

4.5 OP-1 Immunogenicity Report

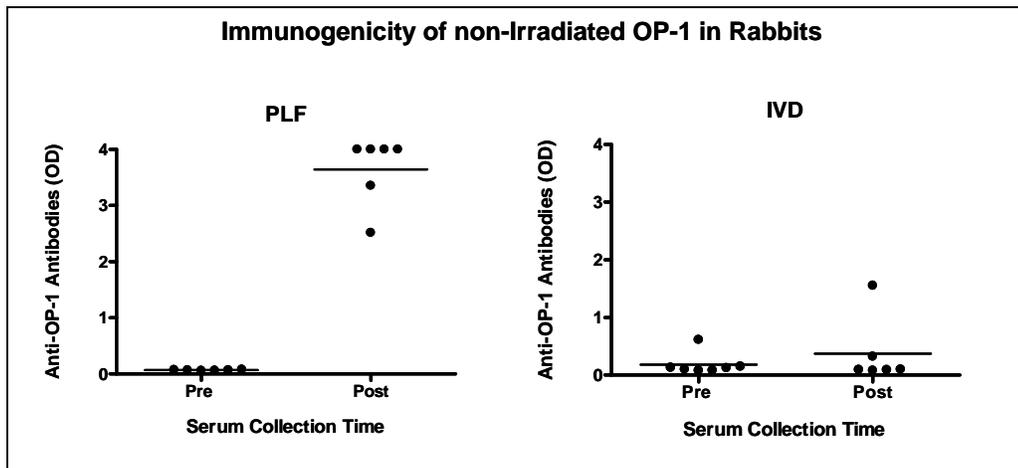
(Content from P060021 Minor Amendment dated February 2009)

OP-1 Immunogenicity

Early research in the BMP field demonstrated that it is essential for the BMP and the collagen matrix to be combined in order to induce bone formation (Sampath and Reddi 1981). In light of this basic research OP-1 and collagen are combined during the OP-1 Putty manufacturing process. Given the particulate nature of the combined product it is necessary to sterilize it with gamma irradiation. While it is hypothesized that sterilization by gamma irradiation is responsible for the differences in immunogenicity observed between OP-1 Putty spine fusion patients and OP-1 degenerative disc patients, there are several lines of evidence that should be considered before coming to this conclusion:

1. Preclinical studies demonstrate clear differences in OP-1 immunogenicity between PLF and IVD administrations:

- Non-irradiated OP-1 induces potent antibody responses in rabbit PLF (Study# 07-012; Memo# 07-043)
- Non-irradiated OP-1 does not induce an antibody response when injected into rabbit IVD (Study# 07-015; Memo# 08-033)



2. The intervertebral disc is an immune-privileged site

- The intervertebral disc is avascular and enclosed with fibrous tissue
- Gene therapy studies with persistent adenovirus demonstrate immune privilege in the IVD (Nishida K et al. 1998; Nishida K et al. 1999)
- Xenogeneic transplantation studies demonstrate immune privilege in the IVD (Wei et al. 2008)
- Fas Ligand mediated mechanism for immune privilege in the IVD demonstrated (Takeda et al. 2002). This mechanism is operative in other immune-privileged sites such as the retina and testes.

3. Preclinical studies demonstrate that the route of administration is an important contributing factor to OP-1 immunogenicity:

- Non-irradiated OP-1 was administered to a number of species using various routes of administration (see Table 1)

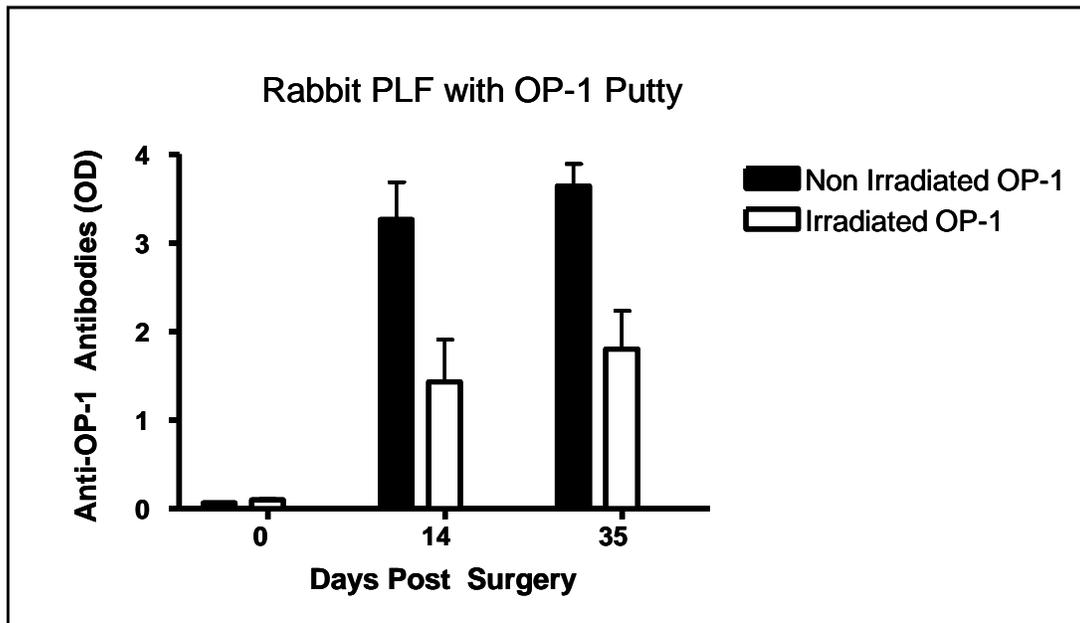
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- Immunogenicity was observed in PLF, intra-articular and subcutaneous delivery
- Immunogenicity was not observed in intervertebral disc and IV administrations

4. Preclinical studies with irradiated and non-irradiated OP-1 Putty in rabbit PLF demonstrate that both formulations induce antibody formulation

- Antibody titers higher in rabbits treated with non-irradiated OP-1 (Study #07-012/Memo# 07-043; Study #08-001/Memo# 08-041)
- Similar fusion results seen with irradiated and non-irradiated products
- Rabbit and human OP-1 amino acid sequences 98% conserved

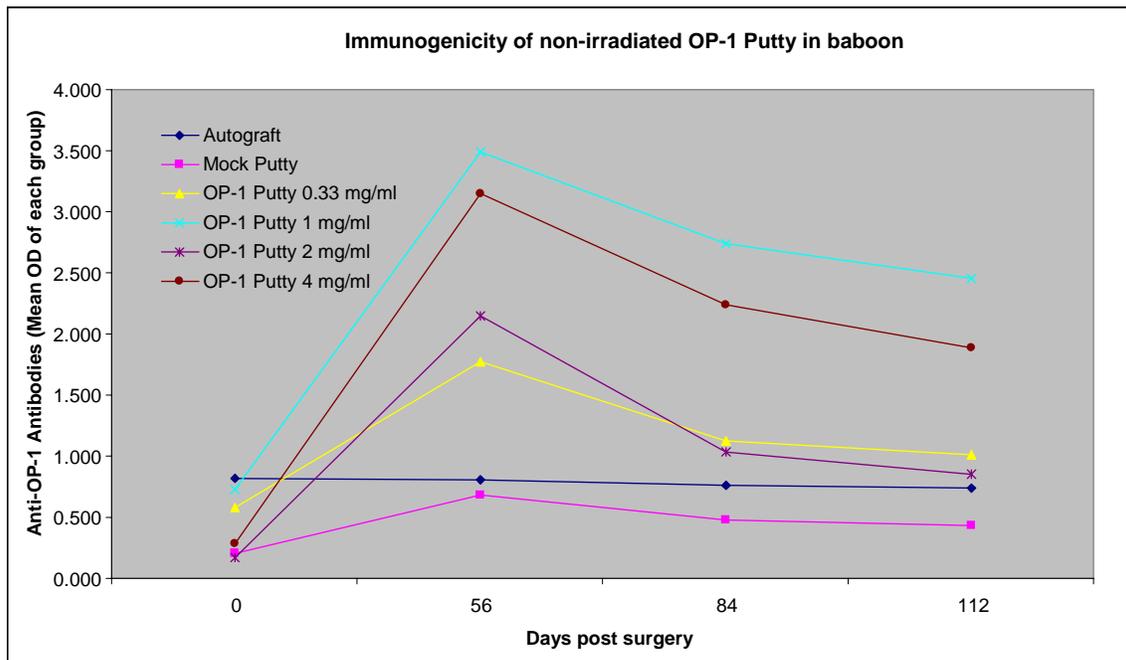


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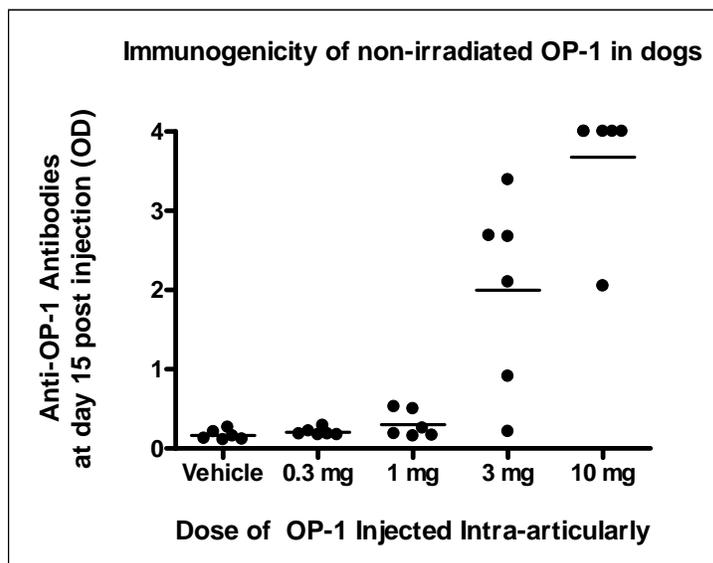
5. Studies in Baboon PLF with non-irradiated OP-1

- Baboons develop anti-OP-1 titers in the same range observed in human PLF patients (Study# 07-003; Memo# 08-016)
- Successful fusions seen in the presence of high antibody titers
- Baboon and human OP-1 amino acid sequences are identical



6. OP-1 Immunogenicity is dose-dependent

- Canine intra-articular dosing study demonstrates dose-dependence of immunogenicity (Study # 05013)
- Antibodies seen only at high doses (3mg, 10mg) but not at lower doses



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7. Clinical doses are different:

- 7 mg OP-1 administered in PLF patients
- 2 mg OP-1 administered into the intervertebral disc in DDD patients

8. Formulations are different

- OP-1 is administered in a complex with bovine collagen in OP-1 Putty
- Soluble OP-1 is administered in a buffered solution to the intervertebral disc

Conclusions

While it is theoretically possible that gamma irradiation of OP-1 is responsible for inducing immunogenicity, a number of preclinical studies with non-irradiated OP-1 suggest otherwise. Non-irradiated OP-1 induces a potent immune response when implanted with collagen in spine fusion studies, while delivery into the intervertebral disc does not. Other factors such as dose, formulation, route of administration, glycosylation, patient characteristics, etc. are potential causes (Mukovozov et al. 2008). Thus, the weight of evidence indicates that removing gamma-irradiation of OP-1 would not eliminate immunogenicity.

References

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Table1Summary of representative preclinical studies investigating the immunogenicity of non-irradiated OP-1 protein.

Route of Administration	Species	Study Nbr	Formulation	Dose	Immunogenicity Observed ^a	Comments	
PLF	Baboon	07-003	OP-1 Putty	0 ug	2/4	Non-irradiated OP-1 is immunogenic in baboon following implantation by PLF surgery	
				2000 ug	3/4		
				6000 ug	4/4		
				12000 ug	4/4		
				24000 ug	4/4		
	Rabbit	07-012	OP-1 Putty (sham, 1, 2, and 4 mg/mL)	0 mg	0/6	Non-irradiated OP-1 is immunogenic in rabbits following implantation by PLF surgery	
				3000 ug	6/6		
				6000 ug	5/6		
12000 ug				6/6			
IVD	Rabbit	04-023	Sham	0 mg/mL	0/12	No immunogenicity observed following IVD injection of non-irradiated OP-1 in rabbits.	
			37C BMP-7	0 mg/mL	0/12		
				10 ug	1/12 ^b		
				100 ug	0/12		
				200 ug	0/12		
	Rabbit	07-015	SF BMP-7	0 ug	0/6		
				20 ug	1/6 ^b		
				100 ug	0/6		
				200 ug	0/6		
				37C BMP-7	200 ug		1/6 ^b
Subcutaneous	Rabbit	04-011 ^e	OP-1 ^c	0 ug	1/5	Non-irradiated OP-1 is immunogenic following sub Q implantation in rabbits	
				1750 ug	10/10		
	Rat	T08-001	OP-1 Implant	0 ug	0/2	Dose dependent immunogenicity is observed following sub Q implantation of non-irradiated OP-1 in rats	
				0.1 ug	0/2		
				0.3 ug	0/2		
				1 ug	0/2		
				3 ug	0/2		
				10 ug	1/2		
				30 ug	2/2		
				100 ug	2/2		
	IA	Cyno	07-023	SF BMP-7	0 ug	0/10	Dose dependent immunogenicity is observed following intra-articular injection of non-irradiated OP-1 in cynomolgous monkeys and in dogs
					100 ug	2/5	
					300 ug	5/10	
37C BMP-7				2500 ug	8/10 ^f		
				300 ug	5/10		
Dog		05-013	37C BMP-7	0 ug	0/6		
				300 ug	1/6		
				1000 ug	2/6		
				3000 ug	5/6		
				10000 ug	6/6		
IV	Cyno	00-003	mature OP-1	0 ug	0/2	No significant immunogenicity observed following IV injection of non-irradiated OP-1 in cynomolgous monkeys	
				20 ug/day	0/2		
				300 ug/day	1/2		
				2000 ug/day	0/2		
	Cyno	07-022	SF BMP-7	0 ug	1/12 ^b		
				200 ug/day	0/7		
				600 ug/day	0/6		
				2000 ug/day	3/10 ^f		

^a Immunogenicity Observed = No. of animals with positive OD values / Total no. of animals^b Animals had positive OD values pre-dose and post-dose.^c OP-1 with Adjuvant (CFA and IFA for boost) was given 5 times^d OP-1 with Adjuvant (CFA and IFA for boost) was given 4 times^e Not all animal serum were run on ELISA; a subset of maternal serum was run.^f one animal had positive baseline values as well as post-dose.

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Table 2: Incidence of Anti-OP-1 Antibodies by Cohort

	Pre-dose	Week 2	Week 6	Week 12	Week 26	Week 52
Samples Tested	24	22	21	14	8	0
Sample Positives	2	1	2	0	0	0
Percent Positive	8.3	4.5	9.5	0.0	0.0	NA

Study reports available upon request