

# BiovaxID, a personalized therapeutic vaccine against B-cell lymphomas

Milan Reiniš

## Address

Institute of Molecular Genetics AS CR, vvi,  
Videňská 1083, Prague 4, CZ-14220, Czech Republic  
Email: reinis@img.cas.cz

*Biovest International Inc (a subsidiary of Accentia BioPharmaceuticals Inc), under license from Stanford University, is developing BiovaxID, a personalized therapeutic vaccine against B-cell lymphomas that, in combination with GM-CSF, exclusively targets cancerous B-cells by raising an immune response to tumor-specific immunoglobulin proteins called idiotypes, for the potential treatment of follicular non-Hodgkin's lymphoma (NHL). Phase I and II clinical trials demonstrated the immunogenicity, safety and therapeutic efficacy of BiovaxID. Phase III clinical trials in NHL were ongoing at the time of publication.*

## Introduction

Non-Hodgkin's B-cell lymphoma (NHL) is expected to be the fifth most diagnosed cancer in the US in 2008, accounting for approximately 4.6% of all cancers (66,120 new cases) and being responsible for approximately 3.4% of cancer-related mortalities (19,160 deaths) [917051]. NHL is a term for a diverse group of malignant lymphoproliferative diseases of several histological types that may be of either indolent or aggressive forms [917058]. The standard and effective treatment modalities of NHL are chemotherapy, radiotherapy [917058] and passive immunization with the anti-CD20 antibody rituximab [533326], [775458]. Indolent B-cell NHL can in a minority of cases undergo spontaneous regression [917058], [917059], suggesting that the immune system plays a role in the control of tumor development. However, most patients with indolent NHL ultimately relapse and cannot be cured with standard therapies as they may become resistant to chemotherapy or mAb treatments in the course of disease progression [917434]. Follicular NHL is associated with the t(14;18) chromosomal translocation, the presence of which can be used as a molecular marker for survival of tumor cells after treatment [937577]. Thus, there is a strong demand on new therapeutic strategies to prevent the growth of recurrent tumors [775453], [917058].

Neoplastic B-cells are considered to be promising targets for specific immunotherapy [912083]. At the molecular level, a number of potential targets for specific immunotherapy have been identified. B-cell lymphomas overexpress on their surfaces several antigens that are specific to different cell lineages, namely CD20, CD22, CD40 and CD80, which represent therapeutic targets [917242]. Passive anti-CD20 immunotherapy has been introduced into clinical practice with the advent of the humanized chimeric anti-CD20 antibody rituximab (Rituxan) [533326], [775452]. CD20 is not a tumor-specific antigen as it is expressed on normal cells of B-cell lineage. The treatment with anti-CD20 antibodies therefore results not

### Therapeutic BiovaxID

**Originator** Stanford University

**Licensee** Biovest International Inc

**Status** Phase III Clinical

**Indications** Follicle center lymphoma, Mantle cell lymphoma, Non-Hodgkin's lymphoma

**Actions** Anticancer, Genetically engineered autologous cell vaccine, GM-CSF receptor agonist, Therapeutic vaccine,

**Technologies** Subcutaneous formulation, Tumor antigen

**Synonym** Vaccine (B-cell lymphoma)

only in depletion of the tumor cells but also in temporary B-cell deficiency; it has been demonstrated that after chemotherapy and administration of rituximab, the B-cell population began to recover at 6 months after treatment and was completely recovered after 1 year [646877]. Efficacy has been demonstrated to be successful in prolonging remissions, and several clinical trials have been conducted with maintenance therapies to prolong remissions in patients with indolent NHL. Longer follow-up studies are required to prove this concept [917107]. Besides anti-CD20 therapy, other antibodies (targeting CD22, CD40 or CD80) have been tested against NHL in clinical trials, some reaching phase III [917242], although none had been approved at the time of publication.

B-cell immunoglobulin variable regions (the complementarity-determining regions [CDRs] of the heavy and light immunoglobulin chains) represent unique and cell-specific antigens called idiotypes (Ids) [770305]. Because neoplastic B-cells are clonal expansions of a single dysregulated lymphoid cell, they share the rearrangement of immunoglobulin genes and express common Ids [775448]. Therefore, because tumor Id proteins differ

from the diverse immunoglobulins expressed on normal B-cells, they are in theory ideal targets for immunotherapy [770305], [775456]. However, their uniqueness is also an obstacle for vaccine development because anti-idiotypic tumor vaccines must be produced for every different individual and are patient- and tumor-specific [770304], [770307]. Vaccine preparation strategies share common steps for the isolation of specific Ids from the tumors but differ in technologies used for the vaccine preparation; in different vaccines this can be heterohybridoma technology [859743], [875427], recombinant antigen preparations [454353], [581577], [912137] or DNA immunization methods [505368]. Id proteins are poorly immunogenic (because they are endogenous human proteins); to increase their immunogenicity various vaccine strategies involve chemical conjugation of the purified Ids with a carrier protein, such as keyhole limpet hemocyanin (KLH) [454495]. In addition, immunization protocols for experimental vaccines utilize a mixture of adjuvant. The use of emulsifying chemical adjuvant agents in early vaccination trials [875427] has been superseded by the use of GM-CSF [454493], [581577], [875426]. GM-CSF is known to promote the recruitment and differentiation of dedicated antigen-presenting cells, in particular dendritic cells, thus increasing the presentation of antigens and resultant induction of antitumor responses [454493], [770303]. Alternative vaccination strategies ensuring more effective antigen presentation utilize dendritic cell-based cellular vaccines or DNA vaccines [454489] based on specific Id sequences conjugated to 'adjuvant' genes that activate the immune system [505368].

This article will focus on BiovaxID, a personalized Id-KLH vaccine produced by the heterohybridoma method that targets idiotype determinants expressed on the cell surfaces of B-cell lymphomas and is administered with GM-CSF. BiovaxID is in development by Biovest International Inc (a subsidiary of Accentia Biopharmaceuticals Inc), in collaboration with and under license from Stanford University [939561]. A phase III clinical trial of BiovaxID for the treatment of NHL was initiated in 2000 [590114], [875425] and preliminary results released in 2008 show improved survival [809823].

## Synthesis and SAR

BiovaxID is a conjugate of a recombinant patient-specific Id protein with KLH that is co-administered with GM-CSF [875426], [875427]. Early clinical trials with Id vaccines (which led directly to the present clinical formulation of BiovaxID) were based on the heterohybridoma fusion method for the preparation of immunoglobulin protein from patients' tumor cells that was developed by Ronald Levy and colleagues at Stanford University in the 1980s [912099]. One clinical report described a method by which lymphoma cells were obtained from tumor biopsies and fused with heterohybridoma K6H6/B5 cells. The hybridomas were screened for production of antibodies matching with those produced by tumors and their identity is additionally characterized genetically by analysis of the third CDR (CDR3) to ensure that it matches that of

the original malignancy [912109]. Ids are purified from hybridoma culture by affinity chromatography and conjugated by a glutaraldehyde method to KLH as a carrier protein [770304], [912109]. The vaccines for all BiovaxID clinical trials to date, including the ongoing phase III trial, were prepared by the heterohybridoma method [912132]; the details of the method have likely been refined over time.

The heterohybridoma fusion method is laborious and typically takes 3 to 6 months to produce a vaccine [912132]. This motivated several research groups to develop recombinant techniques for preparing Id-KLH, which shortened the development time to approximately 2 months. The variable regions of the light and heavy chains of the tumor-specific immunoglobulin were cloned by PCR and inserted into an expression vector for production in a suitable cell type (reviewed in reference [912132]). Two vaccines prepared in this way, mitumprotimut-t (FavID) [750670] and GTOP-99 [862903], reached late-stage clinical development, but are no longer being developed. Given the patient-specific and therefore variable nature of the therapies, it is the means of preparation that sets BiovaxID apart from these strategies. Although the findings of clinical trials with all these vaccines are relevant to idiotype vaccination, and will be discussed in the conclusions, this monograph will discuss data BiovaxID only.

## Preclinical development

Therapeutic Id-based vaccines, such as BiovaxID, are human- and patient-specific structures so experiments in animal models are of limited relevance. However, the principles of anti-idiotypic vaccinations and vaccine optimization have been demonstrated in animal models of B-cell lymphomas.

In a murine BCL1 B-cell lymphoma model, purified tumor-derived idiotype IgM molecules conjugated with KLH were demonstrated to be efficacious therapeutic vaccines. These experiments proved the importance of coupling with KLH to improve immunogenicity, since only Ids coupled with KLH, but not Ids alone, were effective in the treatments [454495], [770307].

Additionally, the effectiveness of adjuvant GM-CSF was proven in a 38C13 lymphoma murine model. Co-administration of GM-CSF significantly increased the antitumor effects of Id immunizations either alone or combined with cyclophosphamide [454493].

## Toxicity

No preclinical toxicity data for BiovaxID were available at the time of publication.

## Metabolism and pharmacokinetics

No metabolism or pharmacokinetics data of BiovaxID were available at the time of publication.

## Clinical development

### Phase I

The first clinical data from humans immunized with an autologous Id-KLH preparation were released from nine patients with B-cell lymphoma [770304]. A series of subcutaneous vaccinations of Id-KLH (0.5 mg Id coupled to 0.5 mg of KLH) were administered together with a chemical adjuvant to patients with minimal residual disease or a complete remission after chemotherapy. Immunological responses were observed in seven patients; humoral responses occurred in two patients, cell-mediated responses in four patients and both responses in one patient. Moreover, complete tumor remissions occurred in two patients who harbored measurable tumors at the start of the trial [770304]. Data from these patients were apparently included in a larger analysis, as follows.

In a long-term phase I, anti-Id vaccination clinical trial in patients ( $n = 41$ ) with NHL in either primary or secondary remission after chemotherapy treatment, 21 patients were in complete remission and 20 patients had residual disease [875427]. The tumors were of follicular low-grade lymphoma (stage III or IV small-cleaved or small and large cell) histology other than one case of diffuse small cell lymphoma. Vaccines were prepared from peripheral lymph node biopsies, using KLH as a carrier protein, and administered with an immunological adjuvant; either 'incomplete' (5% squalane, 2.5% pluronic L121 and 0.2% Tween 80) in nine patients or 'complete' adjuvant supplemented with threonyl-muramyl dipeptide adjuvant in the remaining cases. Five subcutaneous immunizations were administered, on day 0 then at 2, 6, 10 and 20 weeks later; each consisted of 0.5 mg Id coupled to 0.5 mg of KLH and mixed with emulsifying adjuvant. The median duration of follow-up of the patients was 7.3 years after diagnosis and 5.3 years (range 1.2 to 8.6 years) after vaccination. The data revealed that the vaccine was immunogenic and 20 patients (49%) responded specifically against their tumor Ids; of the responders, 85% had antibody responses and 35% had cellular responses. In general, responses were maximal after the fourth or fifth vaccination. The pre-vaccine disease status influenced outcome in that 79% of patients in complete remission responded, but only 25% of those with residual disease did so. Immune responses against KLH occurred in all patients. In the 21 patients with complete remission at the start of the study, 87% of those who responded to vaccination remained tumor-free compared with 33% of nonresponders. In the five patients with residual disease who responded to the vaccine, 40% exhibited disease regression and 20% had stable disease. Only 1 in 15 (~7%) of the nonresponders with residual disease remained tumor-free. Anti-Id immunity correlated with clinical outcome. In patients responding to vaccination, the period of no disease progression and overall survival were significantly longer than in patients who did not mount an immunological response. The period of freedom from progression was 7.9 years in patients who responded immunologically compared with 1.3 years in nonresponders [875427]. In subsequent data from the patient subset

with minimal residual disease before vaccination, after a median follow-up of 5.3 years from last chemotherapy the median duration until disease progression was 4.4 years [912132].

### Phase II

A phase II clinical trial in patients ( $n = 20$ ) with NHL vaccinated with BiovaxID assessed molecular data on eradication of residual t(14/18)+ lymphoma cells before and after chemotherapy, as well as the rate of immunological response [875426]. All qualified patients presented with stage III or IV small-cleaved or mixed-cell follicular lymphoma and had achieved a complete tumor response after chemotherapy. Notably, additional patients who were enrolled on the trial and received vaccination, but were subsequently found to have residual disease were excluded from the analysis. The chemotherapy regimen comprised six or more monthly cycles of prednisone, doxorubicin, cyclophosphamide and etoposide (proMACE), without methotrexate, for two cycles after a complete response was achieved. Vaccination began at least 6 months after chemotherapy to allow recovery of the immune system; it consisted of four monthly subcutaneous injections of BiovaxID (0.5 mg Id protein conjugated with an unspecified amount of KLH) plus 100 or 500  $\mu\text{g}/\text{m}^2$  of free GM-CSF. Additional doses of GM-CSF were administered at the same site on three subsequent days. Malignant cells bearing the t(14/18) marker were detectable (by PCR) in 11 patients before treatment and in the same patients after chemotherapy, despite being in clinical remission, but were absent in 8 of the 11 patients after vaccination. This molecular complete response was sustained for a median of more than 18 months (range 8 to > 32 months). Cytotoxic CD4+ and CD8+ cells specific for the vaccine/tumor Ids were detected in 19 of 20 patients after vaccination. Specific antibodies were also detected in 15 of 20 patients, but were not necessary for molecular remissions [875426]. Additional data on the clinical outcome of patients were published in 2005 [640886], [875430]. After a median follow-up of 9.2 years, the rate of disease-free survival was 45% and overall survival was 95%; the median duration of disease-free survival was 8.0 years. Prolonged disease-free survival was associated with continued absence of the t(14/18) translocation in the subset of patients in which they were detected [640886], [875430]. No additional mortalities had occurred by 2007 [859743].

A phase II clinical trial assessed BiovaxID in patients ( $n = 26$ ) with mantle cell lymphoma who had previously been treated with chemotherapy, including rituximab [646737], [646877]. Patients were previously untreated before receiving six cycles of dose-adjusted treatment with etoposide, vincristine, doxorubicin, cyclophosphamide, prednisone and rituximab (EPOCH-R). This treatment induced complete remissions in 92% of patients and partial remissions in the remaining 8%. Treatment completely depleted peripheral blood B-cells in all individuals (for more than 6 months) although CD4+ T-cell numbers were reduced slightly (and recovered after

~ 3 months) and CD8+ T-cells were not affected. At 3 months after chemotherapy, five cycles of treatment with BiovaxID (0.5 mg of both Id protein and KLH; on months 0, 1, 2, 3 and 5) were administered subcutaneously together with 100 µg/m<sup>2</sup> GM-CSF, followed by GM-CSF on 3 subsequent days. Despite the lack of B-cells as a result of chemotherapy and rituximab treatment, antibody responses against KLH and Id protein were detected in 74 and 30% of the evaluable patients (n = 23), occurring later than in previous trials and correlating with B-cell recovery. CD4+ and CD8+ T-cell responses against the vaccine occurred in 87 and 100% of patients (n = 23), suggesting the activity of professional antigen-presenting cells, such as dendritic cells, in the absence of B-cells. After 46 months median follow-up in patients (n = 26), overall survival was 89%, median event-free survival was 22 months and 19% of patients remained in continuous complete first remission [646877]. Importantly, immunity mediated by both CD4+ and CD8+ was observed in the majority of patients after B-cell depletion by rituximab, and the delayed humoral responses correlated with B-cell recovery.

### Phase III

A phase III, double-blind, randomized clinical trial of BiovaxID (NCT00091676) was subsequently conducted in patients (expected n = 629) with follicular NHL [590114], [640886], [875425], [875430], [940231]. Based on the design of the phase II trial in NHL patients, previously untreated patients with advanced follicular lymphoma were enrolled. Patients who had achieved a verified or unconfirmed complete response after chemotherapy with prednisone, doxorubicin, cyclophosphamide and etoposide (PACE) were randomized in a 2:1 ratio to receive vaccine (as described in reference [875426]) or control (unconjugated KLH plus GM-CSF). Vaccine was administered from 6 months after chemotherapy (or when vaccine preparation was complete, which was up to 12 months) if patients remained in remission. Five doses of vaccine plus 100 µg/m<sup>2</sup> GM-CSF were administered over 6 months; each vaccination was divided into four and injected subcutaneously into the upper and lower extremities, with GM-CSF injected for a further 3 days at all sites. The primary endpoint was the comparison of disease-free survival between vaccinated and control groups [875425]. Interim data were evaluated in 2007. The Kaplan Meier disease-free survival curve showed that 40% of patients (n = 122) remained disease-free between 40 and 70 months [809823]. Encouraging unblinded data were released in July 2008 [926103]. In patients monitored for up to 80 months, vaccination with BiovaxID resulted in a 60% increase in median disease-free survival, from 21.2 months in the control arm to 33.8 months for BiovaxID (p = 0.047). In addition, data from the same patients monitored for 36 months from vaccination showed that BiovaxID treatment improved median disease-free survival by approximately 100% (p = 0.024). Biovest announced the intention to seek regulatory approvals in the US and Europe based on the current unblinded data and follow-up analysis in

August 2008 [926103]. As of May 2008, the trial was ongoing but no longer recruiting patients; the final collection of data for primary outcome measure was expected in March 2009 [940231].

### Side effects and contraindications

In general, vaccination with BiovaxID appears to be safe and nontoxic, although additional investigation is necessary. In phase I clinical trials with BiovaxID or Id-KLH plus adjuvant, the side effects relating to the vaccine were mild injection-site reactions in muscle [770304], [875427]. The more serious adverse effects (local skin reactions, myalgias/arthralgias and fever) observed in the early trial of Id-KLH at Stanford University were linked to the threonine-muramyl dipeptide adjuvant [912132]. In phase II clinical trials, vaccination was generally well tolerated, although mild injection-site reactions (erythema, induration and pruritus) occurred [912132]; other adverse effects were generally those associated with the chemotherapy treatment [646877], [875426]. It was reported that no long-term toxicities or deaths were associated with vaccination in phase II clinical trials [875425].

More detailed safety data were available from the phase III clinical trial [859743]. From patients (n = 231) who had been enrolled to receive chemotherapy, 176 had been randomized to receive vaccine. Of 32 adverse events recorded at a blinded interim analysis, only 2 were classed as to be possibly related to the vaccine (arrhythmia supraventricular and chest pain); 3 were considered unlikely to be related (chest pain, compression fracture and herpes zoster infection); and 1 was unknown (febrile neutropenia). One serious adverse event (cerebral ischemia) that occurred post-vaccination was not considered related to the study agent [859743].

### Patent summary

The intellectual property relating to BiovaxID originated at Stanford University where these technologies were discovered. Patent US-06099846, on which Professor Ronald Levy is an inventor, was granted to Stanford University in August 2000. This patent claims Id/cytokine conjugates for use in vaccines to treat B-cell lymphoma. The conjugates claimed are preferably fusion proteins that comprise the idiotypic region of a B-cell lymphoma-associated immunoglobulin covalently bound to GM-CSF, IL-2 or IL-4. A corresponding filing in Europe appears to have been abandoned.

In WO-2008034074 (filed in September 2006), Accentia claimed the use of high-dose oxazophosphorine drugs (eg cyclophosphamide) in combination with autologous anti-idiotypic vaccines (including BiovaxID) to treat B-cell-derived cancers. No regional equivalents have been published.

### Current opinion

Vaccine strategies for B-cell NHL based on anti-idiotypic antibodies are of particular interest because they target unique structures expressed on tumor cells, but not

healthy cells. Following proof-of-concept of idiotype vaccination in animal models, the immunogenicity, relative safety and therapeutic effects of Id-KLH vaccines have been proven in several clinical trials. In general, anti-Id therapeutic vaccines are able to elicit robust immune responses mediated by CD4<sup>+</sup> and CD8<sup>+</sup> cells, as well as humoral responses and immunological memory (reviewed in references [912083], [912132]). However, there is a disadvantage of the necessity to prepare specific vaccines tailored individually to each patient.

The heterohybridoma method developed at Stanford University has been optimized for preparation of Ids over more than 15 years of development, and vaccines have proven safety, immunogenicity and therapeutic effectiveness in clinical trials. However, its disadvantage is that it is labor intensive and can take over 6 months until the vaccine is ready, after which time the vaccine may not be produced successfully in a minority of cases, as was the case in the clinical trials with BiovaxID [875425], [875426]. In the crucial matter of timing, recombinant technologies seem to offer an advantage over the hybridoma manufacturing procedure used for BiovaxID. The recombinant proteins are then expressed either in bacteria (*Escherichia coli*, as in GTOP-99 [581577]) or in cell lines (eg, baculovirus expression in insect cells, as with FavID [454353]), purified and coupled to KLH. These strategies result in faster vaccine preparations, but vaccines produced by these less established expression methods are expected to raise more regulatory issues, which could delay their clinical use. A novel strategy for rapid generation of vaccines to treat B-cell malignancies was described [912141] by a research group which pioneered various anti-Id vaccine technologies underlying BiovaxID and other NHL vaccine strategies. The vaccine was prepared in 24 h by extracting cell-membrane-bound proteins from lymphoma cells and incorporating them into proteoliposomes, together with IL-2. Such a vaccine was tested in a C3H murine 38C13 B-cell lymphoma model [912141]. So far no clinical trials have been reported. Other promising strategies for effective immunization against lymphoma have been developed (reviewed in reference [912132]). Dendritic cells pulsed with Id proteins as cellular vaccines have been utilized to ensure effective antigen presentation. This strategy has already been tested in clinical trials, but requires *ex vivo* culturing of dendritic cells derived from a patient [454489]. It is questionable whether this would be beneficial compared to Id conjugation with KLH and administration with GM-CSF. Another possibility is to use 'naked' DNA vaccines to express the Id-carrier protein, a strategy that proved immunogenic in an initial clinical trial [505368].

In phase II and III clinical trials with BiovaxID, multiple injections of GM-CSF were administered. This cytokine replaced the emulsifying and immunological adjuvants used in early clinical trials and has become a standard adjuvant treatment, not only in BiovaxID studies, but in clinical trials of Id-based antitumor vaccines (reviewed in references [912083], [912096], [912132]). Its potential boosting effect

is probably related to its capability to attract and induce the differentiation of antigen-presenting cells so they are able to process and present antigens and thus induce stronger immune responses. IL-2, an alternative cytokine of potential adjuvant use, is probably less suitable because of its capacity to induce proliferation not only of effector T-cells, but also of regulatory T-cells that could block effective immune responses [912132].

The BiovaxID vaccine has been tested mainly in patients whose tumor mass has been minimized by chemotherapy. Clinical findings suggest that greater reduction in tumor mass is associated with improved survival likelihood and duration. An important issue in reducing lymphoma mass prior to anti-Id vaccination is the potential to be combined with the effective passive immunotherapy rituximab, alone or with cytotoxic chemotherapy. Rituximab treatment targets not only lymphoma cells, but depletes the entire CD20-expressing B-cell population. Thus, B-cell-mediated immune responses to subsequent Id-KLH vaccination can be induced only after B-cell population reconstitution. It is noteworthy that in clinical trials BiovaxID immunization induced T-cell-mediated immunity, even in the absence of B-cells depleted by anti-CD20 treatment. However, the requirement for B-cells for a proper response to Id-KLH vaccines cannot be excluded.

In the phase II clinical trial, most patients with mantle cell lymphoma treated with rituximab plus chemotherapeutic agents prior to BiovaxID vaccination relapsed in spite of strong T-cell mediated responses in most cases. Additional analysis suggested potential roles of either inadequate T-cell activation or trafficking or the role of immunosuppressive factors produced by the tumor cells [875427]. Data from clinical trials suggest that B-cell populations have recovered sufficiently to start BiovaxID immunization at 3 months after rituximab therapy, although it may be recommended to continue with vaccine boosts after B-cell recovery in order to induce humoral responses. The importance of humoral responses was demonstrated in a clinical trial with another vaccine in which the clinical outcome after Id vaccination was apparently dependent on the presence of specific anti-Id antibodies and correlated with IgG Fc receptor genotype [770316].

The phase III clinical trial with BiovaxID enrolled patients with complete tumor responses after standard chemotherapy, and had an endpoint of the prevention of tumor relapses. If final data from the phase III clinical trial confirm the promising interim findings that showed a 60% increase in median disease-free survival in BiovaxID-treated patients at 80 months, an important consideration will be whether BiovaxID would also be effective in other settings. The use of BiovaxID as a monotherapy against primary and relapse tumors has not been tested (because of ethical constraints of vaccination in treatment-naïve patients who would likely be better treated by existing treatments). Additional clinical trials may also test the effectiveness in patients who responded partially to initial chemotherapy. Results of clinical trials

with other Id-KLH vaccines may answer these questions, as discussed below.

Several different personalized anti-idiotypic vaccine strategies are currently at different stages of development (reviewed in references [912083], [912096], [912132]). In addition to BiovaxID, the two other personalized Id-KLH conjugate vaccines that reached phase III development are Favril's mitumprotimut-t [750670] and Genitope's GTO-99 [862903]. Significantly, however, clinical development of GTO-99 was suspended in March 2008 after a phase III clinical trial failed to meet its primary endpoint [884757] and the development of mitumprotimut-t was likewise discontinued in March 2008 when a phase III clinical trial failed to meet its endpoints [910233]. Although BiovaxID is prepared differently from these vaccines, they all share the conjugation step to KLH and admixture with GM-CSF [912132]. Since all three vaccines are produced from individual patients' malignant B-cells, the strategies are expected to be comparable in terms of immunogenicity.

However, there are several important differences in the BiovaxID phase III clinical trial compared with the mitumprotimut-t clinical trial, so it is possible to speculate that the results of these trials could be different. First, BiovaxID was administered after standard chemotherapy whereas mitumprotimut-t was administered following treatment with rituximab alone, which may hamper immune responses to vaccination. Second, only patients in complete remissions (either confirmed or unconfirmed) were enrolled into the BiovaxID clinical trial, whereas the mitumprotimut-t clinical trial enrolled patients after complete and partial responses or those with stabilized disease. Similarly, patients with only partial responses were also included into the phase III trial of GTO-99. The vaccine-induced immune response would likely be more effective against reduced or minimal disease rather than against bulky tumors; moreover, clinical data from trials with BiovaxID and GTO-99 support this. The primary endpoints were disease-free survival for BiovaxID compared with time to progression for mitumprotimut-t and progression-free survival for GTO-99. It is also

noteworthy that a high percentage (80%) of patients in the BiovaxID phase II clinical trial responded immunologically, suggesting possible therapeutic outcome of the treatment, and survival was also improved significantly.

One additional consideration is that there is mounting evidence that effective use of antitumor therapeutic vaccines should consider the necessity of overcoming the immune suppression that may arise in the course of tumor growth and treatment. For additional optimizations of the anti-idiotypic immunotherapeutic protocols, it seems to be important to focus on elimination of immune suppression effects. It is known that regulatory mechanisms, namely recruitment of regulatory T-cells in the tumor microenvironment, are involved in inhibition of effector T-cell immunity against B-cell lymphomas [912132]. Therefore, an immunotherapeutic setting involving suppression of regulatory cells together with vaccination may substantially increase the immunization effects.

Taken together, the effectiveness of BiovaxID has been proven in phase I/II clinical trials and, if the ongoing phase III clinical evaluation is successful, it may be soon approved for clinical practice. Released data on patients monitored up to 80 months suggested that the vaccine could be effective, and Biovest is already planning to seek accelerated or conditional regulatory approvals.

## Deals

In May 2006, BiovaxID was granted Fast Track status by the FDA for follicular NHL [667065]. In August 2006, BiovaxID was granted Orphan status in the EU by the EU commission [684296]. An interim analysis of the BiovaxID phase III clinical trial, which was designed to support Biovest's plans for accelerated approval in the US and Europe for follicular NHL, was requested by the data monitoring committee [804209], [804211]. Biovest planned to meet with the FDA and EMEA in January 2008 to establish registration pathways by mid-2008 [865560]; at this time, approval was expected in mid-June 2008 and Biovest intended to launch BiovaxID in early 2009 [802223]. The company also planned to file for Swiss approval [839618].

## Development status

| Developer                 | Country            | Status       | Indication               | Date      | Reference |
|---------------------------|--------------------|--------------|--------------------------|-----------|-----------|
| Biovest International Inc | Russian Federation | Phase III    | Follicle center lymphoma | 18-JUL-06 | 678875    |
| Biovest International Inc | US                 | Phase III    | Follicle center lymphoma | 30-SEP-04 | 917445    |
| Biovest International Inc | US                 | Phase II     | Mantle cell lymphoma     | 31-AUG-05 | 646737    |
| Stanford University       | US                 | Discontinued | Non-Hodgkin's lymphoma   | 30-SEP-04 | 917445    |

## Literature classifications

### Chemistry

| Effect studied | Result                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Reference |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Synthesis      | Id protein was prepared from the tumor cells of specific patients by the heterohybridoma fusion method, in which lymphoma cells from tumor biopsies were fused with heterohybridoma K6H6/B5 cells. The hybridomas were screened for production of antibodies matching with those produced by tumors and their identity confirmed by matching their CDR3 with that of the tumor. Ids were purified from hybridoma culture by affinity chromatography and conjugated to KLH as a carrier protein to produce BiovaxID. | 912109    |

## Clinical

| Effect studied                                     | Model used                                                                                                                                                                                                                                                                                                                                                                                                                                            | Result                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Reference |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Efficacy and safety                                | Phase I clinical trial of BiovaxID (five sc immunizations with 0.5 mg Id coupled to 0.5 mg KLH and mixed with emulsifying adjuvant were administered on day 0 and 2, 4, 6, 10 and 20 weeks later) in patients (n = 41) with follicular NHL in primary or secondary remission after chemotherapy treatment.                                                                                                                                            | The vaccine was immunogenic and 20 patients (45%) responded specifically to the Id vaccine; 85% had antibody responses and 35% had cellular responses. In the 21 patients with complete remission, 87% of those who responded to vaccination remained tumor-free whereas 33% of nonresponders to the vaccine were tumor-free. In the five patients with residual disease who responded to the vaccine, 40% exhibited disease regression and 20% had stable disease. Only 1 in 15 (~ 7%) of the nonresponders with residual disease remained tumor-free. The period of freedom from progression was 7.9 years in patients who responded immunologically compared with 1.3 years in nonresponders. Side effects relating to the vaccine were mild injection-site reactions. | 875427    |
| Efficacy, safety and presence of molecular markers | Phase II clinical trial of BiovaxID (four monthly sc injections of BiovaxID plus 100 or 500 µg/m <sup>2</sup> of GM-CSF for 4 days) in patients with follicular NHL (n = 20) in complete remission after six or more monthly cycles of proMACE chemotherapy.                                                                                                                                                                                          | Malignant cells bearing the t(14/18) marker were detectable in 11 patients before treatment and detectable in the same patients after chemotherapy, despite being in clinical remission, but were absent in 8 of the 11 patients after vaccination. This molecular complete response was sustained for a median of more than 18 months (range 8 to > 32 months). Cytotoxic CD4+ and CD8+ cells specific for the vaccine/tumor Ids were detected in 19 out of 20 patients after vaccination. Specific antibodies were also detected in 15 of 20 patients but were not necessary for molecular remissions. Vaccination was generally well tolerated, although mild injection-site reactions (erythema, induration and pruritus) occurred.                                   | 875426    |
| Efficacy and safety                                | Follow-up of phase II clinical trial of BiovaxID (four monthly sc injections of BiovaxID plus 100 or 500 µg/m <sup>2</sup> of GM-CSF for 4 days) in patients (n = 20) with follicular NHL in complete remission after six or more monthly cycles of proMACE chemotherapy.                                                                                                                                                                             | In follow-up data after a median follow-up of 9.2 years, the rate of disease-free survival was 45% and overall survival was 95% (n = 10 and 9 patients, respectively); the median duration of disease-free survival was 8.0 years. Prolonged disease-free survival was associated with continued absence of the t(14/18) translocation in the subset of patients in which they were detected.                                                                                                                                                                                                                                                                                                                                                                             | 640886    |
| Efficacy                                           | Phase II clinical trial of BiovaxID in patients (n = 26) with mantle cell lymphoma in complete remission after six cycles of EPOCH-R chemotherapy. At 3 months after chemotherapy, five cycles of BiovaxID (0, 1, 2, 3 and 5 months sc) were administered together with 100 µg/m <sup>2</sup> GM-CSF; then GM-CSF on 3 subsequent days.                                                                                                               | Despite the lack of B-cells as a result of chemotherapy and rituximab treatment, respective antibody responses against KLH and Id protein were detected in 74 and 30% of the 23 evaluable patients, occurring later than in previous trials and correlating with B-cell recovery. Respective CD4+ and CD8+ T-cell response against the vaccine occurred in 87 and 100% of the 23 patients, suggesting the activity of professional antigen-presenting cells, such as dendritic cells, in the absence of B-cells. After 46 months median follow-up in 26 patients, overall survival was 89%, median event-free survival was 22 months and 19% of patients remained in continuous complete first remission.                                                                 | 646877    |
| Efficacy                                           | Phase III, double-blind, randomized clinical trial (NCT00091676) of BiovaxID in patients (n = 629) with follicular NHL in complete remission after PACE chemotherapy. Five doses of vaccine plus 100 µg/m <sup>2</sup> GM-CSF were administered over 6 months with doses divided into four and injected sc into the upper and lower extremities, with GM-CSF injected for an additional 3 days at all sites (method described in reference [875425]). | In patients monitored for up to 80 months, vaccination with BiovaxID resulted in a 60% increase in median disease-free survival, from 21.2 months in the control arm to 33.8 months for BiovaxID (p = 0.047). In addition, data from the same patients monitored for 36 months from vaccination showed that BiovaxID treatment improved median disease-free survival by approximately 100% (p = 0.024).                                                                                                                                                                                                                                                                                                                                                                   | 926103    |

## Associated patent

**Title** Enhancement of B cell lymphoma tumor resistance using idiotypic/cytokine conjugates.

**Assignee** The Board of Trustees of the Leland Stanford Junior University.

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**Inventors** Levy R, Tao M-H.

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