

2. SCIENTIFIC ABSTRACT

Ovarian cancer is the fifth most common form of cancer (American Cancer Society, 2004). In January 2001, ovarian cancer had an estimated prevalence of 167,002 women in the United States of America (USA) (Ries et al, 2001).

There are three types of ovarian cancer: germ cell, stromal and epithelial. Germ cell cancers start from the cells that produce the ova. Stromal cancers are derived from the connective tissue cells that hold the ovary together and produce the female hormones estrogen and progesterone. Epithelial cell cancers are the most common, accounting for more than 85 - 90% of all ovarian cancers, arising from the cells that cover the outer surface of the ovary. Epithelial ovarian cancer is the most lethal of gynecological malignancies ranking fourth in cancer deaths among women (American Cancer Society, 2004) with an overall five-year survival rate of 28-35% (Hasenburg et al., 2000).

Ovarian cancer is generally asymptomatic in its early stages and unlike mammograms for breast cancer or pap smears for cervical cancer, there is no screening test for early diagnosis of ovarian cancer. It is rarely detected during a routine pelvic examination. Carbohydrate antigen-125 (CA-125) is a cancer cell marker protein found in the blood that suggests the presence of ovarian cancer when present in quantities ≥ 35 U/mL. However, only 50% of early stage ovarian cancer patients have elevated levels of CA-125. In addition pregnancy, endometriosis and uterine fibroids, can contribute to elevated levels of CA-125. Consequently, measurement of CA-125 is not sufficiently sensitive or specific to warrant routine use as an early diagnostic for ovarian cancer in asymptomatic women. Since there is no early diagnostic test and symptoms of ovarian cancer develop late in the disease, 75% of women with ovarian cancer are not diagnosed until the ovarian cancer has spread outside the pelvis to the abdomen (Stage III) or in areas beyond (Stage IV), causing swelling and pain.

Once the presence of ovarian cancer has been confirmed, the patient undergoes laparotomy to determine the staging of the disease. Staging is performed based on the International Federation of Gynecology and Obstetrics (FIGO) and Tumor/Node/Metastasis (TNM) systems (Heintz et al., 2001). Stage I ovarian cancer is confined to either one or both of the ovaries. In Stage II, the ovarian cancer has spread to pelvic organs, such as the uterus and fallopian tubes, but not to the abdominal organs or peritoneum. In Stage III, the ovarian cancer has disseminated into the abdominal organs, such as the liver and bowel. In Stage IV, the ovarian cancer has metastasized outside the abdomen (Heintz et al., 2001). Only 25% of women with ovarian cancer are diagnosed at Stages I or II, when surgical excision results in five-year survival rates of 76-93% and 60-74%, respectively. Most women with ovarian cancer are not diagnosed until Stages III or IV, where the five-year survival rates are 23-41% and 11%, respectively (Berek et al., 2000).

After a diagnosis of ovarian cancer has been made and the stage of ovarian cancer has been determined, all patients undergo surgery, which is the primary treatment for most

patients. Typically initial surgery involves a total hysterectomy and bilateral salpingo-oophorectomy (National Comprehensive Cancer Network, 2004). Surgery is often followed by adjuvant chemotherapy, to reduce the remaining cancerous mass and to prevent recurrence, with possible interval debulking surgery throughout the treatment.

First-line chemotherapy regimens are typically platinum-based combination therapies, usually administered intravenously (IV) for four to six treatments every 21-28 days. Although this first line of treatment has an 88% response rate (Bremer, 1999), 55-75% of women will develop recurrent ovarian cancer within two years (National Institute for Clinical Excellence, 2001).

While traditional chemotherapy regimens kill the fast growing ovarian cancer cells, the goal of cytokine therapies is to enhance the systemic immune response against the ovarian cancer. Interleukin-12 (IL-12) has been demonstrated to be one of the most active cytokines in the induction of potent anti-cancer immunity (Robertson and Ritz, 1996). It is associated with numerous immunomodulatory properties such as T-lymphocyte and natural killer (NK) cell proliferation, activation of cytotoxic T-lymphocytes (i.e., CD8+ lymphocytes) and secretion of interferon gamma (IFN-gamma) subsequently leading to cancer inhibition. Cancer regression by recombinant human IL-12 protein has been induced in several experimental cancer models (Fujiwara and Hamaoka, 1996; Robertson and Ritz, 1996).

Certain cytokines, including IL-12 and IFN-gamma, have demonstrated anticancer activity in cancer patients (Lenzi et al., 2002). Intraperitoneal (IP) administration of recombinant IL-12 (rIL-12) protein in patients with peritoneal cancer (metastasized from ovary or other gastrointestinal malignancies) demonstrated increased concentrations of tumor necrosis factor alpha (TNF-alpha) and IFN-gamma (Lenzi et al., 2002). However, dose limiting toxicity from IP administration of rIL-12 protein was also observed. Due to toxicity concerns, rIL-12 protein is not an approved cancer therapy. As a result, researchers are exploring alternative delivery methods for IL-12.

For optimal efficacy as anticancer agents, cytokines should be present at stable, non-toxic concentrations in the tumor for prolonged periods of time. This pharmacokinetic profile is not achievable with rIL-12 protein because of its short half-life following bolus administration (Nastala et al., 1994; Tan et al., 1996). However, based upon efficacy and safety results from nonclinical studies, IP administration of an IL-12 plasmid into the disseminated ovarian cancer has the potential to provide a sustained local increase in IL-12 and IFN-gamma concentrations, resulting in prolonged infiltration of macrophages and other immune cells in the cancer environment with minimal toxicity to normal cells. This approach to therapy is also less likely to cause the systemic toxicities seen after parenteral administration of rIL-12 protein.

The drug product, EGEN-001 is composed of human interleukin-12 (hIL-12) plasmid (phIL-12-005) containing p35 and p40 genes necessary for producing functional IL-12, formulated with the facilitating agent, polyethyleneglycol-polyethyleneimine-cholesterol (PEG-PEI-Cholesterol or PPC), which is a novel non-viral lipopolymer gene carrier

system. Incorporation of PEG into polyethyleneimine has been shown to enhance gene transfer efficiency while decreasing toxicity (Guo and Lee, 1999; Lenter et al., 2004). Cholesterol promotes cellular uptake of the transfection complexes via low density lipoprotein (LDL) receptor-mediated endocytosis (Furgeson et al., 2003). Thus, the incorporation of cholesterol and PEG into the PPC molecule is designed to improving transfection safety and efficiency.

Polymeric gene carrier systems have been recently used for cytokine gene therapy of cancer. Polymeric carriers have emerged as a viable alternative to the current systems (e.g., viruses and liposomes) due to several distinguishing characteristics such as excellent molecular flexibility that allows for complex modifications and incorporation of novel chemistries with little difficulty, and simple manufacturing schemes which are generally scalable and cost-effective. A functionalized biocompatible water soluble lipopolymer (WSLP) has been described for delivery of the IL-12 gene to CT26 colon carcinoma tumor (Mahato et al., 2001). The WSLP/IL-12 treatment gave higher levels of intratumoral (IT) gene expression than naked DNA. Further, secondary effects of the cytokine IL-12 production, namely IFN-gamma and nitric oxide (NO) levels were also higher in WSLP/IL-12 treated tumors when compared with naked DNA. A single IT injection of WSLP/IL-12 showed significant tumor growth inhibition and improvement in survival. Repeated delivery of the WSLP/IL-12 complexes at four or eight-day intervals enhanced the magnitude of tumor inhibition and improved survival (Yockman et al., 2003). Biodistribution studies following IT injection of WSLP/IL-12 complexes revealed enhanced retention of the complexes within the tumor and limited accumulation in other organs. Intratumoral injection of polyplexes containing IL-12 plasmid and a rapidly degradable water soluble polymer, PAGA, produced partial but significant inhibition of CT26 tumors (Maheshwari et al., 2002).

Nonclinical studies to support the development of IL-12 gene therapy have been conducted in mice with a murine IL-12 DNA plasmid (pmIL-12) formulated in lipopolymer delivery systems, PPC, which parallel the composition of human IL-12 gene therapeutic, EGEN-001. Human IL-12 is not biologically active in rodents. A single intraperitoneal (IP) administration of pmIL-12/PPC complexes into ID 8 ovarian tumor bearing mice resulted in the production of murine IL-12 (mIL-12) and its second messenger, IFN- γ . Weekly IP administration of pmIL-12/PPC reduced the proliferation of peritoneal tumor ascites and improved the animal survival rate in a plasmid dose-dependent manner. The anti-tumor efficacy of pmIL-12/PPC was also observed in IP models of colon and pancreatic cancer and subcutaneous (SC) models of mammary, brain and head & neck cancer. These nonclinical pharmacology and safety/toxicity studies supported IP administration of EGEN-001 in a first-in-humans clinical trial in patients with recurrent epithelial ovarian cancer. (NIH-RAC Protocol Reference # 501-692). This Phase I study is conducted to examine the safety and tolerability of phIL-12/PPC in recurrent ovarian cancer patients. IL-12/PPC delivery is examined at four escalating doses (0.6mg/m², 3mg/m², 12mg/m² and 24mg/m²) administered intraperitoneal by four weekly infusions. The preliminary results demonstrate that phIL-12/PPC is safe for

intraperitoneal administration in ovarian cancer patients. In addition, the IL-12 plasmid delivery is associated with biological and perhaps clinical responses.

A single treatment strategy against cancer is generally ineffective due to the multi-factorial nature of this disease. Various drug combination approaches to maximize anticancer response are being increasingly utilized in recent days. Nonclinical studies were conducted to test the hypothesis that the addition of EGEN-001 to standard chemotherapeutic agent(s) would improve the treatment efficacy against ovarian cancer. The use of pmIL-12/PPC in conjunction with standard Taxol[®]/Paraplatin[®] led to enhanced efficacy without augmenting toxicity in ID8 mouse ovarian cancer model. These nonclinical pharmacology data forms the basis of the proposed Phase I clinical study (Protocol EGEN-001-201) to determine the safety and preliminary efficacy of EGEN-001 in combination with standard chemotherapy in platinum sensitive recurrent ovarian cancer patients.

Protocol EGEN-001-201 is an open-labeled, non-randomized, dose escalation study to evaluate the safety and preliminary efficacy of IP administered EGEN-001 in two stages: First a dose escalation stage (Stage 1) and then a cycle escalation stage (Stage 2). EGEN-001 will be given in combination with carboplatin and docetaxel. The primary objective of Study EGEN-001-201 is to determine the MTD and treatment-related toxicities of EGEN-001 when administered by IP infusion in combination with IV administration of carboplatin and docetaxel in women with recurrent, platinum-sensitive, epithelial ovarian cancer. The secondary objective is to determine the optimal number of EGEN-001 treatments (4, 8 or 12) and treatment-related toxicities of EGEN-001 when administered by IP infusion in combination with IV administration of carboplatin and docetaxel in women with recurrent, platinum-sensitive, epithelial ovarian cancer. Two tertiary objectives are to assess the preliminary efficacy of EGEN-001 when administered in combination therapy by monitoring detectable tumor burden and serum CA-125 levels, and to assess the biological effects of EGEN-001 on circulating and local cytokine levels by measuring interferon gamma (IFN- γ), tumor necrosis factor- α (TNF- α), and interleukin-12 (IL-12) concentrations in the blood and peritoneal fluid. The protocol will exclude patients with intra abdominal disease greater than 5 cm in diameter or who have a life expectancy of less than three months.