymphomas.

1.2.3 Idiotype Vaccine

Studies in experimental animals and patients have demonstrated the utility of the Ig idiotype as a tumor-specific antigen for the treatment of B-cell lymphoma (17). Eisen and co-workers first demonstrated that active immunization against idiotypic determinants on malignant B cells produced resistance to tumor growth in syngeneic mice (18). Since that time, the phenomenon of idiotype-specific tumor resistance, as well as specific anti-tumor therapy against established tumors, has been reproduced in a number of animal models (19-25). These results, taken together, provided the rationale for testing autologous tumor-derived idiotypic surface Ig (Id) as a therapeutic "vaccine" against human B-cell lymphoma

In a series of laboratory and clinical studies, Kwak at et. have shown the feasibility of an idiotype vaccine strategy (26-29). Clinical experience by the Kwak laboratory revealed the induction of particularly potent anti-idiotypic immunity by the addition of free GM-CSF to the Id-KLH formulation as an adjuvant. Furthermore, this effect was dependent on the selective activation of the T-cell arm of the immune response. Results in a subsequent, larger clinical trial of Id vaccination in-patients with follicular lymphoma receiving standard dose chemotherapy (96-C-0133) have confirmed the potency of this cytokine adjuvant in humans. Specifically, 42 previously untreated patients with follicular lymphoma have first been treated with modified ProMACE chemotherapy to induce a minimal residual disease state (complete or partial remission). Twenty patients have subsequently received a series of five monthly subcutaneous vaccinations with autologous Ig-KLH, together with locally administered free GM-CSF (patients are randomized to receive either 500 or 100 mcg/m^2)(30). In contrast to the previous study, local reactions characterize by erythema and induration (at least 8 cm) have been consistently observed at the vaccination sites of all patients, starting with the second vaccination sites of all patients, starting with the second vaccination in the series. These local reactions were subsequently reproduced at distant sites by a challenge with unconjugated autologous Ig alone (without KLH), but not with GM-CSF alone, suggesting that a significant component of this

response is directed against the autologous Ig. In five of six patients formally tested, this response was specific for the individual idiotype, as demonstrated by lack of a reaction to a control, isotype-matched, Ig. These early results provide convincing *in vivo* evidence that it is possible to induce a specific immune response against a self-tumor antigen and that GM-CSF is a potent immune stimulant in human patients. Moreover, CD8+ T cell responses to autologous tumor were demonstrated in 95% (19/20) of patients (31).

An important and unanswered question is the impact of rituximab on the generation of a T-cell response to the idiotype vaccine. This is of practical clinical importance because of the increasing use of rituximab with chemotherapy by treating physicians, as well as of scientific importance. For vaccines which primarily produce an antibody response, there is a concern that rituximab may abrogate the immunological response. However, for vaccines which generate a T-cell response, such as the idiotype vaccine in the present study, recent evidence suggests that rituximab could increase the T-cell response to the vaccine. Elegant experimental evidence in mice has shown that depleting the host of B cells may actually INCREASE anti-tumor immunity elicited by vaccines (32). Specifically, in a murine tumor model, immunization with irradiated tumor cells produced only a humoral response which was non-protective. However, this same vaccination strategy in B-cell deficient mice (genetically engineered knock-out mice) produced potent T-cell mediated protective anti-tumor immunity. The conclusion from these experiments was that the presence of B cells in the priming phase results in disabled CD4+ T-cell help for CD8+, CTL-mediated tumor immunity.

1.2.4 Summary

In light of these observations, the current protocol will evaluate the effect of anti-idiotypic vaccination on the generation of a T cell-based vaccine response after treatment with EPOCH-R chemotherapy using two different methods of idiotype isolation and scale-up. The clinical endpoint of the trial will be to determine if patients on this trial have at least a median PFS of 36 months. Clinically, we consider achieving a PFS of 36 months to represent a significant improvement over standard treatment which has been reported to produce a median PFS of 18 months at the longest.

2. ELIGIBILITY ASSESSMENT AND ENROLLMENT ELIGIBILITY CRITERIA

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion

2.1.1.1 Tissue diagnosis of mantle cell lymphoma (confirmed in Laboratory of Pathology). Blastic cell variant will be eligible.

2.1.1.2 Age ≥ 18 years.

2.1.1.3 Previously untreated with cytotoxic chemotherapy. Patients may have received local radiation or a short course of steroids for control of symptoms.

2.1.1.4 All stages of disease.

2.1.1.5 Lymph node of ≥ 2 cm accessible for biopsy/harvest or > 1000/μl of circulating tumor cells in the blood.

2.1.1.6 ECOG performance status ≤ 3.

2.1.1.7 Adequate major organ function (serum creatinine 1.5 mg/dl or creatinine clearance > 60 ml/min; bilirubin < 2 mg/dl (total) except < 5 mg/dl in patients with Gilbert's syndrome as defined by > 80% unconjugated; ANC > 1000 and platelets > 100,000) unless impairment due to organ involvement by lymphoma.

2.1.1.8 No active symptomatic ischemic heart disease, myocardial infarction or congestive heart failure within the past year. If MUGA is obtained, the LVEF should exceed 40%.

2.1.1.9 Ability to give informed consent.

2.1.2 Exclusion

2.1.2.1 Antibodies to HIV or presence of hepatitis B surface antigen.

2.1.2.2 Pregnant or lactating.

2.1.2.3 Prior malignancy in past 5 years except squamous or basal cell carcinoma or curatively treated in situ of the cervix.

2.1.2.4 Involvement of central nervous system by lymphoma.

2.2 SCREENING EVALUATION

2.2.1 Complete History and Physical examination.

2.2.2 CBC, differential, PT, PTT, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, albumin, calcium, Phosphate, uric acid, creatinine (24 hour creatinine clearance if serum creatinine > 1.5 mg/dl), electrolytes, urinalysis.

2.2.3 HIV antibody and hepatitis B surface antigen.

2.2.4 HCG (urine) in women of childbearing potential.

2.2.5 Electrocardiogram.

2.2.6 Staging: Chest x-ray, CT scan of chest, abdomen and pelvis; colonoscopy if not performed in previous 2 months; SPECT gallium as indicated.

2.2.7 Head CT and lumbar puncture with cytology as clinically indicated.

2.3 REGISTRATION PROCEDURES

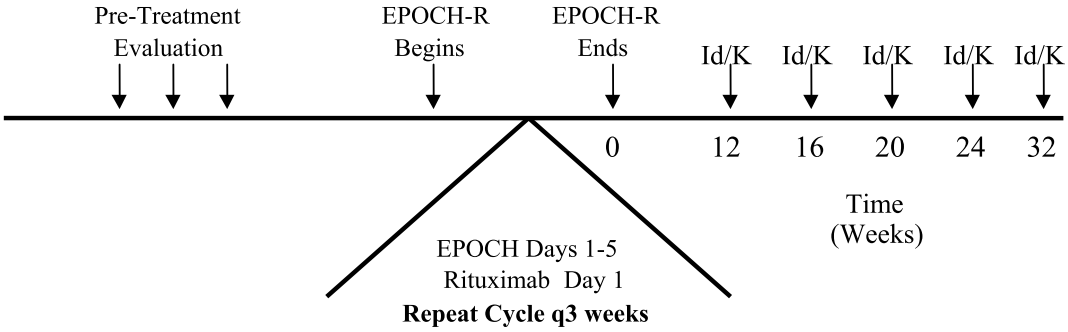
Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3. STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Summary

Patients will receive 6 cycles of EPOCH-R. All patients will receive idiotype vaccine regardless of their response to EPOCH-R unless they require non-protocol chemotherapy or radiation between completion of EPOCH-R and beginning of vaccination. If patients require chemotherapy during vaccination, vaccination will be stopped. The decision to begin non-protocol chemotherapy or radiation will be made by the PI based on good medical practice; generally, major organ system compromise or the presence of systemic symptoms are reasons to begin treatment. Vaccination will begin when the vaccine is available following a minimum of 12 weeks after completion of EPOCH-R.



Administer 6 cycles

3.1.2 EPOCH-R Administration

Infusional agents are administered via central venous access, and cycles are repeated every 21 days. Dose adjustment: To provide optimal dose intensity, doses will be adjusted based on the level of hematopoietic toxicity as follows:

a. Neutrophil

Nadir ANC on previous cycle	Dose Level
$\geq 500/mm^3$	Escalate one level

<500/mm^3 ≤ 2 measurements[1]	No change
<500/mm^3 ≥ 3 measurements[1]	Reduce one level

b. Platelets

Nadir plts on previous cycle	Dose Level
≥ 25K	No change
<25K	Reduce one level

NOTE: [1]MEASUREMENTS BASED ON TWICE WEEKLY CBC

If both unacceptable neutropenia and thrombocytopenia occur, use the lower of the two dose level reduction options. If the patient has extensive bone marrow involvement at the start of therapy, the responsible physician has the option not to attenuate drug doses for unacceptable durations of neutropenia.

Dose Level 1:
Doxorubicin: 10 mg/m^2/day CIV days 1-4 (96 hours).
Etoposide: 50 mg/m^2/day CIV days 1-4 (96 hours).
Vincristine 0.4 mg/m^2/day CIV days 1-4 (96 hours).
Cyclophosphamide: 750 mg/m^2 IV day 5 after infusions.
Prednisone 60 mg/ m^2 P.O. bid days 1-5.
Rituximab 375 mg/m^2 IV day 1 immediately before infusions.
Filgrastim 300 μg/day SC day 6 to post-nadir AGC >5, 000/mm^3**.**

Table of doses per level for adjusted agents:

Drugs	**Drug Doses per Dose Levels**							
	-2	**-1**	**1**	**2**	**3**	**4**	**5**	**6**
Doxorubicin (mg/m^2/day)	10	10	10	12	14.4	17.3	20.7	24.8
Etoposide (mg/m^2/day)	50	50	50	60	72	86.4	103.7	124.4
Cyclophosphamide (mg/m^2/day)	480	600	750	900	1080	1296	1555	1866

- Escalation on subsequent dose levels: Doses of doxorubicin, etoposide, and cyclophosphamide increased by 20% over the immediate preceding level.
- Dose Level (-1)(mg/m^2/day): 20% reduction of cyclophosphamide alone from level I (cyclophosphamide 600 mg/m^2); other drug doses unchanged.
- Reduction on subsequent dose levels: Dose of cyclophosphamide reduced by 20% over the immediate preceding level.
- Duration of therapy: Patients will receive 6 cycles.

3.2 VACCINE ADMINISTRATION

3.2.1 Vaccine Preparation

Two different methods of idiotype isolation and scale-up will be employed in the vaccine manufacture process. The first 13 patients will receive vaccine produced by the somatic hybridization technique (accrual completed) and the second 13 patients will receive vaccine produced by the recombinant method.

3.2.2 Vaccine Administration

Patients will receive a series of 5 Id/KLH vaccinations [0.5 mg tumor-derived Ig conjugated to KLH] beginning 12 weeks (or at such later time, up to 12 months, that the Id vaccine becomes available) after completion (i.e. designated as day 21 of last cycle) of EPOCH-R on the schedule outlined in this section. If patients have progressive disease that requires chemotherapy treatment before or while receiving vaccination, vaccination will either not begin or will be stopped.

3.2.3 Schedule

Id/KLH vaccine and GM-CSF will be administered subcutaneously on the following schedule:

- Day 0: Id-KLH 0.5 mg s.c.
- Days 0, 1, 2, 3 GM-CSF 100 $\mu g/m^2/d$ s.c.

Injection sites are rotated between upper and lower extremities. Each dose of vaccine or GM-CSF is split equally between the two upper or lower extremities. GM-CSF injection is to be given in close proximity (< 5 cm) to the Id/KLH injection site. However, if local reactions to GM-CSF are severe, GM-CSF injections may be given elsewhere. Patients will be observed in the clinic for two hours following Id-KLH and/or GM-CSF administration with vital signs taken before injection and every 30 minutes. Non-steroidal anti-inflammatory drugs and/or steroids should be avoided. Use lymphoma vaccine vital sign sheet. Any local skin reactions will be carefully noted and scored for erythema, induration, pain and disruption of the barrier surface. We will only follow reactions x 4 days while patients are at NIH. If any patient has a reaction suggestive of sensitization, the vaccine may be split into its component parts; specifically, the patient will be tested with Id-KLH alone and then GM-CSF alone. Toxicities will be graded according to the NCI CTC version 2.0 at http://ctep.info.nih.gov.

3.3 TREATMENT MODIFICATIONS

3.3.1 EPOCH-R

- EPOCH dose reductions: Adjustments based on nadir as outlined in Section **3.1.2**. Adjustment based on day 1 counts: If the ANC < $1000/mm^3$ or platelets < 75K on day one of the next cycle, treatment should be delayed by up to 1 week until recovery above these levels with treatment at the last administered dose level. If treatment is deferred due to low ANC, filgrastim 300µg/day may be administered; note that filgrastim should be stopped at least 24 hours before restarting EPOCH. If the counts do not recover by 1 week, reduce EPOCH by one dose level when counts recover. However, if the delayed recovery is felt to be secondary to bone marrow disease, the patient may be treated with reduced counts at the discretion of the principal investigator.

- Rituximab: Side effects of rituximab may be infusion rate related and may be reduced by slower administration or premedication. Thus, dose reductions of rituximab will not be made. Rituximab will be discontinued for the duration of the cycle in patients with grade 4 allergic reactions. At the discretion of the PI, rituximab may be administered on the following cycles using slower infusion rates and pretreatment with benadryl and prednisone using standard medical practice.
- Ileus: All patients should receive stool softeners as indicated to prevent constipation during EPOCH-R. Occasionally, symptomatic ileus may occur and this should be treated with a vincristine dose reduction. Because the degree of ileus may vary and be affected by comorbid events, dose reductions will be based on good medical practice. Generally, a dose reduction of 25% will result in abrogation of the ileus. The vincristine dose may be increased back to full dose if symptoms of ileus do not occur following a dose reduction, based on good medical practice.
- Neurological toxicity:

Toxicity	Grade	Vincristine Dose
Sensory	3	75%
	4	50%
Motor	2	75%
	3	0%

3.3.2 GM-CSF

- Fever: Grade 1-3 fever/chills associated with vaccine and/or GM-CSF administration is treated with acetaminophen and/or meperidine. For grade 4 fever unresponsive to Acetaminophen, hold GM-CSF until < grade 2, and restart at 50% of original dose for remainder of injections.
- Neurologic toxicity: For grade 2-3, hold treatment until symptoms resolved, and then restart at 50% of original dose for remainder of injections. If toxicity recurs or persists, or grade 4 toxicity occurs, GM-CSF will be discontinued.
- Cardiac toxicity: For ≥ grade 3 likely associated with GM-CSF, discontinue GM-CSF.
- Gastrointestinal: For ALT/AST > 10 x normal, hold GM-CSF until < 5 x normal, and then restart at 50% of original dose for remainder of doses. For grade 3 vomiting (unresponsive to therapy), hold GM-CSF until < grade 2 and then restart at 50% of original dose for remainder of doses.

3.4 ON STUDY EVALUATIONS

3.4.1 Pre-Treatment

3.4.1.1 Twenty milliliters of serum for storage.

3.4.1.2 Quantitative immunoglobulin, serum protein electrophoresis.

3.4.1.3 Lymphopheresis: 1 x 10^{10} lymphocytes. Send to Dr. Kopp, Clinical Immunology Services, NCI-FCRDC, Frederick, MD. These samples will be used for baseline studies of T-cell response to Id.

3.4.1.4 Tumor biopsy- Prior to treatment, all patients must undergo a biopsy of a clinically involved lymph node to provide material for idiotype vaccine unless tumor cells available by apheresis. The biopsy should be at least 2 cm square in size. Bone marrow biopsy. Five cc of marrow will be aspirated into 0.5 ml of PFH for PCR analysis. When possible, FACS will be done on the marrow.

3.4.1.5 FACS of peripheral blood for disease detection- send 20 cc in green tops.

3.4.1.6 Lymphocyte subset analysis: Pre-treatment obtain 6 cc green top tube for FACS to Bill Kopp

3.4.2 During EPOCH-R Cycles

3.4.2.1 Each cycle (day 1): Physical exam, CBC/differential, electrolytes, liver function, LDH.

3.4.2.2 All treatment weeks: CBC/differential twice weekly.

3.4.2.3 End of cycle 4 and 6: Whole body CT scans. SPECT gallium, colonoscopy_and bone marrow biopsy if positive on last exam. For cases in which the investigators strongly suspect residual disease (e.g. abdominal masses is gallium avid), imaging-guided needle biopsy may be performed.

3.4.2.4 Pharmacokinetics of etoposide, doxorubicin and vincristine will be drawn on all cycles in which sampling can be obtained. Draw 5 cc green top tube at the following times on each cycle: Time 0 (baseline); Time 22 hours (draw 2 tubes); Time 94 hours (draw 2 tubes). Send tubes to Dr Frank Balis's laboratory in 13C118.

3.4.2.5 Lymphocyte subset analysis: Cycle 6 D21 obtain 6 cc green top tube for FACS to Bill Kopp

3.4.2.6 FACS of peripheral blood for disease detection after cycle 4 and after cycle 6 if positive on cycle 4- send 20 cc in green tops.

3.4.3 During Vaccine Treatment

3.4.3.1 Obtain immediately pre- (within 4 weeks of starting vaccinations) and 4 weeks post-vaccine (after 5 vaccine treatments) tumor measurements and staging disease sites (CT) and gallium/PET if positive on last exam. For cases in which the investigators strongly suspect residual disease (e.g. abdominal masses is gallium avid), image-guided needle biopsy may be performed.

3.4.3.2 Lymphocyte subset analysis: Pre and post-vaccine treatment obtain 6 cc green top tube for FACS to Bill Kopp.

3.4.3.3 PT, PTT, UA and β_2-microglobulin on day 0 of each immunization:

3.4.3.4 Five tiger top tubes of peripheral blood (60-120cc in PFH) for serum and lymphocyte preparation, respectively, for the Clinical Immunology Service on day 0 of each the first immunization, and 2 tiger top tubes on day 0 of subsequent immunizations.

3.4.3.5 Skin Biopsy at the discretion of the Principal Investigator. Obtain near planned immunization site on day 0 of first immunization (baseline sample) and again on

days 1, 2, or 3 of immunization 3 at site of erythema and/or induration. Skin biopsy not required for eligibility.

3.4.3.6 Fine needle aspiration or core biopsy of an enlarged lymph node draining the vaccination sites may be performed to obtain lymphocytes for in vitro assays. FNA or core biopsy of lymph node not required for eligibility.

3.4.3.7 FACS of peripheral blood for disease detection pre and post vaccine- send 20 cc in green tops.

3.4.3.8 CBC with differential and Chem 20 on Day 0, 1, 2, 3 of each immunization.

3.4.3.9 Immune studies on peripheral blood at each post-vaccine follow-up- send 60 cc in PFH and SST tubes.

3.4.4 Post-Therapy Evaluations

3.4.4.1 Following the last immunization, restage all sites of disease by CT scans q 3 months x 4, q 6 months x 4 and yearly thereafter.

3.4.4.2 Studies at each follow-up visit: Hgb, HCT, WBC, and differential, SGOT, alkaline phosphatase, LDH.

NOTE: These may be done less frequently at the discretion of the investigator.

3.5 Criteria for Removal from Protocol Therapy and Off-Study Criteria

3.5.1 Criteria for Removal from Protocol Therapy

- Completion of protocol treatment
- Inability to tolerate therapy or felt to be detrimental to the patient's health.
- Patient voluntary withdrawal
- Institution of or requirement for non-protocol chemotherapy

3.5.2 Off Study Criteria

- Patient does not follow the instructions given by the study team
- Patient voluntary withdrawal
- Death

3.5.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the website **(**http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

4. SUPPORTIVE CARE

4.1 Pneumocystis Jiroveci

All patients will receive prophylaxis during EPOCH-R. Trimethoprim/sulfamethoxazole I DS

P.O bid for three consecutive days each week is preferred. Allergic patients may receive inhaled pentamidine 300 mg once a month.

4.2 TUMOR LYSIS SYNDROME (CYCLE 1 ONLY)

Patients with high tumor burden may be treated with allopurinol 600mg prior to cycle 1 EPOCH-R followed by 300 mg daily for up to 7 days. Hospitalization with aggressive IV hydration and urinary alkalization may be used.

5. BIOSPECIMEN COLLECTION

NOTE: As of Amendment L, no samples are being prospectively collected for this protocol; however, analyses of previously collected samples are ongoing. Sections **5.1.1**-**5.1.7** below represent the original collection information and analyses on the samples and are retained here for historical purposes; Section **5.1.8** represents new analyses. In addition, subjects may be reconsented, if required, to allow the ongoing use of their samples for future research analyses, including genetic testing.

5.1 CORRELATIVE STUDIES

5.1.1 Lymph Node Harvest/Biopsy

Lymph node biopsies will be divided approximately as follows: (a) One-quarter of the specimen will be sent in saline or media to Dr. Elaine Jaffe, Hematopathology Section, Laboratory of Pathology, to be processed for routine histology and for immunophenotypic characterization (i.e. monotypic heavy and light chain expression); (b) One-quarter of the specimen will be sent in cold saline or media to Dr. Staudt for analysis of cDNA microarray and; (c) One-half of the specimen will be sent in sterile RPMI medium to Dr. Kwak's laboratory, NCI-FCRDC, where it will be assigned a unique accession number. Processing into a single-cell suspension and cryopreservation will be performed by Dr. Kwak's laboratory.

5.1.2 Peripheral Blood Harvesting

In patients with adequate circulating tumor cells (> 500 cells/μl) may have tumor cells harvested by apheresis to obtain ≥ 500 x 10^6 tumor cells for production of vaccine. A small aliquot (< 10 x 10^6 cells) will be analyzed by FACS and cytology to confirm presence of tumor cells. Collection of lymphocytes (> 2 x 10^6/kg) by apheresis will also be performed prior to beginning EPOCH-R and prior to beginning vaccine for immunological assays. If an apheresis is done pre-EPOCH-R for collection of tumor cells, this will also suffice for collection of lymphocytes for immunological assays. All cells will be sent to Dr. Kwak's laboratory, NCI-FCRDC, where they will be assigned a unique accession number. Processing and cryopreservation will be performed by Dr. Kwak's laboratory.

5.1.3 Blood and Bone Marrow Samples

Obtain pre- and post-vaccine (within 4 weeks) unilateral bone marrow biopsy and aspirate for assessment of minimal residual disease by pcr. Five cc of marrow will be aspirated into 0.5 ml of PFH for PCR analysis. All peripheral blood and bone marrow aspirate samples will be sent in an expedited manner to Clinical Immunology Services, NCI-FCRDC. Tiger top tubes will be spun down and serum divided into 1 ml aliquots for frozen storage. Peripheral blood mononuclear cells (PBMC) will be isolated prior to freezing by Ficoll-hypaque centrifugation using standard protocols.

5.1.4 Assessment of Immune Function

Lymphocyte subset analysis (B- and T-cells) will be assessed by FACS at the following time points: 1. Pre-EPOCH-R; 2. Post-EPOCH-R (Cycle 6 D21); 3. Pre-Vaccine; 4. Post-vaccine. Send 6 cc in Green Top (Sodium Heparin) to Bill Kopp (301-846-1491) as outlined above.

5.1.5 Assay for Serum Anti-Idiotypic Antibody

In a direct enzyme-linked immunoabsorbent assay (ELISA), pre-immune and hyper-immune serum samples from each patient will be diluted over wells of a microtiter plate that are coated with either autologous immunoglobulin idiotype or a panel of isotype-matched human tumor immunoglobulins of unrelated idiotype.[27] Bound antibody is detected with horseradish peroxidase-goat antihuman light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype (Caltag laboratories, South San Francisco).

5.1.6 Assay for Idiotype-Specific Proliferative Response

When feasible, fresh PBMC will be used on the same day they are obtained for analysis.[27] Stored frozen PBMC will be available as a back-up. PBMC will be washed and plated at a concentration of 4 x 10^5 cells per well in Iscove's modified Dulbecco's medium (IMDM) with 1 percent human AB serum (IMDM-1 percent AB). KLH, autologous Immunoglobulin idiotype, or a panel of isotype-matched immunoglobulins of irrelevant idiotypes at concentrations of 0 to 100 µg per milliliter in IMDM-1 percent AB preparation will be added in triplicate. After the cells are incubated for three days at 37ºC in an atmosphere containing 5 percent carbon dioxide, they will be transferred to a preparation of IMDM and 5 percent fetal-calf serum containing recombinant interleukin-2 (30 U per milliliter). The plates will then be incubated for two days and pulsed for 16 to 20 hours with ^{3}H-labeled thymidine (1 µCi per well). Data are expressed as mean ($\pm$SEM) counts per minute of [^{3}H]thymidine incorporation. Initial five-day cultures of PBMCs established as described above will be expanded in IMDM-5 percent fetal-calf serum containing interleukin-2 (30 U/ml). Harvested cells will be replaced in IMDM-1 percent AB containing autologous immunoglobulin idiotype and fresh irradiated (5000 R) autologous PBMCs (4 x 10^5 cells per well) as antigen-presenting cells for five days, before pulsing with [^{3}H]thymidine.

5.1.7 Definition of a Positive Idiotype T-Cell Response

Procedures and definitions as outlined by Bendandi et al (30).

5.1.8 Detecting Minimal Residual Disease (MRD)

5.1.8.1 Rationale for MRD assessment

Detecting Minimal Residual Disease (MRD) can be a powerful tool to monitor patients' response to treatment and early detection of relapse.

It is of research interest to determine if circulating tumor DNA before, during or after therapy is predictive of long-term disease-free survival. Adaptive Biotechnologies Corp will assess whether immune repertoire data (e.g., B-cell immunoglobulin receptor sequences) from the Human Material can be used as biomarkers that correlate with disease-free survival. Adaptive Biotechnologies Corp will use a proprietary method, Immune Cell Receptor Sequencing (ICRS) platform, for amplifying and analyzing immune cell receptor sequences, allowing unprecedented sensitivity and specificity. Data from experiments conducted by Adaptive Biotechnologies, Corp. using the human material will be provided to NCI and such

data provided by Adaptive Biotechnologies Corp to NCI may be used by NCI for any purpose.

5.1.8.2 Samples to be sent to Adaptive Biotechnologies

Coded, (linked) frozen or formalin fixed and paraffin embedded (FFPE) human tissue and serum and/or plasma samples and data from select patients will be sent, if available.

5.1.8.3 Shipping information

Only coded (linked) samples will be shared as described below; see Section **13.1.1** for process of sample coding, and sample request instructions. The samples and data will be stored in the Clinical Support Laboratory, Leidos Biomedical Research, Inc. and sent in batches to Adaptive Biotechnologies Corp at the address listed below:

Adaptive Biotechnologies Corp.
1551 Eastlake Ave E
Suite 200
Seattle WA 98102

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

All specimens obtained in the protocol are used as defined in the protocol. Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.2.1 Procedures for stored specimens

See Section **13.1.1** for information.

5.2.2 Study Completion, Future Use and Sample Destruction

The study will remain open so long as sample or data analysis continues. Following completion of the planned analyses, samples will remain in storage as detailed above.

Tissue specimens and derived tissue lysates, RNA and DNA collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study that are not expressly stated in the present protocol. However, this research may only be done if the risks of the new questions and the proposed research have undergone prospective IRB review and approval. If new risks are associated with the research the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved; additionally, the samples will be destroyed.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section 7.2.

5.3 GENETIC/GENOMIC ANALYSIS

5.3.1 Description of the scope of genetic/genomic analysis

At any point in the analyses, normal genome could be analyzed for comparison with other testing (e.g., cancer genome).

5.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described (Section **5.1.8.3** and **13.1.1**). In addition, a Certificate of Confidentiality has been obtained for this study.

5.3.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists).

5.3.4 Genetic Counseling

Subjects will be contacted with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH to have genetic education and counseling to explain this result; at the time of any such event(s), these activities will be funded by the NCI/CCR in consideration of the specific circumstances. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6. DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system DCS clinical trials database and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported.

An abnormal laboratory value will only be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study

- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1.**

6.2 DATA SHARING PLAN

6.2.1 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

From the International Workshop to Standardize Response Criteria for non-Hodgkin's Lymphomas. Responses must last for at least 4 weeks off respective treatment (i.e. post-EPOCH-R and post-Id immunization).

- Complete response (CR): Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (e.g. LDH) definitely assignable to the lymphoma. All lymph nodes must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in greatest diameter must have decreased to ≤ 1 cm or by more than 75% in the sum of the products of the greatest diameters (SPD). Spleen, if considered to be enlarge before therapy, must have regressed in size and not be palpable on physical examination. The bone marrow must show no evidence of disease by histology. Flow cytometric, molecular or cytogenetic studies will not be used to determine response.
- Complete response unconfirmed (CRu): As per CR criteria except that if a residual node is > 1.5 cm, it must have regressed by > 75% in SPD. Lymphocyte aggregates within the bone marrow must be negative for B-cell markers (e.g. L26).
- Partial response: ≥ 50% decreased in SPD of 6 largest dominant nodes or nodal masses. No increase in size of nodes, liver or spleen and no new sites of disease. Splenic and hepatic nodules must regress by ≥ 50% in the SPD. Bone marrow is irrelevant for determination of a PR.
- Progressive disease: ≥ 50% increase from nadir in the SPD of any previously involved node or the appearance of any new lesion.

- Stable disease: Defined as less than a PR but not progressive disease.

6.4 TOXICITY CRITERIA

The NCI Common Toxicity Criteria version 2.0 will be used for toxicity and adverse event reporting. A copy of the CTC version 2.0 can be downloaded from http://ctep:info.nih.gov.

7. NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 IND SPONSOR REPORTING CRITERIA

Reporting of Adverse Events to CTEP has not been required since 05/04/2006, as the protocol was completed with CTEP on that date.

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1**will be submitted within the appropriate timelines.

7.5.2 Sponsor

This study was monitored by the Clinical Data Update System (CDUS) version 1.1 until

05/04/2006 when the protocol was completed with CTEP.

8. STATISTICAL CONSIDERATIONS

This is a study intended to provide a small test of a therapeutic strategy in a disease with recognized poor outcomes. The primary objectives of this study are to form an estimate of the progression-free survival (PFS) in patients treated with EPOCH-R and (idiotype vaccine) beginning from the start of chemotherapy.

This study will require a maximum of 26 patients to address the principal objectives posed by the protocol. Estimates of median PFS range from 8 to 19 months for combination chemotherapy, and is 16.2 months for rituximab and CHOP chemotherapy. We are aiming to demonstrate, within a small single arm study, whether the combination of EPOCH-R and idiotype vaccine may demonstrate a potentially superior outcome to other therapeutic approaches with a target median PFS of 36 months.

Because a formal two-stage design will not be practical with 36 months as the time at which the main endpoint is evaluated, this study will use a single stage phase II design in order to estimate the number of patients to enter into the evaluation of PFS as follows. It will be desirable if 50% of patients are able to have a PFS equal to or greater than 36 months (p1=.50). By contrast, if only 25% achieve 36 month PFS (p0=0.25), then this is no better than the best that would have been expected from a cohort treated on other trials, which have a median PFS of up to approximately 18 months (expected PFS would be 25% at 36 months if median were 18 months, assuming an exponential survival distribution). With alpha=0.10 and beta=0.10, 26 patients will be entered. If 10+/26 have 36 month PFS, this will be desirable in order to show consistency with a median of 36 months PFS (33). Confidence intervals about the 36-month PFS point will also be constructed. A 95% one-sided confidence bound beneath 10/26 extends downward to 23%; thus, if 10/26 patients are observed to have 36 month PFS, then there is a 95% chance that the true proportion with 36 month PFS exceeds 23%.

In order to provide a stopping rule based on PFS, in the absence of a formal two-stage design, the following will be used: After the study has been open and has accrued patients for 36 months, the Kaplan-Meier curve for PFS will be constructed, and the 18 month PFS estimate will be calculated, along with its standard deviation. If the 18 month estimate minus one standard deviation includes 50% PFS, then accrual will stop, because this will indicate consistency with the potentially expected median PFS of 50% at 18 months.

It is hypothesized that a factor which may greatly influence the duration of PFS will be production of an immune response. The immune response will be defined as previously published[27]. A prior study of idiotype vaccine showed a 95% frequency of immune response in patients with follicular lymphoma treated with ProMACE chemotherapy[27]. However, the impact of EPOCH chemotherapy, mantle cell histology and/or rituximab on the frequency of a positive immune response is currently unknown. In order to allow the trial to end accrual of patients prior to the planned accrual in the event that the immune response rate is lower than expected, we plan to use an early stopping rule for immune responses. If the observed immune response rate is much less than 70% at an early point, we plan to re-evaluate the trial design. For this purpose, we will employ a simple one-stage stopping rule as follows. Approximately 15 patients will be enrolled in order to expect to have 10 who would be treated with EPOCH-R and receive the full vaccine. If 4+/10 exhibit an immune response, then accrual will proceed until there are 26

patients in order to see the magnitude of PFS as described; otherwise, with 0 to 3 immune responses in 10 patients who have completed all vaccinations, there is only a 1% probability of observing such a rare outcome if the true response proportion were 70%. If this were to happen, accrual would be suspended until an evaluation of the immunological and clinical endpoints is undertaken. Only if there was demonstrated merit in continuing the trial, such as favorable clinical results or information suggesting that the chosen immune response assays do not reflect efficacy, would accrual be reopened. This evaluation would be done in consultation with the IND holder. 5.4.6 It is anticipated that 2 years of accrual will be required in order to enter up to 26 patients onto this trial.

Based on the above estimates and assumptions, up to 25% of entered patients may not have received all of their vaccine treatments due to disease progression. A subset analysis evaluating PFS among patients who received all vaccine treatments will be performed to arrive at an estimate of potential benefit of vaccine in this subset.

9. COLLABORATIVE AGREEMENTS

9.1 MATERIALS TRANSFER AGREEMENT: BIOVEST INTERNATIONAL

There is a Materials Transfer Agreement (MTA) with Biovest International, Inc. **As of Amendment N, this MTA is no longer valid.**

9.1.1 According to the terms of the MTA:

- **As of Amendment N, this section is no longer relevant. It is being kept for historical purposes. Biovest is no longer involved in the study; therefore the collaborators listed below do not have access to any data or samples.** Biovest will have access to the MCL vaccine clinical data and results and raw data in NIH's possession and control as needed to support the registration of the Agent (Id-KLH Vaccine) by the FDA in this disease. Furthermore, Biovest will be provided safety data generated from the use of the Agent (Id-KLH Vaccine) that is in the possession and control of NIH as needed to support the FDA registration. Collaborator's access to and use of said data is limited to use for regulatory filings only.
- Clinical Data and results under NCI Protocol # 00C0133 may be used by Biovest in obtaining FDA approval for the commercial marketing of Agent (Id-KLH Vaccine) products.
- Biovest access to and review of Identifiable Private Information (IPI) shall be only for onsite quality auditing. Biovest will receive IPI only for purposes of satisfying FDA or other health authorities' reporting requirements, and for internal research purposes directly related to obtaining regulatory approval of Agent (Id-KLH Vaccine). Biovest is prohibited from access, review, receipt, or use of such information for other purposes.
- If the Data being provided is coded, the Provider (NCI) will not release, and the Biovest will not request, the key to the code. Biovest will not contact or make any effort to identify individuals who are or may be the sources of Data without specific written approval from Provider.

9.1.2 Contact Information for Responsible Parties at Biovest International, Inc. **As of Amendment N, these collaborators are no longer involved in the study. This information is being kept for historical purposes.**

Carlos F. Santos, Ph.D.
Sr. VP Product Development and Regulatory Affairs
Biovest International, Inc.
324 South Hyde Park Ave
Suite 350
Tampa, FL 33606
www.accentia.net

David Moser
Director of Legal Affairs
324 S. Hyde Park Ave.
Suite 350
Tampa FL 33606
Email: Dmoser@accentia.net

9.2 MATERIAL TRANSFER AGREEMENT: ADAPTIVE BIOTECH

The MTA with Adaptive Biotechnologies Corp has been fully executed to allow the samples described in Section **5.1.8** to be shipped to Adaptive Biotechnologies, Corp.

10. HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

Non-Hodgkin's lymphomas affect all races and genders. However, males are more likely than females to be affected and this will be reflected in the gender distribution of our cases. We have selected mantle cell lymphomas for inclusion in this trial because they are considered to be incurable with chemotherapy. Thus, they would potentially benefit from the use of a novel chemotherapy regimen like EPOCH-R. Additionally, the testing of a novel vaccination approach to potentially stimulate an immune reaction against the lymphoma could result in an improved clinical outcome. Patients under the age of 18 are excluded because mantle cell lymphoma is rare in young patients, and the inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this phase I study. Additionally, patients with HIV infection will be excluded due to the severe immunosuppression of this therapy, and pregnant or nursing mothers are excluded because of the potential teratogenic effects of therapy.

10.2 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent were excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or

cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.3 Evaluation of Benefits and Risks/Discomforts

Patients may obtain direct benefit from treatment with EPOCH-R, a regimen which includes non-experimental cytotoxic agents and rituximab. The risks and benefits of EPOCH-R are comparable to standard treatment approaches. Patients may also potentially benefit from the idiotype vaccine. It is possible but unknown if one technique for idiotype isolation and scale-up is superior to the other one. Risks of idiotype vaccine I previous trials have been due to administrations of GM-CSF with occasional fever, chills and body aches. Although it is possible to have a life-threatening allergic reaction, this has not been observed in previous trials. If this approach is effective, patients may be directly benefited.

The potential risks of genetic testing are as outlined in the informed consent document (included/revised with Amendment L). This study has a Certificate of Confidentiality, which helps to protect patient's research information. The researchers involved in this study cannot be forced to disclose the identity or any information collected in this study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the patient or the researcher may choose to voluntarily disclose the protected information under certain circumstances. Furthermore, federal agencies may review patient's records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act. The Certificate of Confidentiality will not protect against the required reporting by hospital staff of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others. The procedures involved in this protocol, with their attendant risks and discomforts and potential benefits will be carefully explained to the patient.

10.4 Risk/Benefit Analysis

Patients often benefit from administration of CHOP and are likely to derive benefit from EPOCH-R, which contains the same agents. Patients may also benefit from the addition of the idiotype vaccine. A clinical trial of idiotype vaccine in follicular center cell lymphomas suggests that it may stimulate immune-mediated clearly of minimal residual disease as assessed by PCR for the 14-18 translocation. If the idiotype vaccine is able to stimulate immune-mediated tumor clearance, this will be a major and important step in more effectively treating lymphomas. Thus, the knowledge that will be gained from this trial is potentially important and may be of direct benefit to patients.

Given the prospect of direct benefit, this same risks/benefits analysis applies also to adults who may become unable to consent during participation.

10.5 CONSENT PROCESS AND DOCUMENTATION

Informed consent will be obtained in all patients on this trial. There will be no minors enrolled < 18 years old so that assent is unnecessary. The informed consent contains all elements required for consent. In addition, the Principal Investigator or his designee will provide oral consent and will be available to answer all patient questions.

10.5.1 Telephone re-consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented in the medical record.

11. PHARMACEUTICAL INFORMATION

11.1 EPOCH-RITUXIMAB

11.1.1 Cyclophophamide

Commercial pharmacy supply will be used. Available in white crystalline formulation for intravenous injection, in vials containing 100 mg, 200 mg, 500 mg, l gm, and 2 gm. All preparations are stable at room temperature (not to exceed 30'C). Reconstitute with appropriate amounts of sterile water to produce a final concentration of 20 mg/ml. Discard solution after 24 hours at room temperature. Stable up to 6 days if refrigerated (2- 8'C). Administer IV in 100 0.9% NaCl over 15 min. Toxicities: myelosuppression, nausea and vomiting, hemorrhagic cystitis, alopecia. Cystitis can be largely prevented by maintaining a good state of hydration and good urine flow during and after drug administration using the following **Hydration Guidelines:** All patients should received normal saline at the following volumes (based on cyclophosphamide dose levels) and rates with half given before and half given after the cyclophosphamide injection.

- Levels 1-2: 1 liter NS @ 300-500 cc/hr
- Levels 3-5: 2 liter NS @ 300-500 cc/hr
- Levels ≥ 6: 2.5 liter NS @ 300-500 cc/hr

11.1.2 Doxorubicin

Commercial pharmacy supply will be used. Available in 10, 20, 50, and 150 mg vials with 50 mg, 100 mg, and 250 mg of lactose, respectively; the three dosage strengths are reconstituted with 2 ml, 4 ml, 10 ml. and 30 ml of normal saline or sterile water for injection, respectively, giving a final drug concentration of 5 mg/ml. Reconstituted solution is stable for 24 hours at room temperature and for 48 hours under refrigeration and should be protected from sunlight. Toxicities: Myelosuppression, stomatitis, alopecia, nausea and vomiting, and acute and chronic cardiac toxicity, manifested as arrhythmias or a congestive cardiomyopathy, the latter uncommon at total cumulative doses less than 500 mg/m^2. The drug causes local necrosis if infiltrated into subcutaneous tissue.

11.1.3 Vincristne

Commercial pharmacy supply will be used. Available in I mg, 2 mg, and 5 mg vial sizes. Each ml contains I mg of vincristine, 100 mg mannitol, 1.3 mg methylparaben, and 0.2 mg propylparaben. Drug should be stored at 2-8°C and should be protected from light. Toxicities: peripheral neuropathy, autonomic neuropathy, alopecia. Local necrosis if injected subcutaneously.

11.1.4 Etoposide

Commercial pharmacy supply will be used. Available as a concentrate for parenteral use in 100 mg vials; each ml contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg of polyethylene glycol 300, and 30.5% alcohol. Drug should be diluted before administration in D5W or normal saline. Toxicities: myelosuppression, nausea, vomiting, anaphylactic reactions, alopecia, and hypotension if infusion is too rapid.

- ADMINISTRATION OF VINCRISTNE/DOXORUBICIN/ETOPOSIDE. Stability studies conducted by the Pharmaceutical Development Service, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP at concentrations, respectively, of 1, 25 and 125 mcg/mL- 1.4, 35 and 175 mcg/mL; 2, 50 and 250 mcg/mL; and 2.8, 70 and 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also admixtures containing vincristine, doxorubicin and etoposide concentrations of 1.6, 40 and 200 mcg/mL are stable for at least 30 hours at 32 degrees C. In this study, THE DAILY DOSE (i.e. 24 hour supply) of vincristine, doxorubicin and etoposide will be admixed together in 500 mL of 0.9% Sodium Chloride. Injection and delivered with a suitable infusion pump through a central venous access device. The bag will be exchanged daily for each of the four days to complete the 96 hour infusion. Cyclophosphamide will be diluted in 100 mL of D5W or NS and infusion over 15 minutes. Patients will be instructed to drink an adequate amount of fluids and empty their bladders frequently during cyclophosphamide administration.

11.1.5 Prednisone

Commercial pharmacy supply will be used. Available in a large number of oral dosage strengths including pills and liquid formulations. Tablets should be stored in well-closed containers at temperatures between 15-30°C. Toxicities: proximal muscle weakness, glucose intolerance, thinning of skin, redistribution of body fat, Cushingoid facies, immunosuppression, and propensity to gastrointestinal ulceration.

11.1.6 Rituximab

Commercial pharmacy supply will be used. Rituximab is provided 10 ml (100 mg) and 50 ml (500 mg) pharmaceutical grade glass vials at a concentration of 10 mg of protein per ml. Storage: Rituximab for clinical use should be stored in a secure refrigerator at 2 to 8 °C. Reconstitution and Dilution: The antibody should be diluted into a final volume of 0.9% sodium chloride or 5% dextrose in water for final concentration of 1-4 mg/ml. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody. Rituximab solutions for infusion are stable at 2-8 degrees C (36-46 degrees F) for 24 hours and at room temperature for an additional 12 hours. Administration: Rituximab will be administered as an intravenous infusion at 375 mg/m^2 on day 1 of each cycle of EPOCH immediately prior to

the start of the infusions of chemotherapy. Rituximab infusions will be administered to patients in an outpatient clinic setting. Oral pre-medication (2 tablets of acetaminophen and 50-100 mg diphenhydramine hydrochloride) will be administered 30 to 60 minutes prior to starting each infusion of rituximab. A peripheral or central intravenous line will be established. During the rituximab infusion, the patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored every 15 minutes times 4 or until stable and then hourly until the infusion is discontinued. Available at the bedside prior to rituximab administration will be epinephrine for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment for the emergency management of anaphylactoid reactions. The initial dose rate at the time of the first rituximab infusion should be 50mg/hr for the first hour. If no toxicity is seen, the dose rate may be escalated gradually (50 mg/hr increments at 30 minute intervals) to a maximum of 400 mg/hr. If the first dose of rituximab is well tolerated, the starting flow-rate for the administration of subsequent doses will be 100 mg/hr then increased gradually (100 mg/hr increments at 30 minute intervals) not to exceed 400 mg/hr. CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. Toxicities: Common toxicities include fever, chills, nausea, asthenia, headache, angioedema, pruritus and rash. Leukopenia occurs in approximately 10% but grade 3 or 4 neutropenia is uncommon. Hypotension occurred in 10% of patients during rituximab infusion, and serious bronchospasm and urticaria associated with rituximab infusion each occurred in fewer than 10% of patients. Less common toxicities include abdominal pain, vomiting, thrombocytopenia, anemia, myalgia, arthralgia, dizziness, and rhinitis.

11.1.7 Filgrastim (Neupogen®)
Commercial pharmacy supply will be used. It is provided in two vial sizes of 300 and 450 μg/mL. It should be stored at 2-8'C (do not freeze) and is stable for at least I year at this temperature. Toxicities: Rare anaphylactic reactions with the first dose; bone pain at sites of active marrow with continued administration. Local reactions at injection sites. Constitutional symptoms, increased alkaline phosphatase, LDH, uric acid; worsening of pre-existing inflammatory conditions.

11.1.8 Id-KLH Vaccine
Idiotype protein from the individual B cell lymphomas will be obtained using two different methods. The first 13 patients (accrual completed) will receive vaccine manufactured with idiotype isolated using a somatic hybridoma technique. The second 13 patients will receive vaccine manufactured with idiotype manufactured with idiotype isolated by recombinant techniques. Idiotype isolated by either technique will be covalently coupled to keyhole limpet hemocyanin (KLH) using the identical techniques and contractor. All vaccines will be produced according to Good Manufacturing Practices standards and tested for sterility, endotoxin contamination, and general safety prior to its use in any patient. The preparation and quality control/quality assurance testing of the Id-KLH conjugate will be performed by TSI Washington under DCS/NCI contract. The IND for the Id-KLH vaccine will be held by the Drug Regulatory Affairs Section, CTEP. How Supplied: Formulated product for subcutaneous administration contains 0.5 mg if Id and KLH each per ml of normal saline. Id-KLH will be supplied as a 1 ml vial.

- Storage - Prior to administration, Id-KLH will be stored at -20ºC.

- Administration – One of the following investigators will be available for vaccine preparation: Drs. Kwak, Wilson or Fowler. After thawing and gentle agitation, the vial contents should be drawn up using an 18-gauge needle on a syringe. After the entire contents have been drawn up, a 25-gauge needle for injection may replace the 18-gauge needle. This procedure is important to ensure that all particulates (normal components of this vaccine) are obtained from the vial.
- Toxicity - Toxicities described with Id-KLH vaccine administration include local site reactions (erythema, induration, swelling and tenderness), fever, chills, rash, myalgias and arthralgia. Mild elevations in creatinine phosphokinase (CPK) have been observed.

11.1.9 GM-CSF (Sargramostim: NSC #613795; BB-IND 2632)

- Source and Pharmacology - The GM-CSF to be used in this study is glycosylated, recombinant human GM-CSF. This GM-CSF is an altered form of the native molecule; the position 23 arginine has been replaced with a leucine to facilitate expression of the protein in yeast (Saccharomyces cerevisiae).
- Formulation and Stability - The GM-CSF is formulated as a white lyophilized cake and is provided in vials containing 500 µg of the GM-CSF protein as well as 10.0 mg of sucrose, 40.0 mg of mannitol, and 1.2 mg of Tris (Trimethamine). To prepare a vial of GM-CSF for direct subcutaneous use, aseptically inject 1.0 ml of Sterile Water for Injection, USP, into the vial to dissolve the lyophilized cake. The diluent should be directed against the side of the vial to avoid excess foaming. Avoid vigorous agitation of the vial; do not shake. This yields a solution containing 500 µg/ml. The unreconstituted material should be kept refrigerated at 2-8^{o}C and is stable for at least eighteen months. Once reconstituted, the solution is stable for at least 24 hours at 2-8^{o}C or at 18-25^{o}C. Because the product does not contain a preservative, vials should be treated as unit-dose containers; reconstituted solution should be held at 2-8^{o}C and discarded after no more than six hours. Do not freeze GM-CSF.
- Supplier - Manufactured by Immunex. Will be provided by CTEP.
- Route of Administration - The appropriate total dose is withdrawn into and administered from a plastic tuberculin syringe. The GM-CSF will be injected subcutaneously as close as possible to the Id-KLH injection site. All GM-CSF doses for each patient will be administered by the nursing staff in the outpatient unit.
- Toxicity - Toxicities described in patients receiving GM-CSF include: Fever, chills, diaphoresis, myalgias, fatigue, malaise, headache, dizziness, dyspnea, bronchospasm, pleural effusion, anorexia, indigestion, nausea, vomiting, diarrhea, injection site tenderness, urticaria, rash, pruritus, hypersensitivity reaction, bone pain, thromboembolic events, phlebitis, hypotension, peripheral edema, leukocytosis, thrombocytosis or thrombocytopenia, hepatic enzyme abnormalities, and bilirubin elevation. The first administration of GM-CSF has provoked a syndrome of dyspnea and hypotension within two hours after GM-CSF injection in a single patient receiving yeast-derived GM-CSF; this type of reaction has more frequently been observed in patients receiving GM-CSF produced in E. coli. One report of a vascular leak-like syndrome occurring after autologous bone marrow transplant in a patient receiving continuous IV infusion of GM-CSF has been recorded.

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13. APPENDICES

13.1 APPENDIX 1: PROCEDURES FOR STORED SPECIMENS

NOTE: See Section 5.2 for more information on request and use of stored specimens.

13.1.1 Apheresis Products, Tissue, and Serum Specimens

The Clinical Support Laboratory, Leidos Biomedical Research, Inc., processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All laboratory personnel with access to patient information annually complete the NIH online course in Protection of Human Subjects. The laboratory is CLIA certified for CD4 immunophenotyping and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:

- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
    - The database resides on a dedicated program server that is kept in a central, locked computer facility.
    - The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
    - Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
    - The database sample entry program itself is accessed through a password protected entry screen.
    - The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Beginning October 1, 2006 inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage.
- Access to stored clinical samples is restricted. Investigators establish sample collections under “Source Codes” and the investigator responsible for the collections, the protocol Principal Investigator, specifies who has access to the collection. Beginning October 1, 2006 specific permissions will be required to view, input or withdraw samples from a collection. Prior to that date sample input was not restricted and restrictions were limited to specimen withdrawal.

- Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. Beginning October 1, 2006 the repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.
    - The Clinical Support Laboratory does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

13.1.2 Bloods for Pharmacokinetic analysis

The blood that was collected for PK analysis is currently stored as plasma in screw-capped polypropylene vials in a -70°C degree freezer which is located in a common freezer room in the 1W lab area of the Pediatric Oncology Branch. The freezer temperature is continuously monitored for +/- temperature differences of 10 degrees C. Samples are stored by patient initials as well as date and time of sample in individual boxes within this freezer. Records of samples stored are catalogued in a Filemaker database which is maintained by Pharmacology and Experimental Therapeutics research nurses and is password protected so that only members of the section have access to patient sample information. Results from the analyses of drug levels from these samples are maintained in an Excel database maintained on a password-protected computer in the laboratory of Dr. Frank Balis. Both computers containing patient sample information are backed on a regular (at least one time per week) basis.

NOTE: These analyses are no longer ongoing. Accordingly, Dr. Balis no longer involved in the study; however, his name is retained for historical reasons.