



Safety and Toxicity Following Intraperitoneal Injection of Murine Interleukin-12 Plasmid Formulated with a Novel Polymeric Delivery System

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ABSTRACT [287]

Ovarian Cancer is the most lethal gynecologic malignancy and the fifth most common malignancy affecting women. Advanced disease is often characterized by the widespread dissemination into the peritoneal cavity. We have previously shown that repeated intraperitoneal delivery of an Interleukin-12 (IL-12) plasmid formulated into nanocomplexes with a novel polymeric delivery system (PPC) has shown efficacy in treating a variety of disseminated peritoneal malignancies in mice, including disseminated epithelial ovarian cancer. As part of a pre-clinical development plan the safety and toxicity of four weekly IP doses of murine IL-12 formulated with PPC were evaluated in mice. For this GLP study 168 mice (males and females) were used. The weekly administrations were given at three dose levels of 10, 50 and 250 µg (total DNA/mouse) and correspond to ~1.5, 7.5 and 38 mg/m² respectively. These doses represent significant multiples of the starting dose currently being used in Phase I clinical testing. Specifically the low, medium and high doses are approximately 2.5, 12.5 and 60 fold higher than the starting dose in humans based on a 25 g mouse and a 70 kg human. Regular clinical observations were made during the dosing phase of the study. One week and one month following the final dose the animals were euthanized and samples obtained for histopathology and clinical pathology analysis. There were no apparent treatment related effects on mortality, clinical signs, body weight, food consumption and gross necropsy findings. A dose dependent increase in spleen weight was observed. Additionally, test article related microscopic findings consisting of granulomatous to pyogranulomatous inflammatory reaction were seen in multiple organs and tissues of the peritoneal cavity. These effects were considered mild to moderate and were resolved by one month after the last treatment. Based on the observation of some inflammation in the animals at the 250 µg dose the no-observed-adverse-effect-level (NOAEL) was considered to be the 50 µg dose level. In summary, IP delivery of mL12/PPC was well tolerated. The effects associated with delivery were inflammatory in nature and not considered adverse but expected physiologic/pharmacologic responses associated with Interleukin-12 immunotherapy. The results of this safety/toxicity study have supported the current clinical testing of IP delivered hIL-12/PPC for treating recurrent ovarian cancer.

INTRODUCTION

Immunotherapy treatment strategies for cancer rely on stimulating the body's own immune system and have gained acceptance during the last decade. Interleukin-12 (IL-12) has proven to be one of the most active cytokines for inducing potent anti-cancer immunity (Robertson and Ritz, 1996). Interleukin-12 is associated with immunostimulatory properties including T-lymphocyte and natural killer (NK) cell proliferation and cytotoxic activation and secretion of interferon-gamma (IFN-γ) subsequently leading to tumor inhibition. Interleukin-12 also potentiates T helper cell differentiation and development of cytotoxic CD8⁺ T cell responses against viruses and tumor cells. Despite initial aggressive testing in the clinic, use of recombinant hIL-12 therapy has not advanced to an approved therapy due primarily to systemic toxicity concerns, which has led researchers to explore alternative means of IL-12 delivery (Imboden et al., 2003; Kang et al., 2001).

We have developed a non-viral IL-12 gene delivery approach that incorporates the use of a novel polymeric delivery system PEG-PEI-CHOL (PPC). Results from in-vivo studies in mice have indicated that this system is useful for treating a variety of solid tumors and peritoneal malignancies.

The goal of this study was to evaluate the potential toxicity of repeated intraperitoneal administration of murine IL-12 plasmid (p2CMVml-12) formulated with PPC in mice. The results of this study have been used to support initiation of Phase I clinical trial evaluating IP delivery of human IL-12/PPC in women with recurrent ovarian cancer.

MATERIALS/METHODS

Plasmid

The murine IL-12 plasmid (p2CMVml-12) consists of the p35 and p40 genes each under control of a separate CMV promoter/enhancer and each containing the SV40 late polyadenylation signal sequences (Figure 1A).

Polymer

PPC (Figure 1B) is a cationic polymer that is composed of a low molecular weight branched polyethyleneimine (PEI) that has functional groups of methoxyethylmethacrylate (PEG) and cholesterol (CHOL) attached at an approximate 2.5:1:1 ratio (PEG:PEI:CHOL) described by Fewell et al. (2005).

Formulation

Plasmid and PPC were formulated at a 1:1:1 nitrogen to phosphate molar ratio and lyophilized. Prior to use the final solutions were reconstituted at 0.5 mg/ml, 0.1 mg/ml or 0.02 mg/ml with water for injection dependent on the dose to be administered. A constant volume of 500 µl was used for all IP injections.

Experimental Design

The design of the study is outlined in Table 1 and Figure 2. The study was performed by Geneligo, Inc (Gaitherburg, MD) following an approved IACUC protocol and conforming to GLP guidelines. The DNA doses evaluated (10 µg, 50 µg and 250 µg) correspond to approximately 1.5 mg/m², 7.5 mg/m² and 38 mg/m² respectively and are 2.5, 12.5 and 60-fold higher than the starting dose (0.6 mg/m²) of hIL-12/PPC (EGEN-001) used in the Phase I clinical trial.

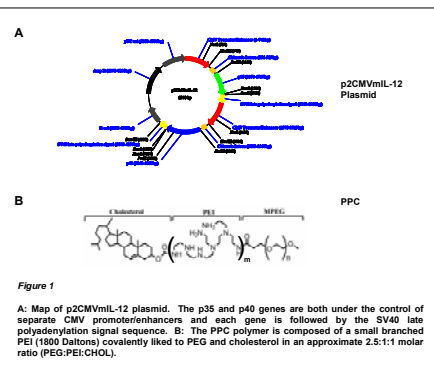


Figure 1

A: Map of p2CMVml-12 plasmid. The p35 and p40 genes are both under the control of separate CMV promoter/enhancers and each gene is followed by the SV40 late polyadenylation signal sequence. B: The PPC polymer is composed of a small branched PEI (1800 Daltons) covalently linked to PEG and cholesterol in an approximate 2.5:1:1 molar ratio (PEG:PEI:CHOL).

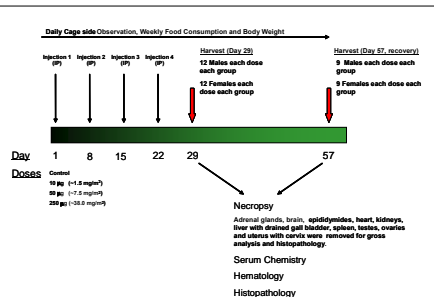


Figure 2

Experimental design schematic. Mice were injected weekly with either control article or 1 of 3 escalating doses of p2CMVml-12/PPC for a total of four injections. The animals were harvested for analysis on study days 29 and 57 which were one week and five weeks after the final treatment.

Table 1.

Experimental Design: Safety/Toxicity Study												
Group	Treatment	Route	Dose Level	Main Phase				Recovery Phase		Recovery Phase		
				µg/ml	mg/m ²	M	F	M	F	M	F	
1	Control article	IP	0	0	8	8	4	4	6	6	3	3
2	p2CMVml-12/PPC	IP	10	1.5	8	8	4	4	6	6	3	3
3	p2CMVml-12/PPC	IP	50	7.5	8	8	4	4	6	6	3	3
4	p2CMVml-12/PPC	IP	250	38.0	8	8	4	4	6	6	3	3

F=Female; M=Male; p2CMVml-12/PPC = Murine Interleukin 12 plasmid; IP=Intraperitoneal; PEG=Polyethylene glycol; PEI=Polyethylenimine; CHOL=Cholesterol; (a) mg/m² was based upon use of 0.00666m² as the surface area of a mouse.

(b) Separate animals were assigned for prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests

RESULTS

>There were no apparent test related effects on mortality, clinical signs, body weight, food consumption and gross necropsy.

>A small (but significant) dose dependent increase in spleen weight was noted in males following IP administration (Figure 3). In females, a similar increase was seen following repeated subcutaneous administrations. This effect may be attributed to the hematopoietic effects of IL-12 in vivo. No other changes in organ weights were noted.

>Some indication (not statistically significant) of a dose dependent decrease in WBC was noted (Figure 4). Loss of absolute lymphocytes was most pronounced. This result is consistent with cell mobilization and/or the myelosuppressive effects that are associated with recombinant IL-12 protein administration.

>No significant changes were detected in any serum chemistry parameters (Figure 5).

>Test article related microscopic findings following IP dosing consisted of granulomatous to pyogranulomatous inflammatory reaction occurring in a dose-dependent manner in multiple organs and tissues of the peritoneal cavity (not shown). The most severe reaction (mild) was noted in the serosal surface lining in 5 of 8 female mice at the 250 µg dose level.

>Histopathological observations were diminished in the recovery group of animals and suggested resolution of the lesions.

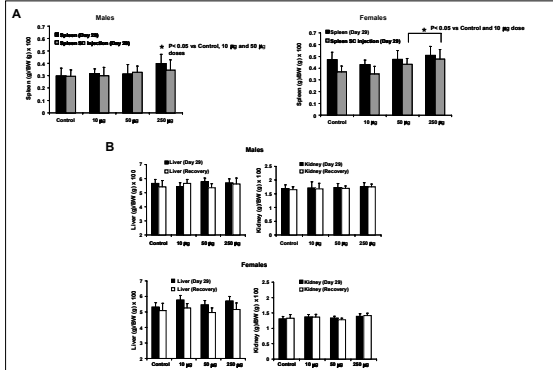


Figure 3

Organ weights of animals injected with p2CMVml-12/PPC or control vehicle. A: Spleen weights of male and female mice indicating a dose dependent increase in weight following IP injection in males. Results of a parallel study, where p2CMVml-12/PPC was injected subcutaneously, are also shown indicating a small but significant spleen weight increase in females following this delivery modality. B: A partial representation of other organs that were analyzed showing no significant changes. Values are means ± SD with n=8 (day 29) and n=6 (day 57, Recovery).

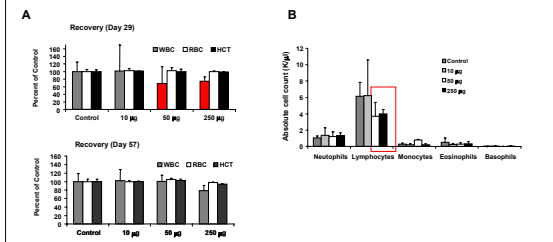


Figure 4

Hematology data of male mice. A: Subset of the CBC data showing a trend toward a dose dependent decrease in WBC of animals treated with p2CMVml-12/PPC. A normalization is seen in the recovery groups (day 57). All other hematology B: The decrease in WBC at the higher doses appears to be due to a decrease (not significant) in absolute lymphocyte count.

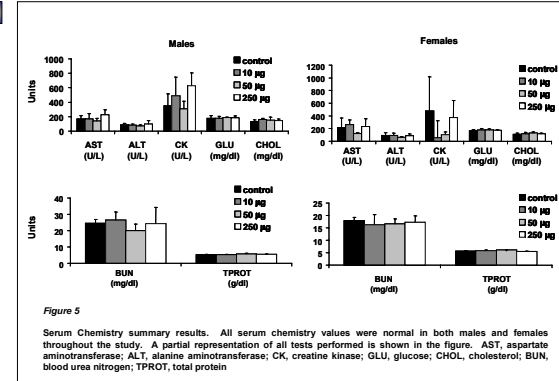


Figure 5

Serum Chemistry summary results. All serum chemistry values were normal in both males and females throughout the study. A partial representation of all tests performed is shown in the figure. AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; GLU, glucose; CHOL, cholesterol; BUN, blood urea nitrogen; TPROT, total protein

CONCLUSIONS/SUMMARY

>The administration of a plasmid encoding for murine IL-12 formulated with the PPC delivery system has been shown to be well tolerated in mice following repeated IP administration.

>All of the effects associated with IP administration were mild to moderate and inflammatory in nature and were not considered adverse but expected responses following IL-12 administration. Most effects that were observed at day 29 had completely resolved by recovery (day 57).

>Some evidence of inflammation was observed in animals receiving 250 µg at the end of the recovery interval, therefore, the no-observed-adverse-effect level (NOAEL) of pmIL-12/PPC administered by the intraperitoneal route was considered to be 50 µg/animal.

>The results of this study have been used to support initiation of a Phase I clinical trial evaluating IP delivery of human IL-12/PPC in women with recurrent ovarian cancer.

CLINICAL STATUS

A plasmid encoding for human IL-12 and formulated with PPC (EGEN-001) is currently being evaluated in an open label Phase I clinical trial in patients with recurrent ovarian cancer. In this trial escalating doses of plasmid are being administered IP on a weekly basis for up to four weeks. Recruitment is ongoing but preliminary results will be presented Sunday June 4th.

REFERENCES

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