

**TROPISM MODIFIED CONDITIONALLY  
REPLICATIVE ADENOVIRUSES (CRAds)  
FOR OVARIAN CANCER THERAPY**

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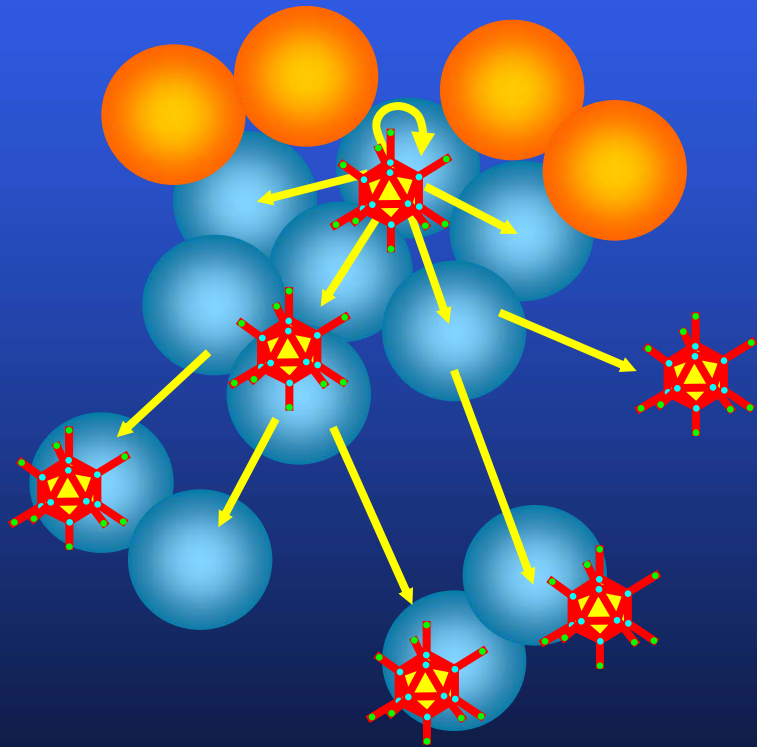
# Presentation Overview

- **Rationale for vector tropism modification**
- **Ad5- $\Delta$ 24RGD ovarian cancer preclinical data**
- **Review preclinical safety study plans**
- **Review clinical trial design**
- **Address issues raised by reviewers**

# What have we learned from initial gene therapy trials for ovarian cancer?

- Although various vectors can be used, gene transfer remains limited
- Various gene therapy approaches appear feasible but are constrained by vector limitations
- Clinical trials have yet to identify appropriate dosages and schedules
- Nonspecific toxicity continues to exist
- Correlative studies have not been optimized

# Replicative oncolytic viruses (CRADs) for cancer gene therapy

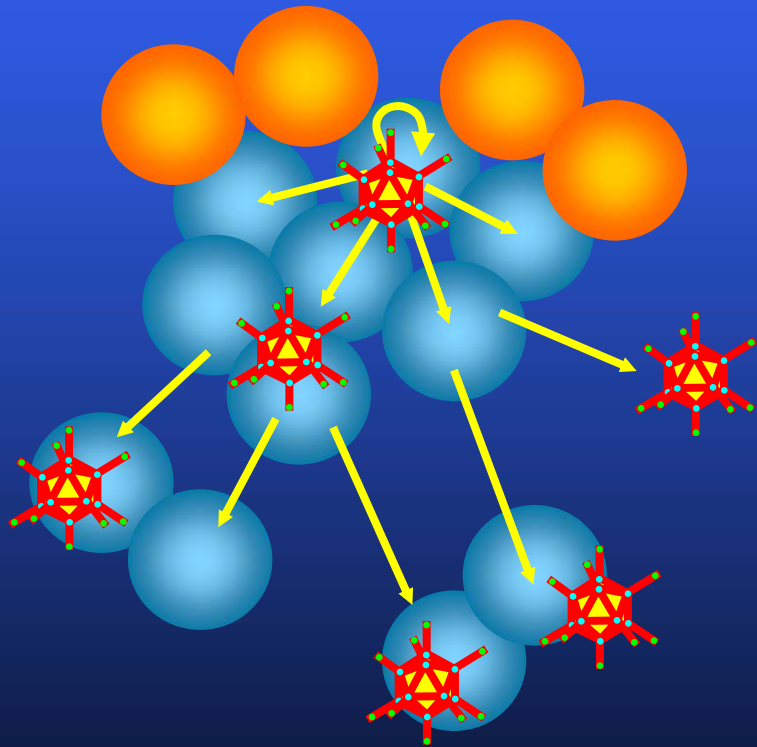


- CRAd replication induces oncolysis
- Tumor cell burst allows CRAd dispersion and penetration of solid tumor
- Potential treatment option for metastatic disease

# Ovarian cancer clinical trial experience with ONYX-015

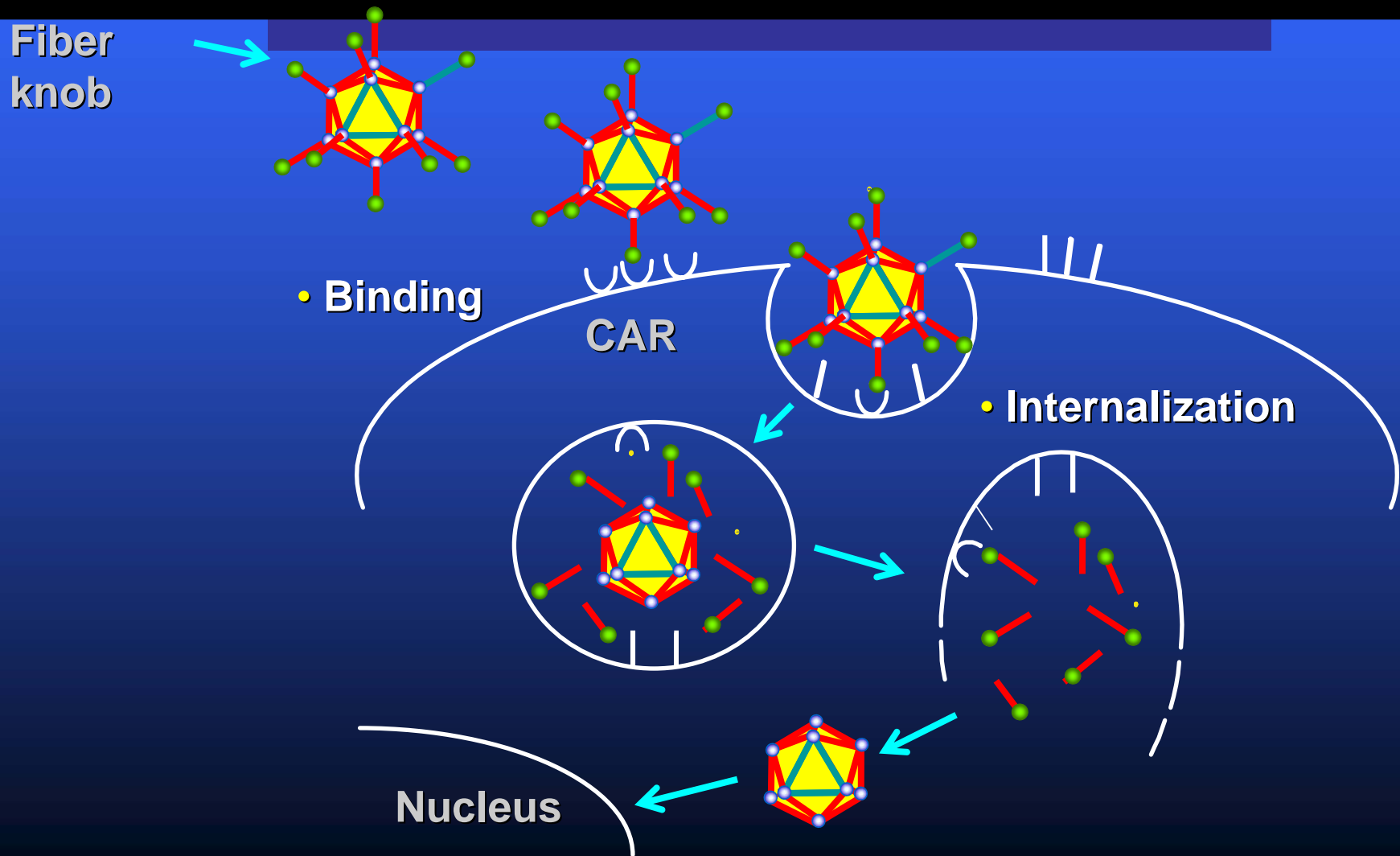
- Toxicities – fever, abdominal pain, N&V, diarrhea
- DLT –  $10^{12}$  particles in bulky tumor,  $10^{13}$  particles in non-bulky tumor (**given daily x 5 q3wk, range 1-4 cycles**)
- Limited evidence of viral replication (7 of 25 cell free peritoneal samples in 8 patients PCR positive)
- 6/13 patients with Nab
- No clinical efficacy (0/16) when administered alone (one response when given in combination with chemotherapy)

# CRADs - Biologic Determinants of Efficacy

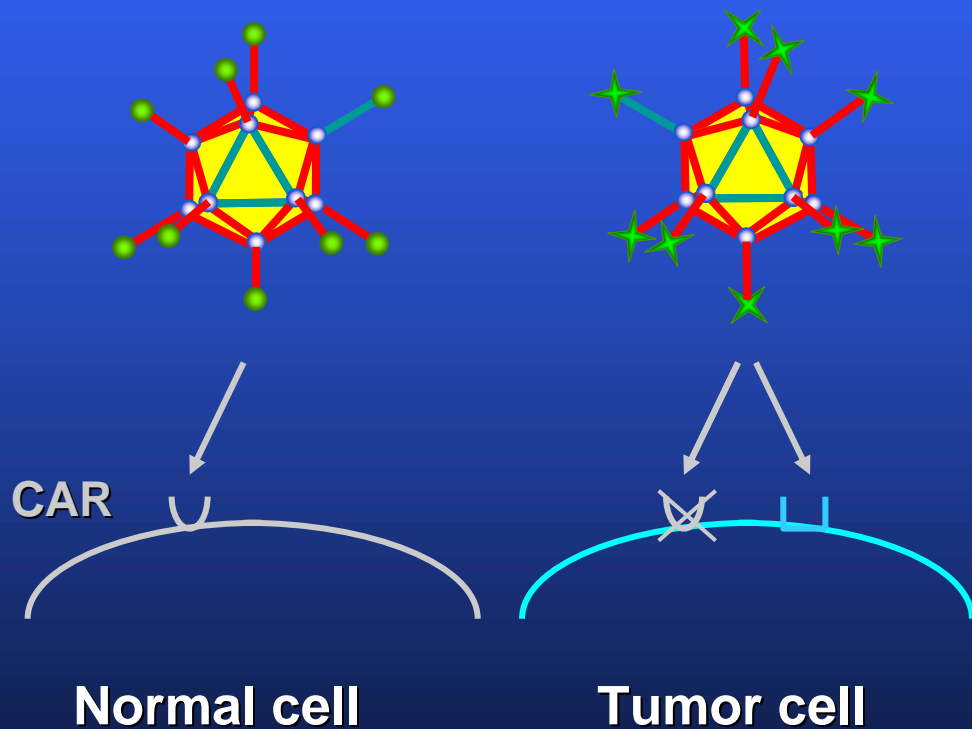


- **Efficient tumor cell infection**
- **Tumor cell specific replication**
- **Lateral dispersion**
- **Avoidance of immune recognition**

# Biological Determinants of Adenovirus Infectivity



# Vectorology: Expansion of Ad/CRAd Tropism

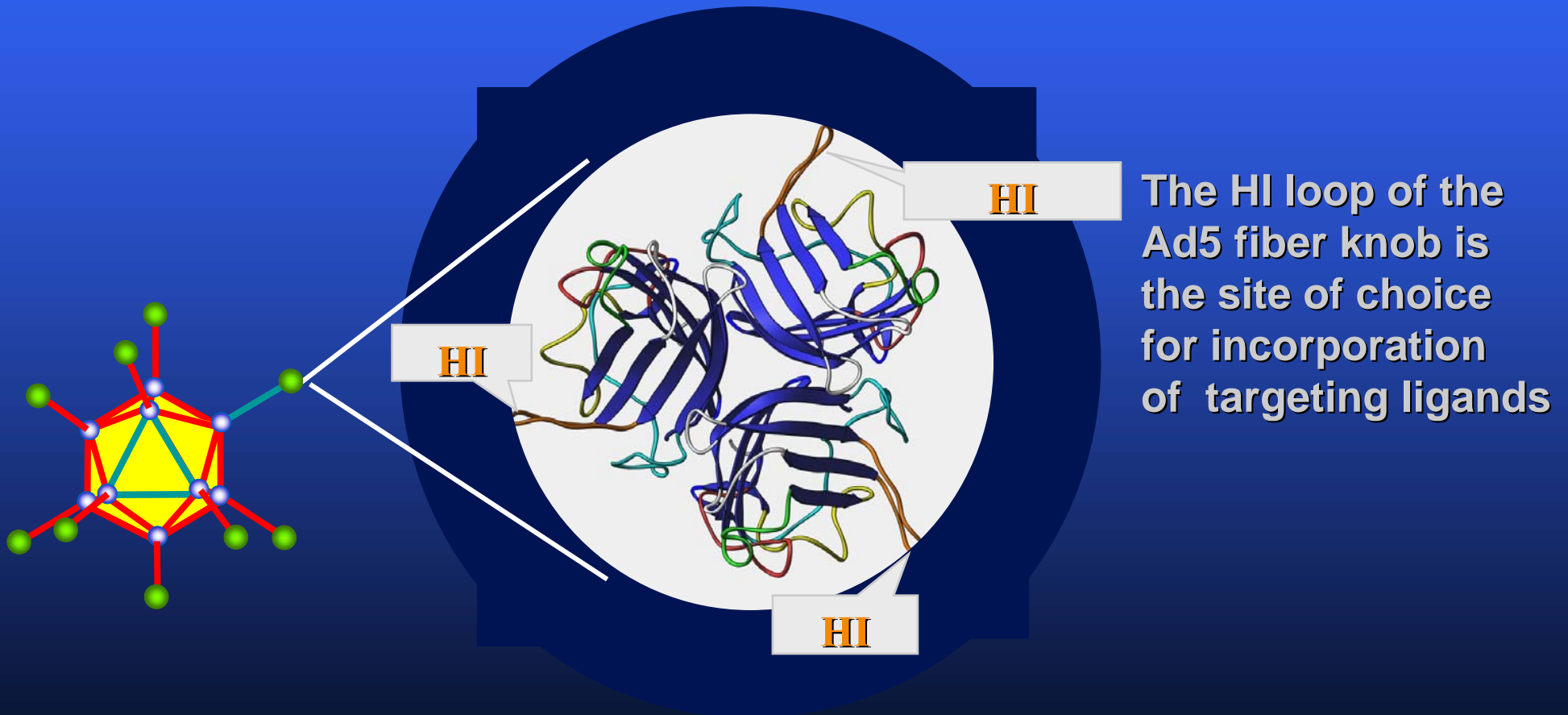


**Goal:**

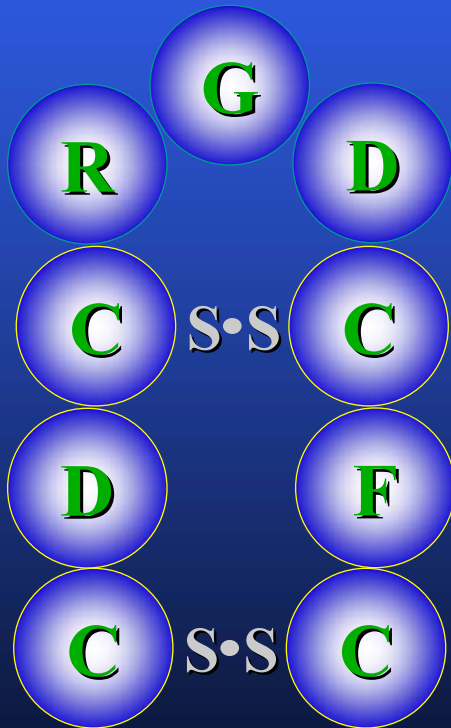
CAR-independent gene transfer to overcome CAR deficiency of tumor cells



# Expansion of Ad Tropism via Genetic Capsid Modifications



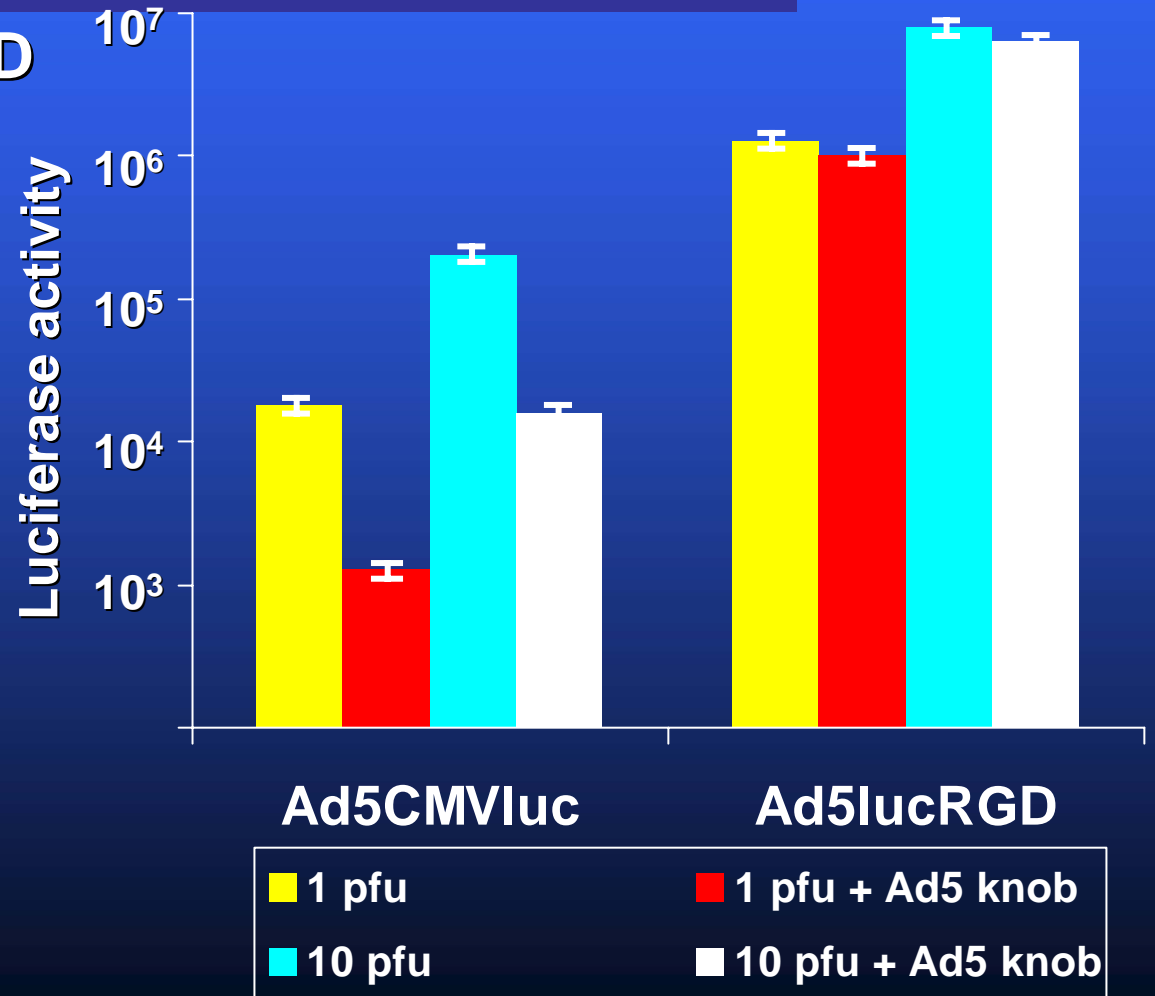
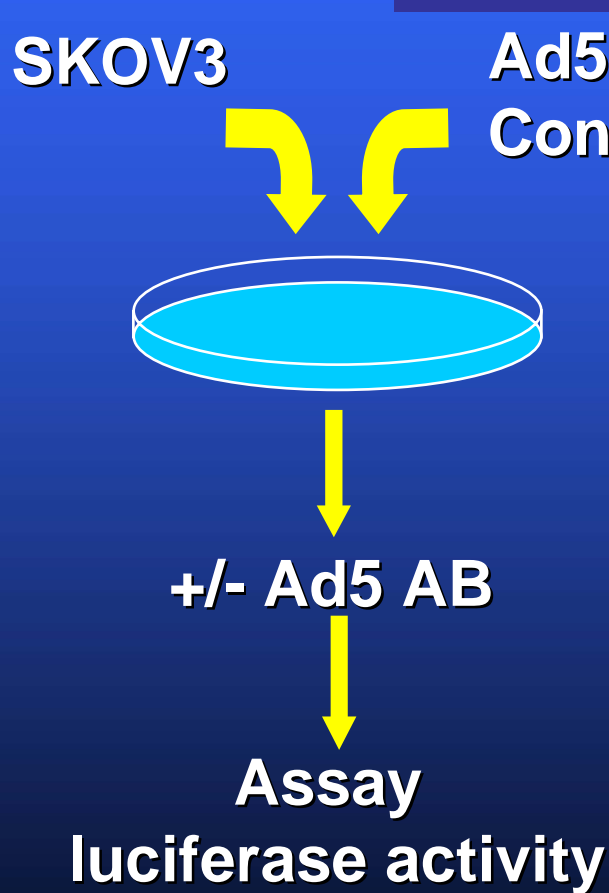
# Expansion of Ad Tropism via Genetic Incorporation of RGD-4C Peptide into the HI Loop of the Fiber Knob



## ***Rationale:***

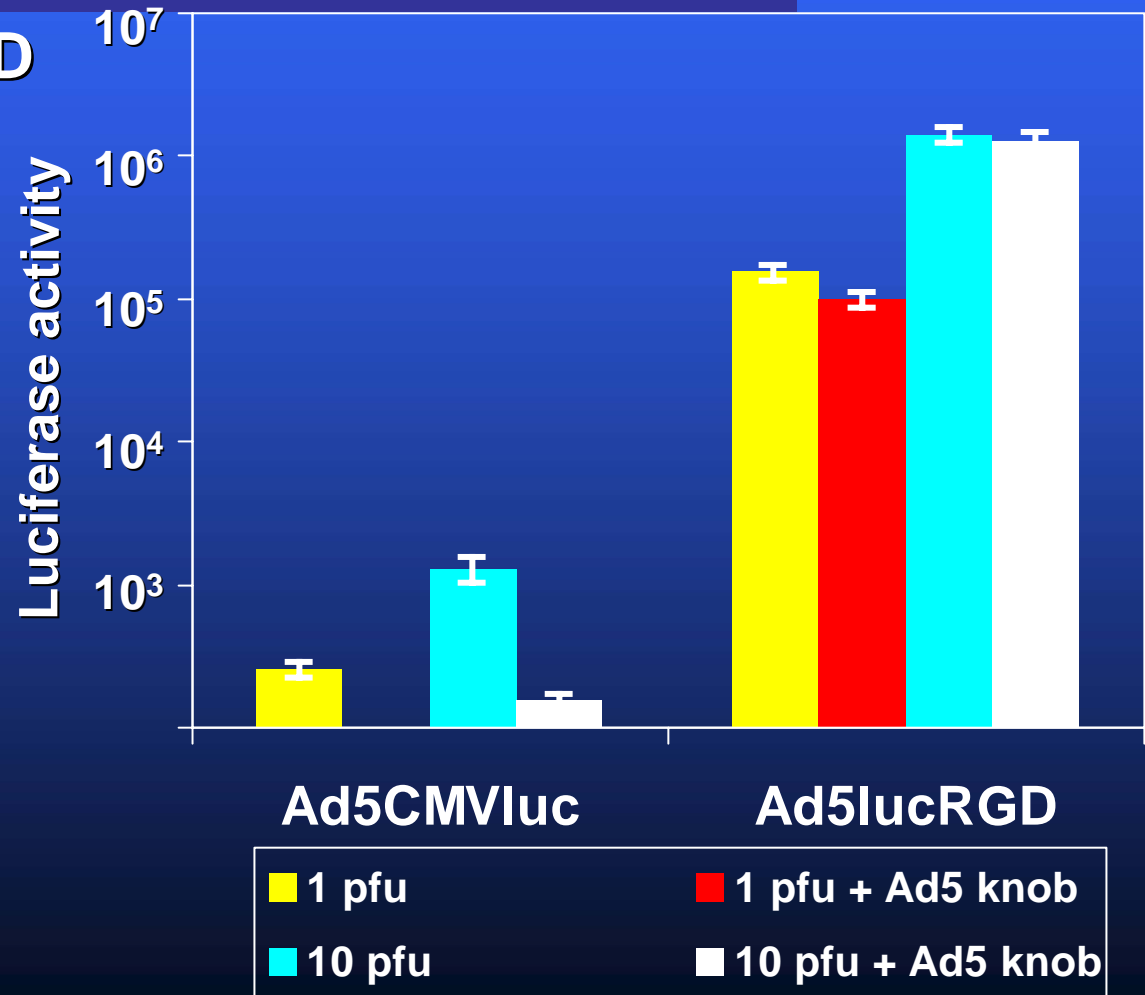
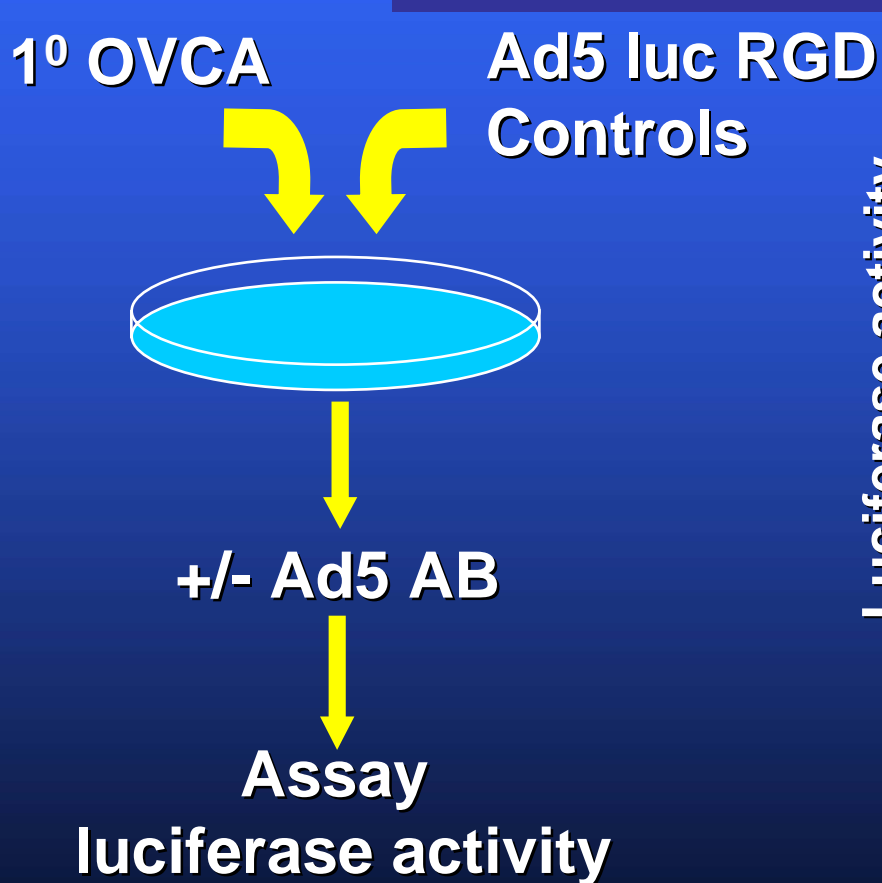
- Small size - 9 amino acids
- High affinity to integrins which are overexpressed in various cancer cells
- Ability to target to cancer cells *in vivo*

# Genetically Modified Vectors for Ovarian Cancer Gene Therapy



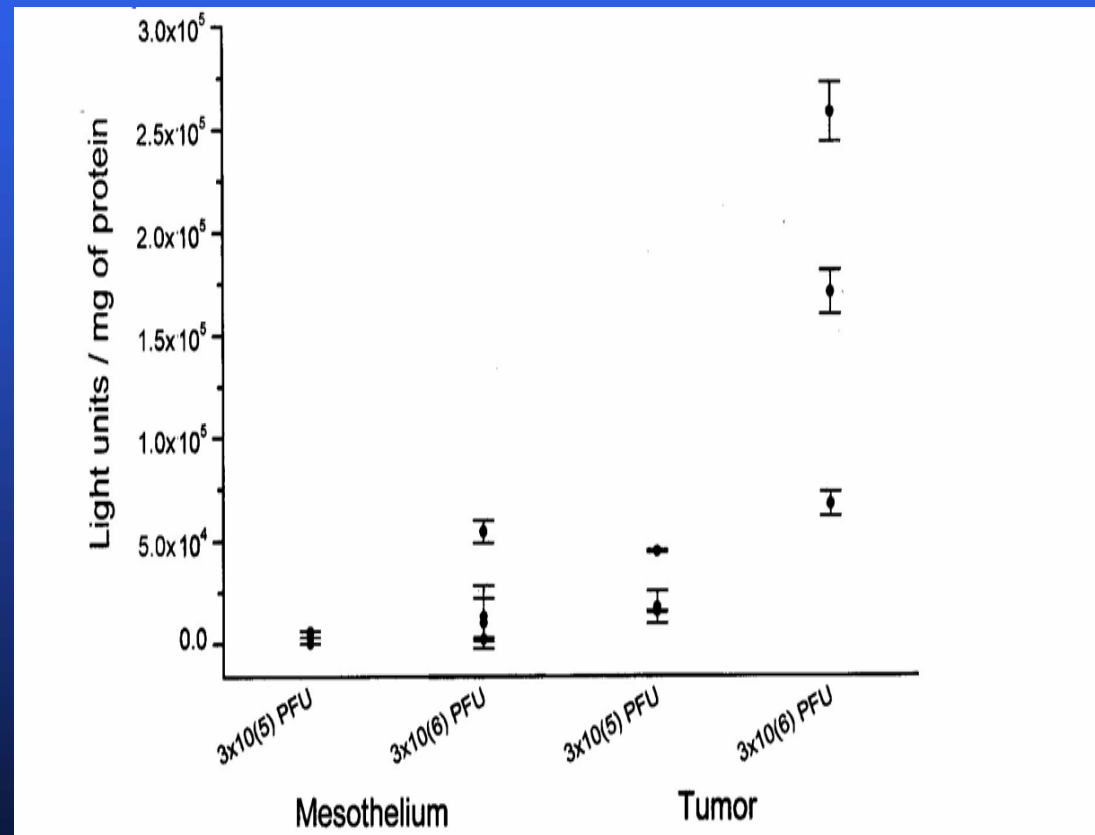
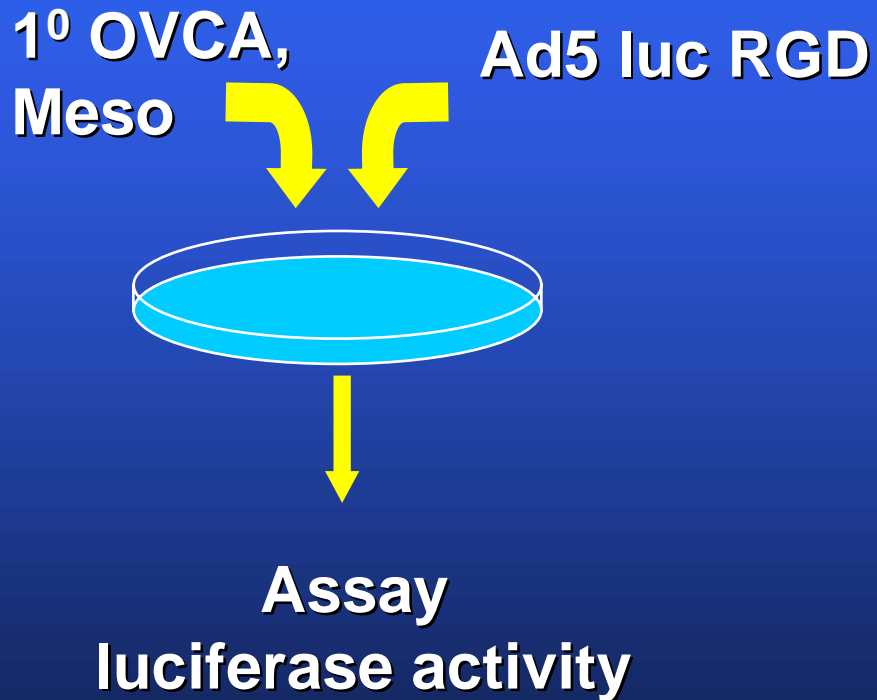
*Dmitriev et al. J Vir, 1998*

# Genetically Modified Vectors for Ovarian Cancer Gene Therapy



*Dmitriev et al. J Vir, 1998*

# Transfection of Mesothelium with RGD Modified Adenoviruses

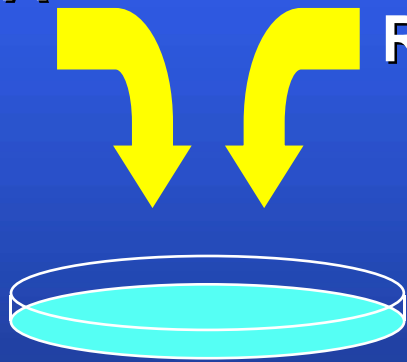


*Vanderkwaak et al*  
*Gyn Onc, 1999*

# Genetically Modified Vectors Circumvent Ascites Inhibition of Gene Transfer

1° OVCA

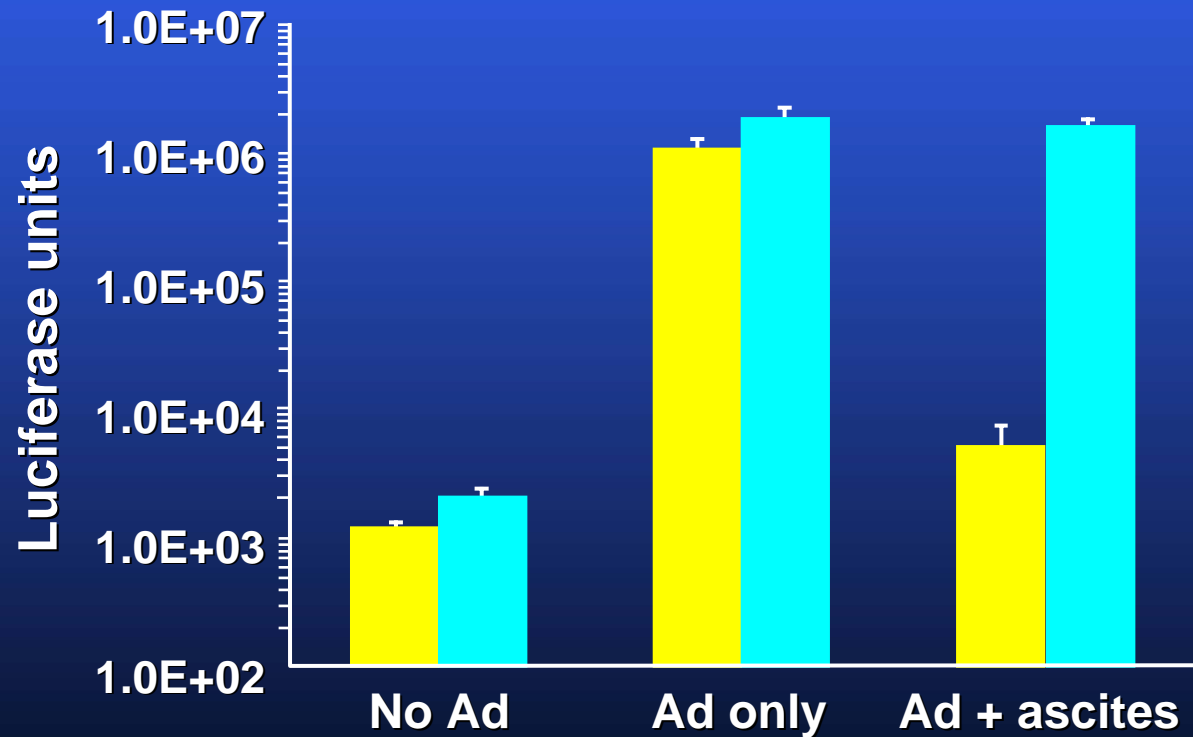
Ad-Luc  
RGD Ad Luc



± Ascites

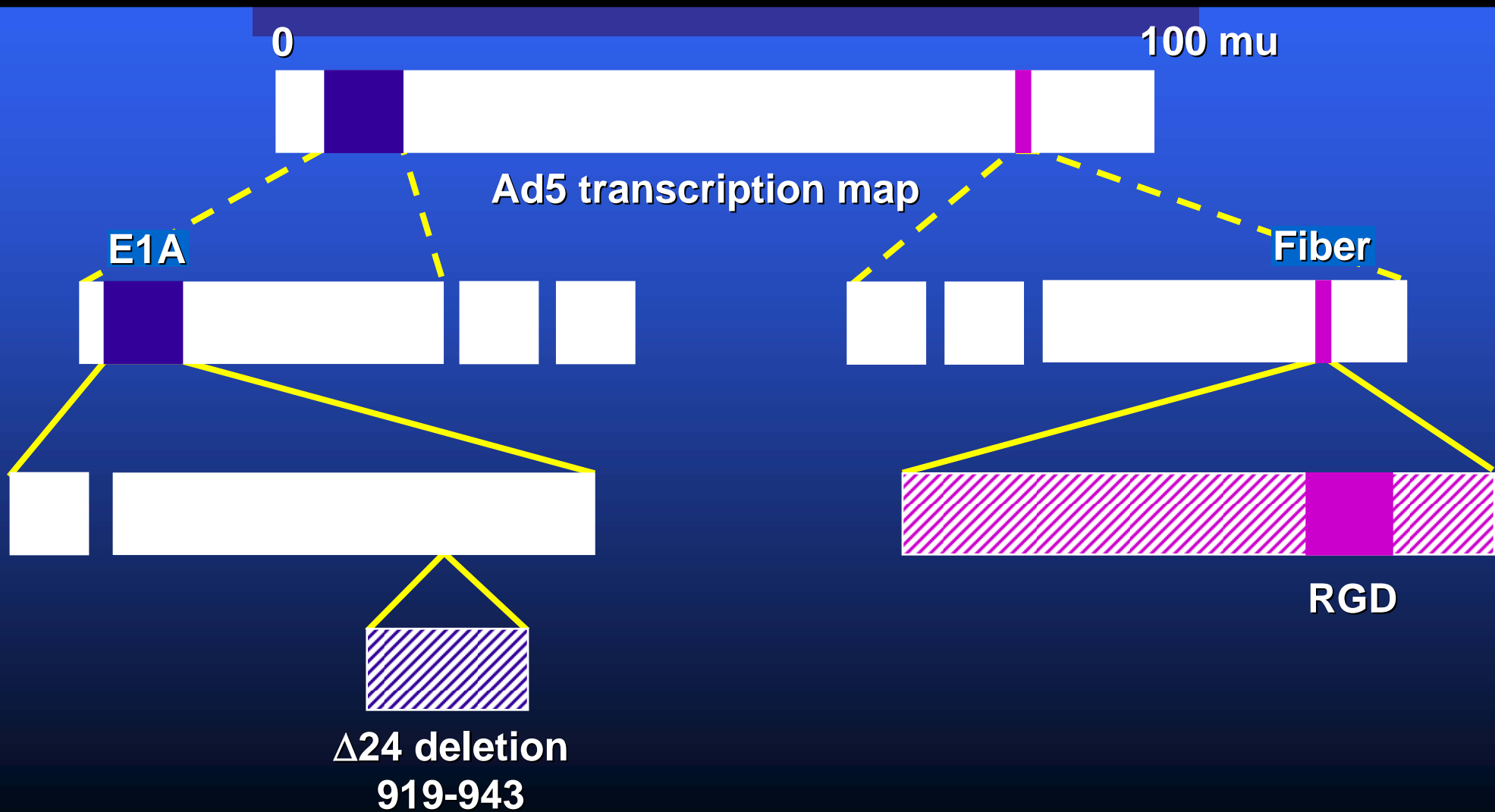
Assess luciferase expression

■ Ad5CMVLuc ■ Ad5CMVLuc-RGD

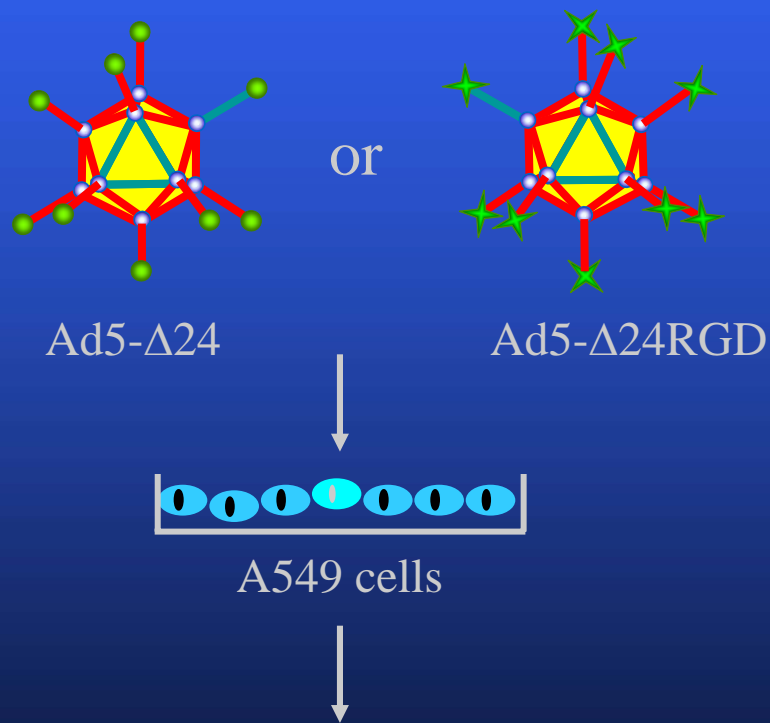


*Blackwell et al, Human Gene Ther, 2000*

# Construction of Ad $\Delta$ 24RGD

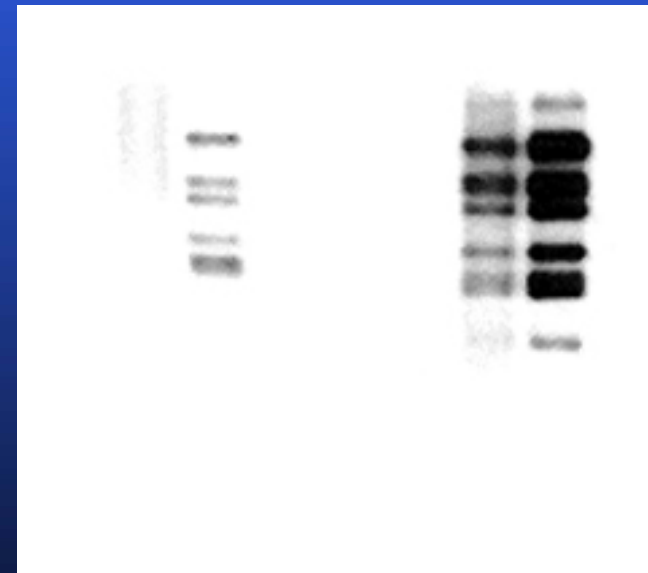


# Infectivity Enhanced CRAd Shows Greater DNA Replication *in vitro*



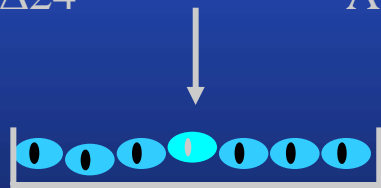
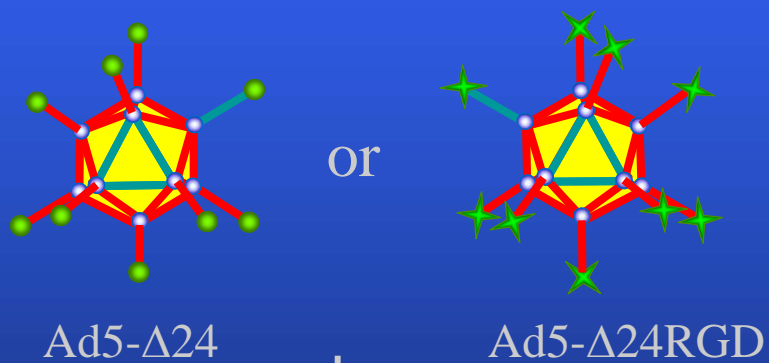
Incubate in presence of BrdU  
Southwestern blot with anti-BrdU antibody

Day	Ad5- $\Delta$ 24				Ad5- $\Delta$ 24RGD			
	2	4	6	8	2	4	6	8



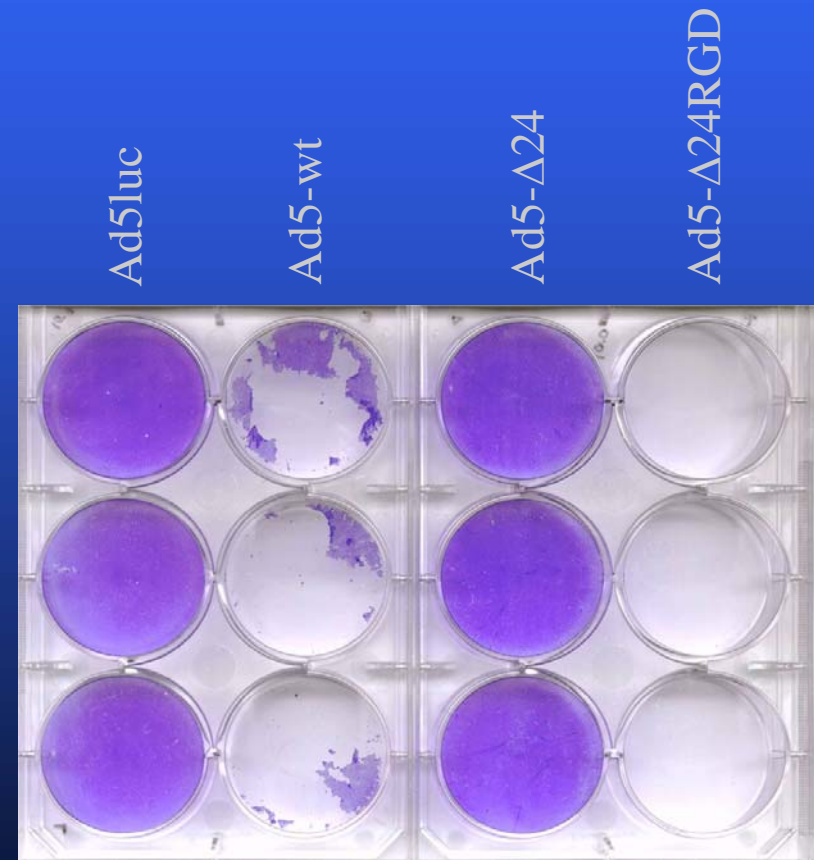


# Expansion of Ad Tropism Allows Enhanced *in vitro* Efficacy of CRADs



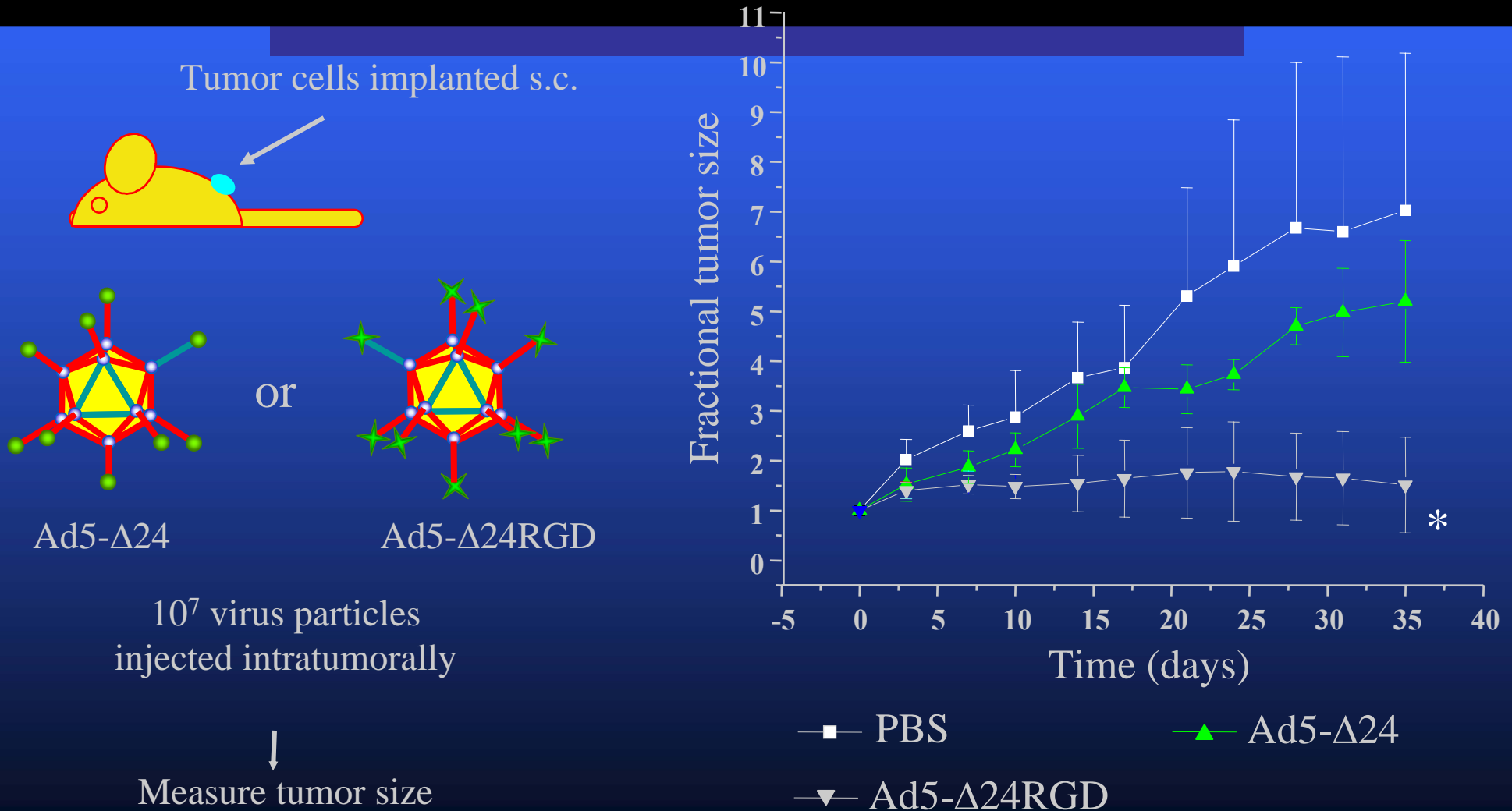
A549 cells

Incubate 8 days  
Crystal violet assay

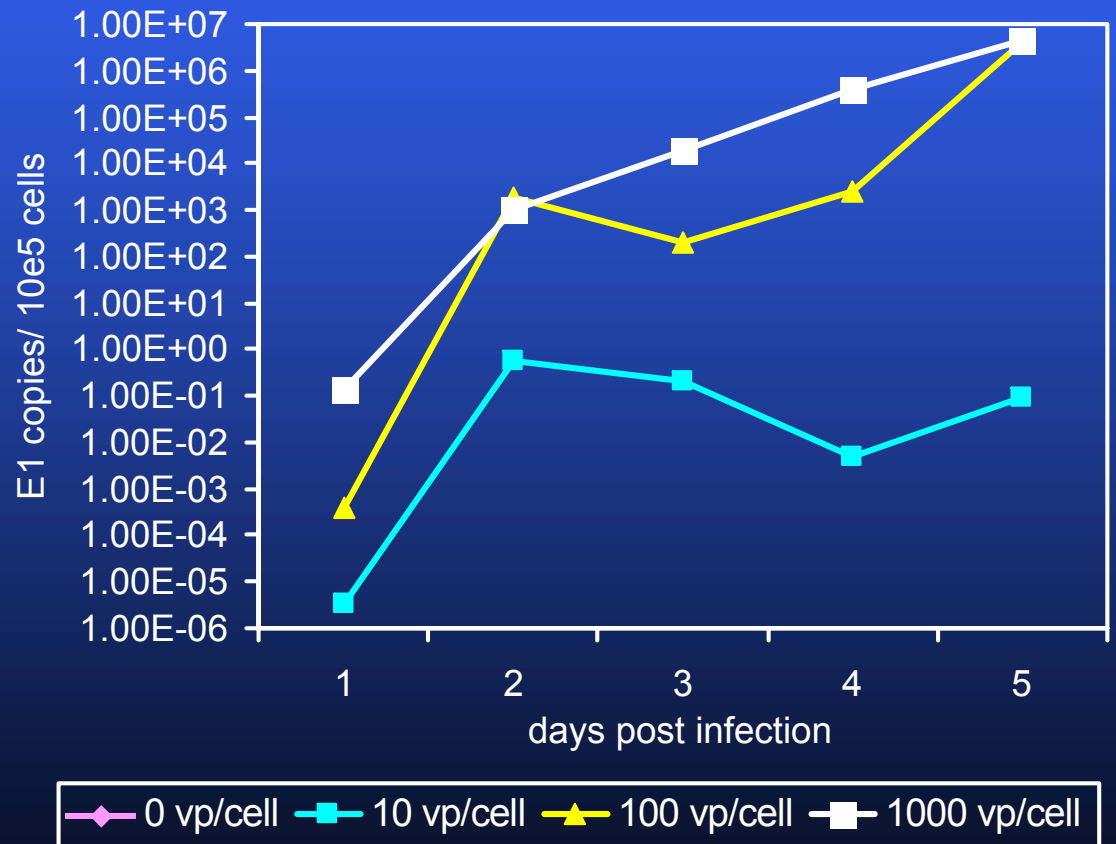
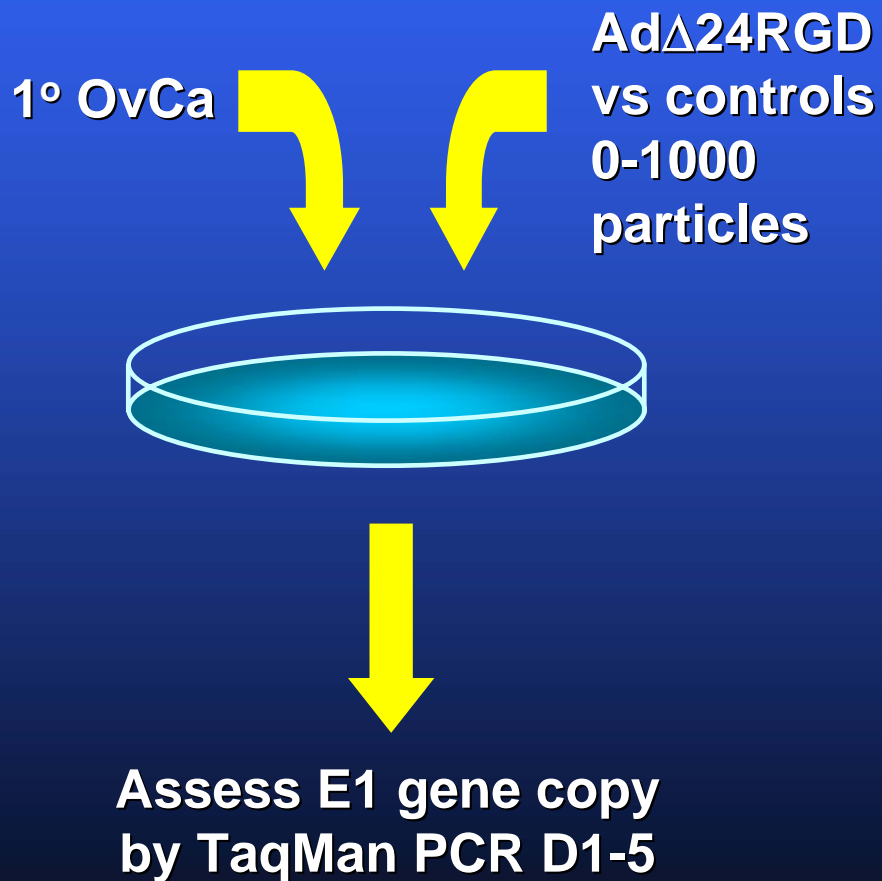


Initial inoculum: 0.01 viral particles per cells

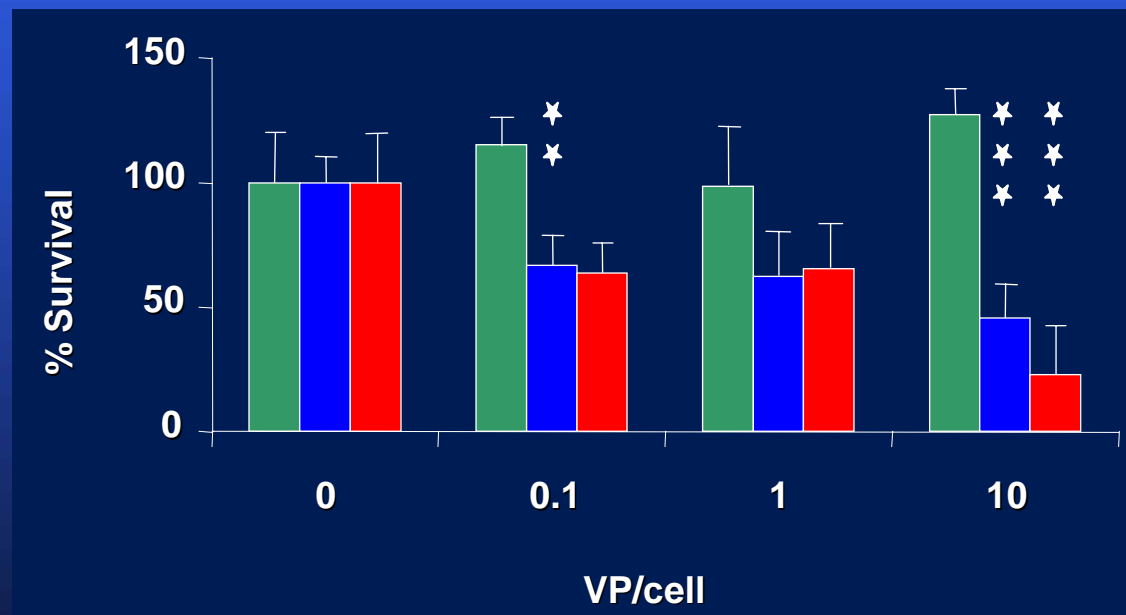
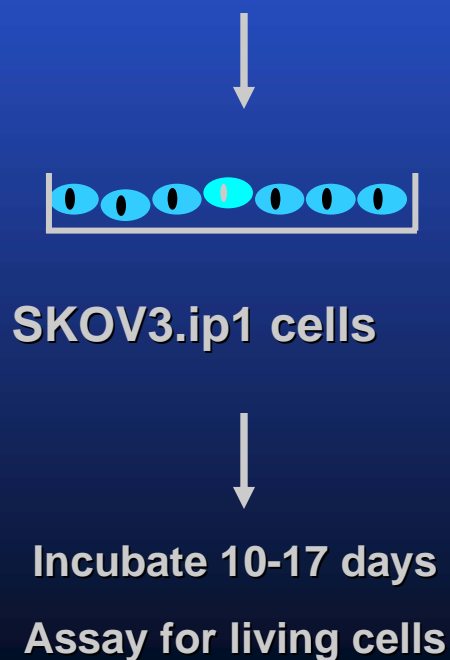
# Expansion of Vector Tropism Allows Enhanced *in Vivo* Efficacy of CRADs



# Evidence of Ad $\Delta$ 24RGD Replication in Ovarian Cancer



# Ad5- $\Delta$ 24RGD Causes Oncolysis in Ovarian Cancer Cell Lines

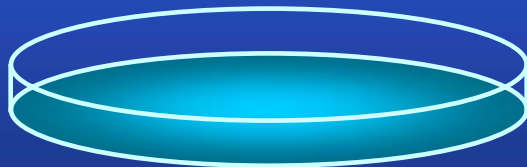


(\*\*p<0.01; \*\*\*p <0.001)

# Evidence of Ad $\Delta$ 24RGD Oncolysis in Ovarian Cancer

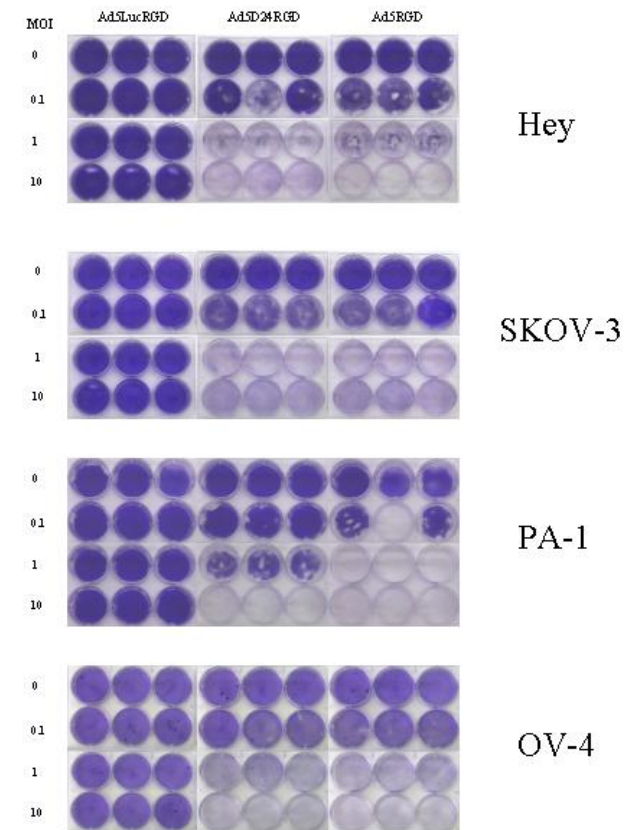
Various  
OvCa  
Cell lines

Ad $\Delta$ 24RGD  
vs controls  
0-10 MOI



Assess cellular viability  
by crystal violet assay

D24RGD validation in OvCa



# In Vivo Efficacy of Ad $\Delta$ 24RGD

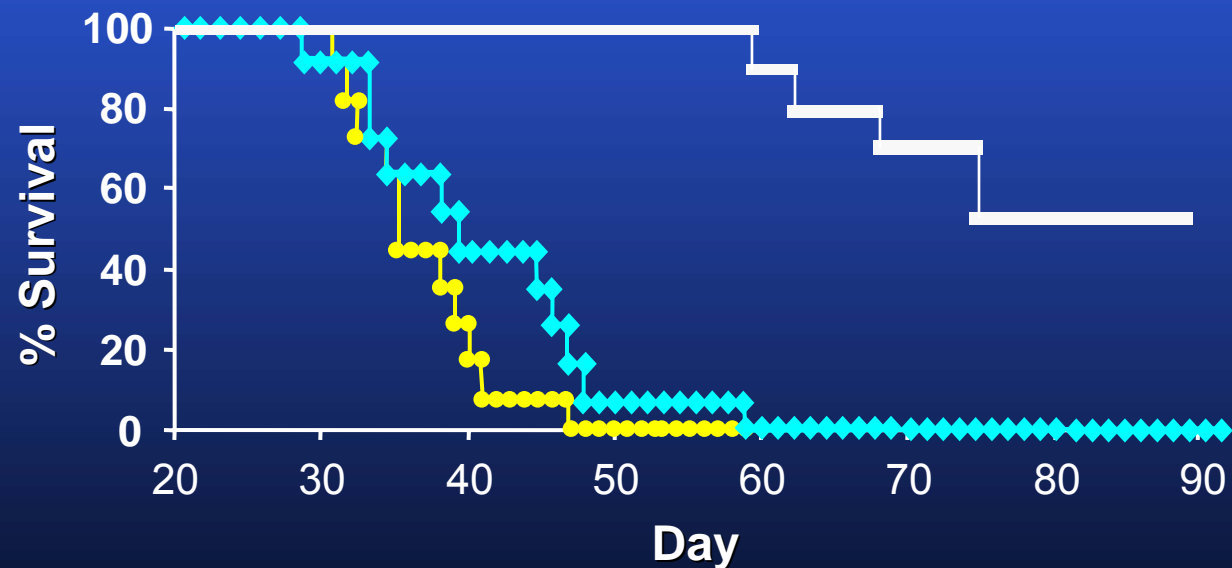
SKOV3.ipl

Ad $\Delta$ 24RGD  
Control D4-6  
 $5 \times 10^8$  VP/d



Assess survival

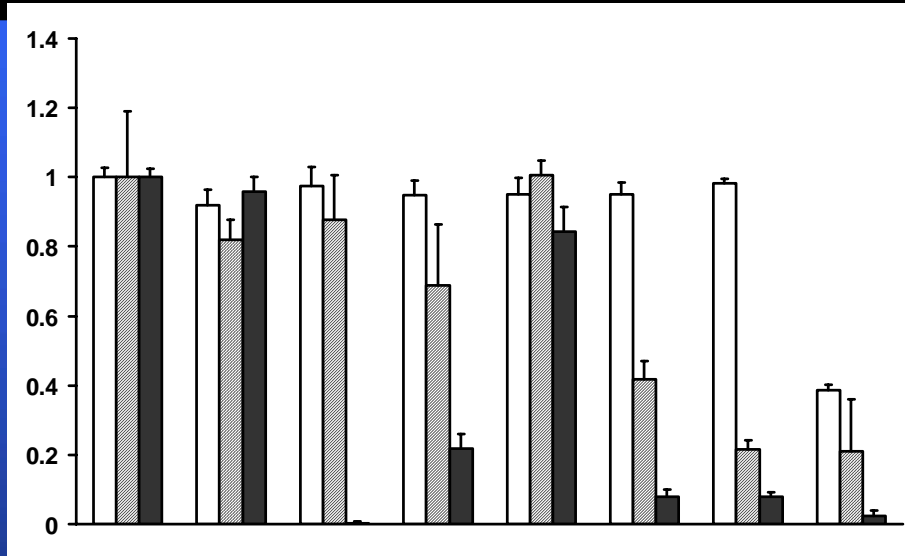
■ Ad5-D24RGD  
◆ Ad5LucRGD  
● Mock



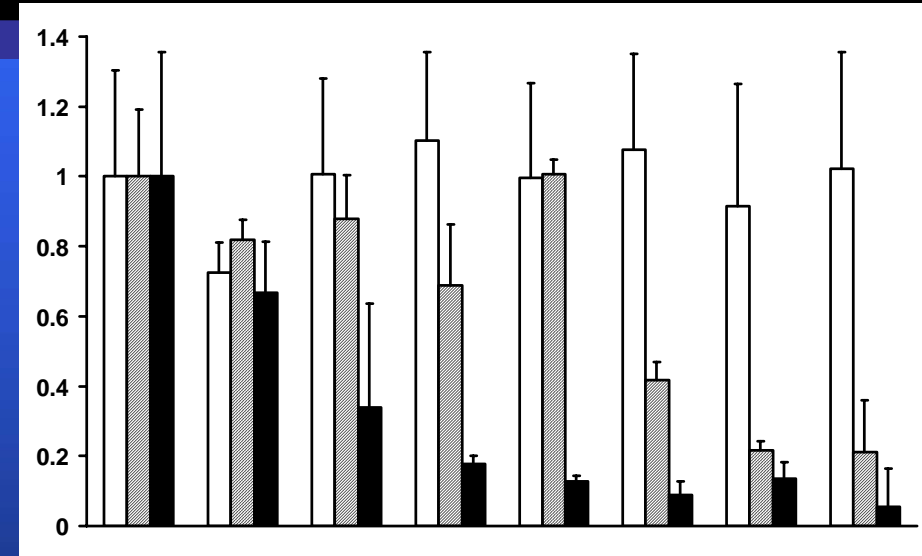
Bauerschmitz, Ca Res, 2002

# Quantifying CRAAd Mediated Oncolysis

## Monolayers



## Spheroids



Mock

E1-deleted

wild type

RGDCRADcox-2R

Ad5VEGFE1

Ad5/3VEGFE1

Ad5-Δ24RGD

Ad5/3-Δ24

Mock

E1-deleted

wild type

RGDCRADcox-2R

Ad5VEGFE1

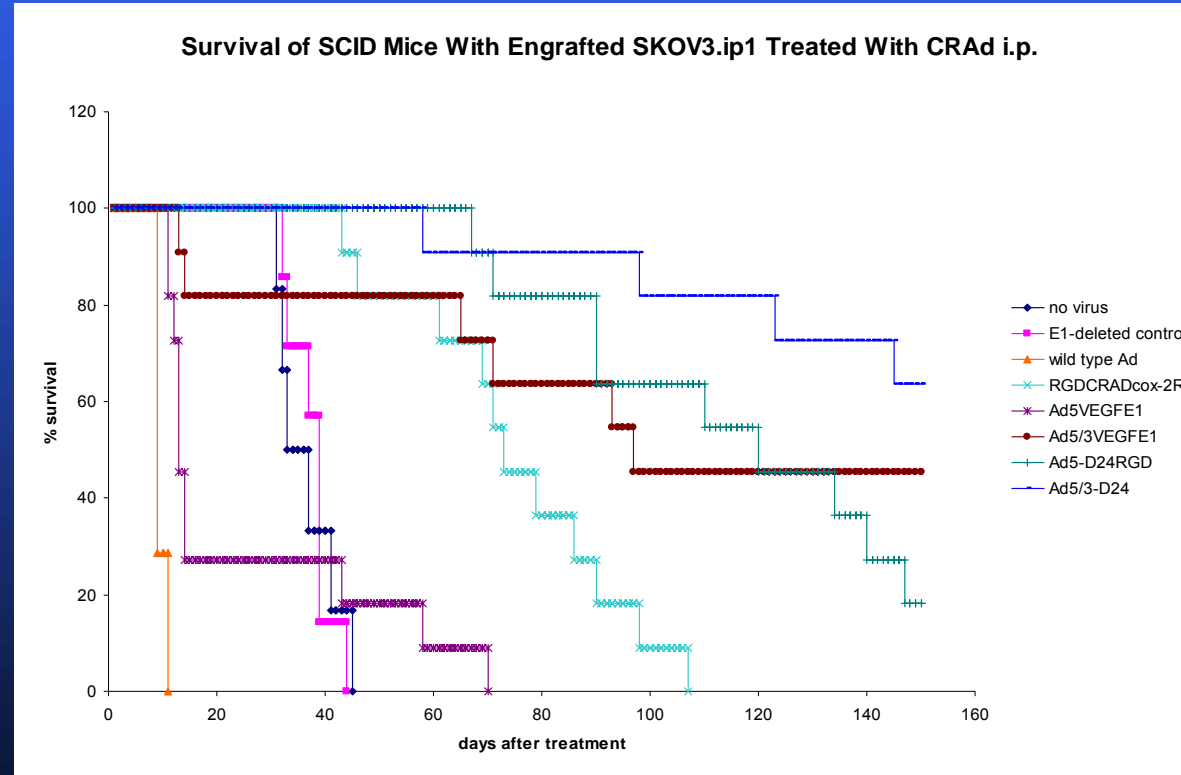
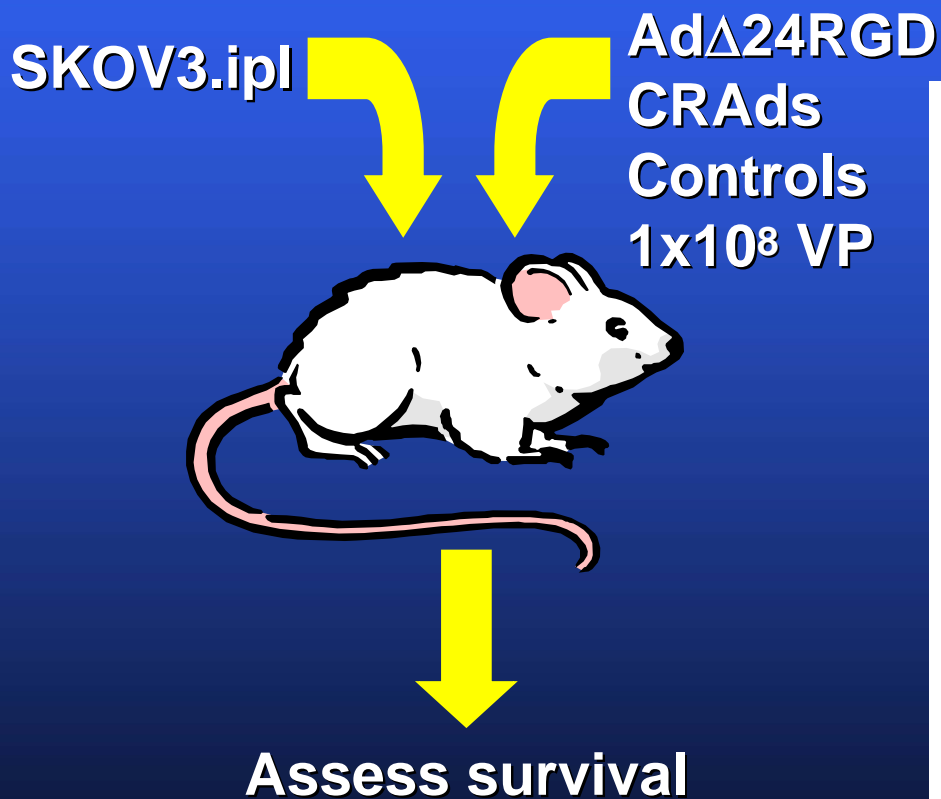
Ad5/3VEGFE1

Ad5-Δ24RGD

Ad5/3-Δ24

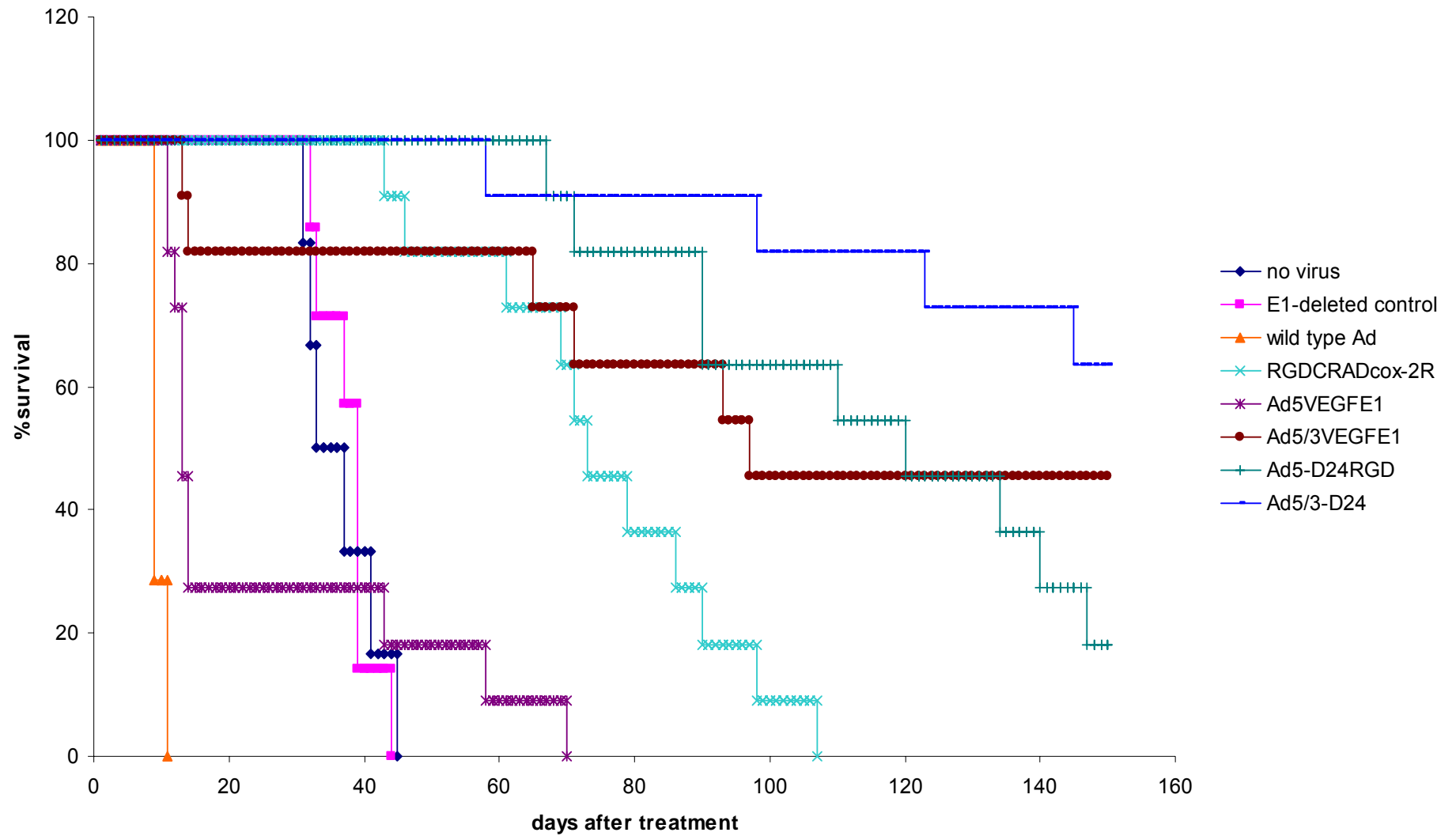
100 VP/cell  
D4,8,20

# Quantifying CRAAd Therapeutic Effect





## Survival of SCID Mice With Engrafted SKOV3.ip1 Treated With CRAd i.p.



# Summary of Preclinical Studies

- RGD modified adenoviruses infect ovarian cancer cells more efficiently than unmodified adenoviruses
- Ad5- $\Delta$ 24RGD replicates and achieves oncolysis in established and primary ovarian cancer cells in vitro
- IP Ad5- $\Delta$ 24RGD achieves a survival benefit in in vivo models of ovarian cancer

# Planned Preclinical Safety Studies Relevant to Ovarian Cancer Trial

- In vitro infectivity studies
- Biodistribution study – IP/IV rodent model
- Toxicology study – IP rodent model
- Pharmacokinetic study – IP/IV primate model
- Toxicology study – IP primate model

Vectors to be tested - Ad5- $\Delta$ 24RGD, Ad5- $\Delta$ 24,  
wtAd5-RGD, wtAd5

# Phase I Trial of IP Ad5- $\Delta$ 24RGD



Ad $\Delta$ 24.RGD



## Objectives:

1. Determine MTD and toxicity
2. Determine biologic effects
3. Determine immunologic effect

# Phase I trial of IP Ad5- $\Delta$ 24RGD



Ad $\Delta$ 24.RGD



## Eligibility:

1. Recurrent epithelial ovca
2. Disease within abdominal cavity
3. Adequate organ function, PS

# Phase I trial of IP Ad5- $\Delta$ 24RGD



Ad $\Delta$ 24.RGD



Treatment plan:

1. Vector: Ad5-  $\Delta$ 24RGD
2. Dose:  $10^8$  –  $10^{12}$  particles
3. Days/cycles: 3 days/1 cycle
4. Route: IP

# Phase I trial of IP Ad5- $\Delta$ 24RGD



Ad $\Delta$ 24.RGD



## Study Endpoints:

1. Toxicity
2. Clinical efficacy
3. Molecular analysis of peritoneal aspirates, tumor biopsies
4. Evaluation of Nab

# Addressing Concerns of Reviewers

- **Demonstrated activity of Ad5- $\Delta$ 24RGD in additional preclinical studies**
- **Expansion of planned preclinical safety studies**
- **Plan for revisions to Informed Consent document**
- **Address clinical trial design issues**



# Collaborators

## Gene Therapy Center

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## Colleagues at MD Anderson and the RAID Program