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Safety toxicity study of plasmid-based IL-12 therapy in Cynomolgus monkeys

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Abstract

We have investigated the potential toxicity of hIL-12 DNA plasmid formulated with 5% polyvinylpyrrolidone (PVP) administered twice weekly via subcutaneous injections to Cynomolgus monkeys for four weeks, and have evaluated recovery from any effects of the test article over a four-week treatment-free period. Doses of the formulated hIL-12 plasmid were selected based on anti-tumour efficacy studies previously conducted in mice. The duration of the study and the frequency of dosing were designed to support clinical trials. No clinical signs indicative of an adverse effect of administration of formulated hIL-12 plasmid were observed. There were no apparent effects of the formulated hIL-12 plasmid on body weights or on serum chemistry, haematology, coagulation or urinalysis parameters. No treatment-related ocular abnormalities were evident. In addition, examination of the electrocardiograms from all monkeys at the pre-study, week-4, and week-8 time points did not reveal any treatment-related effects. No treatment-related gross lesions were noted at days 28 or 57. Slight histopathological changes associated with high doses of PVP vehicle were observed at both time points. These results suggested that the administration of formulated hIL-12 plasmid at a dose level up to 18 mg kg^{-1} dose twice per week for four weeks to experimentally naïve Cynomolgus monkeys did not result in significant toxicity. These results support further testing of this gene therapy in clinical trials.

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Introduction

With over 220 investigational new drug applications currently active, gene therapy represents one of the fastest growing areas in biotherapeutic research (Pilaro & Serabian 1999). We have evaluated the safety of human IL-12 DNA plasmid formulated with 5% polyvinylpyrrolidone (PVP), when given by a series of subcutaneous injections in Cynomolgus monkeys. This study was conducted to support further testing of this novel gene therapy in human clinical trials for the treatment of solid tumours.

Cytokine gene therapy for cancer is a strategy used to target the expression of cytokines locally to sites within and around the tumour lesion to break the putative operational immune tolerance typically observed in many tumours. Such therapy may eventually lead to a systemic and therapeutic antitumour immune response in the tumour-bearing host (Tahara et al 1996; Parker et al 1999). The local expression of cytokines may be less toxic than their systemic delivery as recombinant proteins (Mier & Atkins 1993; Mendiratta et al 1999). While a number of cytokines have been tested for cancer immunotherapy, IL-12 has proven to be one of the most

effective in the induction of potent antitumour activity (Nastala et al 1994; Brunda et al 1995; Cavallo et al 1997; Rakhmilevich et al 1997). IL-12, formerly termed T-cell stimulating factor, natural-killer cell stimulatory factor, or cytotoxic lymphocyte maturation factor (Lotze 1996), is a heterodimeric cytokine with potent immunoregulatory activity (Atkins et al 1997), that has been demonstrated to have antitumour activity in animal models.

Studies of the recombinant protein in patients have been limited in their ability to demonstrate efficacy due to the occurrence of a dose-limited toxicity. A gene therapy approach may allow the administration and generation of more therapeutically relevant levels, at the tumour site, without the associated systemic toxicity observed with the recombinant protein. Preclinical studies in several tumour-bearing mouse models (Mendiratta et al 1999) have demonstrated the significant antitumour effect of the IL-12 plasmid/PVP formulation (IL-12 gene medicine) after intratumoral injection. Such therapy has the potential also to eliminate local, regional, and distant tumours by stimulating a systemic and anamnestic memory host immunoresponse.

To develop a safe and effective plasmid-based IL-12 therapy, the in-vivo toxicology status of the formulated plasmid must be determined. The purpose of this toxicological study was to establish a potential dose response, to determine target organs, to look for reversibility of any toxicity, and for any cumulative toxicity, and to characterize any exaggerated pharmacological effects (Pilaro & Serabian 1999; Serabian & Pilaro 1999).

The objective of this study was to investigate the potential toxicity of human IL-12 plasmid formulated with 5% polyvinylpyrrolidone administered twice weekly by subcutaneous injections to Cynomolgus monkeys for four weeks, and to evaluate recovery from any effects of the test article over a four-week treatment-free period.

Materials and Methods

Plasmid composition and structures

The plasmid-based IL-12 gene medicine consisted of two components: a gene expression system and a gene delivery system. The two components were mixed together at an appropriate ratio using a defined process, then lyophilized. The lyophilized single vial product was supplied to the investigative site for rehydration with 0.9% sodium chloride for injection (U.S.P.) immediately before administration.

The gene expression system was a closed circular plasmid that contained two coding sequences to direct expression of human IL-12. It contained two expression cassettes directing expression of the IL-12 subunits (p35 and p40). The plasmid backbone also contained an expression cassette for neomycin phosphotransferase to confer kanamycin resistance to allow growth selection during the manufacturing process. The origin of replication sequence allowed plasmid replication in bacteria only and not in mammalian cells. The plasmid is shown schematically in Figure 1.

The gene delivery system comprised polyvinylpyrrolidone (PVP), a synthetic PINC (protective, interactive, non-condensing) polymer with a molecular weight of approximately 50000 (Mumper et al 1998). The chemical formula was $(C_6H_9NO)_n$, where n was approximately 500. PVP has been widely used in pharmaceuticals products (Mumper et al 1996) and it can function as an in-vivo gene delivery agent to facilitate uptake and expression of plasmid-based therapeutics in solid tissue.

After rehydration of the lyophilized IL-12 gene medicine, the final product consisted of a non-covalent complex between the plasmid and PVP in 0.9% sodium chloride for injection (U.S.P.). The plasmid concentration in the final product was 3 mg mL^{-1} and the concentration of PVP was 5%.

Animal studies

Single dose study of the biodistribution of hIL-12 gene medicine in mice

The purpose of this study was to determine the biodistribution and clearance of hIL-12 plasmid DNA in mice after a single subcutaneous injection. Two groups of 18 CD-1 mice (Charles River, NC), nine per sex, per group, of approximately seven weeks of age received a single subcutaneous injection of approximately 50 μL of either control or test formulation on the back of the neck. The total dose of hIL-12 plasmid DNA for the test group was approximately 6 mg kg^{-1} per animal. Mice were maintained on freely available feed and water at 23°C, 40% humidity, and a 12-h light–dark cycle. Animals were killed at approximately six hours, two days, and seven days after injection. Three animals per sex were killed at each time point per group. A series of tissues were collected from the animals in the following order: blood, gonads, spleen, liver, kidneys, heart, lung, bone marrow from right femur, mandibular lymph nodes, brain and injection site skin and tissue. Extensive precautions were taken to avoid potential cross-contami-

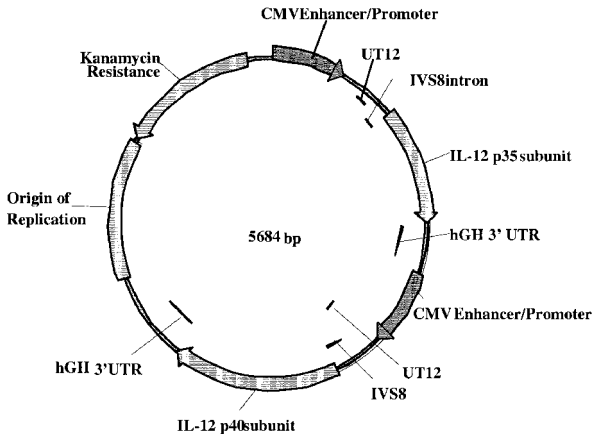

Figure 1 The IL-12 double-barrel expression plasmid. The coding sequence for IL-12 p35 and p40 subunits were subcloned into a plasmid containing a human CMV promoter upstream of each sequence and a bovine growth hormone polyadenylation signal at the 3′ end of each coding region (modified from Blezinger et al (1999)).

nation of tissue samples with plasmid DNA. These included extensive decontamination with 10% bleach solution and soaking instruments in bleach solution for at least 30 s between harvesting each tissue. All animals were handled in accordance with the regulations of the Institutional Animal Care and Use Committee of Valentis, Inc., and with generally acceptable animal guidelines. Total DNA isolation from each collected tissue was performed in the Molecular Analysis facility at Valentis, Inc. according to written protocols to minimize potential contamination. Each DNA sample was analysed for this hIL-12 plasmid DNA (Valentis, Inc. plasmid identification number: pIN1143) using a quantitative polymerase chain reaction (qPCR) assay specific for this plasmid. This assay had been shown previously to specifically detect hIL-12 DNA in the presence of mouse DNA isolated from each of the studied tissues. Spiking studies confirmed that the tested tissues did not inhibit the qPCR assay. As performed in this study, the hIL-12 qPCR assay was quantitative to approximately 800 plasmid copies per μg of total DNA (1600 copies/μg for lymph nodes). Below that level, the assay could reliably detect (but not accurately quantify) the presence of as little as 40 copies per μg of total DNA (80 copies/μg for lymph nodes).

Non-human primate safety toxicity study

A four-week toxicity study for formulated hIL-12 plasmid administered by subcutaneous injection to Cynomolgus monkeys (*Macaca fascicularis*) was conducted at Sierra Biomedical, Incorporated (Sparks, NV), in compliance with Good Laboratory Practices Procedures of the United States Food and Drug Administration and following Standard Operating Procedures established at Sierra Biomedical, Incorporated. Animals selected in this study were as uniform in age and weight as possible. They were generally pre-pubertal to young adults (approximately 3–6-years of age). At the time of assignment, their body weights ranged from 2.4 to 4.0 kg for the males and from 2.4 to 3.4 kg for the females. This route of administration was chosen to be the closest to the intended intratumoral administration in the human clinical trials of this novel gene therapy. This study was conducted to investigate the potential toxicity of the hIL-12 gene medicine when administered by repeated subcutaneous injections. Sierra Biomedical, Inc., Animal Care and Use Committee approved the study protocol.

The animals were housed individually in stainless-steel cages. Primary enclosures were as specified in the USDA Animal Welfare Act and as described by the Guide for Care and Use of Laboratory Animals. All animals were kept in one room with no other species housed in the same room. The room was well ventilated with 100% fresh air and a 12-h light–dark photoperiod was maintained.

Animals received eight bi-weekly injections over a 28-day period. A subset of the animals was killed on day 29, while the remaining animals underwent an additional treatment-free 28-day recovery period and were killed on day 57 (Table 1). Dose levels were selected based on anti-tumour efficacy studies conducted in mice at Valentis, Inc. (Mendiratta et al 1999). The duration of the study and frequency of dosing were designed to support clinical trials after intratumoral injections in cancer patients.

A total of 48 animals, experimentally naive and weighing 2.4– 4.0 kg at outset of the study were assigned to six treatment groups. Each animal received a dose of test or control formulation twice weekly via subcutaneous injection for four weeks as described in Table 1. The animals were temporarily restrained for the dosing procedure. The first day of dosing was designated day 1 and subsequent doses were given on days 4, 8, 11, 15, 18, 22 and 25 (total of eight doses). The animals were observed twice daily for clinical signs of toxicity and body weights were measured weekly. Ophthalmic examinations were conducted and electrocardiograms were recorded before the study and in weeks 4 and 8. Blood samples for evaluation of serum chemistry, haematology, and coagulation parameters were collected from all animals pre-study and on days 14 and 28. Blood samples were collected from recovery animals on days

Table 1 Group assignments and dose levels.

Group	Number of males/females	Dose level (mg kg^{-1})*	Dose volume (mL kg^{-1})	Dose solution DNA concn (mg mL^{-1})*	Number killed on day 29	Number killed on day 57
1	2/2	0 (saline control)	6	0	1/1	1/1
2	4/4	0 (vehicle control)	6	0	2/2	2/2
3	5/5	0.36	0.12	3	3/3	2/2
4	5/5	1.2	0.4	3	3/3	2/2
5	3/3	6.0	2.0	3	3/3	
6	5/5	18.0	6	3	3/3	2/2

*Expressed as mg DNA per kg body weight or per mL of dosing solution.

42 and 56. Urine samples for standard analysis were collected by bladder puncture during necropsy.

Throughout the study animals were observed within their cages at least twice daily by the veterinary staff. Particular attention was paid to the appearance of the injection sites. Body weights were measured before the first dose and weekly thereafter. Food consumption was qualitatively assessed daily for each animal as part of cage-side observations, beginning at least five days before the first dose.

Thirty animals (1/sex from group 1, 2/sex from group 2, and 3/sex from groups 3–6) were killed on day 29. The remaining 18 animals (1/sex from group 1, and 2/sex each from groups 2, 3, 4, and 6) were maintained over an additional four-week treatment-free period and killed on day 57. Toxicologic analysis included analysis of blood samples for evaluation of serum chemistries, haematologic parameters, and coagulation parameters. Urine samples were collected at necropsy for urinalysis. At the time of death a gross necropsy was performed. A complete necropsy was performed on all animals, and selected organs (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary (post fixation), spleen, testes, thymus and thyroid with parathyroids were collected and weighed for calculation of organ to body weight ratios.

At necropsy, a large panel of tissues (47 tissues) was collected for histopathologic evaluation. A bone marrow smear was made for microscopic evaluation.

Results

Biodistribution study of hIL-12 on CD-1 mice

Skin from the injection site was the only tissue that contained high levels of plasmid DNA. At 6 h, plasmid DNA levels averaged 1.4×10^9 copies/μg in males, and 2.3×10^7 copies/μg in females. Total calculated recoveries of plasmid DNA from the injection sites at 6 h averaged approximately 4 μg in males and 0.1 μg in females. At two days, plasmid DNA levels at the injection site dropped more than 1000-fold, averaging 2.6×10^5 copies/μg in males, and 1.4×10^4 copies/μg in females. At seven days, plasmid DNA levels at the injection site averaged 2.5×10^4 copies/μg in males, and approximately 4.4×10^4 in females.

Low levels of plasmid DNA were reproducibly seen in blood at 6 h. Levels averaged 2.1×10^4 copies/μg in males and 1.9×10^3 copies/μg in females. Plasmid DNA was cleared from blood of all animals at day two and day seven. Gonads, liver, spleen, kidney, heart, brain, and mandibular lymph nodes of most animals contained traces of plasmid DNA at 6 h. Levels were typically below the limit of quantitation, although a few samples contained several thousand plasmid copies/μg. Plasmid DNA was cleared from all peripheral tissues, including gonads at day two and day seven. The only exceptions were that traces of plasmid DNA were observed in one male spleen and one male brain at day seven. No plasmid was detected in any bone marrow sample at any time.

Only four of 198 samples from animals treated with the control article tested positive for plasmid DNA (a calculated false positive rate of 2.0%). All four false positive results were very close to assay background. The key results from this study are shown in Table 2.

Safety/toxicology study of hIL-12 in Cynomolgus monkey

No animals died during the course of the study, and no clinical signs indicative of an adverse effect of administration of formulated hIL-12 plasmid were observed. There were no apparent effects of the test article itself on body weight or on serum chemistry, haematology, coagulation or urinalysis parameters. Changes in clinical

Table 2 Results from the biodistribution study for animals receiving IL-12 gene medicine.

Animal	Sex	Time	log of hIL-12 plasmid copies per μg of total DNA[1]									
			Injection site skin	Blood	Gonads	Liver	Lung	Spleen	Kidney	Heart	Brain	Bone marrow	Mandibular lymph nodes
3131	M	6 h	9.24	3.71	+	+	+	+	+	+	+	−	+
3132	M	6 h	8.84	3.70	−	+	+	+	+	+	+	−	−
3133	M	6 h	9.27	4.64	−	+	+	−	−	+	+	−	−
3134	F	6 h	7.73	−	+	+	3.41	2.93	+	3.17	+	−	+
3135	F	6 h	5.93	3.69	+	+	+	+	+	+	+	−	−
3136	F	6 h	7.13	3.14	+	+	+	+	+	+	+	−	3.64
3143	M	2 days	5.52	−	−	−	−	−	−	−	−	−	−
3144	M	2 days	5.57	−	−	−	−	−	−	−	−	−	−
3145	M	2 days	4.79	−	−	−	−	+	−	−	−	−	−
3146	F	2 days	4.47	−	−	−	−	−	−	−	−	−	−
3147	F	2 days	3.89	−	−	−	−	−	−	−	−	−	−
3148	F	2 days	3.60	−	−	−	−	−	−	−	−	−	−
3155	M	7 days	3.60	−	−	−	−	−	−	−	+	−	−
3156	M	7 days	3.80	−	−	−	−	−	−	−	−	−	−
3157	M	7 days	3.95	−	−	−	−	−	−	−	−	−	−
3158	F	7 days	3.97	−	−	−	−	−	−	−	−	−	−
3159	F	7 days	5.09	−	−	−	−	−	−	−	−	−	−
3160	F	7 days	+	−	−	−	−	−	−	−	−	−	−

[1]Samples marked "+" were positive for hIL-12 DNA at levels below the limit of quantitation. The estimated range for these samples was between 40 and 800 copies/μg (80–1600 copies/μg for lymph nodes). Samples marked "−" were negative for hIL-12 DNA. Based on the assay limit of detection, these samples contained $<$ 40 copies/μg ($<$ 80 copies/μg for lymph nodes).

pathology parameters due to administration of the vehicle, polyvinylpyrrolidone (PVP), were seen, including dose volume-dependent decreases in serum globulin (also reflected by total protein) and cholesterol, and an increase in activated partial thromboplastin time. An indication of the baseline values was obtained from the saline control values (Table 3). High dose and vehicle control groups showed similar changes at the same time points providing confirmation that the effects seen were PVP related. Additionally, values were similar to the saline values at the lowest dose (0.36 mg kg^{-1}). No treatment-related ocular abnormalities were evident. In addition, examination of the electrocardiograms from all monkeys at the pre-study, at week four, and at week eight time points did not reveal any treatment-related effects.

Test formulation-related gross lesions were not identified in animals killed on day 29. Histopathological evaluation of tissues from animals killed on day 29 revealed changes in Kupffer cells, including hyperplasia, hypertrophy and cytoplasmic basophilic material accumulation in animals from groups 4–6. Although this incidence was suggestive of a possible relationship with administration of formulated hIL-12 plasmid, similar morphologic alterations in other histiocytic cell populations, as well as in the Kupffer cells of recovery animals killed on day 57, were clearly associated with the PVP vehicle. Thus, the contribution of formulated hIL-12 plasmid to the Kupffer cell changes, if any, was considered minor.

Minimal to mild inflammation of the subcutaneous tissue consisting of macrophages and lymphocytes seen at the injection site on day 29 was attributable to a vehicle (PVP) effect. Other histologic alterations at the injection site that were related to the PVP vehicle included the accumulation of basophilic material in the cytoplasm of cutaneous phagocytic cells and subcutaneous fibrosis. The basophilic material identified in the cytoplasm of affected cells was presumed to be PVP.

Vehicle-induced alterations were also identified in the axillary, mandibular and mesenteric lymph nodes of animals killed on day 29, and consisted of sinus histiocytosis and the accumulation of basophilic material in histiocytic or phagocytic cells. Histiocytic cell alterations were also identified in the kidney and brain, and were characterized by aggregations of histiocytic cells in renal glomeruli and histiocytic cells in the stroma of the choroid plexus of the brain. The incidence and severity

Table 3 Select clinical chemistry and haematology parameters in Cynomolgus monkeys following repeated subcutaneous administration of hIL-12 formulated with 5% PVP. Data from days 14, 28 (except group 5), and 42 are not shown.

Group	Group 1 saline control (n = 4)		Group 2 PVP control (n = 8)		Group 3 0.36 mg kg^{-1} (n = 10)		Group 4 1.2 mg kg^{-1} (n = 10)		Group 5[1] 6.0 mg kg^{-1} (n = 6)		Group 6 18 mg kg^{-1} (n = 10)	
Time points	Pre-study	Day 56	Pre-study	Day 56	Pre-study	Day 56	Pre-study	Day 56	Pre-study	Day 28	Pre-study	Day 56
Na (mEq L^{-1})	147	146	146	147	148	150	151	146	145	146	147	146
K (mEq L^{-1})	6.1	4.9	5.2	5.3	5.2	5.5	5.2	4.8	5.0	4.9	5.4	5.2
Cl (mEq L^{-1})	110	104	110	108	112	109	112	107	111	110	113	107
Ca (mg dL^{-1})	10.6	10.5	10.6	10.5	10.8	10.6	11.2	10.5	10.6	10.7	10.6	10.1
P (mg dL^{-1})	6.1	6.7	6.7	6.2	6.9	5.9	7.4	6.5	6.8	6.3	6.5	6.2
BUN (mg dL^{-1})	19	21	22	22	22	21	23	24	18	19	20	21
Creatinine (mg dL^{-1})	0.8	0.9	0.9	0.8	0.9	0.8	1.0	0.9	0.8	0.7	0.9	0.9
Glucose (mg dL^{-1})	61	79	65	76	71	72	76	83	62	69	70	78
Cholesterol (mg dL^{-1})	130	141	136	121	147	144	134	143	144	117	133	127
T. Protein (g dL^{-1})	6.8	6.9	6.9	6.0*	6.9	6.6	7.0	6.5	6.8	6.3	6.8	6.0*
Albumin (g dL^{-1})	4.7	4.6	4.9	4.6	4.8	4.7	5.0	4.8	4.8	4.5	4.8	4.4
Globulin (g dL^{-1})	2.1	2.3	2.0	1.5	2.1	1.9	2.0	1.8	2.0	1.8	2.0	1.5
Albumin/globulin ratio	2.4	2.1	2.5	3.1	2.3	2.5	2.5	2.8	2.6	2.6	2.5	3.3
T. Bilirubin (mg dL^{-1})	1.1	0.3	0.4	0.2	0.4	0.3	0.5	0.1	0.4	0.2	0.3	0.2
AST (U L^{-1})	44	68	71	49	46	41	44	46	47	39	42	60
ALT (U L^{-1})	29	43	58	34	37	44	33	49	38	36	33	38
WBC (10^3 mm^{-3})	9.8	11.2	10.6	9.2	11.3	11.7	11.3	11.3	10.8	10.4	10.7	10.9
Neutrophils (%)	30.9	32.0	30.8	40.0	26.4	40.1	33.5	34.0	19.7	24.7	35.0	30.0
Lymphocytes (%)	62.5	58.0	63.3	51.6	67.2	51.2	60.3	59.7	73.8	67.6	58.7	58.7
Monocytes (%)	4.33	6.39	4.04	5.32	4.16	5.80	4.35	3.41	4.12	4.36	3.38	6.33
Eosinophils (%)	0.91	1.64	0.63	1.90	0.69	1.72	0.80	1.89	1.30	2.20	1.65	3.04
Basophils (%)	1.34	1.96	1.22	1.09	1.52	1.22	1.04	0.92	1.07	1.00	1.32	1.27
RBC (10^6 mm^{-3})	5.90	6.06	5.86	5.69	5.89	5.68	5.91	5.89	5.57	5.38	5.88	5.77
Haematocrit (%)	43.6	44.9	43.5	43.3	43.4	42.7	44.3	45.1	42.7	41.7	43.1	42.5
Platelets (10^3 m^{-3})	427	284	456	426	387	396	397	442	451	462	349	324
PT	11.5	12.7	11.5	11.1	11.4	12.0	11.3	12.3	11.2	11.4	11.1	11.7
APPT	20.6	26.9	20.7	48.4*	21.1	24.1	20.8	28.7	21.0	47.9*	20.7	56.1*

*P value is less than or equal to 0.050 for Dunnett's test (groups 2, 3, 4, 5, or 6 compared with group 1 – saline control on same time point).
[1]All animals were killed before day 56. Blood urea nitrogen (BUN); aspartate aminotransferase (AST); alanine amiotransferase (ALT); white blood cells (WBC); red blood cells (RBC); prothrombin time (PT); activated partial prothrombin time (APPT).

of these histiocytic cell alterations correlated with administration of the larger volumes of PVP.

There were no treatment-related gross observations noted in the day 57 (recovery) animals. Histologic lesions related to PVP vehicle administration were identified in the liver, injection site, lymph nodes, kidney and brain on day 57. In general, the lesions seen were similar in character, frequency and severity to those described in animals on day 29. A reduction in the severity of injection site inflammation and the confinement of inflammation to only groups 2 (vehicle control) and 6 (18 mg kg^{-1}/dose) indicated a substantial recovery from the effects seen at day 29. The distribution of the lesions in the liver and lymph nodes was more wide-ranging and the severity slightly greater than that seen at day 29, consistent with a persistent effect of PVP. Alterations attributed to hIL-12 plasmid were not identified in day 57 animals.

There were no test article-related changes in organ weights noted at either the day 29 or day 57 (recovery) kill time points. However, thymus weight was slightly decreased on day 29 in those groups that received the largest volume of PVP vehicle (groups 2 and 6), but treatment-related microscopic alterations were not clearly evident in this organ.

Discussion

The biodistribution study of hIL-12 in CD-1 mice indicated that the plasmid DNA was cleared rapidly from peripheral organs, thus limiting any possibility of marked systemic toxicity related to the plasmid.

Administration of formulated hIL-12 plasmid twice a week for four weeks at a dose level of up to 18 mg kg^{-1}/dose to experimentally naïve Cynomolgus monkeys was well tolerated. No test-article effects were evident from evaluation of clinical signs, body weight, serum chemistry, haematology, coagulation, and urinalysis parameters, ECGs, ophthalmic indices, and organ weights.

Effects of the PVP vehicle included decreases in globulin and cholesterol, an increase in activated partial thromboplastin time, and a slight decrease in thymus weight. There were no clearly evident sex-specific differences in these changes.

Treatment-related gross findings at necropsy were not evident. The only possible contribution of formulated hIL-12 plasmid to any histologic alteration was identified at day 29 and was limited to Kupffer cell hyperplasia, hypertrophy, and cytoplasmic basophilic accumulation in the liver. However, similar number, size and morphologic alterations in other histiocytic cell populations were more clearly associated with the PVP vehicle (particularly with respect to Kupffer cell changes in animals killed on day 57). Thus, these alterations were attributed primarily to the PVP vehicle and the contribution of hIL-12 plasmid, if any, was considered minor. A number of additional histological lesions attributable to the PVP vehicle were identified. Injection site lesions included minimal to moderate inflammation of the subcutaneous tissue and accumulation of basophilic material in the cytoplasm of cutaneous phagocytic cells, and subcutaneous fibrosis. Vehicle-induced alterations identified in the lymph nodes included sinus histiocytosis and the accumulation of basophilic material in histiocytic or phagocytic cells. Histiocytic cell alterations were also identified in the kidney and brain. The histologic lesions seen at the recovery kill were generally similar in character, frequency and severity to those described for the terminal kill and may reflect persistence of the PVP vehicle.

In general, the histological changes induced by the vehicle seemed to reflect a clearance process, which, judging from the absence of degenerative changes in any of the affected organs, appeared to be relatively benign. The changes were generally dose-volume dependent and tended to be of minimal severity in the lower dose groups. The persistence of these PVP-related alterations in recovery animals was suggestive of a slow clearance process, which is not uncommon for material removed by the mononuclear phagocytic system. Similar PVP-related effects had been observed previously in an IFN-α gene medicine (INF-α plasmid formulated with 5% PVP) safety and toxicology study by the same overall study design (unpublished results).

Conclusion

The administration of formulated hIL-12 plasmid DNA twice a week at a dose level of up to 18 mg kg^{-1} in Cynomolgus monkeys was well tolerated. Effects of PVP vehicle were well apparent, and were consistent with the known, natural process of lysosomal destruction following ingestion of foreign materials. Dose levels used for this study were chosen to reflect the proposed doses to be administered in the phase I/II (3, 6, and 9 mg/dose) dose ranging study. The dose levels chosen for this study were 0.36, 1.2, 6.0 and 18 mg kg^{-1} given by subcutaneous injections twice per week for four weeks. These doses were 2.4-, 8-, 40-, and 120-times higher on a mg kg^{-1} basis than the highest dose (9 mg) proposed for use in the phase I/II clinical study, providing a large safety margin. On the basis of this preclinical toxicology study it is not anticipated that drug-related adverse events of a serious nature will occur in the clinical study.

This study suggested that human IL-12 plasmid DNA formulated in 5% PVP was a safe formulation potentially useful in the treatment and control of local tumours.

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