



THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

*Medical Graphics
& Photography*

Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD-4C) for Recurrent Malignant Gliomas

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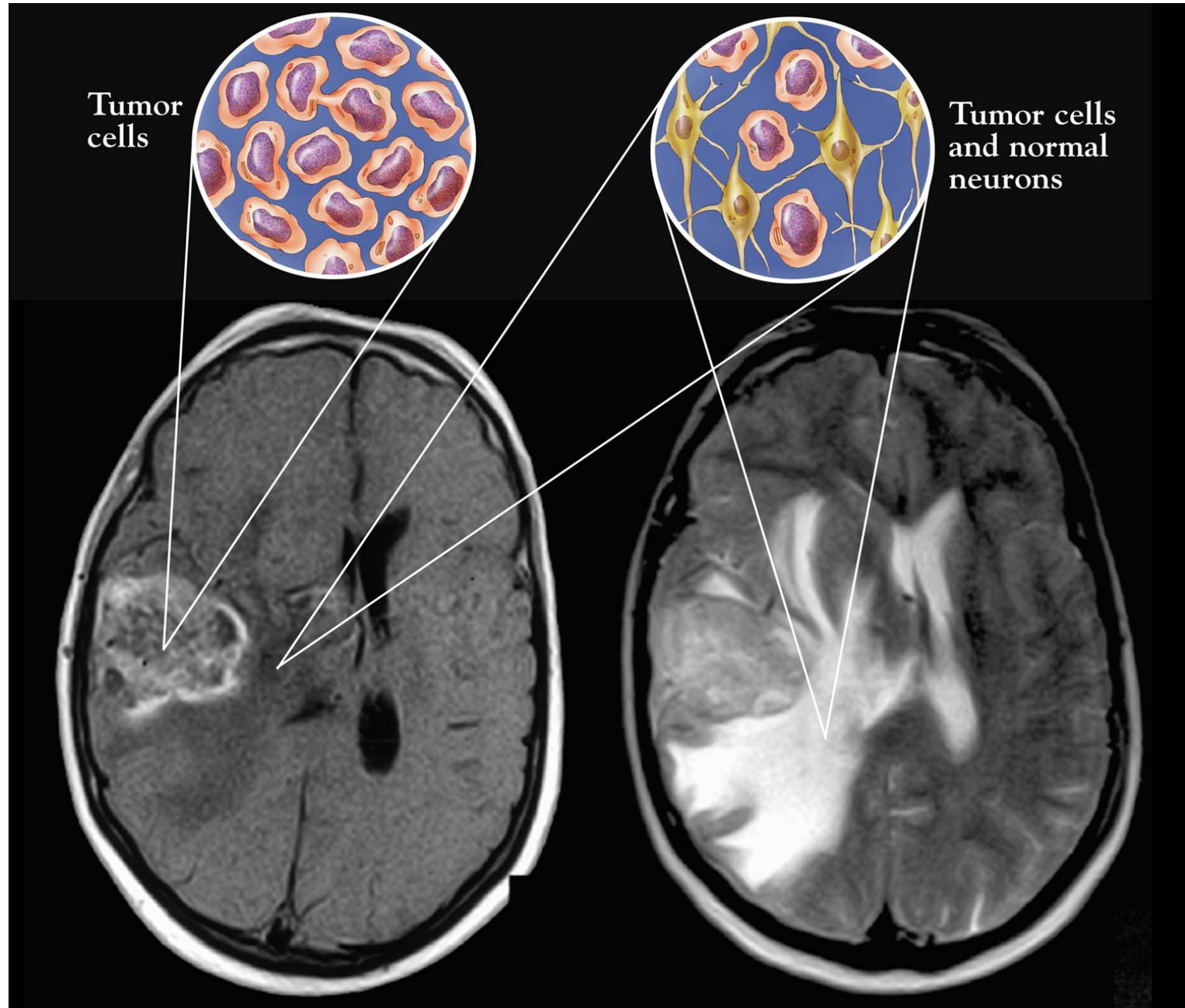
W. K. Alfred Yung, M.D. – Co-investigator

NIH Recombinant DNA Advisory Committee
Review (March 10, 2004)

Goals

- Rationale/ Background
 - General concepts: biology of gliomas
 - Experience with Ad-p53 clinical trial
- Development of Delta-24
- Enhancing tumor cell tropism: RGD-4C
- Clinical trial

Glioblastoma Multiforme



Molecular Alterations in Gliomas

Self-sufficiency in growth signals

- EGF/EGFR
- PDGF/PDGFR
- Ras

Sustained Angiogenesis

- VEGF/VEGFR
- bFGF
- HIF-1a
- EGF/EGFR

Evading apoptosis

- p53 mutation
- bcl-2
- PI3 Kinase-AKT
- PTEN
- c-Myc

Limitless Replicative Potential

- Telomerase reactivation

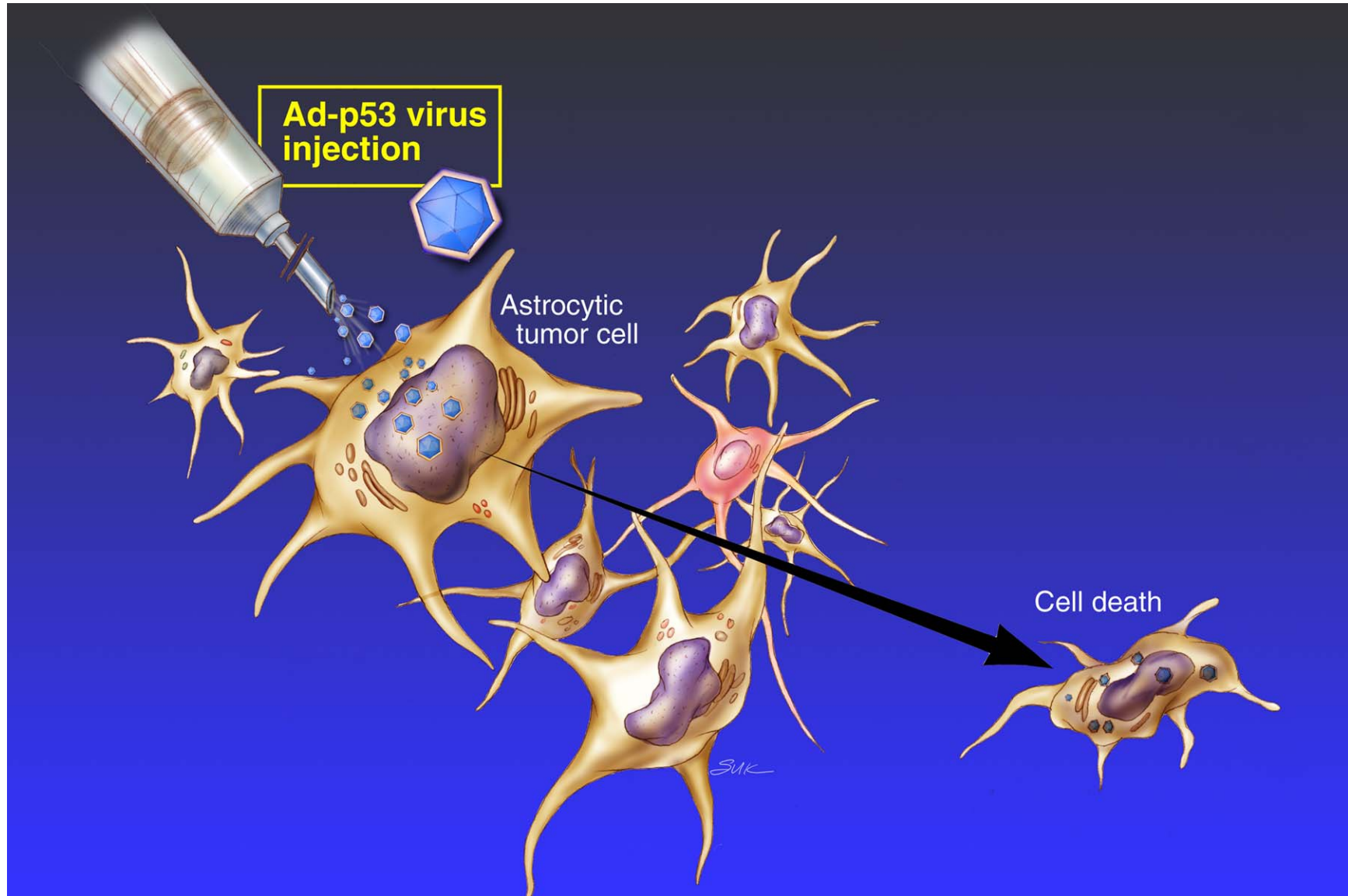
Insensitivity to Growth inhibitory signals

- pRb
- p16
- TGF β

Tissue Invasion

- Matrix Metalloproteases

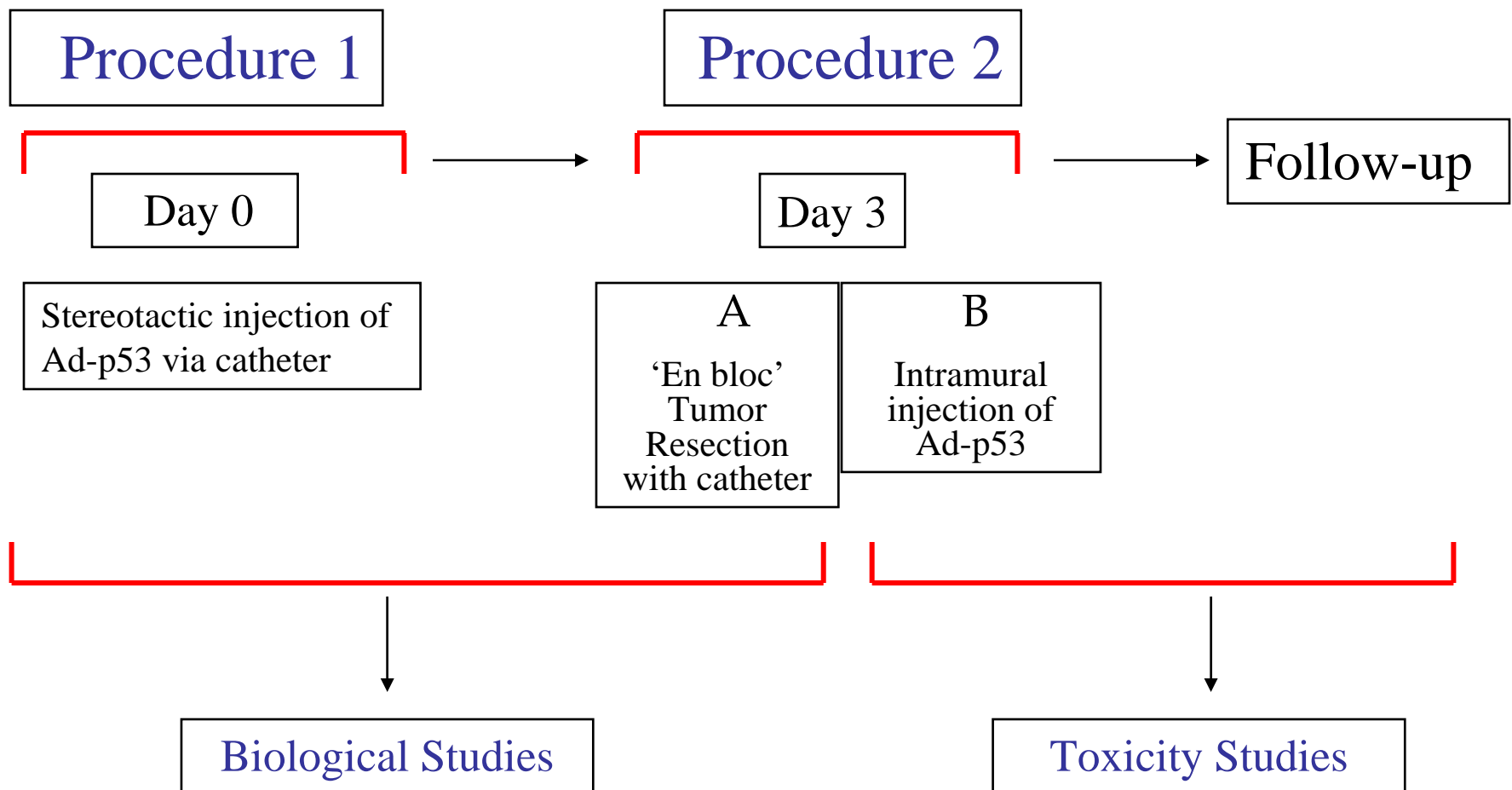
Replication Incompetent Adenoviral Vectors



Ad-p53 Trial: Objectives

- To determine the qualitative and quantitative **toxicity** of Ad-p53 administered by intratumoral injection.
- To determine the **maximum tolerated dose** (MTD) of Ad-p53 administered by intratumoral injection in patients with recurrent malignant gliomas.
- To determine the **biological effects** at the molecular level of intratumoral administration of replication-deficient adenovirus vector containing wild-type p53 gene (Ad-p53) in human malignant gliomas by analyzing the expression and distribution of exogenous p53 protein.

Ad-p53 Clinical Trial for Recurrent Malignant Gliomas: Treatment Schematic



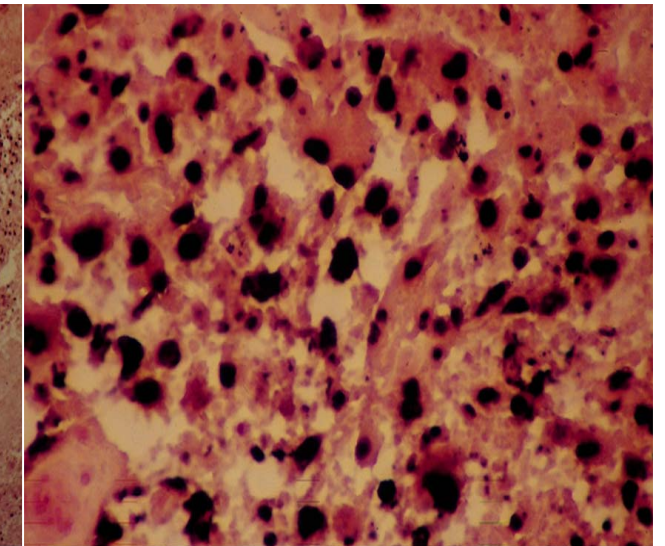
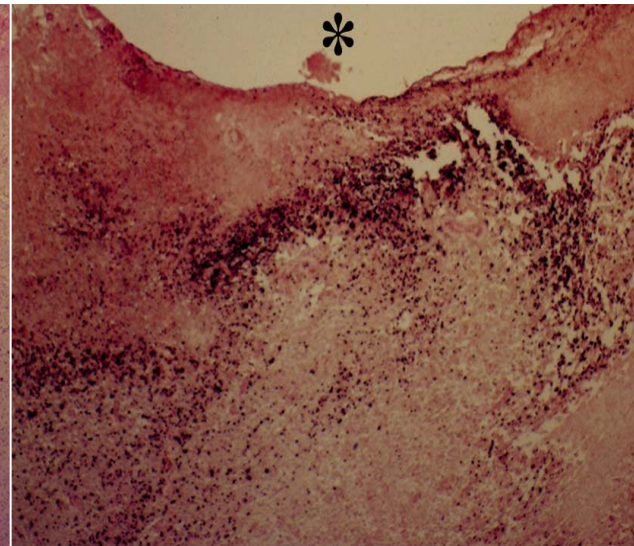
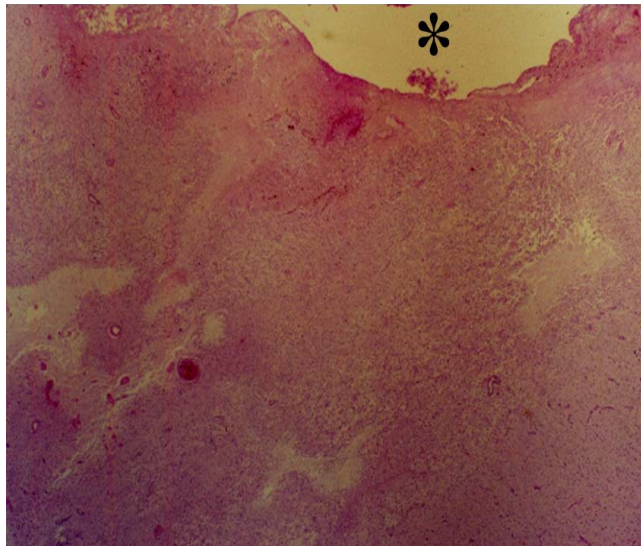
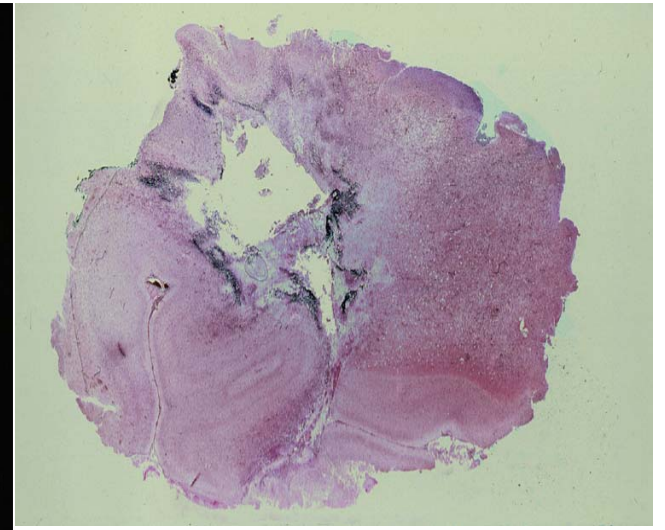
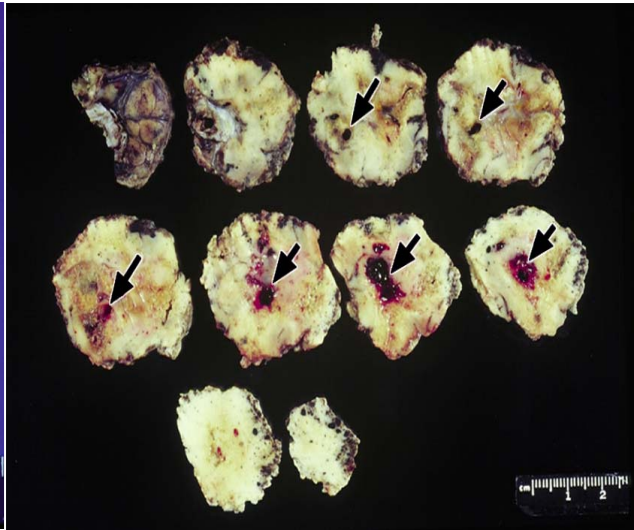
Clinical Studies: Toxicity

Table 3. Nonsurgical Adverse Events¹

Adverse Event	Grade	Relationship to Treatment		Total
		Possible (No. of events)	Probable/definite (No. of events)	
CNS hemorrhage	3	1	0	1
Confusion	2	1	0	1
Fatigue	1	5	0	5
	2	1	1	2
Fever without neutropenia	1	3	1	4
Granulocytopenia	1	1	0	1
Headache	1	7	1	8
	2	2	0	2
	3	1	0	1
Leukopenia	1	2	0	2
Motor dysfunction	2	1	0	1
Nausea alone	1	2	0	2
Pyramidal tract dysfunction	2	1	0	1
Seizure	2	2	0	2
	4	1	0	1
Speech impairment	1	1	0	1
	2	3	0	3
	3	2	0	2
Viral-like syndrome	2	0	1	1
Vomiting	1	3	0	3
	2	1	0	1
Total		41	4	45

¹ Results are reported as independent events. More than one event may have occurred per patient or the same event may have occurred more than once in a given patient.

Ad-p53 Phase I Trial: Biological Studies



Biological Studies: Summary

Table 2. Biological Analysis

Patient No.	Dose Level	Pretreatment biopsy		Post injection specimen					
		p53 IHC	p53 mutational status ¹	p53 IHC				p21 IHC	TUNEL
				Nuclear staining	Cytoplasmic staining	Hypoplastic vessels	Maximum Distance (mm)	Nuclear	
1	I	Rare	ND	+	-	-	4.0	+	ND
2	I	+	Wt	+	-	-	5.5	+	+
3	I	-	Wt	+	-	-	5.0	ND	ND
4	II	ND	ND	+	-	-	4.5	+	ND
5	II	-	Wt	+	+	-	5.0	+	+
6	II	ND	ND	NE	NE	NE	NE	NE	ND
7	III	-	Wt	+	-	-	5.0	+	ND
8	III	-	Wt	+	+	-	6.0	-	+
9	III	-	Wt	+	+	-	5.0	+	+
13	IV	ND	ND	NE	NE	NE	NE	NE	ND
14	IV	-	Wt	+	-	-	1.0*	+	ND
15	IV	ND	ND	+	+	Rare	8.0	ND	ND

Abbreviations: IHC, immunohistochemistry; ND, not done; Wt, wild-type; NE, not evaluable; * Intracystic injection

¹ Patients 10, 11, 12 (see Table 1) were also analyzed for p53 mutation use the pretreatment biopsy specimen. Mutations were identified in patient 10 [codon 234 (tyr/stop) and codon 157 (val/phe)] and patient 11 [codon 248 (Arg/Gln)].

Ad-p53 Clinical Trial: Lessons Learned

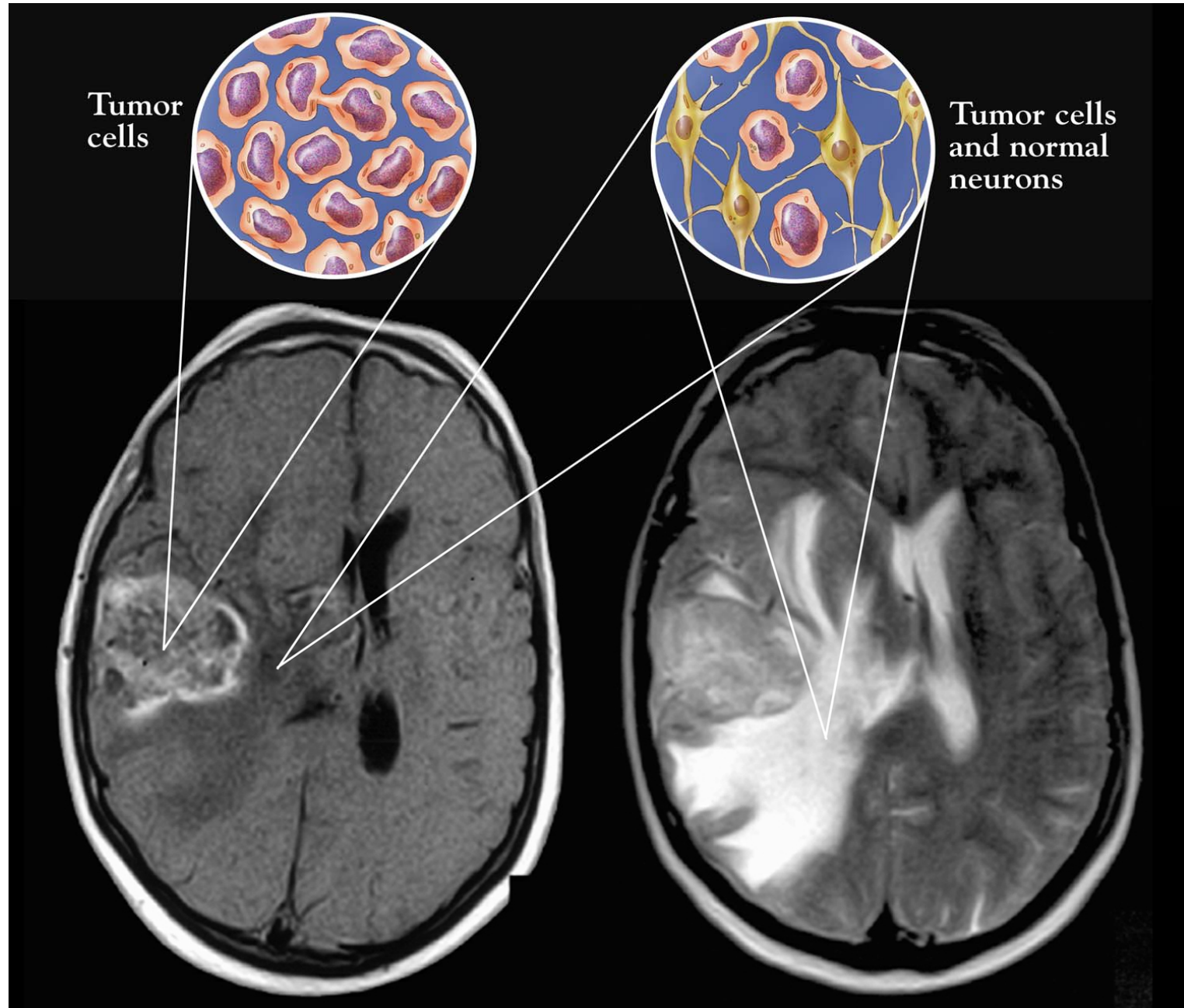
- No significant toxicities were observed
- Maximum Tolerated Dose (MTD) was reached at 1×10^{12} viral particles
- Two-stage design was well tolerated and without complications
- No Ad-p53 virus was detected systemically
 - (blood, urine, sputum, or feces)

Ad-p53 Clinical Trial: Lessons Learned

Selectivity: Ad-p53 can infect and transduce exogenous p53 in both normal and tumor cells.

Delivery: With the injection technique used, p53 distribution is limited to 5-6 mm from injection site.

Glioblastoma Multiforme

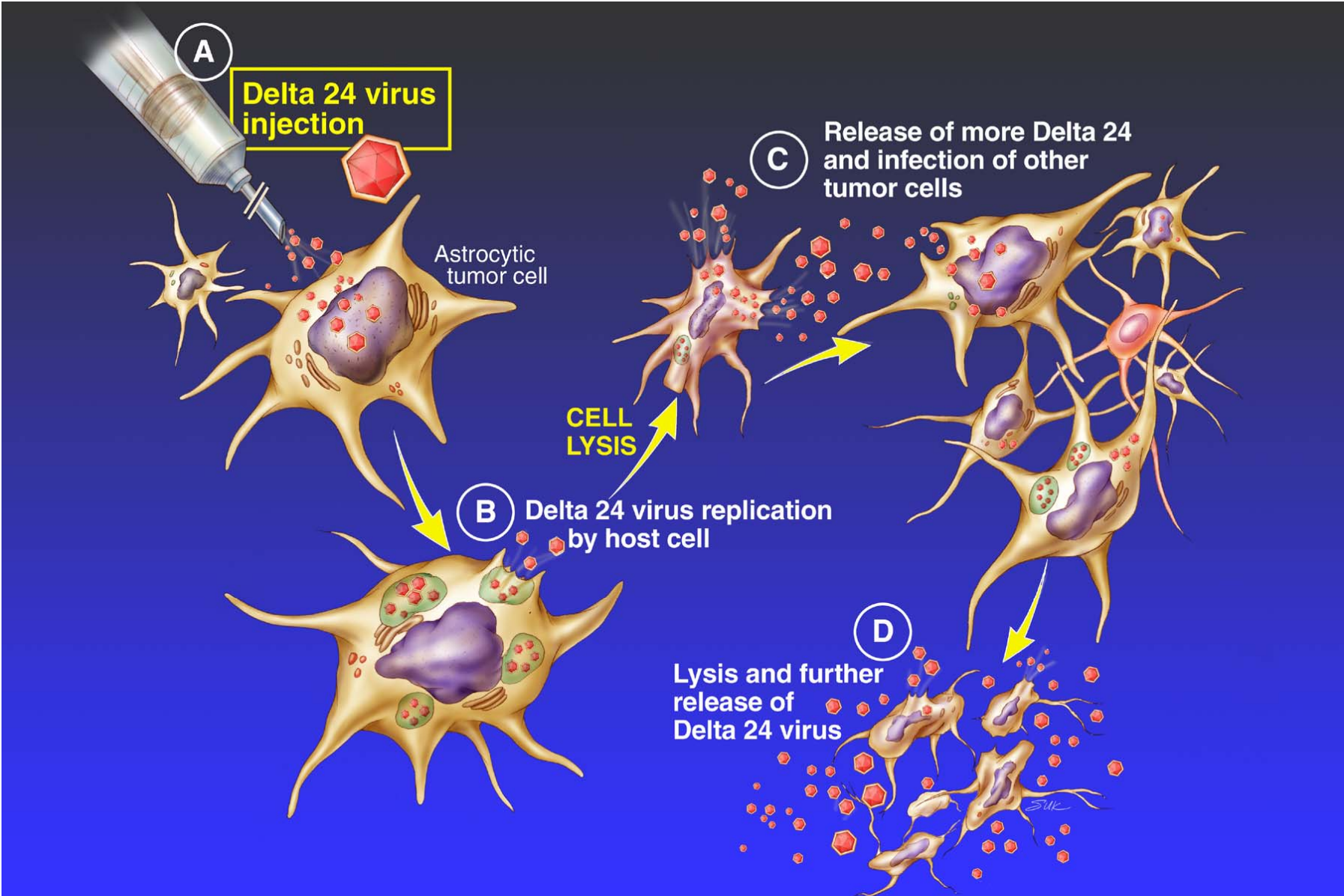


Conditionally Replication-Competent Adenoviruses

- **dl1520 (ONYX-015)**
 - Contains a deletion in E1b 55kD gene
 - Replication depends on p53 status

- **Delta-24**
 - Contains a partial deletion in E1a gene
 - Replication depends on Rb/p16 status of the cell

Replication Competent Adenovirus

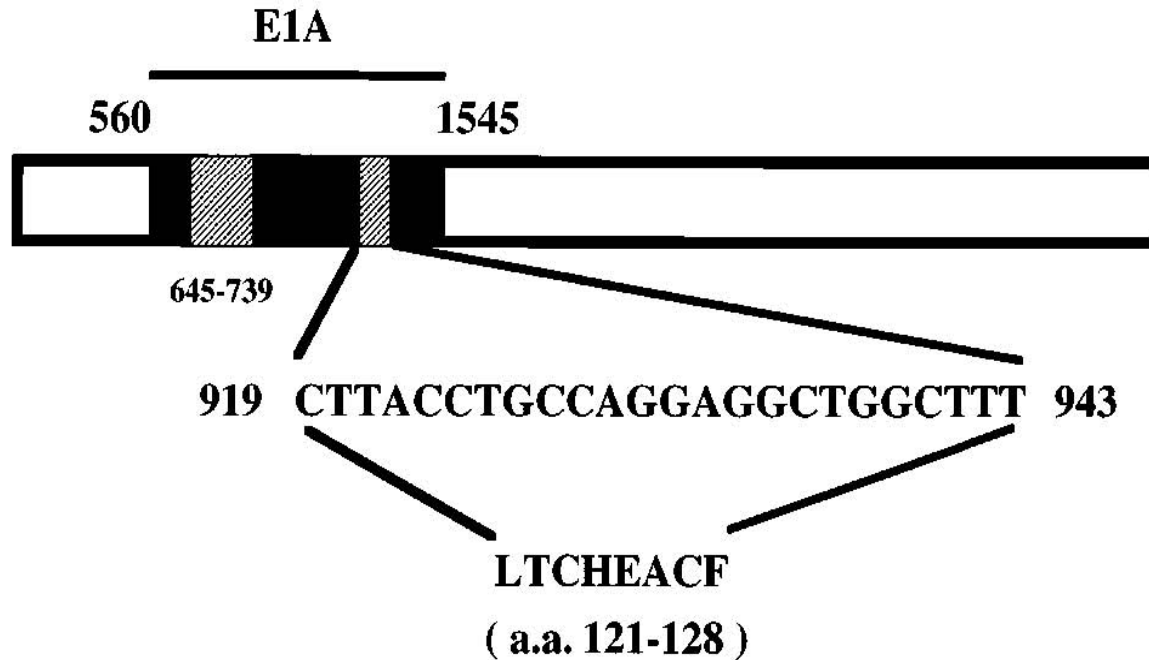


**A mutant oncolytic adenovirus targeting
the Rb pathway produces anti-glioma
effect in vivo**

**Fueyo J, Gomez-Manzano C, Alemany R, Lee PS,
McDonnell TJ, Mitlianga P, Shi YX, Levin VA,
Yung WK, Kyritsis AP**

Oncogene. 2000;19:2-12

Construction of Delta-24

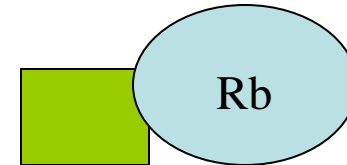
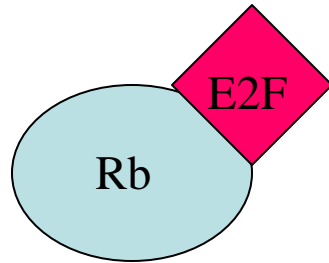


Delta-24 has a deletion of 24 DNA base pairs within the E1A gene. This deletion renders the E1A protein incapable of binding to and inactivating Rb

(Oncogene. 2000;19:2-12)

Selective Mechanism of Delta-24

Normal Cell



Free E2F

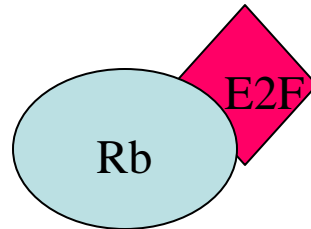
Forced Cell Cycle Entry (S-phase)

Cell cycle Control

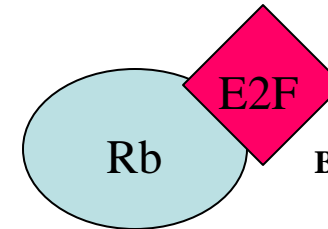
Normal Cell



Delta-24 E1a



Cell cycle Control



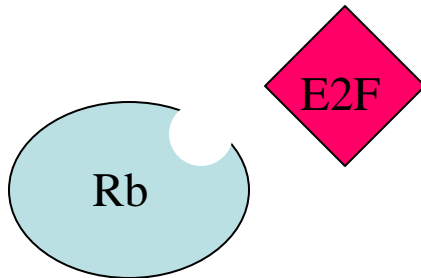
Controlled Cell cycle

Bound E2F

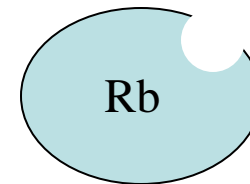
Tumor Cell



Delta-24 E1a



Uncontrolled Cell Cycle

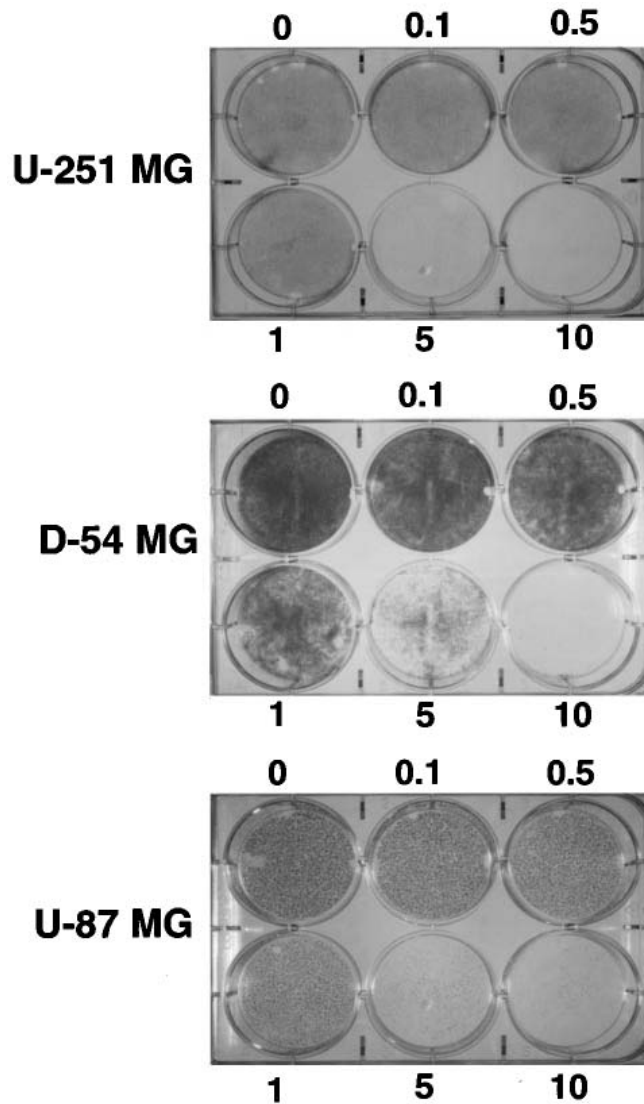


Uncontrolled Cell Cycle (S-phase)



Free E2F

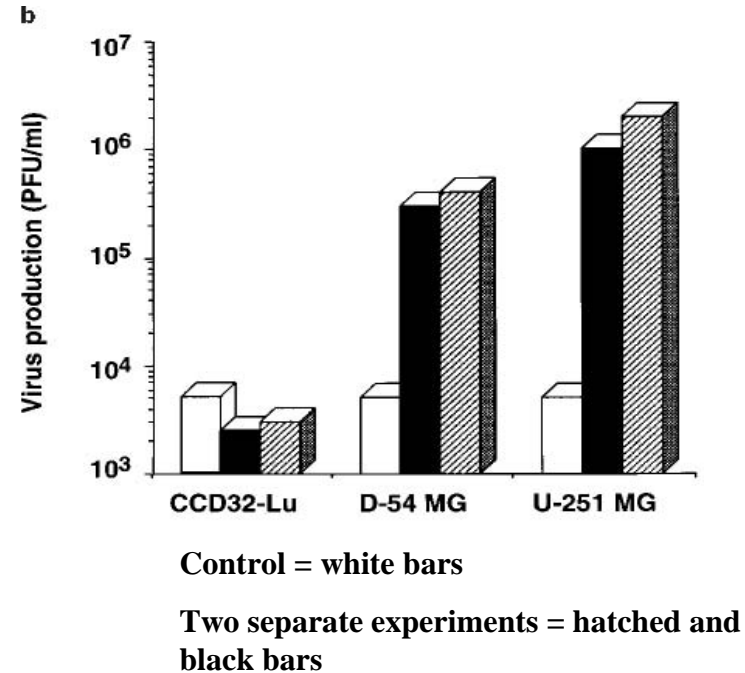
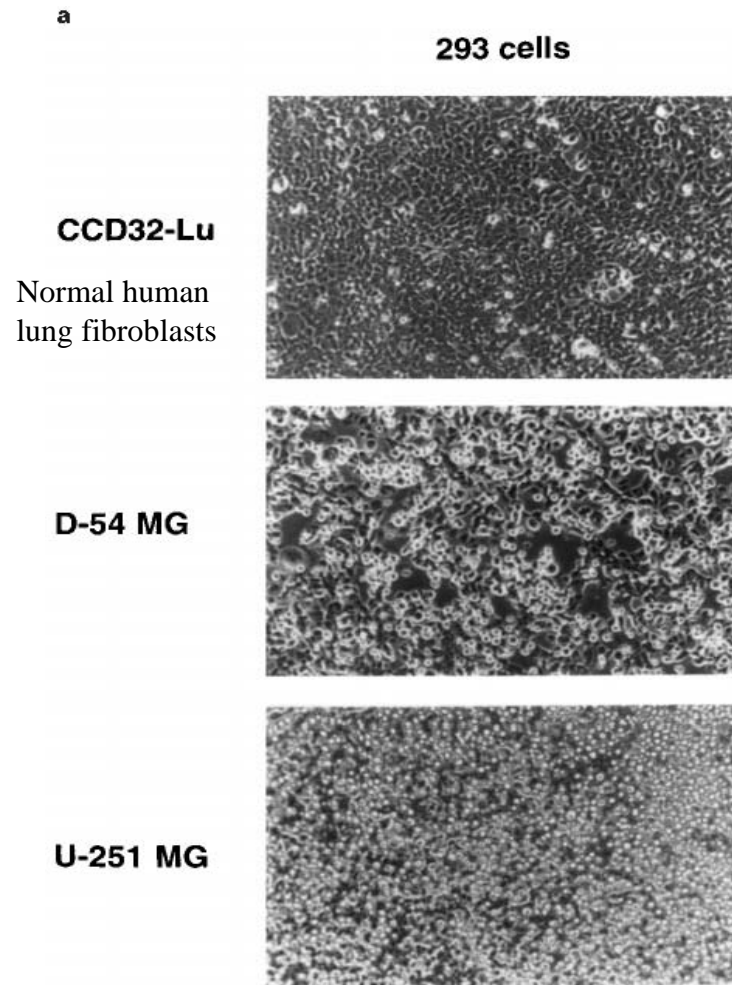
Delta-24 Effect: In Vitro Studies



**Crystal violet staining of
cell culture plates
demonstrating
“cytopathic effect” (CPE)**

**Glioma cell lines are permissive to
replication of Delta-24 and are
therefore killed (lysed) by this agent**

Delta-24 Effect: Normal Vs. Tumor



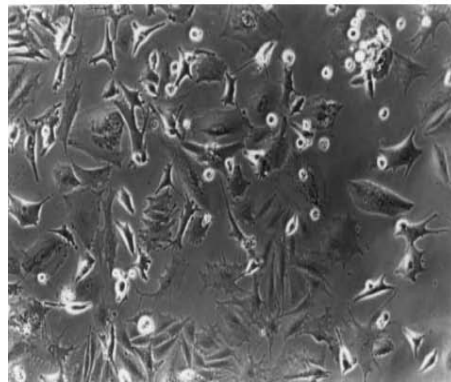
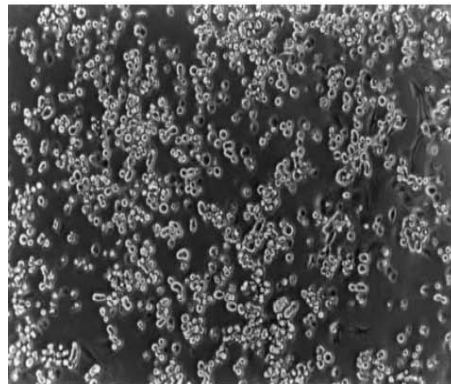
Titration values of virus progeny produced from the infection of Delta-24 in three cell lines

Infection of 293 cells with the supernatant from infection cell lines

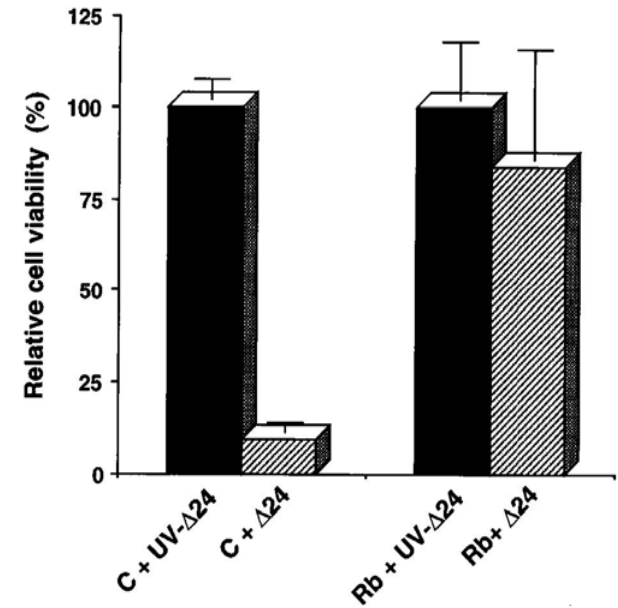
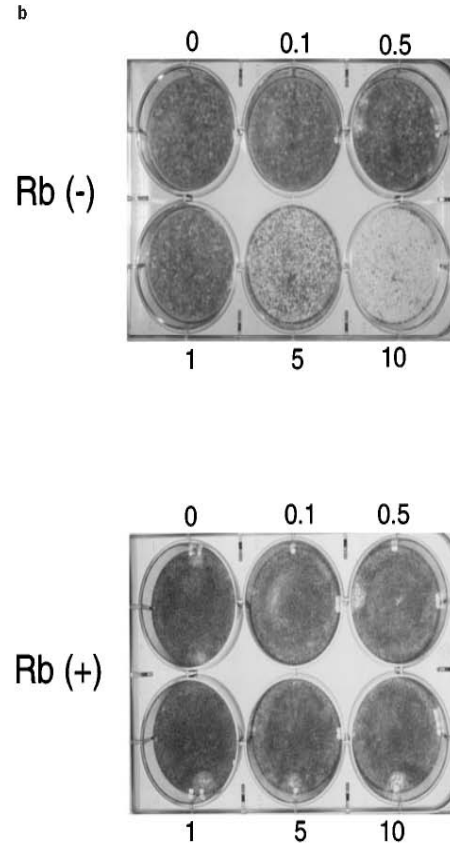
Oncogene. 2000;19:2-12

Delta-24 Effect: Rescue by Rb

Treatment with either
Ad5CMV-pA or Ad5CMV-Rb



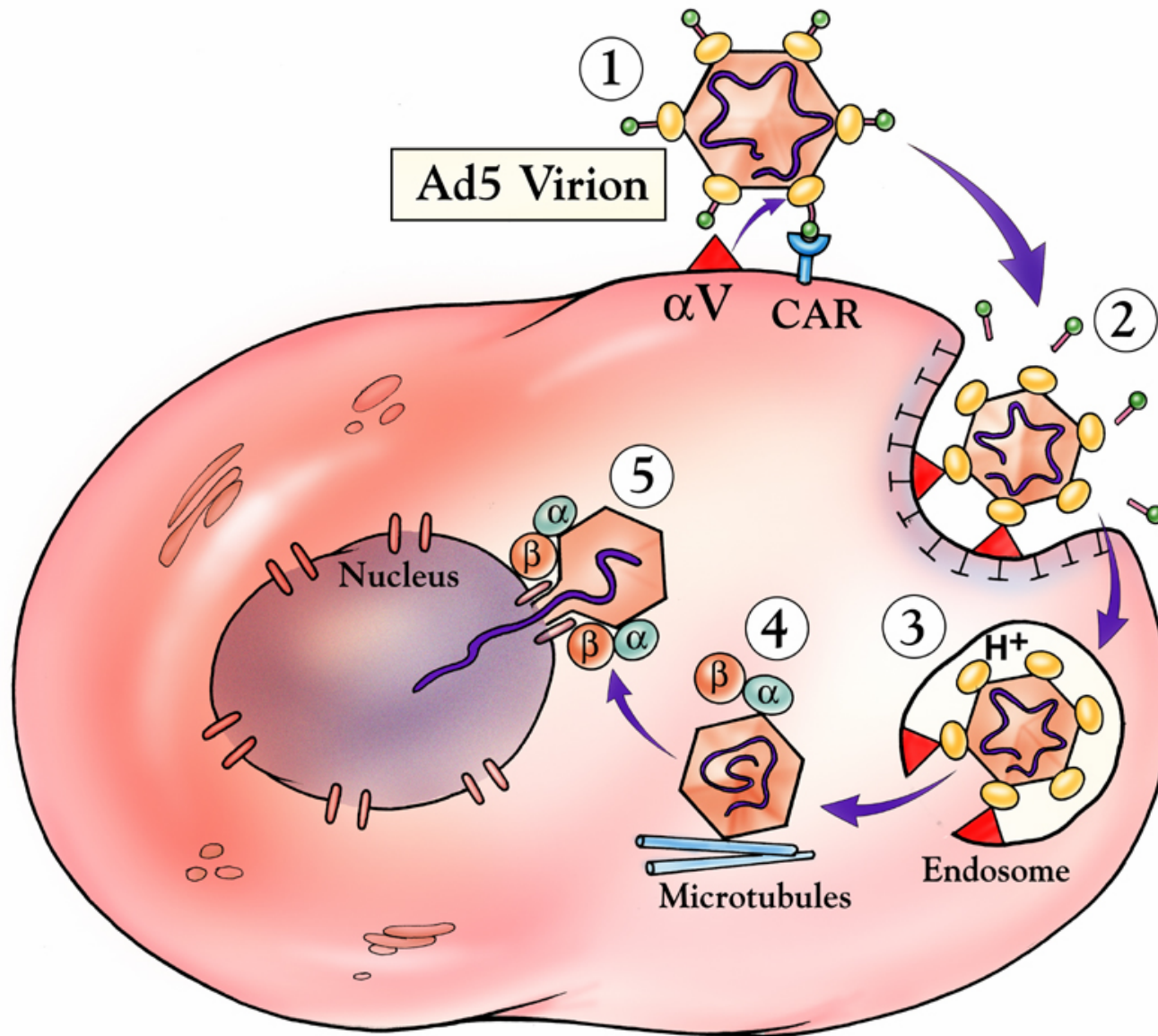
Saos-2 cells



Protection against cell lysis in Rb+ cells

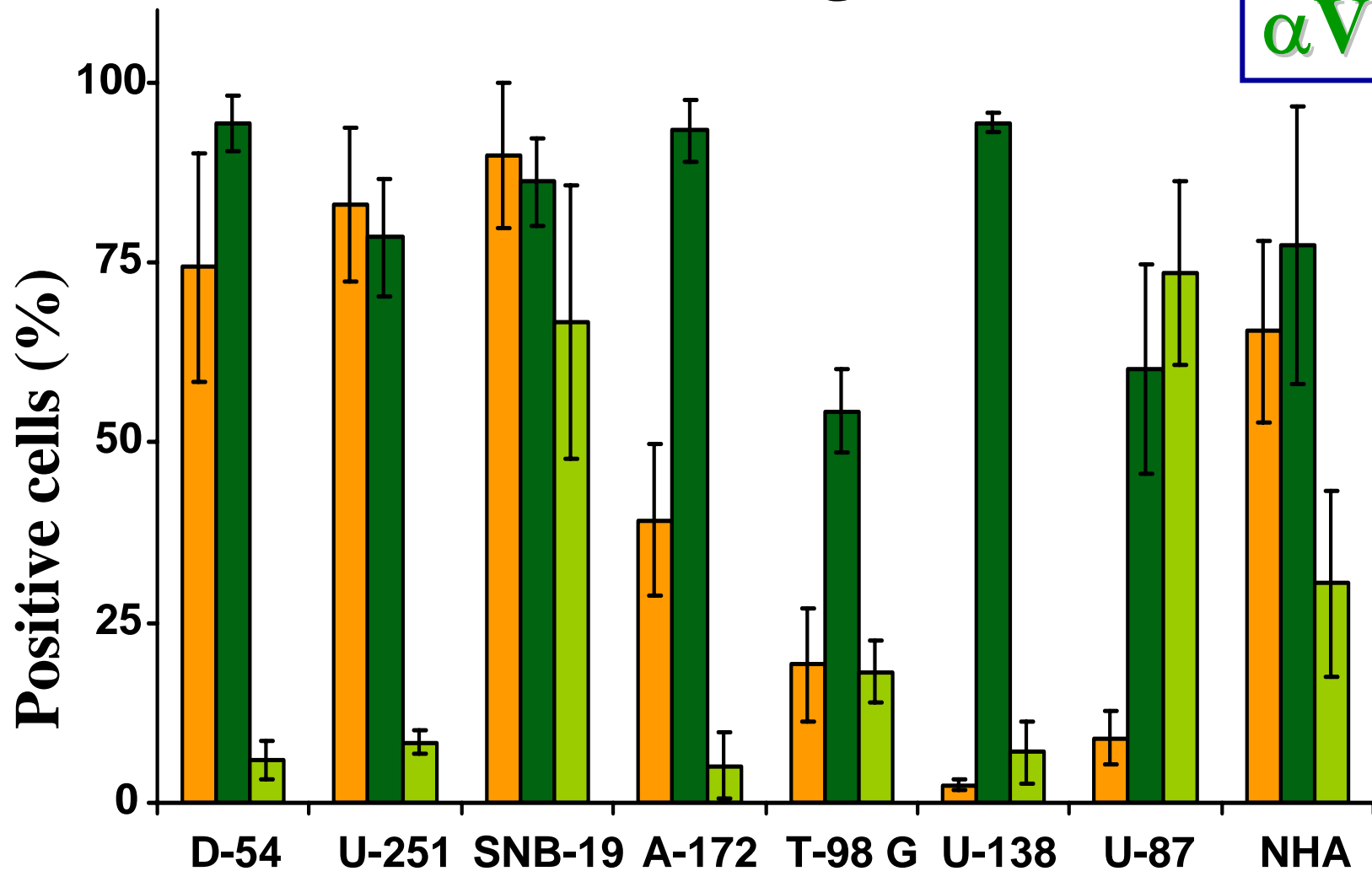
Oncogene. 2000;19:2-12

Adenovirus Life Cycle



Cell Surface Expression of CAR and αV Integrins

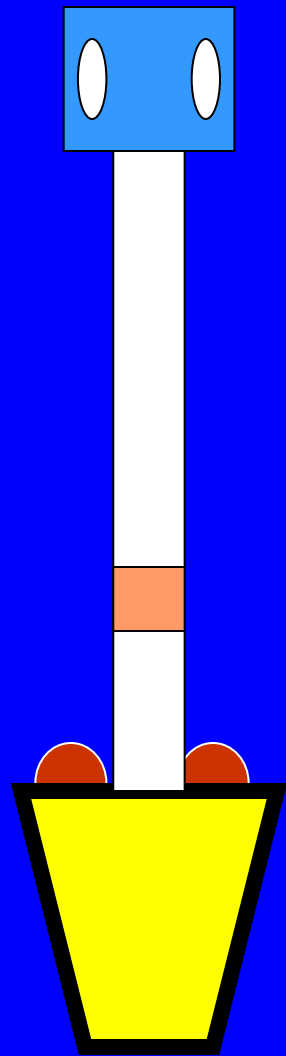
CAR
 $\alpha V\beta 5$
 $\alpha V\beta 3$



A Conditionally Replicative Adenovirus with Enhanced Infectivity Shows Improved Oncolytic Potency

**Kaori Suzuki, Juan Fueyo, Victor Krasnykh,
Paul N. Reynolds, David T. Curiel
and Ramon Alemany**

Clin Cancer Res. 2001;7:120-6.



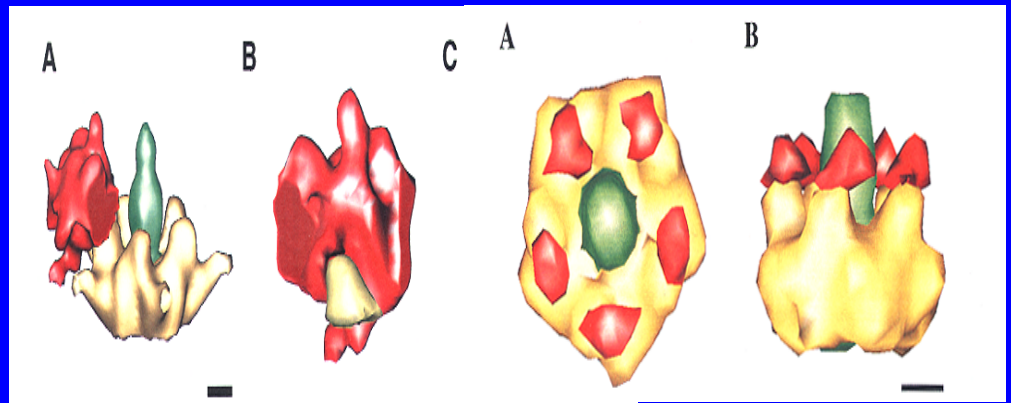
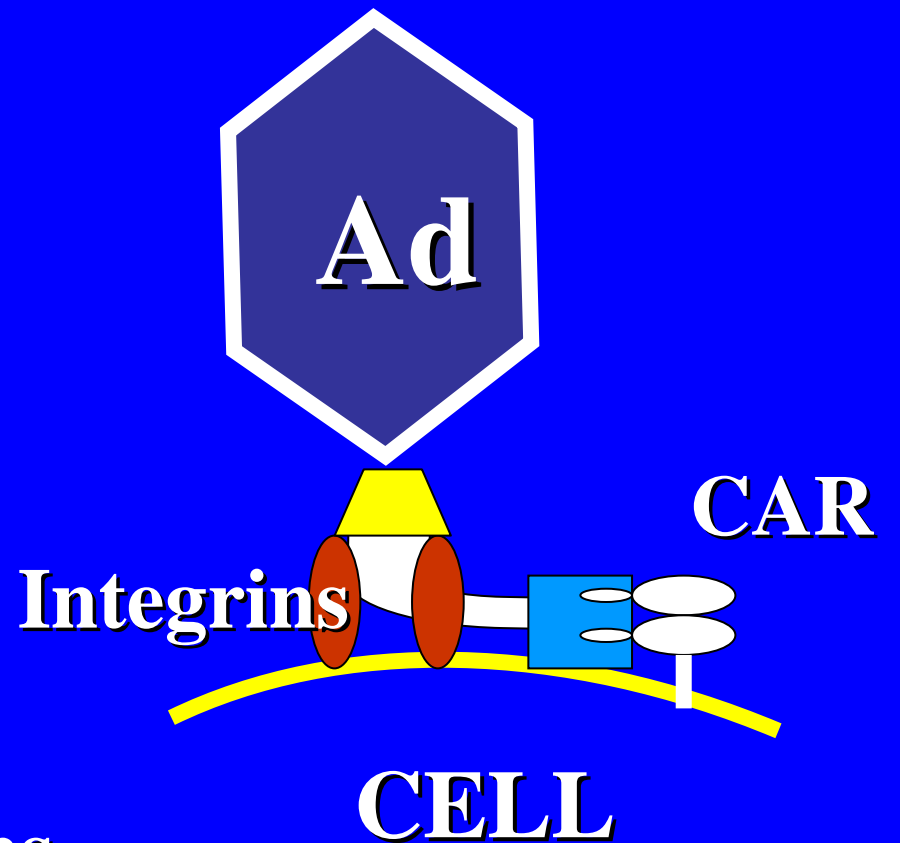
Knob

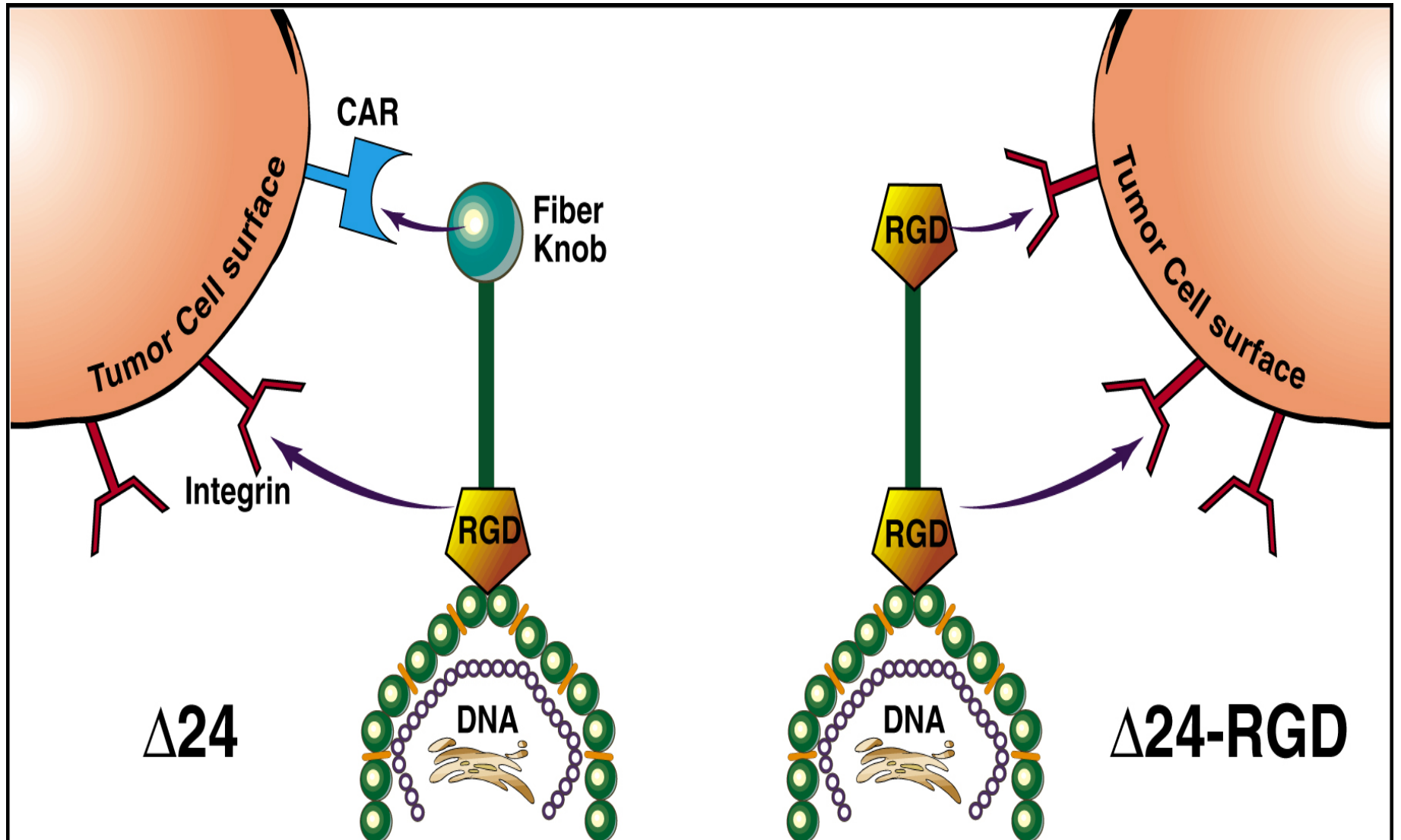
Shaft

Kink

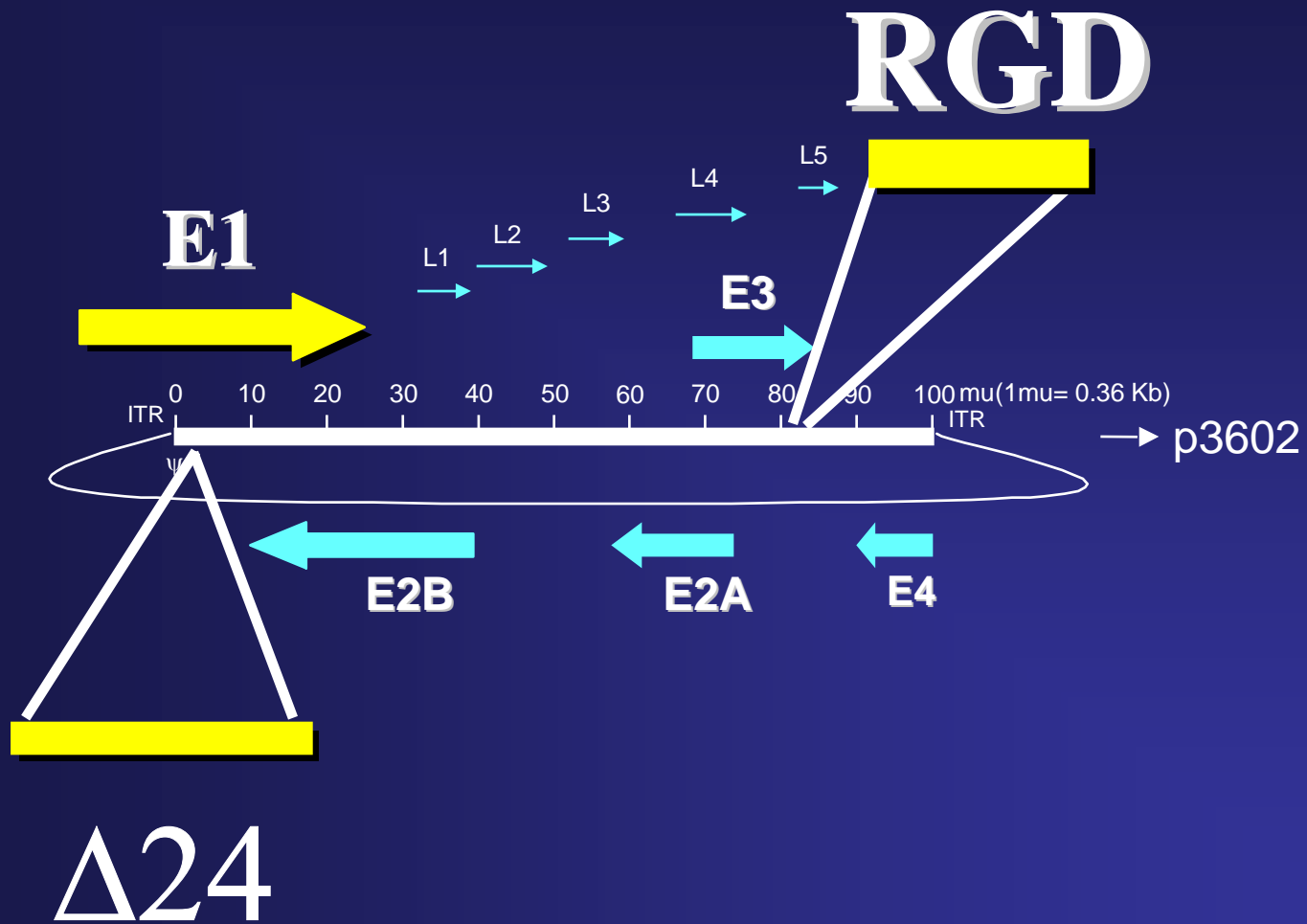
RGD protrusions

Penton base





$\Delta 24$ -RGD Adenovirus

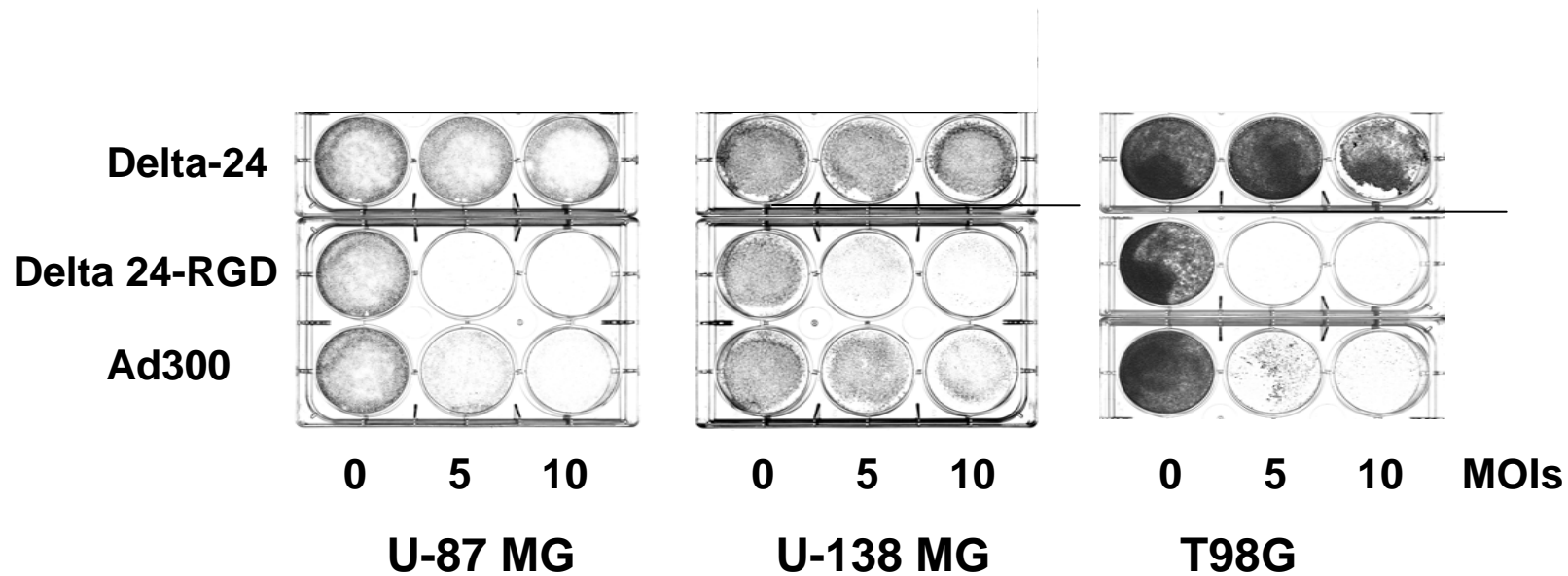


Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway

**Fueyo J, Alemany R, Gomez-Manzano C,
Fuller GN, Khan A, Conrad CA, Liu TJ,
Jiang H, Lemoine MG, Suzuki K, Sawaya R,
Curiel DT, Yung WK, Lang FF.**

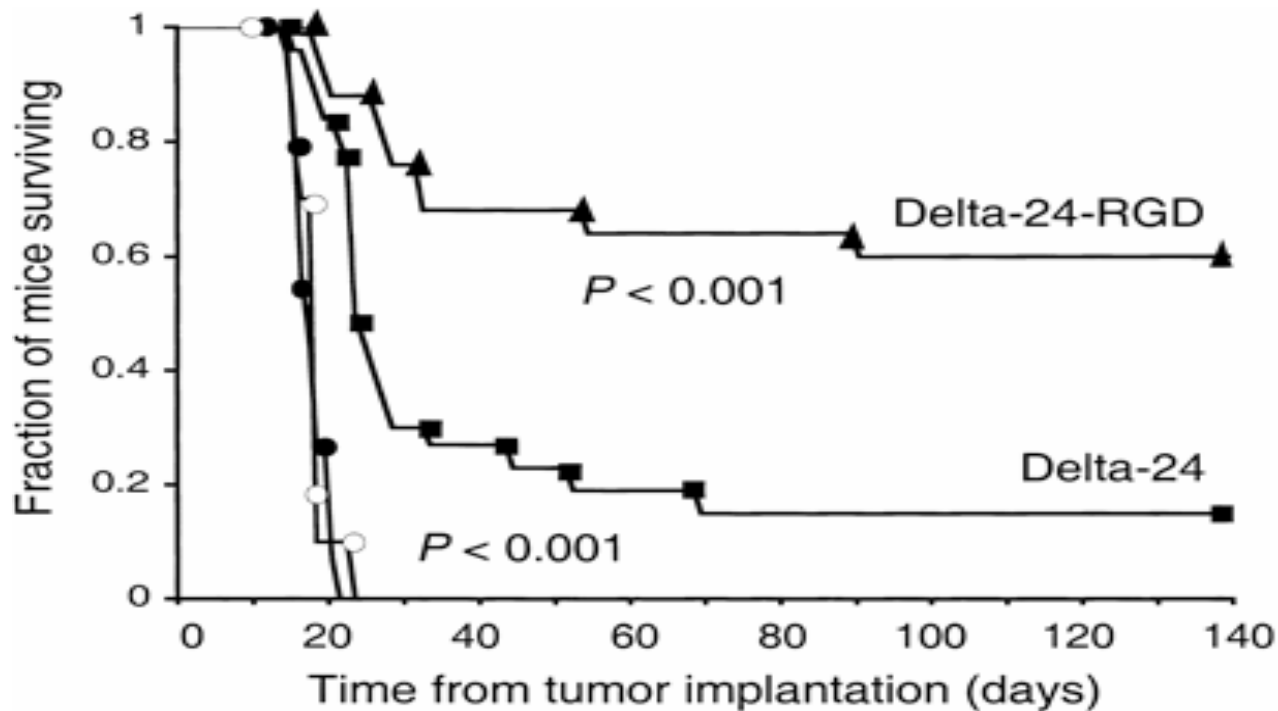
J Natl Cancer Inst. 2003;95:652-60.

Delta-24 Vs. Delta-24-RGD: Effect of Low CAR Expression

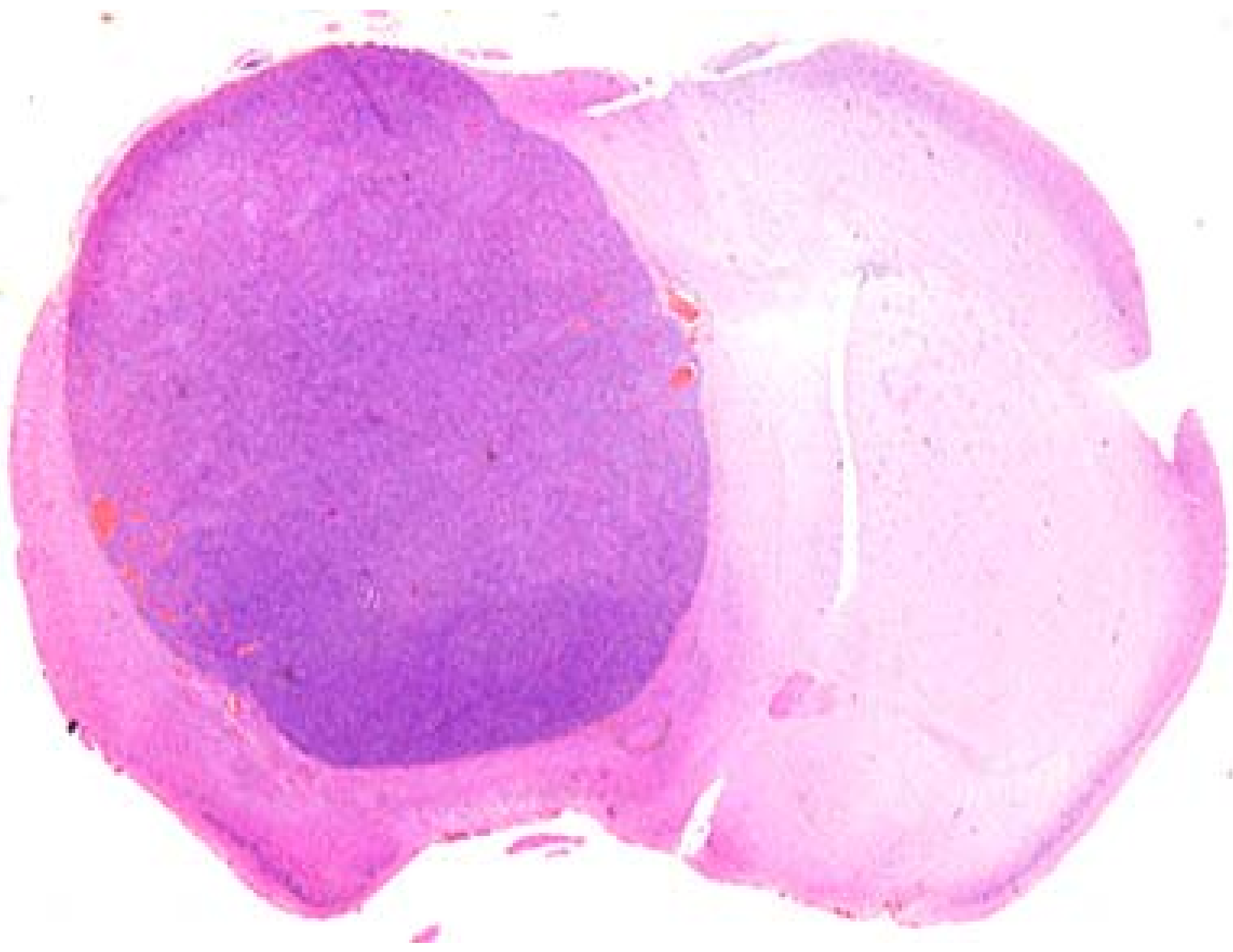


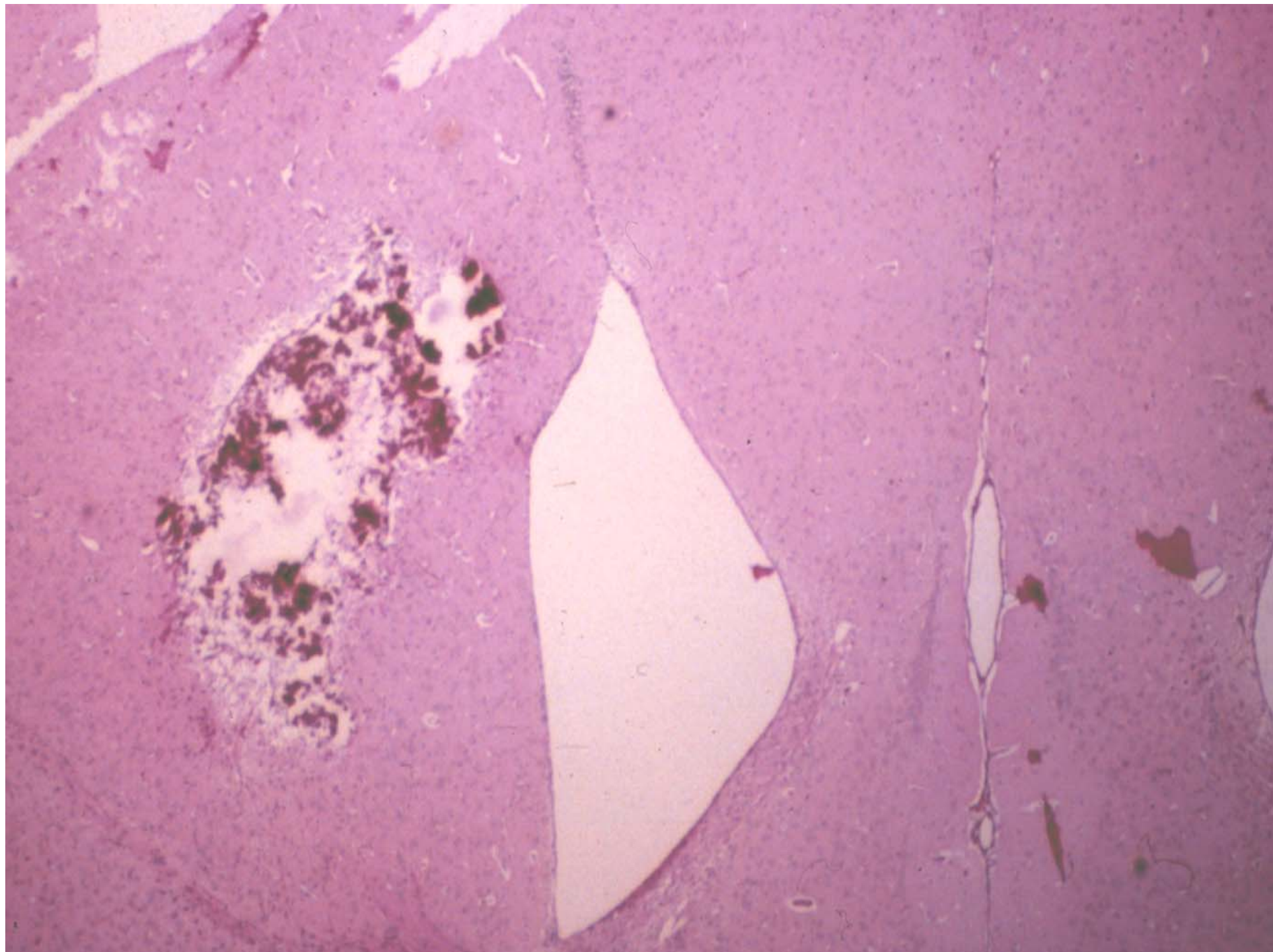
Low CAR expression

Kaplan-Meier Survival Curves for Delta-24 and Delta-24-RGD

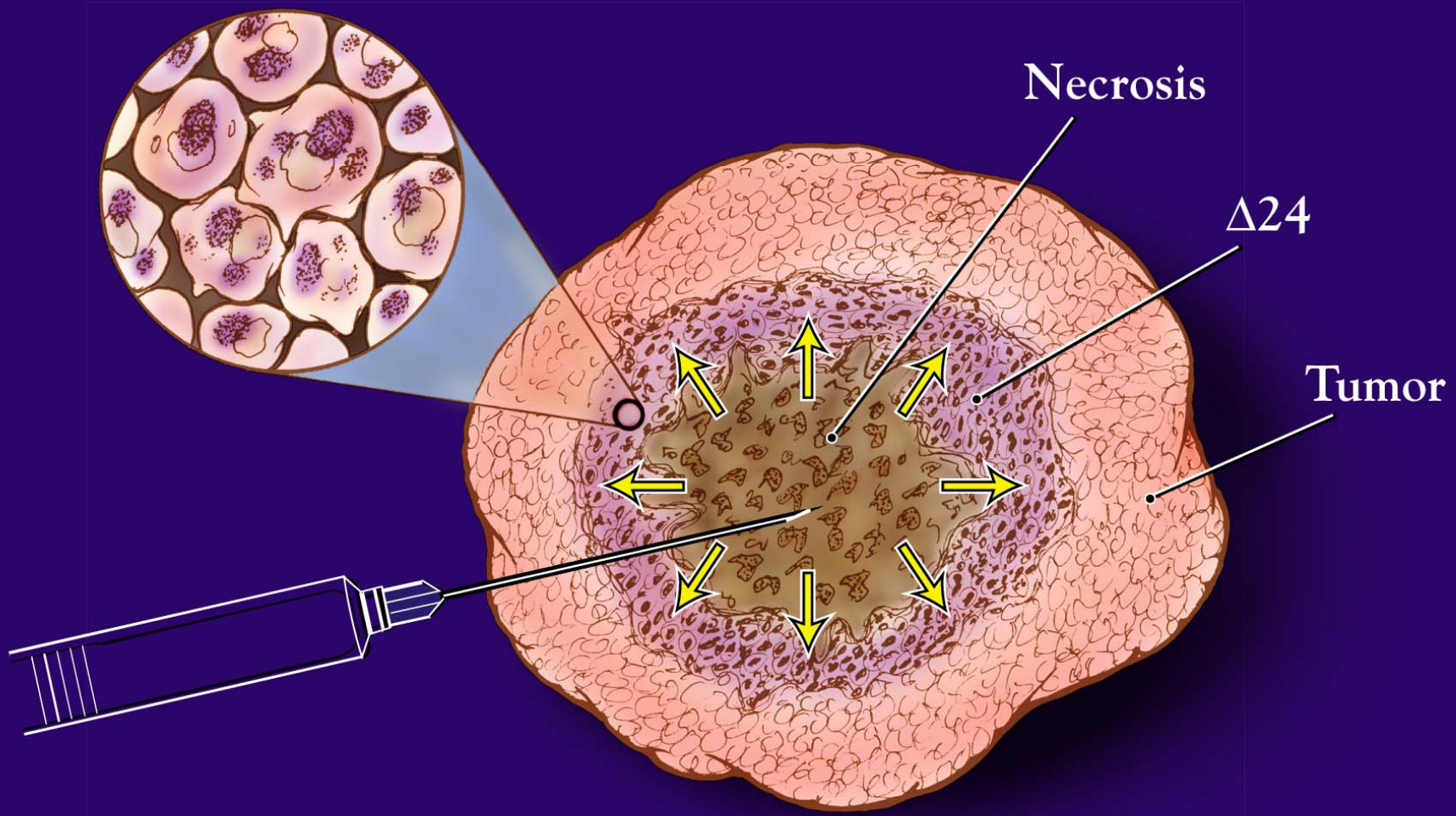


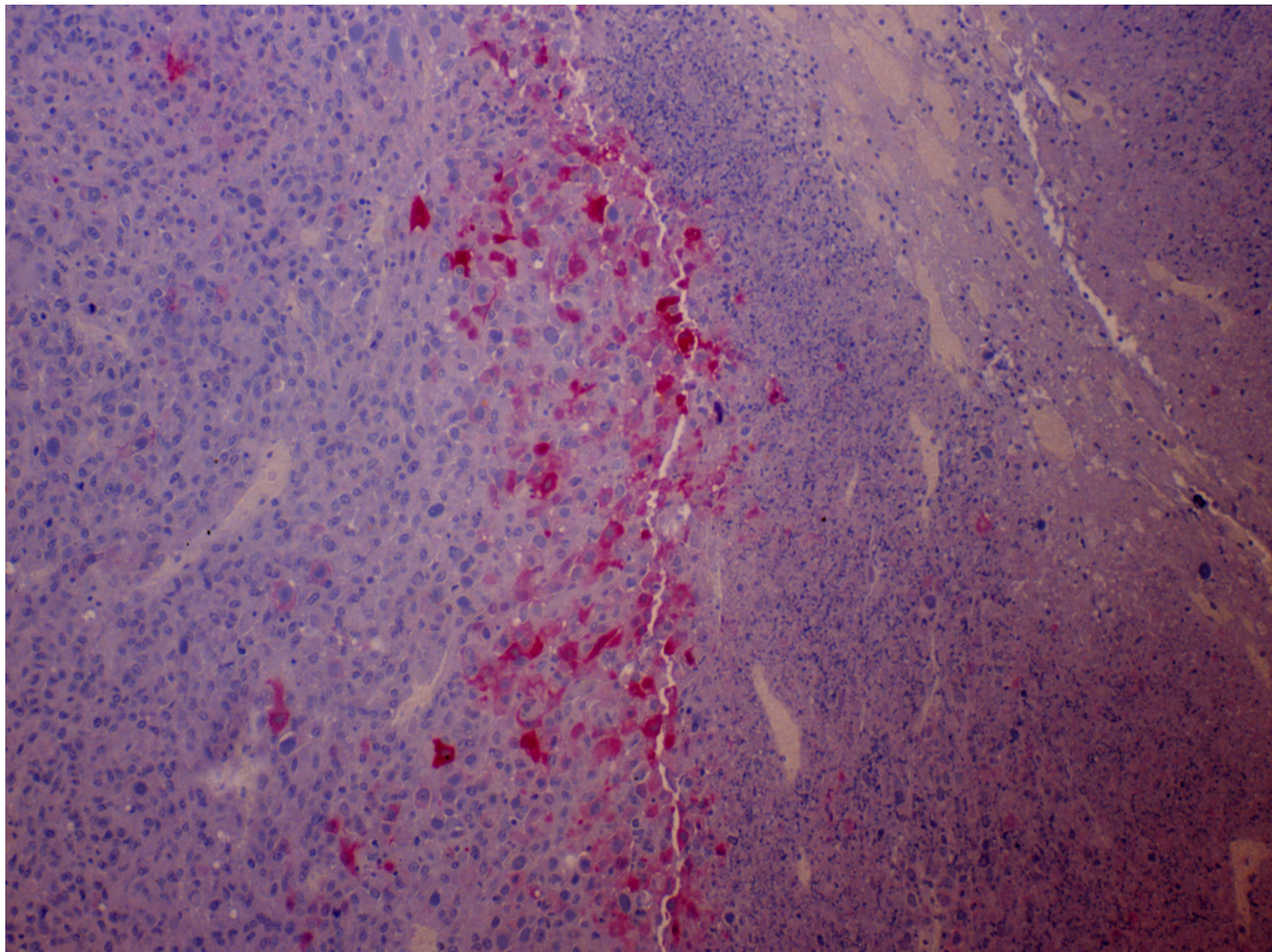
Treatment	n	mean (days)	95% CI (days)
Delta-24-RGD	25	131	100 to 162
Delta-24	26	50	30 to 70
UV-i Delta-24-RGD	26	18	18 to 19
PBS	20	19	18 to 20



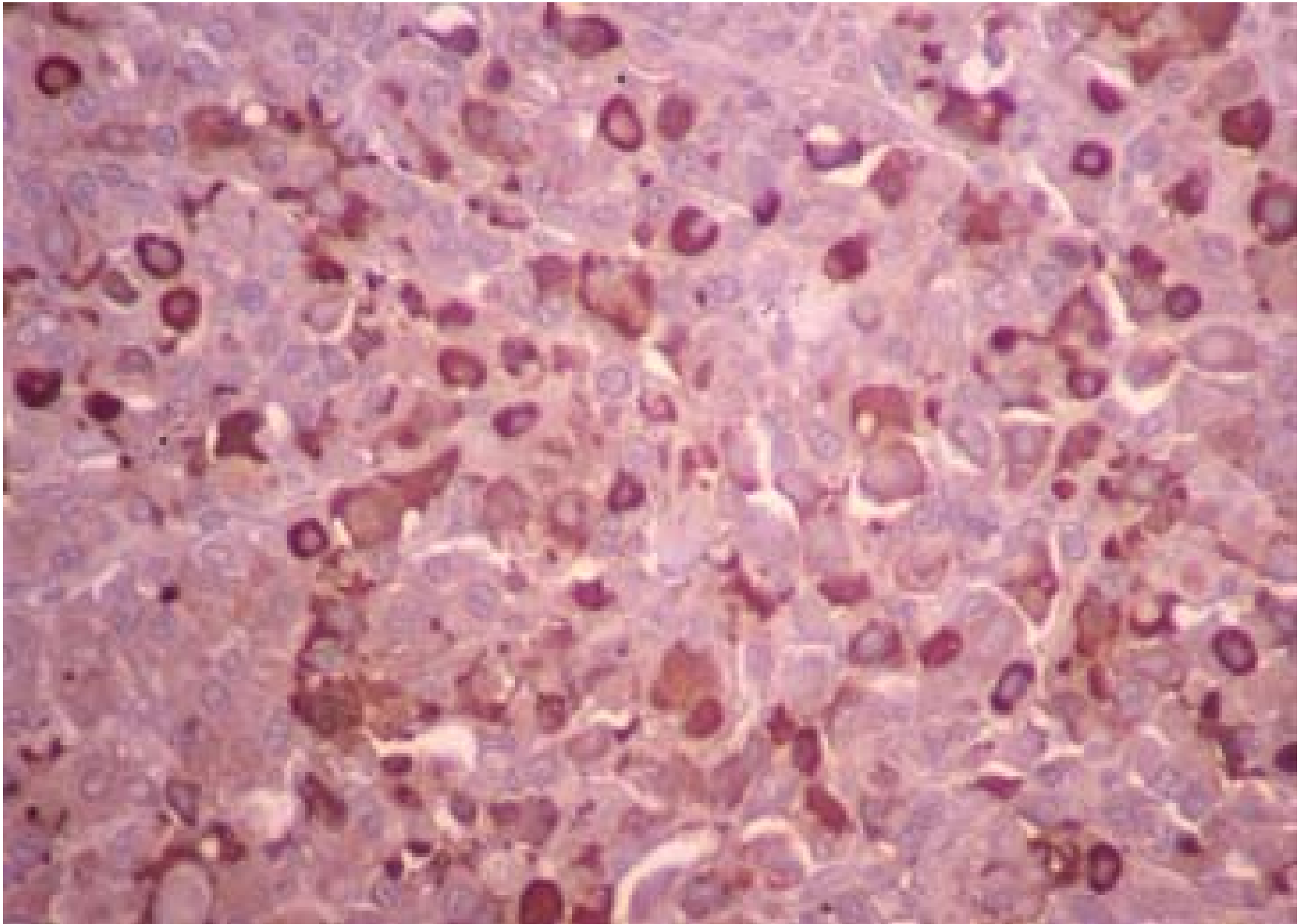


Predicted Spread of $\Delta 24$ within Gliomas





“Late” Viral Protein Expression (Hexon)



Effect of Delta-24 and Delta-24-RGD on Normal Vs. Tumor Cells:

Adenovirus	NHA (pfu/mL)	U-251 MG (pfu/mL)	U-87 MG (pfu/mL)
<hr/>			
Delta-24			
Exp. 1	1.9×10^2	6.3×10^5	3.1×10^2
Exp. 2	0	7.9×10^4	7.9×10^2
Delta-24-RGD			
Exp. 1	2.5×10^3	4.0×10^7	1.0×10^7
Exp. 2	5.0×10^3	3.2×10^6	2.0×10^7
Ad300			
Exp. 1	1×10^6	2.5×10^7	1.5×10^5
Exp. 2	5×10^6	3.1×10^6	1.5×10^5

*Data represent the final viral titer in plaque-forming units (pfu)/mL from two independent experiments. Viral titers were determined by the tissue culture infection dose50 method (15) from lysates of cells plated in low confluence, maintained in serum-starved conditions (0.5% fetal bovine serum, no growth supplements), and infected with 2×10^4 pfu/mL of Delta-24, Delta-24-RGD, or Ad300 (a wild-type adenovirus used as a control). Exp. = experiment.

The expression of alpha-V integrins is common in gliomas and uncommon in normal brain

Bello L et al., Alpha(v)beta3 and alpha(v)beta5 integrin expression in glioma periphery. Neurosurgery. 2001;49:380-9

Pasqualini et al., Alpha(v) integrins as receptors for tumor targeting by circulating ligands Nat. Biotechnol. 15:542-546, 1997

Paulus et al., Characterization of integrin receptors in normal and neoplastic human brain. Am J Pathol. 1993;143:154-63.

“Normal brain astrocytes expressed alpha 2, alpha 3, alpha 6, beta 1, and beta 4 chains ... but they were consistently negative for other integrins examined (alpha 4, alpha 5, alpha V, alpha L, alpha M, alpha Z, beta 2, and beta 3)”

Conclusion of Pre-clinical Data

Delta-24-RGD infects glioma cells with low expression of CAR, a common feature of human tumors, by binding alpha-V integrins.

Importantly the expression of alpha-V integrins is common in gliomas and uncommon in normal brain.

Clinical Trial

Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD-4C) for Recurrent Malignant Gliomas

Charles Conrad, M.D. – Co-P.I.
Frederick Lang, M.D. – Co-P.I.

Objectives

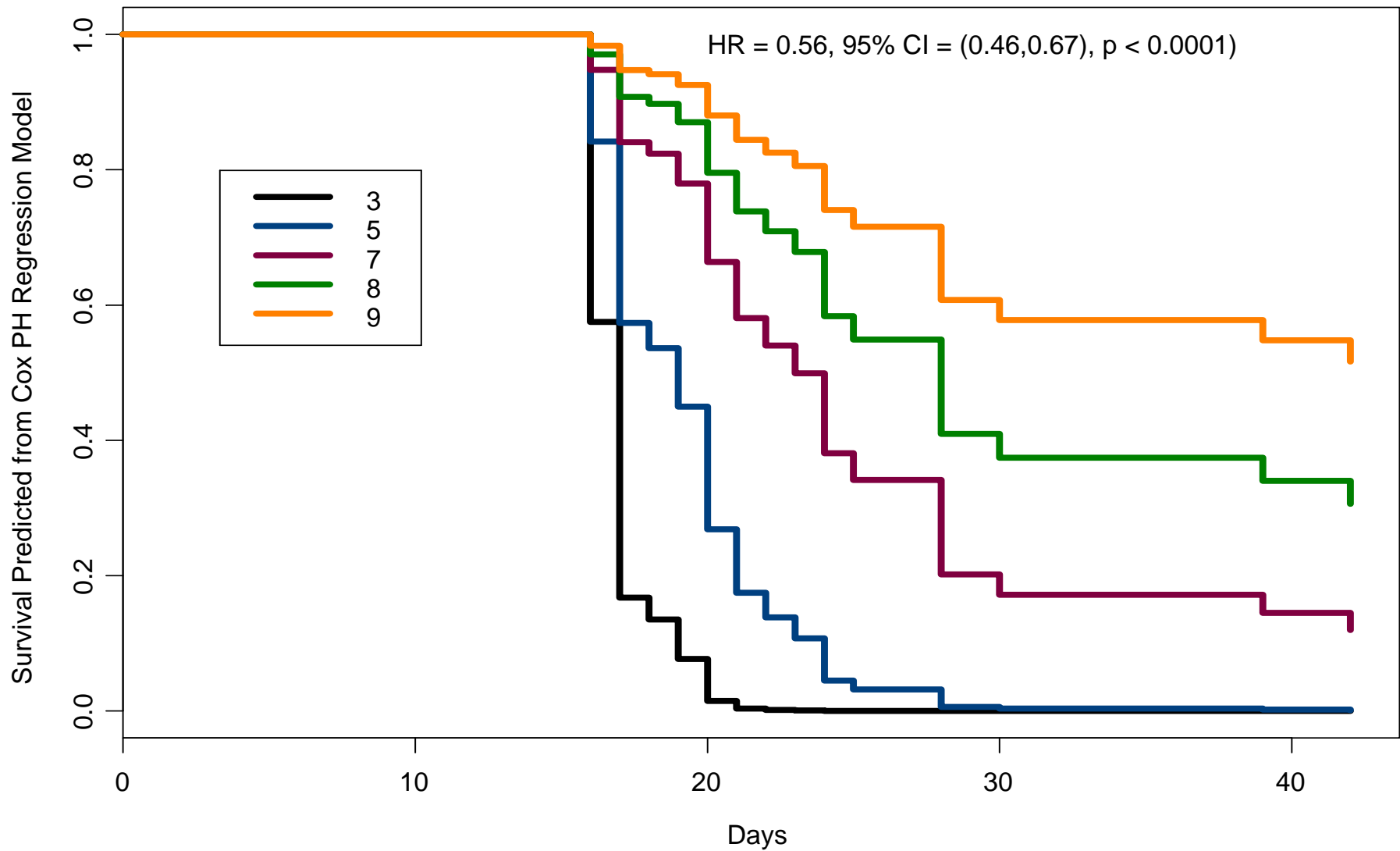
- To determine the qualitative and quantitative **toxicity** of Delta-24-RGD administered by intratumoral injection.
- To determine the **maximum tolerated dose** (MTD) of Delta-24-RGD-4C administered by intratumoral injection in patients with recurrent malignant gliomas.
- To determine the **biological effects** at the molecular level of intratumoral administration of replication-competent adenovirus Delta-24-RGD in human malignant gliomas by analyzing; viral distribution, expression of “late” viral genes, evidence of oncolysis, characterization of immune responses, evaluate for the presence of viral shedding.

Dose escalation

<u>Dose Level</u>	<u>Dose Delta-24-RGD (viral particles)</u>
1	1×10^9
2	3×10^9
3	1×10^{10}
4	3×10^{10}
5	1×10^{11}

Dose Dependence Experiments

RGD Dosing Experiment, 10 Animals per Dose



Treatment Plan: Group A

Procedure 1: Stereotactic
Tumor Injection



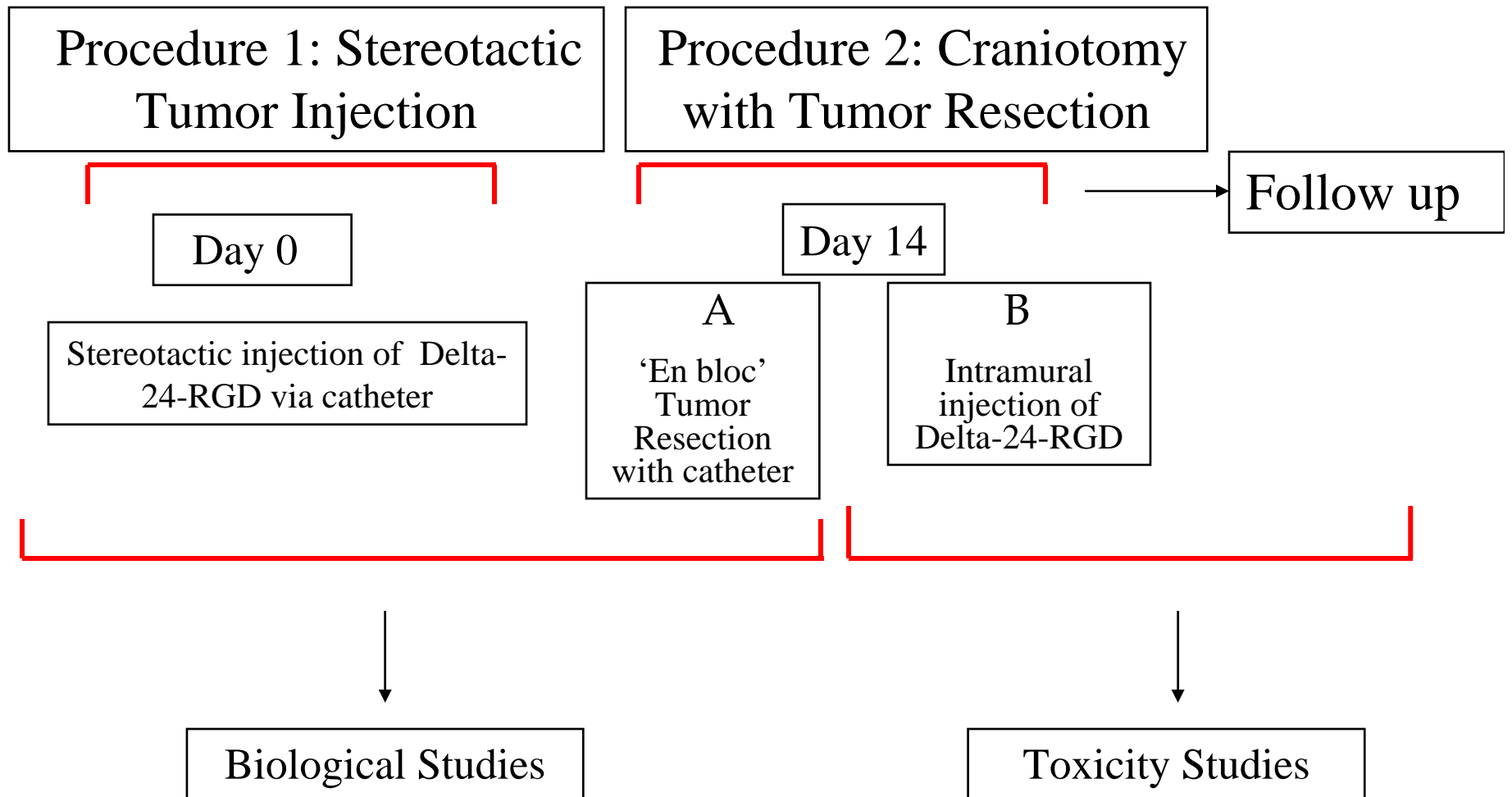
Follow

Day 0

Stereotactic injection of Delta-
24-RGD via catheter

Toxicity Studies

Treatment Plan: Group B



Additional Components Regarding the Clinical Trial

1. IRB approval has been obtained with MDACC for this phase I protocol.
2. The current clinical trial has plans to perform pre- and post-treatment serum antibody titers for adenovirus.
3. The clinical trial will also perform biodistribution studies on serum, sputum, urine and feces.
4. Specific studies to identify local immune reactions around the injection site within the tumor (in Group B patients) will be further defined.
5. A pre-IND meeting with the FDA has been completed.
 - Suggested studies with Cotton rats are in the planning stages.
 - A discussion of the suggested toxicology, pharmacokinetic, and biodistribution studies is ongoing.
6. The Manufacture and quality control of the clinical-grade (GMP-virus) is being provided through the RAID program.
7. Formal plans for the animal toxicology, pharmacokinetics, biodistribution, and specific neurotoxicity studies is being provided through the RAID program with concurrent discussions with the FDA.