

I concur with this review memo. I Wu 3/31/20

**FOOD AND DRUG ADMINISTRATION  
Center for Biologics Evaluation and Research  
Office of Tissues and Advanced Therapies  
Division of Clinical Evaluation and Pharmacology/Toxicology  
Pharmacology/Toxicology Branch**

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BLA NUMBER:	STN #125700.000
DATE RECEIVED BY CBER:	September 3, 2019
DATE REVIEW COMPLETED:	January 19, 2020
PRODUCT:	ADSTILADRIN® (nadofaragene firadenovec-nrpl) suspension, for intravesical use
APPLICANT:	FKD Therapies Oy
PROPOSED INDICATION:	Treatment of high-grade, Bacillus Calmette-Guérin (BCG) unresponsive non-muscle invasive bladder cancer (NMIBC)
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**EXECUTIVE SUMMARY:**

Intravesical administration of rAd-IFN with the excipient Syn3 was evaluated in an orthotopic mouse model of human bladder cancer (b) (4). Anti-tumor activity was observed following intravesical administration of rAd-IFN/Syn3 at  $1 \times 10^{10}$  viral particles/mouse (rAd-IFN concentration of  $1 \times 10^{11}$  vp/ml in 1 mg/mL Syn3). A dose-dependent increase in IFN $\alpha$ 2b concentration was observed in urine and bladder tissue of healthy animals. Urine IFN $\alpha$ 2b concentration declined more rapidly following repeat administrations as compared to a single administration in healthy (b) (4) rats, with concentrations sustained longest for a dosing interval of 90 days compared to shorter intervals.

Safety was assessed in cynomolgus monkeys receiving intravesical administration of rAd-IFN/Syn3 at dose levels of  $2.5 \times 10^{11}$  vp/animal ( $1 \times 10^{11}$  vg rAd-IFN/mL in 1 mg/mL Syn3),  $1.25 \times 10^{13}$  vp/animal ( $5 \times 10^{11}$  vg rAd-IFN/mL in 1 mg/mL Syn3), Syn3 alone (1 mg/mL), or placebo control on Days 1 and 91, followed by a two month observation period in a GLP toxicology study. Histopathology changes in rAd-IFN/Syn3 and Syn3 groups were noted in the urethra, ureter, and urinary bladder at Days 8 and 98. Findings in the urethra and ureter included mononuclear cell infiltration, urothelial hyperplasia, cytoplasmic vacuolation, and were mostly mild in severity. Findings in the urinary bladder included mononuclear cell infiltration, inflammation, cytoplasmic vacuolation, urothelial hyperplasia, ulceration, and chronic inflammation, and were minimal to mild except for a few animals with moderate ulceration. Severity and incidence were increased at the higher rAd-IFN/Syn3 dose level. Most of the changes were trending towards resolution after the two month observation period following the second dose, with remaining findings of minimal cytoplasmic vacuolation and mononuclear cell infiltration observed in the urinary bladder and urethra, and minimal inflammation and fibrosis in the urinary bladder. Development of neutralizing antibodies to the adenovirus and anti-IFN $\alpha$ 2b antibodies occurred in the majority of animals in the high dose group and to a lesser extent in the low dose group, and antibody titers remained detectable throughout the entire observation period.

Biodistribution analysis indicated the presence of rAd-IFN DNA in the bladder on Days 8 and 98. Vector DNA was detected in the blood of most high dose animals and a few low dose animals within the 24-hour post-instillation period, and was not detectable at subsequent time points evaluated starting at Day 8. In the urine, animals in both groups had detectable vector DNA for 2-3 days following dosing. In several animals, lower levels of vector DNA were also detected in the liver, kidney and gonads. No rAd-IFN DNA was detected in the reproductive tissues at the end of the observation period. Human IFN $\alpha$ 2b was detected in serum and urine, and declined in most animals by Day 15 post-dose.

Safety pharmacology, pharmacokinetics, toxicology, and genotoxicity studies were also conducted for the excipient Syn3. Carcinogenicity and reproductive and developmental toxicity studies were not performed for rAd-IFN/Syn3. These studies are not warranted based on the product characteristics, results from the toxicology studies, and target patient population.

#### **PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:**

Based on review of the pharmacology and toxicology data presented in STN 125700/0, there are no outstanding requests for additional nonclinical data. The nonclinical data provided in this submission support the approval of this biologics license application.

#### **Formulation and Chemistry:**

ADSTILLADRIN is an adenovirus vector-based gene therapy product, comprised of rAd-IFN, a replication-deficient recombinant type 5 adenovirus (Ad5) vector expressing the interferon alfa-2b (IFN $\alpha$ 2b) transgene driven by the (b) (4) the excipient [N-(3-cholamidopropyl)-N-(3-lactobionamidopropyl)]-cholamide (Syn3). rAd-IFN (b) (4). Additional excipients include: citric acid monohydrate, sodium citrate dihydrate, polysorbate 80, hydroxypropyl-beta-

cyclodextrin, sodium dihydrogen phosphate dihydrate, tromethamine, sucrose, magnesium chloride hexahydrate, and glycerol.

ADSTILLADRIN is suspension supplied in a 20 mL extractable volume in 4 single-dose 30 mL vials for a single administration at  $3.0 \times 10^{11}$  vp/mL.

### Abbreviations

Ad5	adenovirus type 5
BCG	bacillus calmette-guerin
BD	biodistribution
BQL	below quantification limit
CNS	central nervous system
CPE	cytopathic effect
ECG	electrocardiogram
(b) (4)	
GLP	good laboratory practices
(b) (4)	
hIFN	human interferon
	(b) (4)
IFN $\alpha$ 2b	interferon alpha-2b
IHC	immunohistochemistry
IND	investigational new drug
IU	international units
IV	intravenous
MOI	multiplicities of infection
NMIBC	non-muscle invasive bladder cancer
NOAEL	no observed adverse effect level
PK	pharmacokinetics
(b) (4)	
PK	pharmacokinetics
QWBA	quantitative whole body autoradiography
rAd	recombinant adenovirus
ROA	route of administration
SNF	serum neutralizing factor
VEGF	vascular endothelial growth factor
WBA	whole body autoradiography

### Related File(s)

**IND #12547**; FKD Therapies Oy; Adenovirus Vector Expressing Interferon alpha-2b Gene (rAd-IFN, SCH 721015) with Syn3 (SCH 209702) for treatment of transitional cell carcinoma of the bladder; ACTIVE

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## INTRODUCTION

ADSTILLADRIN is a gene therapy product indicated for the treatment of patients with high grade Bacillus Calmette-Guerin (BCG) unresponsive NMIBC. It is comprised of an adenoviral vector, rAd-IFN, a replication-deficient recombinant Ad5 vector expressing the human IFN $\alpha$ 2b transgene (b) (4) . rAd-IFN (b) (4)

Recombinant IFN $\alpha$ 2b protein (Intron A®) is currently approved for several oncology indications including hairy cell leukemia, malignant melanoma, follicular lymphoma, and AIDS-Related Kaposi's Sarcoma. (b) (4)

The recommended dose level for ADSTILLADRIN is  $2.25 \times 10^{13}$  vp rAd-IFN/subject (b) (4) mg/mL Syn3 to be administered in a total volume of 75 mL (equivalent to a concentration of  $3.0 \times 10^{11}$  vp rAd-IFN/mL) by intravesical instillation every three months. Nonclinical studies were conducted to evaluate the bioactivity, biodistribution, and safety of rAd-IFN and rAd-IFN with Syn3 prior to administration to humans. In addition, the novel excipient Syn3 was tested for pharmacokinetics (PK) and safety.

## NONCLINICAL STUDIES

Note: In this review memo, the term rAd-IFN is used to denote the adenoviral vector, Syn3 is the excipient, and rAd-IFN/Syn3 (b) (4) . In the nonclinical study reports submitted in the BLA, rAd-IFN is used interchangeably with SCH 721015, Ad-IFN $\alpha$ 2b, and IACB, and Syn3 is used interchangeably with SCH 209702.

## PHARMACOLOGY STUDIES

### Summary List of Pharmacology Studies

#### Primary Pharmacology Studies

The following pharmacology studies were conducted to support the scientific rationale for the intravesical administration of rAd-IFN/Syn3 for human bladder cancer. These studies are reviewed under *Overview of Primary Pharmacology Studies*.

Study Number	Study Title / Publication Citation	Report Number
1	(b) (4)	
2		

### Supporting Pharmacology Studies

The following supporting pharmacology studies evaluated the effects of (b) (4) on IFN $\alpha$ 2b (b) (4) (Study #3), effects on (b) (4) in bladder cancer cells (Study #4), IFN $\alpha$ 2b expression in relation to dose levels, volumes, and dosing regimens (Studies #5-10), and antitumor activity of intravenously administered rAd-IFN (Studies #11-12). These studies are briefly summarized under *Overview of Supporting Pharmacology Studies*.

Study Number	Study Title / Publication Citation	Report Number
3	(b) (4)	
4		
5		
6		
7		
8		
9		
10		
11		
12		

7 pages have been determined to be not releasable: (b)(4)

**PHARMACOKINETIC STUDIES**

Biodistribution studies for rAd-IFN were conducted as part of the toxicology studies in cynomolgus monkeys (Toxicology Study #1, Report No. 03245) and rats (Toxicology Study #2, Report No. D47454) and are reviewed in the TOXICOLOGY STUDIES section of the memo. Pharmacokinetic studies for Syn3 are reviewed in the SYN3 STUDIES section of the memo.

**TOXICOLOGY STUDIES****Summary List of Toxicology Studies****Primary Toxicology Study:**

The following toxicology study was conducted to evaluate the safety of rAd-IFN/Syn3 and is reviewed under *Overview of Primary Toxicology Studies*.

Study Number	Study Title / Publication Citation	Report Number
1	Intravesical Toxicity and Toxicokinetic Study of SCH 721015 (rAd-IFN In SCH 209702) in Cynomolgus Monkeys with a Two Month Post-Dose Period	03245

**Supporting Toxicology Studies**

The following preliminary toxicology studies were conducted for rAd-IFN/Syn3 and are briefly summarized under *Overview of Supporting Toxicology Studies*.

Study Number	Study Title	Report Number
2	Pilot Toxicology and Biodistribution of rAd-IFN/ SCH 209702 in Rats	D47454
3	Pilot Experiments on the Intravesical Gene Therapy of SCH 721015 in Cynomolgus Monkeys	D47457

***Developmental and Reproductive Toxicology Studies:***

Developmental and Reproductive Toxicity studies were not conducted using rAd-IFN/Syn3 or Syn3 alone based on the transient rAd-IFN DNA levels detected in the gonads in the biodistribution analysis of rAd-IFN/Syn3, as well as the transient (b) (4) detected in the gonads from whole body (b) (4).

***Carcinogenicity/Tumorigenicity Studies:***

Carcinogenicity of rAd-IFN/Syn3 was not conducted given the target patient population, existing data that have not reported development of cancers in human subjects administered type 5 adenoviral viruses, and the limited systemic exposure to Syn3.



Overview of Primary Toxicology Study**Study #1**

<b>Report Number</b>	03245			
<b>Date Report Signed</b>	January 12, 2006			
<b>Title</b>	Intravesical Toxicity and Toxicokinetic Study of SCH 721015 (Rad-IFN In SCH 209702) in Cynomolgus Monkeys with a Two Month Post-Dose Period			
<b>GLP Status</b>	Yes			
<b>Testing Facility</b>	(b) (4)			
<b>Objective(s)</b>	<ul style="list-style-type: none"> <li>- Assess the systemic toxicity of rAd-IFN and Syn3 following repeat intravesical administration to cynomolgus monkeys on study Days 1 and 91;</li> <li>- Assess the toxicokinetics of rAd-IFN and Syn3;</li> <li>- Evaluate recovery from and/or persistence of any effects over a period of two months following the last administration (recovery).</li> </ul>			
<b>Study Animals</b>	<b>Strain/Breed</b>	Cynomolgus monkey ( <i>Macaca fascicularis</i> ), supplied by (b) (4)		
	<b>Species</b>	Non-human primates		
	<b>Age</b>	Males - 2.6 to 5.9 years old; Females - 2.8 to 6.1 years old		
	<b>Body Weight</b>	Males - 2.4 to 5.0 kg; Females - 2.0 to 2.7 kg		
	<b>#/sex/group</b>	3-5 monkeys/sex/group		
	<b>Total #</b>	60 monkeys		
<b>Test Article(s)</b>	rAd-IFN (Batch No. (b) (4)) mg/mL Syn3 (Batch No. (b) (4))			
<b>Control Article(s)</b>	SCH 528679 placebo*, Lot No. (b) (4) mg/mL Syn3 (Batch No. (b) (4)); Syn3 excipient control			
<b>Route of Administration</b>	Intravesical administration			
<b>Description of the Administration Procedure</b>	<p>All study animals were fitted with a (b) (4) (b) (4) Catheter to facilitate filling and voiding of the bladder. Monkeys were anesthetized with IV injection of 2.5 mg Propofol via an infusion catheter, followed by a continuous infusion of Propofol at a rate of 0.4-1.0 mg/kg/min for the duration of the dosing procedure. The bladder was flushed and voided using warm PBS. Monkeys were then administered control or test material by intravesicular administration in a volume of 25 mL (Note: the 25 mL volume capacity in a monkey bladder is equivalent to a 75 mL volume capacity in a human bladder). The instilled dosing formulation was left in the bladder for 60 minutes (dwell time). After 60 minutes from the end of the bladder fill time, the instillation material was voided, collected, and the collection volume was determined.</p>			
<b>Study Groups and Dose Levels</b>	<b>Group</b>	<b>rAd-IFN (vp/animal)</b>	<b>Syn3 (mg/animal)</b>	<b>Volume (mL)</b>
	C1 – Placebo control	0	0	25
	C2 – Syn3 control	0	25	25
	T1 – Low dose	2.5x10 <sup>11</sup>	25	25
	T2 – High dose	1.25x10 <sup>13</sup>	25	25
<b>Note:</b> rAd-IFN/Syn3 concentrations administered were 1x10 <sup>11</sup> vg/mL for T1 and 5x10 <sup>11</sup> vg/mL for T2				

<b>Dosing Regimen</b>	Single administration groups – Day 1 Repeat administration groups – Day 1 and Day 91			
<b>Randomization</b>	Yes			
<b>Description of Masking</b>	Not described			
<b>Scheduled Sacrifice Time Points</b>	Single administration groups – Day 8 Repeat administration groups – Day 98 and Day 148			
	<b>Group</b>	<b>Single Dose Day 8</b>	<b>Repeat Dose Day 98</b>	<b>Repeat Dose Day 148</b>
	C1 – Placebo control	3M/3F	3M/3F	--
	C2 – Syn3 control	3M/3F	3M/3F	2M/2F
	T1 – Low dose	3M/3F	3M/3F	2M/2F
	T2 – High dose	3M/3F	3M/3F	2M/2F

Note: According to IND #12547.039, the composition of the rAd-IFN (b) (4)

*Key Assessments and Evaluations:*

- Clinical observations: Twice daily
- Body weights: Weekly
- Ophthalmoscopic examinations: Days 5 and 95
- General veterinary examinations: Days 4 and 94
- ECG: Day 5, Week 12 and Day 95
- Hematology: Days 5 and 15, during Week 12, and on Days 95, 105 and 148
- Coagulation: Days 5 and 15, during Week 12 and on Days 95, 105 and 148
- Serum chemistry: Days 5 and 15, during Week 12 and on Days 95, 105 and 148
- Urinalysis/urine chemistry: Days 5 and 15, during Weeks 7 and 12, and on Days 95, 105 and 148
- Organ weights: Upon sacrifice
- Macroscopic observations: Upon sacrifice
- Microscopic observations: Upon sacrifice<sup>3</sup>
- Anti-adenovirus antibodies: Days 15, 22, 36, during Week 12, and on Days 98, 105, 112, 126 and 148
- Antibodies against IFN- $\alpha$ 2b: Days 15, 22, 36, during Week 12, and on Days 98, 105, 112, 126 and 148
- IFN $\alpha$ 2b protein in serum: During Week 12 and on Day 91 (0.5, 1, 2, 3, 4, 6 and 24 hours after dwell period), and on Day 98
- IFN $\alpha$ 2b protein in urine: Days 1, 2, 3, 4 and 15, during Week 12 and on Days 91, 92, 93, 94 and 105

<sup>3</sup> A comprehensive list of tissues was examined microscopically for all groups at scheduled necropsy except for Group T1 and the necropsies on Day 148. For Group T1, only tissues with gross findings were examined microscopically. For samples from Day 148, only bladders, target organs and gross findings were examined.

- Toxicokinetics of Syn3: Week 12 and on Day 91 (0.5, 1, 2, 3, 4, 6 and 24 hours after dwell period), and on Day 98
- Biodistribution analysis for rAd-IFN-specific DNA: The following samples were analyzed by (b) (4): urinary bladder, kidney, liver, and gonads from Groups C1 and T2 for Day 8 and T2 for Day 148.
- (b) (4) analysis for rAd-IFN-specific DNA in urine: Days 1, 2, 3, 4 and 15, during Week 12, and on Days 91, 92, 93, 94 and 105

### *Key Results:*

Clinical Observations: There was no test article-related or incidental mortality. There were no clinical observations that were attributed to the administration of test articles or controls.

Body Weights: There were no test article-related effects on body weights.

Ophthalmoscopic Examinations: There were no test article-related ophthalmoscopic findings.

General Veterinary Examinations: There were no test article-related physical examination findings.

ECG: There were no test article-related ECG findings.

Hematology: There were no test article-related effects on hematology parameters.

Coagulation: There were no test article-related effects on coagulation parameters.

Serum Chemistry: There were no test article-related effects on the standard serum chemistry parameters.

Urinalysis/Urine Chemistry: No test article-related effects on urinalysis or urine chemistry parameters were noted.

Organ Weights: There were no test article-related effects on organ weights.

### Macroscopic Observations:

- At Day 8, test article findings were limited to the urinary bladder in the C2, T1 and T2 groups. There was red discoloration/foci of the urinary bladder in one C2 male (Syn3 control), one T1 male (low dose rAd-IFN/Syn3) and one T2 male (high dose rAd-IFN/Syn3). The same macroscopic changes were found in most females in the C2 and T2 dose groups and in one female in the T1 group. Transmural red foci were found in one male in the T1 group and one female in the T2 group. There was an increased bladder thickness in one female in each of the C2 and T2 groups and in one male in each of the T1 and T2 groups.
- At Day 98, there was focal discoloration of the urinary bladder in most males in the C2 group, one female in the T1 group and in all males in the T2 group.

- At Day 148, there were no test article-related findings observed during necropsy.

#### Microscopic Observations:

- At all scheduled necropsies, test article-related findings were primarily in the urinary bladder with fewer, less severe findings in the urethra and the ureters.
- Microscopic findings at Day 8:
  - Urethra – 1) minimal mononuclear cell infiltration in the lamina propria and/or submucosa in at least two monkeys each in the C2, T1 and T2 groups; 2) urothelial hyperplasia and urothelial mononuclear infiltration both present in one female in the C2 group.
  - Urinary bladder – 1) minimal to mild submucosal mononuclear cell inflammation in all monkeys in the C2, T1 and T2 groups, with slightly greater severity (i.e., mild) in the T2 group, suggesting a dose response; 2) focal/multifocal ulceration (mild/moderate) in at least one monkey in all groups exposed to Syn3 (C2, T1, T2). The inflammation and ulceration correlated with the macroscopic findings in the urinary bladder at the interim sacrifice.
- Microscopic findings at Day 98:
  - Urethra – 1) minimal cytoplasmic vacuolation in one male in the T2 group; 2) mononuclear cell infiltration in one male in each of the groups exposed to Syn3; 3) minimal or mild mixed inflammatory cell infiltration in 2/3 males in the C2 group and one male in the T2 group; 4) mild mononuclear cell infiltration of the lamina propria/ submucosa in one female of the T2 group.
  - Ureter at the opening into the urinary bladder – minimal cytoplasmic vacuolation in the urothelium of the ureter and minimal mononuclear cell infiltration of the lamina propria both present in one male in the T1 group.
  - Urinary bladder – 1) inflammation was generally similar in incidence and/or severity to the Day 8 findings; 2) mild focal/multifocal ulceration in 1-2 monkeys in the T1 and T2 groups, and one T2 monkey of moderate severity, but not in any C2 monkeys; 3) minimal or mild urothelial hyperplasia in one male in the C2 group, 3/6 monkeys in the T1 group and in 5/6 monkeys in the T2 group; 4) minimal to mild cytoplasmic-vacuolation in one monkey in each of the C2 and T1 groups and in 4/6 monkeys in the T2 group; 5) minimal mononuclear cell infiltration of the urothelium in 4/6 monkeys each in the T1 and T2 groups and in one female in the C2 group; 6) minimal to moderate chronic inflammation of the muscularis in all males of the T2 group.

#### **Reviewer Comments:**

- *For the animals sacrificed on Day 98, there was a slight increase in incidence/severity in the microscopic findings for the urinary bladder as the dose level of rAd-IFN increased, suggesting a dose-response relationship for the findings observed.*
- *There was a slight increase in the severity of inflammation and/or mixed inflammatory cell infiltration in the T1 and T2 groups compared to the C2 group for the Day 98 sacrifice.*

- Microscopic findings at Day 148:
  - Urethra – 1) minimal mononuclear cell infiltration of the urethral urothelium was found in one monkey in each of the C2, T1 and T2 dose groups; 2) minimal mononuclear cell infiltration of the lamina propria/submucosa was found in all males in the T2 group;
  - Urinary bladder - 1) minimal urothelial cytoplasmic vacuolation in one monkey in each of the C2 and T1 groups and in two monkeys in the T2 group; 2) minimal mononuclear cell infiltration in one female in the T1 group and two females in the T2 group; 3) minimal submucosal mononuclear cell inflammation in one male in the T2 group; 4) minimal fibrosis in the lamina propria of one male of the T1 group and one female of the T2 group.

#### **Reviewer Comments:**

- *The study report states that the reversal of findings was nearly complete in the Day 148 group with residual minimal findings primarily in the urothelium of the urethra and urinary bladder.*
- *The microscopic finding of 'cytoplasmic vacuolation' in the urothelium was a new finding noted at the Day 98 and Day 148 sacrifices and is potentially reflective of the 'clean-up process' of the cells over time.*
- *The sponsor cited an incidental finding of multifocal mineralization of the 'umbrella-type' cells of the urothelium lining the lumen of the urinary bladder in 1/2 Group C2 males. This finding appears to be incidental.*

#### Assessment of Anti-Adenovirus Antibodies:

- Results from a (b) (4) assay using serum samples showed detectable (b) (4) activity at all post-dose time points evaluated (Day 15 through Day 148) at a range of  $2.5 \times 10^7$  to  $3.3 \times 10^{10}$  vp (b) (4) serum. The majority of the monkeys in the high-dose group (T2) had quantifiable levels of (b) (4) to rAd-IFN after the first dose, whereas half of the monkeys in the low-dose group (T1) had quantifiable levels. All monkeys in both groups had quantifiable levels after the second dose.
- Increased anti-adenovirus binding antibodies were detected by (b) (4) following the first dose for both T1 and T2, with higher average (b) (4) in Group T2 at every time point from Day 15 through Day 98 and similar (b) (4) at subsequent time points. (b) (4) for T2 were reached on Day 36 (b) (4) and the (b) (4) for T1 were reached at Day 98 (b) (4).

#### Assessment of Antibodies against IFN $\alpha$ 2b:

- The majority of the monkeys in T2 developed antibodies against IFN $\alpha$ 2b following a single dose compared to about half of the monkeys in the T1 group based on a (b) (4). Some monkeys that were negative after a single dose became positive after a second dose. The antibody response after the second dose was more consistent and sustained in monkeys in T2 compared to T1.

Interferon Protein Assessment in Serum:

- Human IFN $\alpha$ 2b was detected in the serum using an (b) (4) assay in all monkeys in T2 and in 9/16 monkeys in T1 starting on Day 2. Maximum (b) (4) were detected on Day 2 for T2 and T1, respectively. Maximum (b) (4) declined to (b) (4) by Day 4 for T2 and T1, respectively.
- After the second dose, human IFN $\alpha$ 2b was detected in the serum in about half of T1 and T2 monkeys. One monkey in T2 had a (b) (4) on Day 92, however the next highest was (b) (4) for T2 animals. The maximum serum level in the low-dose group was (b) (4) on Day 92. All monkeys had serum levels near or below background on Day 98.
- The (b) (4) of the IFN $\alpha$ 2b as measured by a (b) (4) assay generally correlated with levels of IFN $\alpha$ 2b detected.

Interferon Protein Expression in Urine:

- Human IFN $\alpha$  (b) (4) was detected in urine by (b) (4). Increased IFN $\alpha$ 2b was detected in the urine of 11/16 animals in T1 with levels between (b) (4) at Day 2. All 16 animals in the T2 group had detectable IFN $\alpha$ 2b above background levels at (b) (4) on Day 2.
- The average (b) (4) value was (b) (4) for T1 and 238,313 pg/mL for T2 on Day 2.
- IFN $\alpha$ 2b (b) (4) was detected in urine on Days 1 through 4 with (b) (4) declining after Day 2. In a few samples, IFN $\alpha$ 2b was detected at 15 days at lower (b) (4), and was detected in only one T1 sample at Week 12.
- Following repeat administration, 5/10 monkeys in T1 had measurable amounts of IFN $\alpha$ 2b on Days 92-94, with (b) (4); 9/10 monkeys in T2 group had IFN $\alpha$ 2b (b) (4). By Day 105, no measurable levels of IFN $\alpha$ 2b were detected in any samples.

Toxicokinetics of Syn3:

- (b) (4)

Biodistribution Analysis for rAd-IFN-specific DNA:

- *Blood:* vector DNA was detected in the blood in the 0.5-24 hour period following instillation in 11/16 animals in T2 (range of  $1 \times 10^4$  –  $3.41 \times 10^6$  copies/mL blood) and 2/16 animals in T1 (range of  $1.08 \times 10^4$  –  $4.80 \times 10^4$  copies/mL blood). Similar levels were

detected in the 24 hour period following the second dose. The DNA levels in the blood for the majority of study animals were below quantifiable levels for the remaining intervals (Days 8, 15, 98 and 148).

- *Bladder*: vector DNA was detected in the bladder tissue for all monkeys in T1 and T2 on Days 8 and 98 ( $4 \times 10^4$  to  $9 \times 10^5$  copies/ $\mu$ g gDNA in T2 and  $2 \times 10^2$  to  $4 \times 10^4$  copies/ $\mu$ g gDNA in T1). The DNA copies were below quantifiable levels in the bladder tissue of T2 animals on Day 148. However, vector DNA was detected in the bladder of 2/4 monkeys of the T1 group on Day 148 ( $7 \times 10^2$  –  $1 \times 10^5$  copies/ $\mu$ g gDNA).
- *Liver, kidneys, gonads*: vector DNA was detected in a limited number of monkeys in the liver, kidney and gonads at Days 8 and 98. No vector DNA was detected on Day 148.
  - o In group T1, vector DNA was detected in the kidney of one monkey (905 copies/ $\mu$ g gDNA, Day 148) and the liver of two monkeys (24.55 to 174 copies DNA/ $\mu$ g gDNA, Day 8).
  - o In group T2, vector DNA was detected in the liver (3054.58 to 8761.53 copies DNA/ $\mu$ g gDNA in 2/6 monkeys on Day 8, 29.32 to 1547.66 in 3/6 monkeys on Day 98), testis (11.4 in 1/3 monkeys on Day 8), ovary ( $1.15 \times 10^4$  in 1/3 monkeys on Day 8), and kidney (119.98 to 2515.08 in 2/6 monkeys on Day 8, and 34.9 in 1/6 monkeys on Day 98).
    - The monkey with the highest DNA levels in the kidney (2515 copies/ $\mu$ g gDNA) also had the highest whole blood and liver DNA levels after a single dose ( $3.41 \times 10^6$  and 8762 copies, respectively). This monkey also had DNA in the ovary on Day 8 ( $1.15 \times 10^4$  copies/ $\mu$ g gDNA).
    - The DNA level in the kidneys of the other monkeys was considerably lower ( $\leq 120$  copies) and did not clearly correlate to systemic DNA levels. The potential for retrograde exposure from the bladder (site of dose administration) to the kidney could not be ruled out.
    - One male that was positive for the DNA levels in the testis on Day 8 also had the second highest whole blood and liver DNA levels after a single dose ( $2.08 \times 10^6$  and 3055 copies/ $\mu$ g gDNA, respectively).
    - The DNA level in the liver of another monkey was 1548 copies on Day 98. This monkey had the highest whole blood DNA levels ( $6.34 \times 10^6$  copies/ $\mu$ g gDNA) after the second dose.
    - Analysis of the testis and ovary was not conducted at Day 98 but was done at Day 148 and no vector DNA levels were detected in these tissues.
- *Urine*: The majority of monkeys in T1 and T2 had detectable vector DNA in the urine on Days 1 and 91 (dosing days), and remained positive for the next two to three days. There were a few monkeys that were positive on Days 15 and 105 and none of the monkeys were positive on Day 84.

#### Reviewer Comment:

- *The dose levels evaluated in this study  $2.5 \times 10^{11}$  vp/animal ( $1 \times 10^{11}$  vp/mL) and  $1.25 \times 10^{13}$  vp/animal ( $5 \times 10^{11}$  vp/mL) extrapolate to dose levels of  $7.5 \times 10^{12}$  vp/subject and  $3.75 \times 10^{13}$*

*vp/subject for humans using the instillation volumes in monkeys (25 mL) and humans (75mL). The rAd-IFN dose levels evaluated bracket the recommended clinical dose level of  $2.25 \times 10^{13}$  vp/subject (concentration of  $3 \times 10^{11}$  vp/mL).*

- *The resulting data demonstrated no unscheduled deaths, adverse findings in clinical observations, body weight changes, ophthalmic examinations, electrocardiograms, or clinical pathology.*
- *Histopathology findings include mononuclear cell infiltration in the urethra, urothelial hyperplasia, submucosal mononuclear cell inflammation and multifocal ulceration in the urinary bladder. These findings were mostly reversed after the 2-month recovery period.*
- *Biodistribution data demonstrate the detection of rAd-IFN DNA in the kidney and liver of several animals, indicating systemic exposure and a potential for retrograde distribution from the bladder to the kidney. Vector DNA was detected in the ovary and testis in a few animals in the high dose group on Day 8 post-dose and were not detected at the Day 148 sacrifice.*
- *There were no rAd-IFN/Syn3 related effects on urinalysis or urine chemistry parameters.*
- *Antibody titers to rAd and IFN $\alpha$ 2b were detected after the first administration, and remained measurable throughout the study duration.*

#### Overview of Supporting Toxicology Studies

##### ***Study #2. Pilot Toxicology and Biodistribution of rAd-IFN/ SCH 209702 in Rats (Report No. D47454, corresponding to Study Number 03-509)***

**Date Report Signed:** May 21, 2005

**Testing Facility:** (b) (4)

**Objective:** To evaluate toxicity and biodistribution of the rAd vector in response to intravesical administration of rAd-IFN

**Study Animals:** Female (b) (4) rats, adult, body weight 200-250 g

**Test Article(s):** rAd-IFN/Syn3, Lot (b) (4),  $8.24 \times 10^{11}$  P/mL; (b) (4)/Syn3 (Lot # (b) (4),  $1.1 \times 10^{12}$  P/mL); (b) (4) (Lot (b) (4),  $1 \times 10^7$  IU/5mL)

**Note:** (b) (4) uses the same vector (b) (4) as rAd-IFN but expresses (b) (4), whereas the human IFN $\alpha$ 2b (b) (4)

**Control Article(s):** Syn3 (Lot # (b) (4))



**Route of Administration:** Single intravesical administration  
**Study Groups and Dose Levels:**

Group	Vector dose (vp/animal)	Syn3 (mg/animal)	(b) (4) (IU/animal)	Volume (mL/animal)	Day 4	Day 14
1 – Untreated	0	0	0	0	n = 1	n = 1
2 – Syn3 control	0	0.5	0	0.5	n = 1	n = 2
3 – rAd-IFN/Syn3	5x10 <sup>10</sup>	0.5	0	0.5	n = 3	n = 3
4 – (b) (4)/Syn3	5x10 <sup>10</sup>	0.5	0	0.5	n = 3	n = 3
5 – (b) (4)	0	0	1x10 <sup>6</sup>	0.5	n = 2	n = 1

*Key Evaluations and Assessments:*

- Urine samples were collected from each animal at 0-4 hours (Group 5), or 2 days post administration (Groups 3 and 4) to determine the human IFN levels by (b) (4) with a below quantification limit (BQL) of (b) (4)
- Animals were sacrificed on Days 4 and 14 post administration, and blood samples were collected by heart puncture into (b) (4). Whole blood samples were used for (b) (4) analysis for rAd-IFN DNA. Blood plasma was used for serum chemistry analysis.
- Tissues from the bladder, liver and kidney were collected for histopathology
- Blood samples, liver, and kidney tissues were prepared for (b) (4) analysis

*Key Results:*

- In Group 5 (b) (4) IFN $\alpha$ 2b concentration in the urine dropped >3-4 logs at 4 hours post-dose and was at baseline two days post-dose.
- In Group 3 (rAd-IFN/Syn3), urine IFN $\alpha$ 2b concentrations were between 2,988 to 34,244 pg/mL at Day 2 post-dose as compared to levels below the quantitation limit for the other groups.
- There were no significant differences in clinical chemistry among the study groups. There were no significant differences in hematology among Groups 1, 2, 3, and 5. Platelet counts were slightly lower in Group 4 (b) (4)/Syn3) as compared to the controls.
- There were no significant differences in histopathologic findings in the liver and kidney samples among the study groups. Inflammatory lesions in the bladder were observed in all study groups.
- Viral DNA copy numbers were not detected in any blood and liver samples at 4 and 14 days post-dose. DNA levels were detected in the kidney samples (1.1 x and 2.9 x 10<sup>2</sup> copies/mg, in 2/9 samples), indicating possible retrograde distribution during intravesical administration.

***Study #3. Pilot Experiments On The Intravesical Gene Therapy Of SCH 721015 In Cynomolgus Monkeys (Report No. D47457, corresponding to Study Number 03-590)***

**Date Report Signed:** May 21, 2005

**Testing Facility:** (b) (4).

**Objectives:** To evaluate: 1) the volume required to fill the monkey bladder facilitating adenovirus attachment to the entire urothelium; 2) Syn3 mediated enhancement of transgene expression following intravesical instillation of rAd-IFN; and 3) potential adverse local and systemic effects

**Study Animals:** Three female cynomolgus monkeys (b) (4)

(b) (4), 2-6 years old, body weight 2-5 kg, experimentally naive

**Test Article:**  $1 \times 10^{11}$  vp/mL rAd-IFN in 1 mg/mL Syn3 or in vehicle

**Route of Administration:** Intravesical administration.

#### *Methods:*

To test for instillation volume, 25 mL was determined to completely fill the bladder without overdistension of the urothelium. Animal # (b) (4) received 25 mL of warm PBS into the bladder using the (b) (4) catheter-based procedure and the volume was retained in the bladder for one hour with minimal leakage, indicating the catheter and procedure was appropriate.

Monkey (b) (4) received  $2.5 \times 10^{12}$  vp/animal rAd-IFN/Syn3 in 1mg/mL Syn3 and monkey (b) (4) received the same dose level of rAd-IFN in vehicle in a total volume of 25 mL. The administered dose was retained in the bladder for 60 minutes. Then, 2 mL of the instillation material was collected for transgene expression analysis before animals were allowed to void. The administration procedure was repeated on Days 1, 30, 62 and 90. Animal (b) (4) received  $2.5 \times 10^{12}$  vp/animal rAd-IFN/Syn3 at Day 90 only.

#### *Key Evaluations and Assessments*

- Immediately after each administration, animals were evaluated for bladder size, reduction in urine volume, body weight losses, or other signs of discomfort, as well as transgene expression.
- In-life parameters included daily clinical observations, body weight (baseline and weekly thereafter), clinical pathology (baseline and at sacrifice), blood and urine samples for rAd-DNA levels by (b) (4). Blood collection was conducted for baseline, immediately following each dosing, and on Day 98. Urine collection was conducted for seven consecutive days starting 24 hours following administration and on Days 14 and 21 post-dose.
- (b) (4) were assayed at baseline, immediately following dosing, and prior to sacrifice.
- Animals were sacrificed on Day 98, and histopathology analyzed on lung, liver and kidney samples.

#### *Key Results:*

- Recovery of rAd-IFN from instillation material following administrations on Day 62 was  $4.53$  to  $7.71 \times 10^{10}$  vp/mL in two monkeys, and following Day 90 dosing recovery was  $4.55$  to  $9.23 \times 10^{10}$  vp/mL in three monkeys.

- There was no reduction in urine volumes observed in the study monkeys.
- After the initial dose, Monkey (b) (4) which received rAd-IFN/Syn3 showed significantly increased levels of IFN $\alpha$ 2b in urine (e.g. 50,000 pg/mL gradually declining to >1000 pg/mL during Days 1-5 post-dose) as compared to the baseline levels (about 200 pg/mL) and IFN $\alpha$ 2b levels in Monkey (b) (4) (about 200 pg/mL) that received rAd-IFN in vehicle. However, IFN $\alpha$ 2b concentrations in the urine decreased after a second dose and were undetected after a third dose at all time points evaluated.
- Following repeat administration, there were no adverse findings or clinical signs, changes in food consumption, or reduction in urine volumes.
- (b) (4) were increased in all the three monkeys throughout the study duration.
- In Monkeys (b) (4), viral DNA was not detected in the blood at 60 minutes after the first dose (only time point tested), while increased IFN $\alpha$ 2b levels (363-618 pg/mL) were detected in serum on Days 1 and 2 post-dose, indicating a possibility of absorption of IFN $\alpha$ 2b from the bladder to serum. Serum hIFN $\alpha$ 2b levels were not detected after Day 2. In Monkey (b) (4), low viral DNA level (8.75 copies in 0.2 mL) were found in the blood at 60 minutes post-dose after single administration, and serum IFN $\alpha$ 2b was detected at 7 days post-dose, which indicates systemic exposure to the virus.
- Viral DNA levels were detected in the voided urine within the first day post-dose only.
- There were no abnormal findings in clinical pathology analysis.
- There were no macroscopic findings or histopathology findings in the liver, lung and kidneys. However, acute cystitis was found in the urothelium of all three animals, which was more severe in animals receiving four intravesical administrations than single administration.





**SYN3 STUDIES****Summary List of Studies for Syn3**

Study Number	Study Title / Publication Citation	Report Number
Safety Pharmacology		
1	(b) (4)	
2		
3		
4		
Pharmacokinetics		
5	(b) (4)	
6		
7		
Toxicology		
8*	(b) (4)	
9*		
10		
11		
12		
13		
14		
15		
Genotoxicity		
16	(b) (4)	
17		
18		
Other Studies		
19	(b) (4)	

\*Studies #8 and #9 are not reviewed in this memo due to the preliminary nature of the studies.


Overview of Safety Pharmacology Studies of Syn3

(b) (4)




Overview of Pharmacokinetics Studies of Syn3

(b) (4)




(b) (4)

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Overview of Toxicology Studies of Syn3

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Overview of Genotoxicity Studies of Syn3:

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Overview of Other Safety/Toxicology Studies of Syn3:

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**OTHER STUDIES****Studies for Other Impurities**

Study Number	Study Title	Report Number
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(b) (4)

(b) (4)

**Analytical Methods and Validation Reports**

Study Number	Study Title	Report Number
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(b) (4)

**Note:** Study No. 7 was reviewed by CMC. The remaining studies in the table are not summarized in this review memo, as they are primarily assay development reports for analytical testing methods for blood, tissues, and urine samples from the nonclinical studies. These assays are acceptable for the respective measurements.

#### **APPLICANT'S PROPOSED LABEL**

Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14)<sup>5</sup>.

Section 13 ('Nonclinical Toxicology') should be revised to reflect the toxicology study data (Section 13.2; 'Animal Toxicology and/or Pharmacology').

#### **CONCLUSION OF NONCLINICAL STUDIES**

Review of the nonclinical studies did not identify any safety concerns that could not be adequately addressed in labeling (see above recommendations regarding the label). The nonclinical data support approval of the license application.

#### **KEY WORDS/TERMS**

Gene therapy, ADSTILADRIN®, bladder cancer, type 5 adenovirus, Ad5, interferon alfa 2b, mice, rats, monkeys, Syn3, anti-tumor activity, human IFN protein, pharmacokinetic, biodistribution, single dose toxicity, repeat dose toxicity.

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<sup>5</sup> Pregnancy and Lactation Rule (PLLR), at:

<http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/actsrulesregulations/ucm445102.htm>.