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Tovaxin, radiation-attenuated, patient-specific T-cells for the therapeutic vaccination of multiple sclerosis

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Opexa Pharmaceuticals Inc is developing a trivalent formulation of attenuated myelin-peptide-reactive T-cells, named Tovaxin, for the potential treatment of multiple sclerosis. Tovaxin is being evaluated in phase II clinical trials. Opexa was previously investigating Tovaxin for the potential treatment of rheumatoid arthritis; however, no development has been reported for this indication since December 2002.

Originator Baylor College of Medicine

Licensee Opexa Pharmaceuticals Inc

Status Phase II Clinical

Indications Multiple sclerosis, Rheumatoid arthritis

Actions Anti-inflammatory, Genetically-engineered autologous cell vaccine, Therapeutic vaccine

Technologies Cell therapy, T-lymphocyte

Introduction

Multiple sclerosis (MS) is a chronic and debilitating disease that affects the CNS. Specifically, MS destroys the oligodendrocytes that create and maintain the myelin sheath that facilitates the effective transmission of signals along axons in the 'white matter' of the brain and spinal cord. Reduction or damage to the myelin sheath results in the clinical manifestations of MS, often causing progressive physical and cognitive disability [624193]. The incidence of MS is between 2 and 150 per 100,000 individuals, depending on the country or specific population, and onset usually occurs in young adults. MS is more prevalent in women [890337].

Based on elaborate animal studies and *ex vivo* human studies, myelin peptide-reactive T-cells (MRTCs) are considered to have a critical role in the pathogenesis of MS. These autoreactive T-cells are thought to migrate to the CNS, where they are reactivated upon encountering their specific antigen presented on resident antigen-presenting cells.

Consequently, MRTCs spread into the white matter parenchyma and produce proinflammatory cytokines, such as TNF α and IFN γ . These cytokines are pivotal in orchestrating immune and inflammatory cell-cell interactions that are noxious to oligodendrocytes and to the myelin sheath [890261]. During the neuroinflammatory events, chemokines are released and the expression of adhesion molecules on endothelial cells is increased. This results in the recruitment of other immune cells, including monocytes, T-cells, mast cells and B-cells, which are thought to be actively involved in demyelination, astrogliosis, and loss of oligodendrocytes and neurons in MS lesions [624193], [890261].

While MRTCs are also present in healthy individuals, numerous studies have demonstrated that these cells are clonally expanded and persist in the blood of MS patients, [890275], [892904], [892910]; however, the manner in which the cells differ and become pathogenically relevant is not understood. Several reports indicate an impaired immune regulation by CD4CD25^{hi} regulatory T-cells in the peripheral blood of MS patients [888795], [888798]. In addition, subtle functional alterations of myelin specific T-cells have been observed in MS patients, including impaired susceptibility to apoptosis [890262], cytotoxic potential [888800], changes in co-stimulation requirements [888802], IL-7 sensitivity and avidity for the autoantigen [888804].

In addition to treatment of acute attacks with immune suppressive drugs (eg, glucocorticoids) and symptomatic therapy, there are currently six FDA-approved agents that modify the course of MS: Three preparations of IFN β (IFN β 1a [Avonex and Rebif] or IFN β 1b [Betaseron]) have shown efficacy in relapsing-remitting MS (RR-MS) [893092]. Glatiramer acetate (Copaxone) significantly reduces the relapse rate, extends time to first exacerbation and decreases the number of MRI lesions [890265]. More recently, mitoxantrone (Novantrone) [890266] and natalizumab (Tysabri) [503604] have been approved for the treatment of MS based on the significant reduction in active MRI lesions. However, the efficacy of MS therapies such as IFN β , glatiramer acetate and immunosuppressive agents is limited. Although the ultimate benefit of these drugs has yet to be established, it is likely that, at best, they will slow the progression of MS [893062]. In addition, serious side effects (such as flu-like symptoms with IFN β treatment [893092]) and safety concerns (eg, cases of the rare and often fatal infection progressive multifocal leukoencephalopathy [890267] and liver toxicity [883048] with natalizumab) make long-term application unlikely. Therefore, therapeutic approaches have switched from general immunosuppression to more specific targeting, even at the level of the T-cell receptor (TCR) peptide–MHC complex. These T-cell-based experimental therapies include TCR peptide vaccination, induction of oral tolerance, altered peptide ligands (APL), MHC-peptide blockers, mAbs against leukocyte surface molecules (eg, CD3, CD25, CD40L or B7) and cytokine modulators (eg, TNF α antagonists, IL-10 or TGF β) [890302]. A T-cell vaccine Tovaxin (Opexa Pharmaceuticals Inc) belongs to this new generation of specific immunotherapies; it was designed to selectively target myelin-specific T-cells without affecting other T-cell populations [890358]. The methods that led to the development of Tovaxin were based on studies by Jingwu Zhang and colleagues at Baylor College of Medicine, who also conducted early clinical trials together with Piet Stinissen and Jef Raus (Diepenbeek, Belgium) in the early 1990's that provided proof-of-concept for T-cell vaccination (TCV) in MS [877240], [888854], [892728].

Tovaxin is a trivalent therapeutic vaccine composed of autologous inactivated MRTC lines. T-cells are derived from the peripheral blood of individual patients and the vaccine is generated *ex vivo* using T-cells reactive to one of the proteins of the myelin sheath – myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) [890358], [892551]. Tovaxin targets circulating MRTCs in the blood of MS patients by inducing or boosting regulatory CD4⁺ and CD8⁺ T-cells, which in turn suppress or eradicate the potentially pathogenic autoreactive T-cells. At the time of publication, Tovaxin had completed a phase I/II dose-escalation clinical trial and a phase I/II extension clinical trial in MS patients [890358]. A subsequent phase IIb, multicenter clinical trial had completed recruitment and administered a first dose of Tovaxin to all participants (n = 150) by November 2007 [847741].

Synthesis and SAR

Tovaxin consists of a mixture of MRTC lines that are generated from the peripheral blood of individual patients [877240], [890358]. PBMCs are isolated from diluted heparinized blood by means of Ficoll gradient density centrifugation. Subsequently, cells are stimulated with synthetic myelin peptides that correspond to immunodominant epitopes within the major candidate autoantigens for MS; namely MBP, PLP and MOG [890358], [892551]. After the selection of responsive T-cell cultures, individual MRTCs are further expanded to sufficient amounts through repeated peptide stimulation rounds as previously described [877240].

Various activating peptides have been used for the generation of T-cell vaccines in clinical TCV trials and improvements in the choice of peptides (designed to represent a greater proportion of autoreactive T-cell epitopes) define the clinical evolution of T-cell vaccines that led to Tovaxin. Early clinical trials by Zhang and colleagues employed human brain-derived MBP [888854] and subsequently, various panels of synthetic 15-amino acid peptides representing MBP [877240], [892728]. A later trial tested a vaccine comprising cells reactive to immunodominant epitopes of MBP (three synthetic peptides) and MOG (two synthetic peptides) [877239]. In the phase I/II trials that are described by Opexa as the first trials with Tovaxin, the vaccine comprised T-cells reactive to any of six synthetic peptides representing MBP, PLP and MOG [892728]. In the latest phase IIb trial with Tovaxin, a prescreening assay was performed to allow for selection of relevant peptides based on a patient's specific reactivity profile [892551]. A panel of mixtures of synthetic peptides spanning the full length of MBP, PLP and MOG proteins allows detection of an individual's T-cells that are reactive to more than 80% of the protein content of the myelin sheath [892551].

The resulting T-cell lines are CD4⁺CD8⁻ and exhibit a T-helper (Th)₁-like phenotype, producing IFN γ and TNF α , but little or no IL-4 and IL-10 [877238]. This phenotype resembles that of rodent MRTCs that are able to transfer disease (experimental autoimmune encephalomyelitis; EAE) to naive recipient mice or rats. Each MRTC line is then cryopreserved until the time of vaccine preparation. Two weeks before immunization, MRTC are thawed and expanded with phytohemagglutinin. The final vaccine consists of equal amounts of each MRTC, which are suspended in saline with 4% of human serum albumin and subsequently inactivated by γ -irradiation [890358].

Preclinical development

No preclinical studies with Tovaxin have apparently been published by Opexa or by Zhang and colleagues; however, their approach was based on earlier preclinical studies by several research groups, an overview of which follows.

Early studies into TCV

Irwin Cohen of the Weizmann Institute in Israel is the originator of TCV. In the early 1980s, he demonstrated that Lewis rats injected intravenously with attenuated encephalitogenic T-cells were protected from subsequent induction of EAE [888840]. The protection was specific and long lasting. In addition, the disease resistance could be transferred from vaccinated animals to naive recipients by T-cells raised against the vaccinating clone [888844]. TCV is applicable in many other autoimmune disease models, including adjuvant arthritis, murine lupus and non-obese diabetic mice [616294], [890301], [890303]. Although the exact mechanisms by which TCV ameliorates autoimmune disease in animal models remain unclear, indirect evidence suggests that the antivaccine T-cell responses specifically target the immunizing T-cell clones by recognition of a clonotype specific determinant (the 'idiotype'), most likely the TCR [888844]. In addition to these anti-idiotypic T-cell responses, other regulatory mechanisms are thought to contribute to the protective immunity induced by TCV. Cohen and colleagues observed that anti-clonotypic T-cells might induce autoreactive T-cells to shift from Th₁ to Th₂ subtype, which would be expected to reduce inflammation [895837]. Lohse *et al* further demonstrated that anti-ergotypic T-cell responses directed at activation markers (the 'ergotope') may account in part for the suppression of activated autoreactive T-cells after vaccination [888848]. In addition, TCV in EAE induced humoral responses that inhibited the proliferation of vaccine cells [888851]. Collectively, these findings reveal that TCV in EAE induces a complex immune response that results in the neutralization of pathogenic T-cells.

Toxicity

No toxicity studies with Tovaxin have apparently been published.

Metabolism and pharmacokinetics

No formal metabolism studies have been published for Tovaxin. As the vaccine is administered subcutaneously, the general concept is that antigen presenting cells, such as dendritic cells or macrophages, will take up the irradiated MRTCs and present components, such as idiotopes or ergotopes, to the immune system. This will induce a strong anti-vaccine response. While anti-idiotypic responses have been shown to be long lasting, ergotypic responses have a transient effect [888856].

Clinical development

Early TCV trials

The first pilot trial with a T-cell vaccine produced using the techniques underlying Tovaxin was conducted by Zhang and colleagues in a small number of MS patients. Patients (n = 8) with RR-MS or chronic progressive MS were

immunized with three doses of activated, irradiated, autologous MBP-specific T-cell clones at intervals of 2 to 4 months [888854]. Subcutaneous inoculations of autologous vaccine clones were demonstrated to be well tolerated and caused no adverse effects, supporting further development. Clinical data suggested a moderate clinical improvement in five out of eight MS patients, which was demonstrated by a reduced rate of exacerbations, stabilization of Expanded Disability Status Scale (EDSS) scores and MRI data on brain lesions [888855]. The vaccines induced an anti-idiotypic T-cell response, specifically recognizing the vaccine clones, accompanied with a progressive depletion of circulating MBP-reactive T-cells in all patients [888854]. An open-label trial in MS patients (n = 49) revealed that MBP-reactive T-cells remained undetectable for one to two years after vaccination in most patients, following three doses of a vaccine, comprising up to six anti-MBP clones, administered at 2-month intervals [888856].

Zhang and colleagues performed similar extended preliminary clinical trials in MS patients (total n = 54; n = 28 RR-MS patients and n = 26 secondary progressive MS [SP-MS]). These trials confirmed that vaccination with MBP-reactive T-cells induced immune responses, resulting in the depletion or suppression of circulating MBP-reactive T-cells. Clinical results indicated that these enhanced immune responses coincided with a prolonged time-to-progression in both RR- and SP-MS patients compared with the progression of untreated MS [888858].

A subsequent trial in RR-MS patients (n = 20) assessed a vaccine comprising T-cells reactive to MOG protein in addition to MBP (four activating peptides) [877239], which is described by Opexa as the vaccine that preceded phase I studies with Toxavin [890358]. After three vaccinations at 6- to 8-week intervals, the annualized relapse rate (ARR) of MS decreased by 55% (p = 0.026) compared with the incidence of relapse in the previous year. EDSS scores improved (p = 0.118), and the number and volume of MS lesions (imaged by MRI) decreased significantly in vaccinated patients compared with pretreatment values [877239].

For a T-cell vaccine to be effective, it should induce T-cell cytotoxic and/or regulatory immune responses against the pathogenic T-cells. Studies have indicated that vaccination of MS patients with the Toxavin approach resulted in the *in vivo* induction of CD8+ cytotoxic T-cells and CD4+CD25+FoxP3+ regulatory T-cells specific for the T-cell vaccine [877238]. The induction of anti-idiotypic cytotoxic CD8+ effector T-cells and anti-ergotypic CD4+CD25+FoxP3+ regulatory T-cells is believed to provide a therapeutically effective dual mechanism of protection in vaccinated patients. The observed regulatory immune responses collectively correlate with clinical improvement in treated patients [877238].

Phase I/IIa

The first phase I/II clinical trial with Toxavin employed an open-label, dose-escalation design in MS patients (n = 10). The vaccine (6 to 9 million, 30 to 45 million or 60 to 90 million MRTCs sc) was administered at 0, 4, 12 and 20 weeks, and patients were assessed for one year [585053], [605670], [780344]. At one year after treatment with the vaccine, a reduction in ARR of 90% occurred overall (p = 0.0039) [690973] and a 100% reduction in ARR occurred in patients receiving the mid-dose of the vaccine [780344].

A second phase I/II clinical trial enrolled RR-MS or SP-MS patients (n = 8) who had been treated with an earlier T-cell vaccine in a clinical trial conducted by Zhang and colleagues approximately five years previously. The one-year reduction in ARR in patients who received two doses of 30 to 45 million MRTCs, 8-weeks apart, was 92% (p = 0.0078). MRTC counts were reportedly reduced by 84 and 72% at 6 and 12 months after vaccination, respectively [807084].

In data consolidated from both trials, EDSS scores improved by a mean of 1.58 points (range 0.5 to 3.5) over the trial period. Reductions in specific MRTC counts reportedly correlated with clinical outcome [890358]. Opexa has only published summary findings of these trials. The vaccine dose selected for the following trial was 30 to 45 million MRTCs [890358].

Phase IIb/III

A phase II, randomized, double-blind, placebo-controlled clinical trial (TERMS trial) in patients (n = 150) with clinically isolated syndrome (a less severe demyelinating syndrome that can progress to MS) and early RR-MS began in May 2006 [606430], [780907]. Patients were to receive subcutaneous injections of the vaccine at 0, 4, 8, 12 and 24 weeks and be assessed over one year, with an optional one-year extension under a different protocol. The primary endpoint was to be the number of gadolinium-enhanced lesions on MRI images at weeks 28, 36, 44 and 52. Secondary endpoints were the number of new gadolinium-enhanced lesions at weeks 28 to 52, the change in T2-weighted lesion volume and the ARR [631261]. All of the patients had received the first vaccine dose by November 2007 [847741]. Data from the trial were to be expected in the second half of 2008 [795697].

Side effects and contraindications

All phase I/II clinical trials with TCV have indicated that TCV has a high safety profile. Both phase I/II trials of Tovaxin found the vaccine to be acceptably tolerated, with no severe adverse reactions related to TCV. Mild injection site reactions increased with increasing dosage, but resolved within 48 h [605670].

In the ongoing phase IIb trial, no safety issues were reported after more than 60% of the total 750 vaccine doses had been administered, as of September 2007 [829399].

Patent summary

The EPO announced its intention to grant the main Tovaxin patent application by Opexa, EP-01416956, in August 2007. Two equivalent applications were still pending grant in the US (US-20030091578 and US-20050186192). The sole inventor on these applications, Jingwu Zhang, was associated with Baylor, which is named as coassignee on EP-01416956.

Opexa and Baylor also appear as coassignees on a later application by Zhang, WO-2004015070, which claims improved methods for the production of autologous T-cell vaccines; as of February 2008, equivalents EP-01546719 and US-20060105336 were still pending grant. Different inventors claimed 'an improved T-cell vaccine' in WO-2007131210, an application assigned solely to Opexa.

Current opinion

All of the TCV trials performed worldwide thus far, including those with Tovaxin, strongly indicate a clinical benefit of a reduction in MRI-imaged lesions and a decreased relapse rate. The ongoing, double-blinded, TERMs, phase IIb clinical trial is the first trial that will demonstrate whether this can be confirmed in a larger sample of patients. The outcome of this study is pivotal to the further development of TCV as a therapy for MS. The design and approach taken in the TERMs trial is very accurate and accounts for several pitfalls expected for a patient-specific cell therapy approach (ie, the inconsistency and labor-intensive nature of the vaccine production) by using a semi-automated, GMP-controlled production and delivery procedure, resulting in lower costs [890358]. The prescreening for relevant myelin epitopes will certainly enhance the success rate of Tovaxin compared with previous versions of the vaccine. In addition, for other T-cell-directed MS drugs under development (eg, ATX-MS-1467, Apitope Technology (Bristol) Ltd; in phase II [872396]), the inclusion of a prescreening assay is considered to exclude/identify potential treatment failures. Nevertheless, it seems unlikely that all MS patients can benefit from TCV. In most trials, including those with Toxavin, the majority of patients treated were of the RR-MS type, so it remains to be tested whether different clinical manifestations are susceptible to TCV. Weiner and colleagues performed a TCV trial in patients (n = 84) with moderate-to-severe SP-MS and found no significant treatment effect in this patient subgroup [893091].

Several current TCV trials, including the phase II clinical trial with Tovaxin, aim to treat MS patients as early as possible by recruiting probable MS patients and patients with clinically isolated syndrome. One major challenge for the future will be to identify markers that will help discriminate potential responders from non-responders. In light of this, a simple assay that allows monitoring of responses to the vaccine is not currently available. The development of such an assay would aid greatly in assisting clinical trials that test efficacy and allow discrimination between responders and non-responders. In the next three to five years, it will be important to test efficacy of TCV in a sufficiently large population of MS patients. Although major efforts have been undertaken to plan double-blinded, placebo-controlled studies at academic centers, in our opinion these large-scale vaccination studies should be performed in a GMP-controlled company setting, as is done by Opexa, where more funding, better facilities and greater standardization of protocols are possible.

In addition, preclinical animal models and human trials are needed to further understand the complex immune networks triggered by TCV. Although different immune subsets are activated after vaccination, it is unclear how any of these regulatory cells are involved in the depletion of autoreactive T-cells *in vivo* and how they may alter disease course. One way to test this would be to boost one specific component of the vaccine-induced immune response. This rationale was applied in TCR peptide vaccination aimed at specifically boosting anti-idiotypic T-cells [266918]. This

approach was effective in an EAE model where encephalitogenic T-cells display limited V-gene diversity. Similar approaches could be undertaken when more data are available on the identity of candidate ergotopes that boost anti-ergotypic T-cells. These matters require further basic research, and could ultimately lead to more simplified, cell-free vaccines and thus eliminate several limitations of whole-cell preparations.

Besides Tovaxin, there are currently several other vaccination approaches directed against autoreactive T-cells under evaluation in phase I or II trials. These include a vaccine containing soluble synthetic peptide tolerogens derived from MBP (ATX-MS-1467, Apitope Ltd) [872396]; a therapy comprising a MBP-encoding plasmid and a cytokine-encoding plasmid (BHT-3009; Bayhill Therapeutics Inc) [895812] and a combination vaccine of three T-cell receptor peptides (BV5S2, BV6S5 and BV13S1) in incomplete Freund's adjuvant (NeuroVax; Orchestra Therapeutics Inc) [813711]. The major advantage of these three approaches as compared with Tovaxin is that all are cell-free preparations that can be applied in a non-patient-specific manner, allowing easy upscaling of production and consequently wider applicability. The comparative advantage of BHT-3009 over the peptide formulations is the stability of the product (both *in vitro* and *in vivo*), as it is a DNA-based vaccine. In addition, this DNA vaccine expresses the full length MBP, encompassing all potential MBP epitopes and thus allowing for a broader possible population of responders to the drug. While no safety problems were observed in clinical trials with BHT-3009 performed so far, safety aspects of DNA vaccination require further study. As for the developmental stage, BHT-3009 [888295] and NeuroVax [772052] – like Tovaxin – are currently undergoing or are slated to begin double-blinded, placebo-controlled efficacy studies in large groups of patients (n > 150), in contrast to ATX-MS-1467 that was so far only tested in a phase I pilot trial (in 8 patients). These studies will determine the success rate and impact of vaccination on MS therapy for the coming years.

Licensing

Opexa Pharmaceuticals Inc

By December 2002, Opexa had licensed the worldwide rights to Tovaxin from the Baylor College of Medicine [473919], [882819].

Development status

Developer	Country	Status	Indication	Date	Reference
Opexa Pharmaceuticals Inc	US	Phase II	Multiple sclerosis	12-DEC-02	473919
Opexa Pharmaceuticals Inc	US	No development reported	Rheumatoid arthritis	12-JUN-04	-
Baylor College of Medicine	US	Discontinued	Multiple sclerosis	12-DEC-02	882819

Literature Classification

Chemistry

Study type	Result	Reference
Synthesis	PBMCs are isolated from the blood of individual patients by Ficoll gradient density centrifugation. T-cells are stimulated with synthetic peptides that correspond to immunodominant epitopes within the MBP, PLP and MOG myelin proteins. After selection of responsive T-cell cultures, individual MRTCs are further expanded to sufficient amounts through repeated rounds of peptide stimulation.	890358

Clinical

Effect studied	Model used	Result	Reference
Efficacy and safety	A phase I/II dose-escalation trial in RR-MS patients (n = 10) administered Toxavin (6 to 9 million, 30 to 45 million or 60 to 90 million MRTCs sc) over a period of 20 weeks (administered at 0, 4, 12 and 20 weeks).	At one year after vaccination, there was a reduction in ARR of 90% overall (p = 0.0039) and a 100% reduction in ARR in patients receiving the mid-dose of the vaccine. Vaccination was well tolerated, causing no serious adverse reactions at any dose level.	780344
Efficacy and safety	A phase I/II trial in previously treated RR-MS and SP-MS patients (n = 8) administered two doses of Tovaxin (30 to 45 million MRTCs sc), at an 8 week interval.	The reduction in one-year ARR was 92% in vaccinated patients (p = 0.0078). MRTC counts were reportedly reduced by 84 and 72% at 6 and 12 months after vaccination, respectively. No safety issues occurred.	807084

Associated patent

Title Autologous T-cell vaccines materials and method.

Assignee Opexa Pharmaceuticals Inc, Baylor College of Medicine.

Publication WO-03024393 27-MAR-03

Priority US-2001952532 14-SEP-01

Inventors Zhang J.

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772052 **IRC starts European phase II study of NeuroVax for MS** The Immune Response Corp *PRESS RELEASE* 2007 March 07

780344 **Opexa Therapeutics reports positive top-line data in phase I/II dose escalation trial with Tovaxin for multiple sclerosis.** Opexa Therapeutics Inc *PRESS RELEASE* 2007 April 03

780907 **NCT00245622: Autologous T-cell vaccine (TCV) for multiple sclerosis.** *CLINICALTRIALS.GOV* 2007 February 08

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