Phase I Dose Escalation Trial of Intratumoral Injection with Oncolytic Adenovirus Vector INGN 007 (VRX-007) in Patients with Advanced Solid Tumors

Principal Investigator: John Nemunaitis, M.D.

Presenters:

William Wold, Ph.D. (Preclinical Principal Investigator)

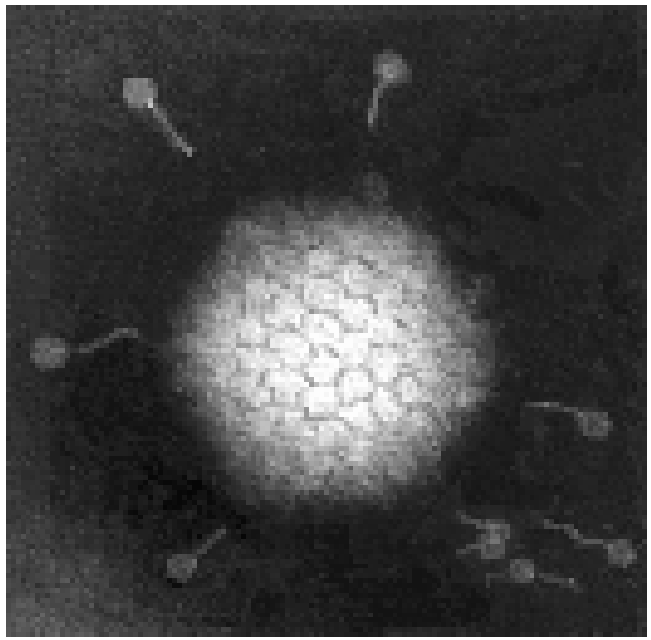
Neil Senzer, M.D. (Clinical Sub-Investigator)

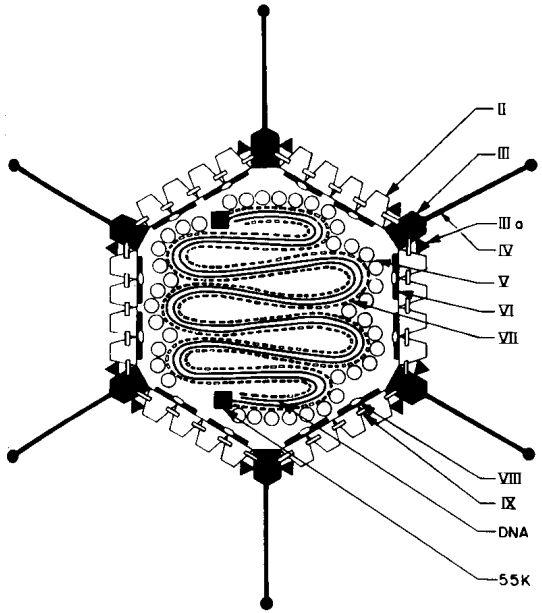
Sponsor: Introgen Therapeutics, Inc.

Overall Summary

- **INGN 007, which overexpresses ADP, has greater oncolytic activity than other replicating adenovirus vectors**
- **Syrian hamster is an appropriate permissive immunocompetent model for safety studies with INGN 007**
- **Preclinical data suggest that INGN 007 will not be more transmissible than wild-type Ad5**
- **INGN 007 is well-tolerated with the No Observable Adverse Effect Level at the highest dose proposed in the clinical trial**

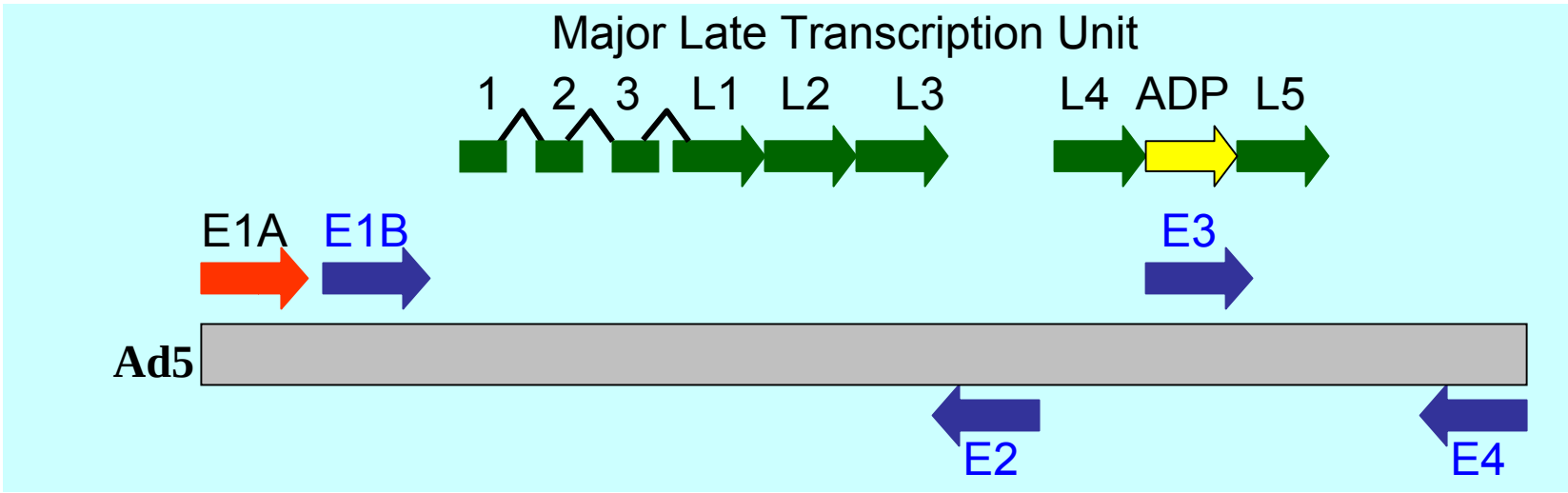
Adenovirus

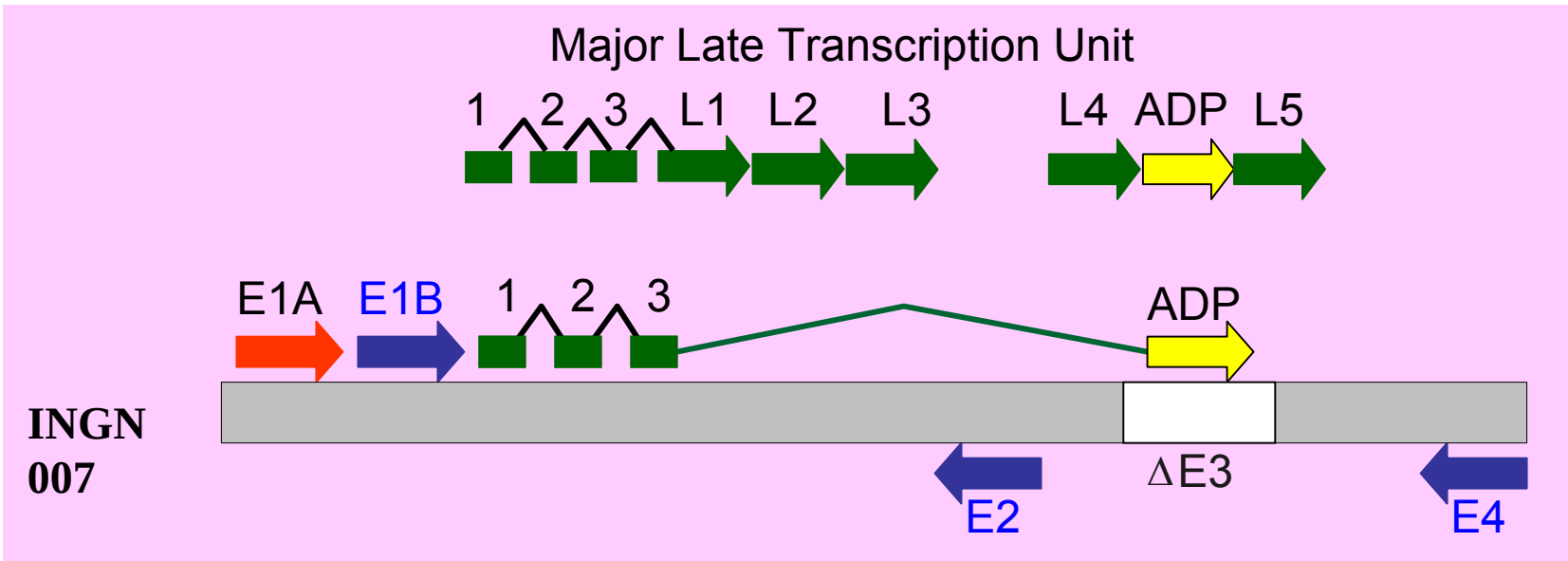




- DNA genome, 34 genes; protein shell (capsid)
- Serotype 5—ubiquitous, causes mild upper respiratory tract infections in infants; lifelong immunity
- No significant disease in healthy adults
- Safe live virus vaccine used in many military recruits

Genome Structure of Natural Adenovirus (Ad5) and Vector INGN 007

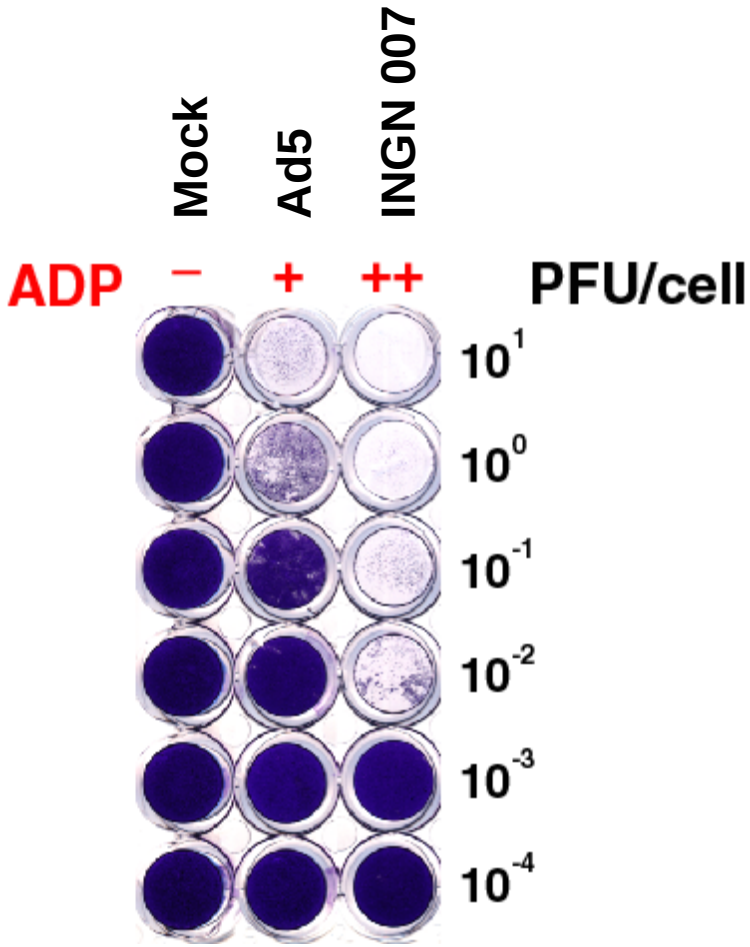




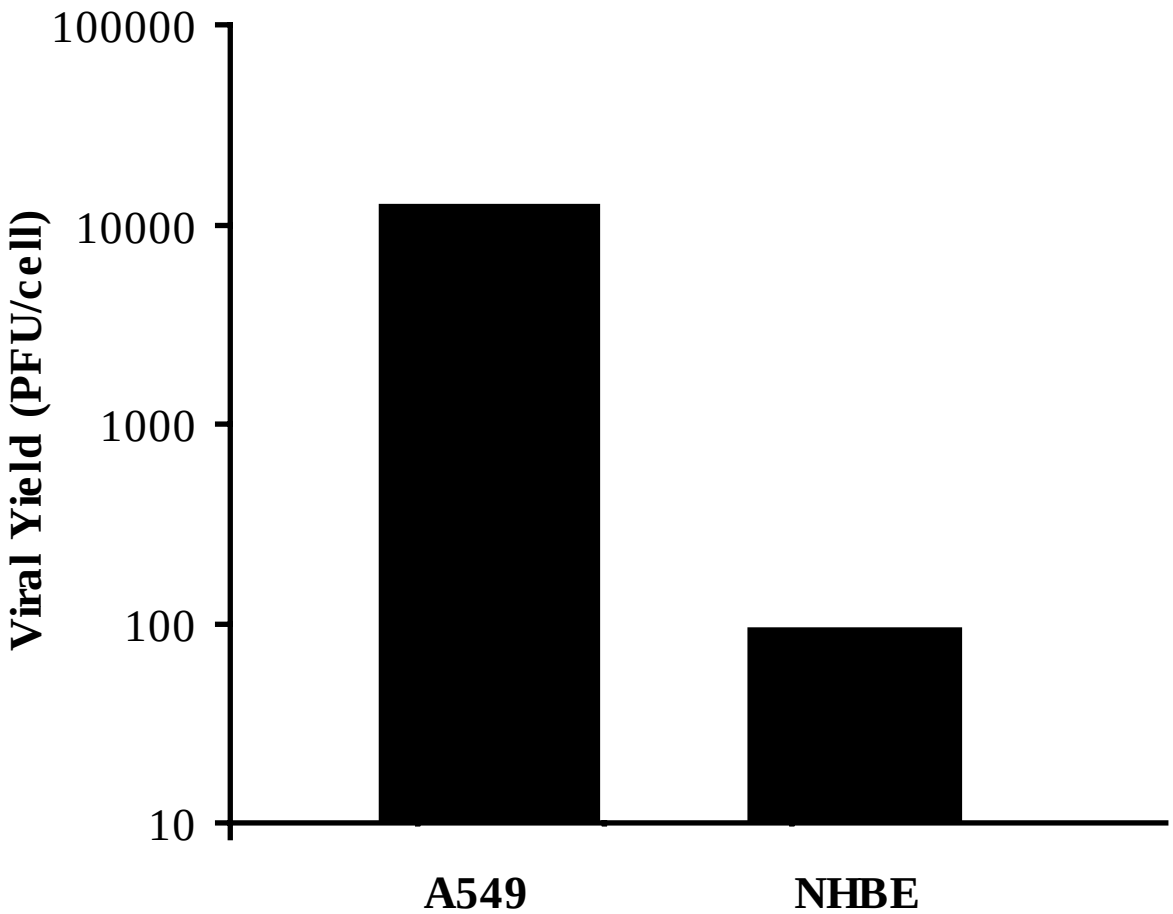
INGN 007 Kills Cancer Cells Better than Wild-Type Ad5 Because INGN 007 Overexpresses ADP

(A549 Human Lung Cancer Cells)

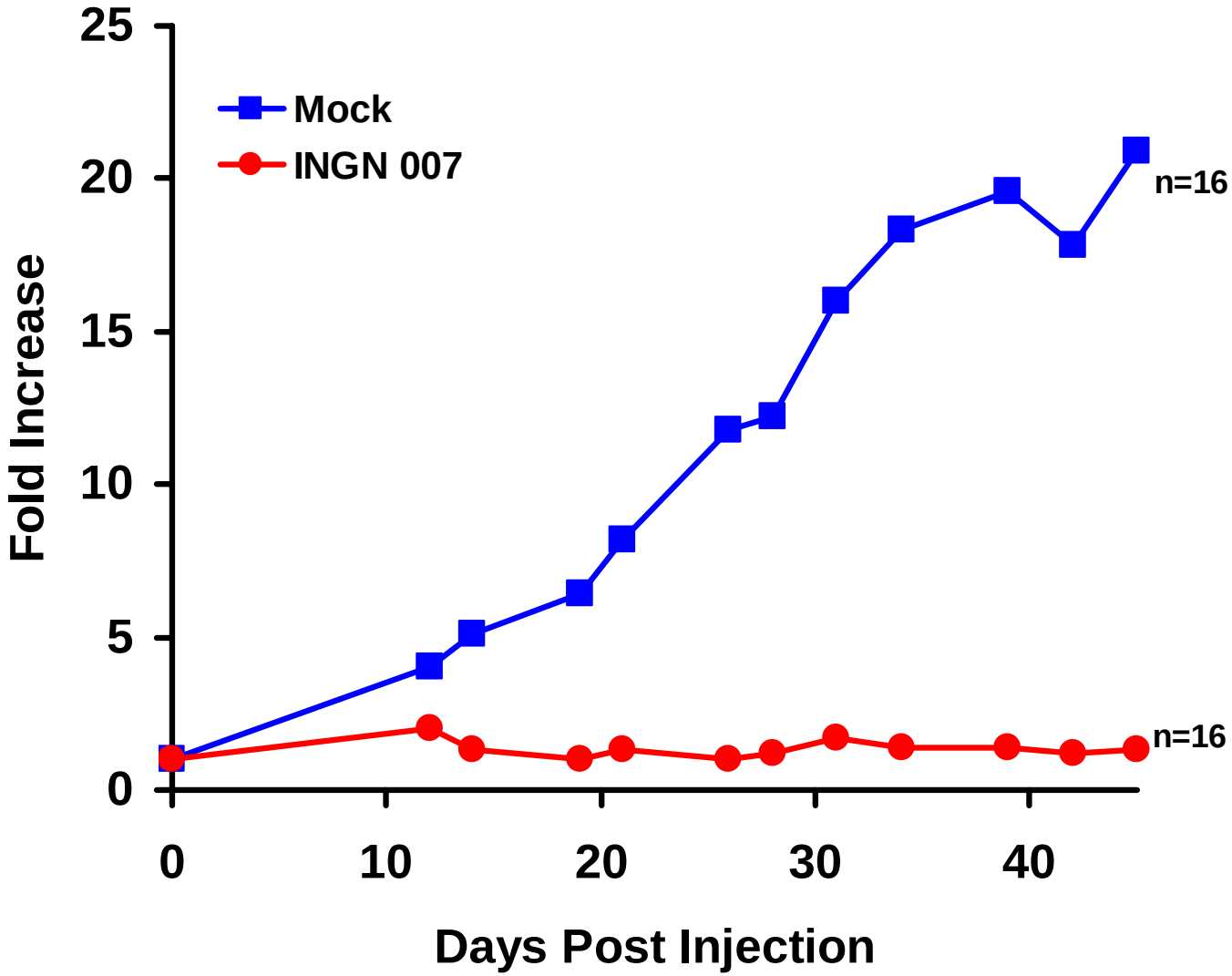
Stained with crystal violet 8 days p.i.



INGN 007 Replicates 100-fold Better in Human A549 Lung Cancer Cells than in Primary Human Bronchial Epithelial Cells (NHBE)



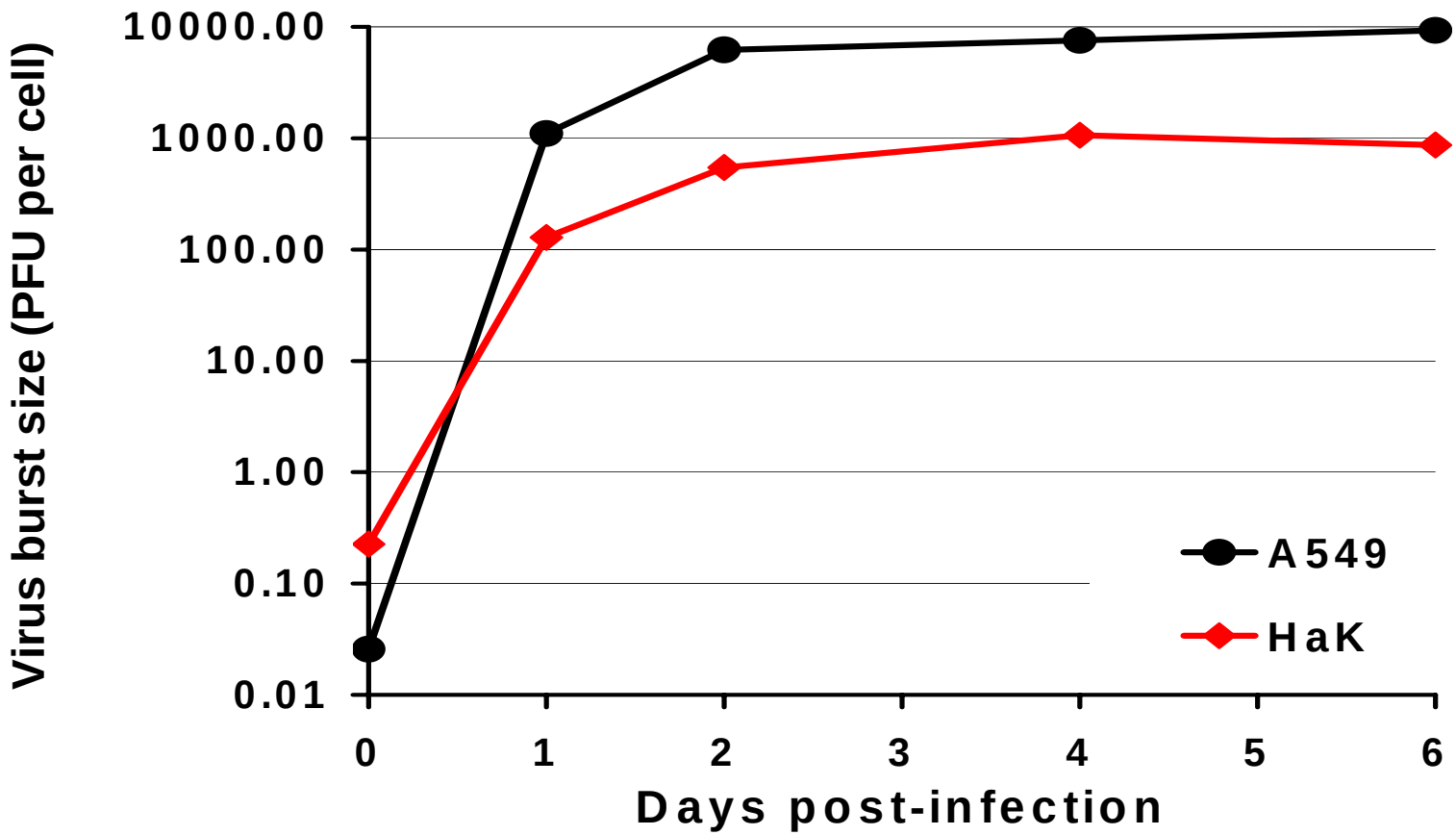
INGN 007 Suppresses A549 Human Lung Tumors in "Nude" Mice



Characteristics of Syrian Hamster Model for Safety Studies

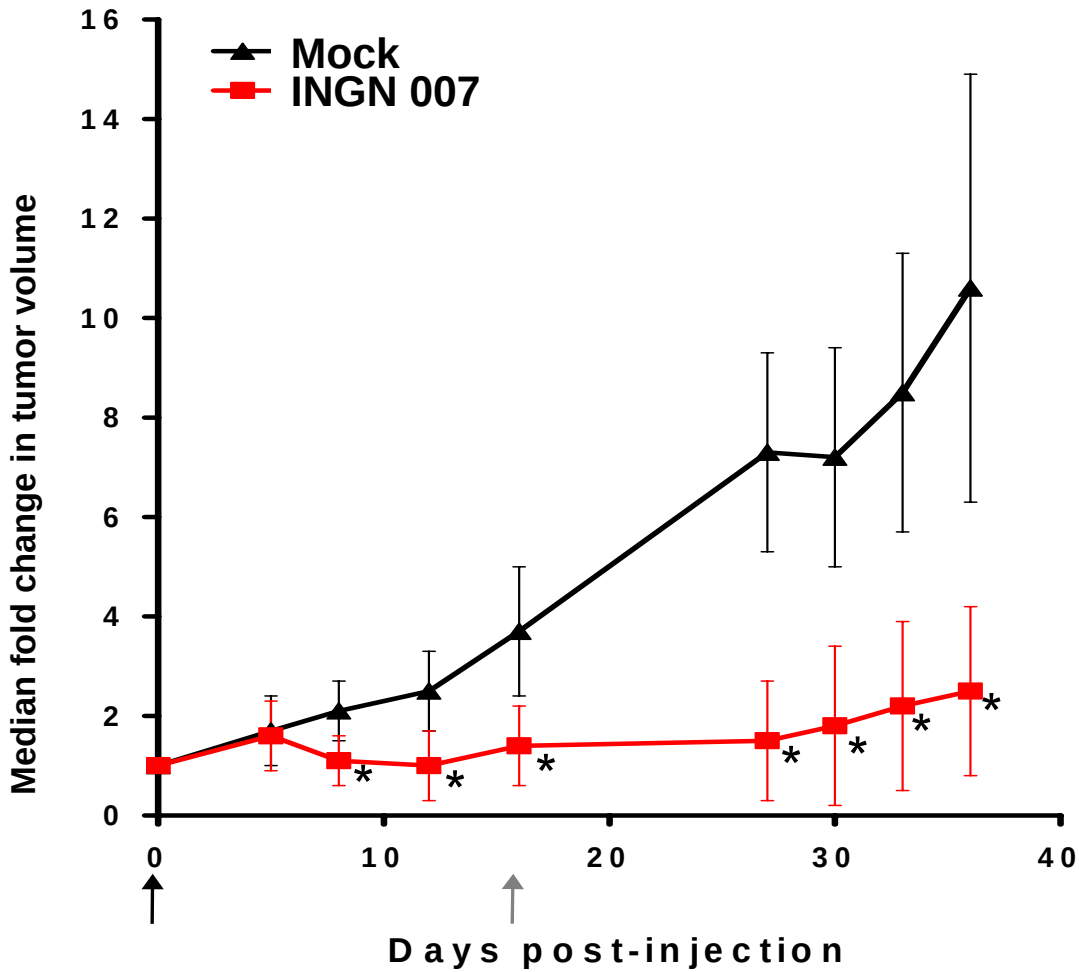
- **INGN 007 replicates in hamster cancer cells nearly as well as in human cancer cells**
- **Hamster lungs and liver are permissive for INGN 007**
- **Hamster has intact immune system**

INGN 007 Grows Nearly as Well in Hamster Cancer Cells as in Human Cancer Cells



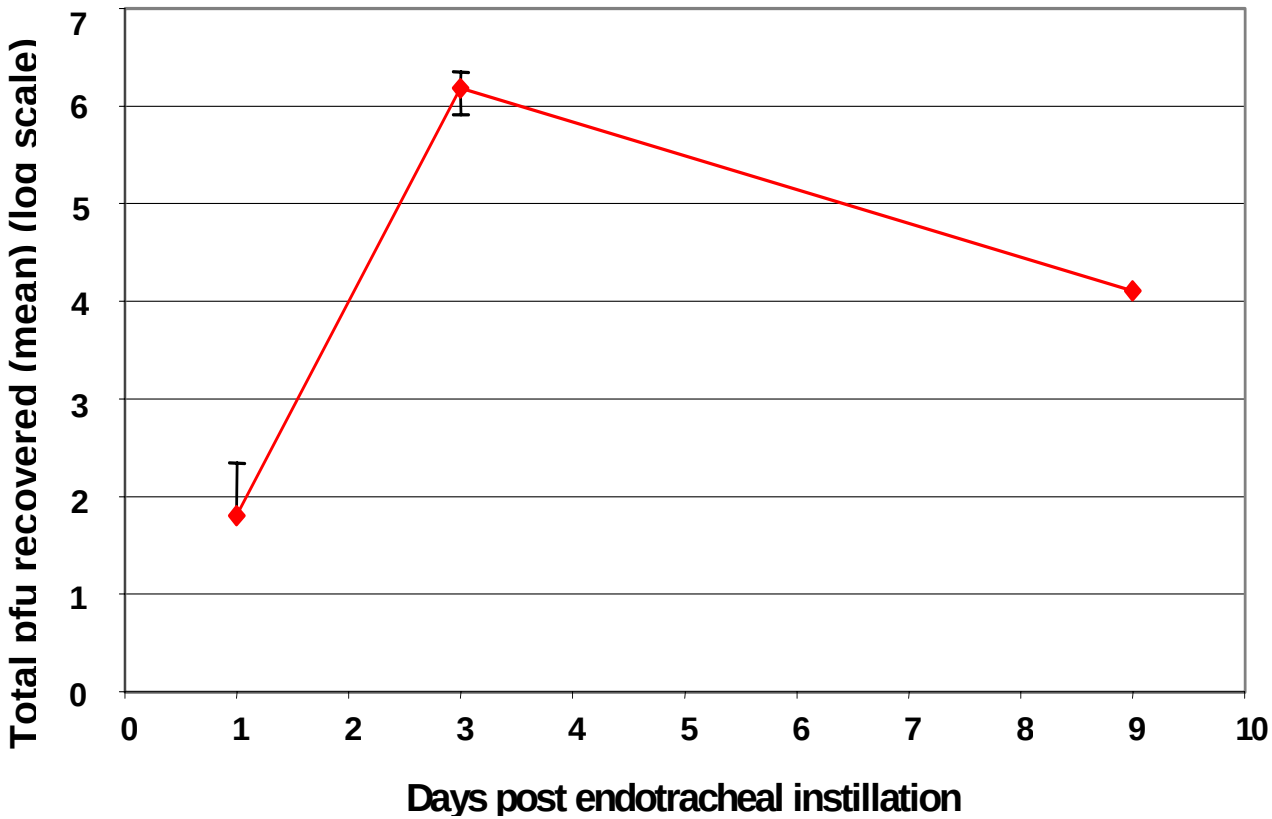
INGN 007 Suppresses the Growth of Subcutaneous Hamster HaK Kidney Cancer Tumors in Hamsters

(n = 18)



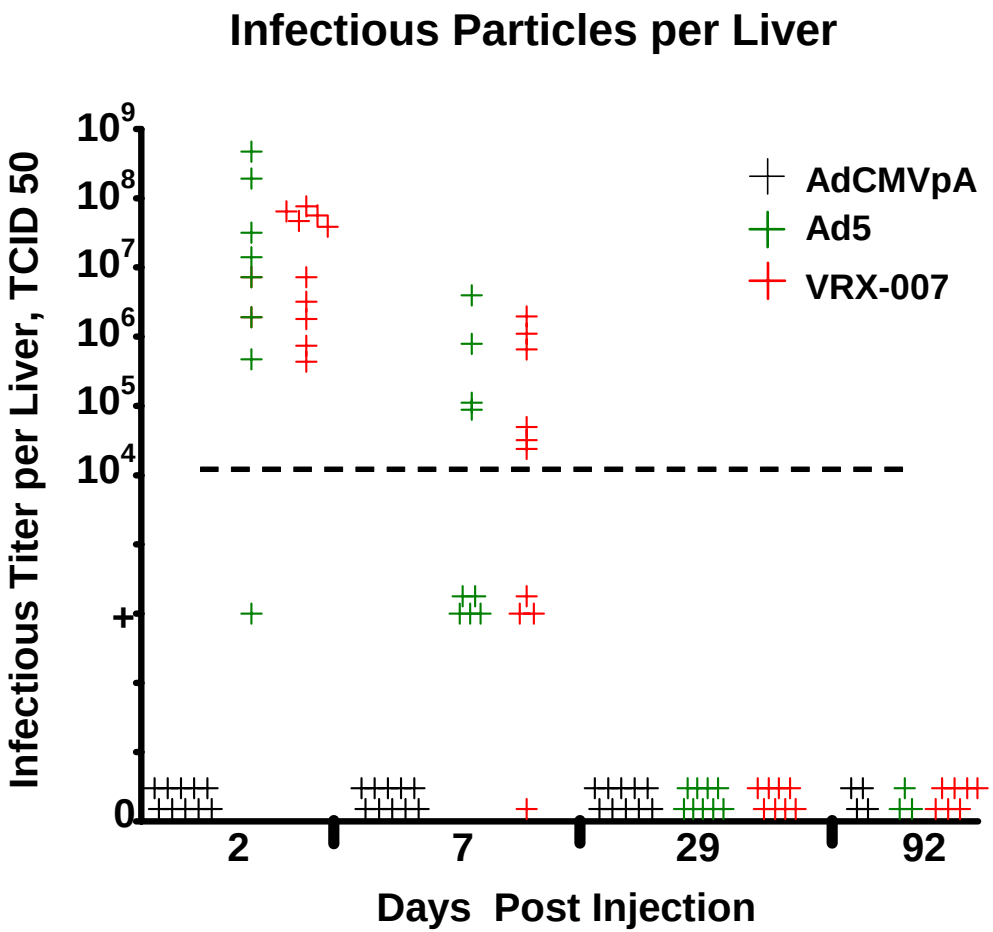
Wild-type Adenovirus Replicates in the Lungs of Syrian Hamsters

10^7 PFU were administered by intratracheal instillation. Virus was extracted from lungs and titered on A549 cells



INGN 007 and Ad5 Replicate in the Syrian Hamster Liver

1.9 x 10^{12} vp/kg (1.9 x 10^{11} vp) was injected i.v.



Toxicology Study Design in Hamsters

- **5 animals/sex/group**
- **Single dose, intravenous**
- **Three doses of INGN 007 tested:**
  - **Low dose, 3 x 10^9 vp/kg**
  - **Mid dose, 3 x 10^{10} vp/kg, equivalent to maximum human dose**
  - **High dose, 1.9 x 10^{12} vp/kg, 60-fold higher than MHD**

Toxicology Study, cont'd

- **Control groups were:**
  - **Wild-type Ad5 (at high dose)**
  - **A replication-defective adenovirus (at high dose)**
  - **Vehicle**
- **Sacrifice at days 2, 7, 29**
- **Examine typical clinical signs, clinical chemistry, hematology, and histopathology**

Summary of Hamster Toxicology Study

- **INGN 007 NOAEL is 3 x 10^{10} vp/kg, the maximum dose in our clinical trial**
  - **No adverse effects in the liver**
  - **No effects in the lung**

- **INGN 007 and Ad5 have virtually identical effects; replication-defective adenovirus less toxic at highest dose tested**

Summary of Preclinical Results

- **INGN 007 is effective in suppressing human tumor xenografts in nude mice and hamster tumors in hamsters**
- **Hamster is an appropriate permissive immunocompetent model to test the safety and phamacokinetics of INGN 007**
- **Our preclinical data suggest that INGN 007 should not be more infectious or transmissible than wild-type Ad5**

INGN 007 Clinical Trial Doses Compared to Safety Study Doses

Clinical Trial Cohort	Clinical Trial Intratumoral Dose	Syrian Hamster IV NOAEL Dose
1	2×10^8 vp (3×10^6 vp/kg)	10,000-fold below NOAEL
2	2×10^9 vp (3×10^7 vp/kg)	1,000-fold below NOAEL
3	2×10^{10} vp (3×10^8 vp/kg)	100-fold below NOAEL
4	2×10^{11} vp (3×10^9 vp/kg)	10-fold below NOAEL
5	2×10^{12} vp (3×10^{10} vp/kg)	NOAEL 3×10^{10} vp/kg

i.t. injection in the clinical trial vs i.v. injection in the hamster study

Phase I Dose Escalation Trial of Intratumoral Injection with Oncolytic Adenovirus Vector INGN 007 in Patients with Advanced Solid Tumors

PI: John Nemunaitis M.D.
Sub-investigator: Neil Senzer M.D.
Mary Crowley Medical Research Center
Dallas, TX 75246
www.mcmrc.com

INGN 007 Phase I Dose-Escalation

- **Primary objectives**
  - Evaluate Safety of Intratumoral (IT) injection into accessible lesions
  - Determine Maximum Tolerated Dose (MTD) (Up to $2x10^{12}$ vp)
- **Secondary objectives**
  - Assess INGN 007 pharmacokinetics
    - Measure viral particles in tumor, blood and urine by qPCR-DNA and CPE (infectious particles) assays
  - Evaluate clinical anti-tumor responses

INGN 007 Phase I Dose-Escalation

- Additional evaluations
  - INGN 007 pharmacodynamics (d 8, 28) in tumor (Ad proteins, histopathology)
  - Immune response (e.g., anti-capsid, neutralizing Ab, cytokines, FACS analysis of T, B and NK cells)

INGN 007 Protocol Entry Criteria

- Confirmed carcinoma with accessible lesions post-failure standard therapy
- KPS ≥70% (i.e., cares for self, but limited normal activity and work); age ≥18 yrs; life expectancy ≥3 mn
- Adequate organ/hematologic function
- Negative HIV 1, $HpBAg_s$ and HpCAb; no viral syndrome within 2 weeks
- No current immunosuppressives; no hematologic malignancy
- IRB approved consent

INGN 007 Study Design

- Five 5-patient cohorts

- Single IT dose starting 4 logs lower than pre-clinical 'No Observed Adverse Effect Level' with IV administration

- Dose escalate $2x10^8 \rightarrow 2x10^{12}$ vp total inoculum (@ 1 log increments); 2-week interval between any cohort d 28 and next cohort

INGN 007 Study Design

In each cohort of 5 patients:

- For safety and efficacy: In 3 of 5 patients inject one lesion and evaluate clinically safety and efficacy
- For Pharmacodynamics Studies : In 2 of 5 patients (who require at least 2 lesions)
  - One lesion is resected prior to treatment and the remaining lesion is treated
  - The treated lesion is resected on day 8 in one patient and on day 28 in the second patient

INGN 007 Dose Limiting Toxicity (DLT) and Safety Assessment

- DLT (NCI Common Toxicity Criteria)
  - Any Grade 4 toxicity; Grade 3 hematological toxicity attributable to Rx >3 days; Grade 2 neurotoxicity; “flu” >10 days post-Rx

- If DLT observed; 3 additional patients enrolled at ≥ 2-week intervals, if 2nd DLT observed then prior dose level defines MTD

INGN 007 Safety Assessment

Pulmonary side effects unlikely:

1. Absence of pulmonary toxicity in permissive animal model

2. Adenoviral respiratory infections are well tolerated in the normal and cancer populations

3. Safe use of enteric-coated wild type adenoviral vaccines in millions of military recruits

INGN 007 Respiratory Monitoring

- Patients will be followed (d 2, 4 and weekly) with pulmonary function tests (PFT) and scans
- If symptomatic grade 2 pneumonitis and CXR infiltrate $\rightarrow$ bronchoscopy with aspirate/biopsy
- If not tumor or non-viral infection then PCR for viral identification. No new entry until assessment complete.
- INGN 007+ or grade 3 pneumonitis (affecting daily activities or requiring O_2) initiate anti-viral cidofovir (or ribovirin)

Summary-Phase I Study of INGN 007

- Starting IT dose in our clinical trial is 4 logs lower than the IV NOAEL in hamsters
- Maximum IT dose in our clinical trial is equal to the IV dose NOAEL in hamsters
- Proposed clinical trial and dose escalation schedule is supported by these preclinical data in permissive animal model