

# Tocagen Presentation to the RAC

June 17, 2009



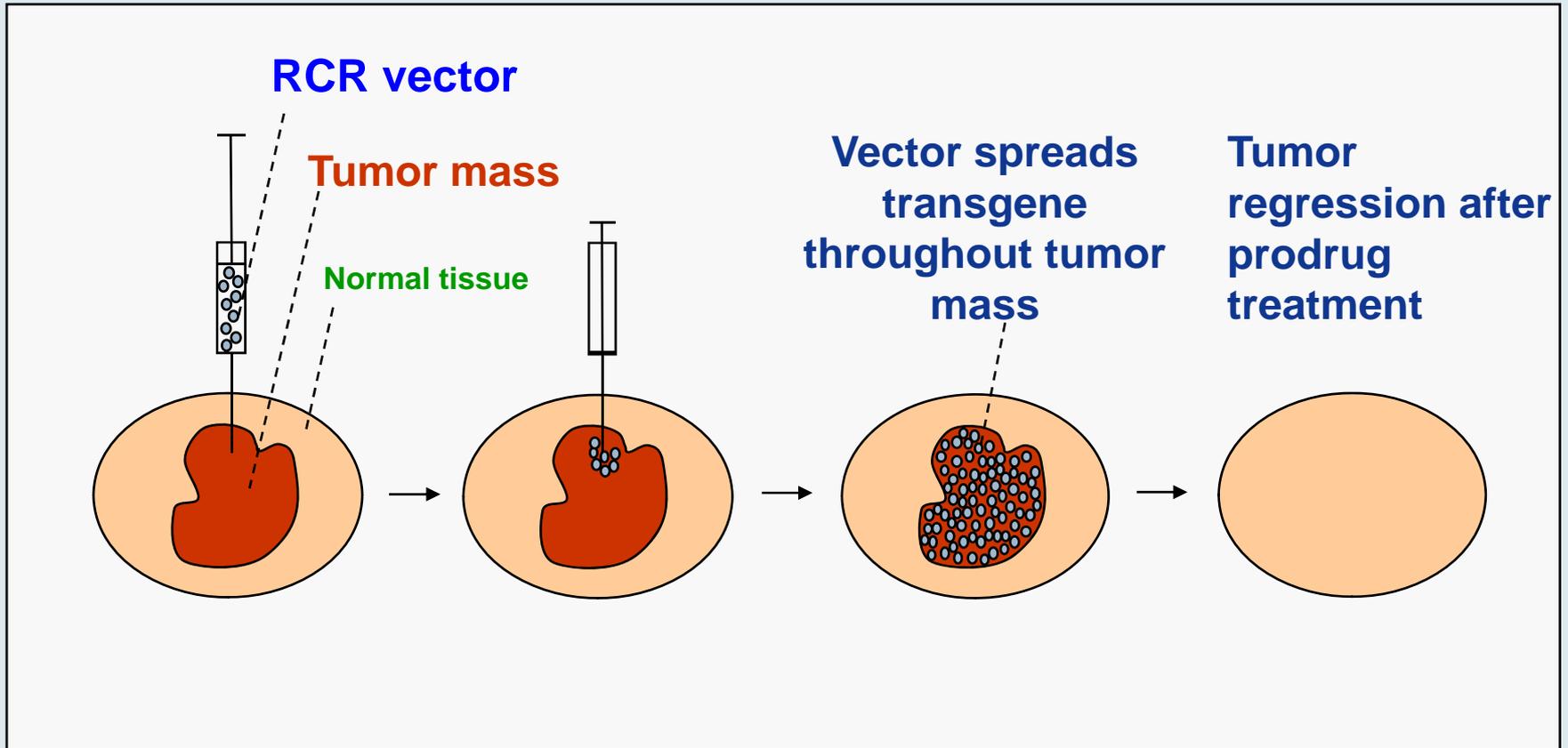
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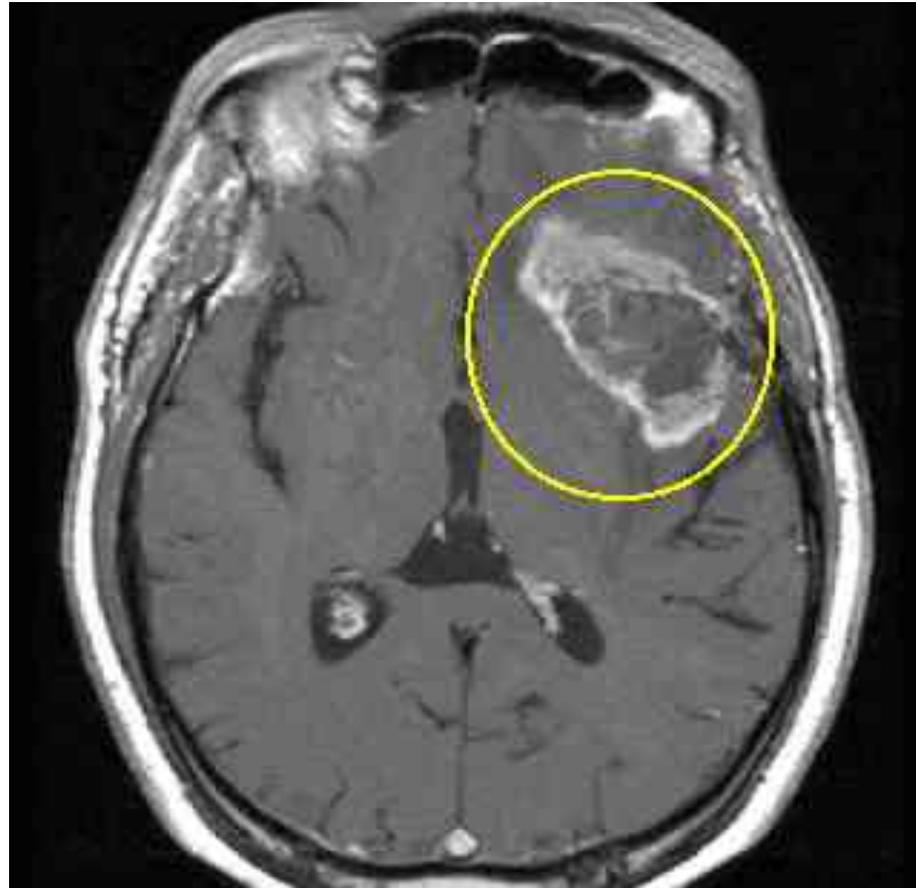
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Sr. Vice President, Research & Development  
Tocagen

# Cartoon of the Replication-Competent Retrovirus prodrug activator approach



# MRI of Glioblastoma



# GBM remains a clinical problem



- $\approx$ 22,000 primary brain tumors Dx in U.S. in 2008
- $\approx$ 25% were GBM (grade IV glioma)
- GBM is most aggressive 1<sup>o</sup> malignant brain tumor
- Tends to affect people in middle life
- Maximal initial Rx = surgery, radiation, TMZ
  - Median survival 14 months
  - $<$  10% survive two years

# Challenges have made GBM an attractive gene transfer target



- Aggressive tumor with early invasion of surrounding normal brain structures
  - Renders surgery and RT palliative rather than curative
- Cellular/molecular heterogeneity
  - Renders chemoRx and targeted Rxs variably effective
  - TMZ only benefits  $\approx$  40% of patients
- Blood Brain Barrier
  - Limits efficacy of many systemic therapies

# Previous gene transfer studies in 1° brain tumors showed limited efficacy



Review by Aghi and Chiocca in 2006 lists 18 studies

- Non-replicating viruses failed to infect majority of tumor cells
  - 7 retrovirus
  - 4 adenovirus
  - 1 combination retrovirus and adenovirus
- Oncolytic viruses were either too attenuated or cleared by the immune system
  - 5 herpes virus
  - 1 Newcastle Disease virus

# 1<sup>st</sup> generation platform: non-replicating retrovirus

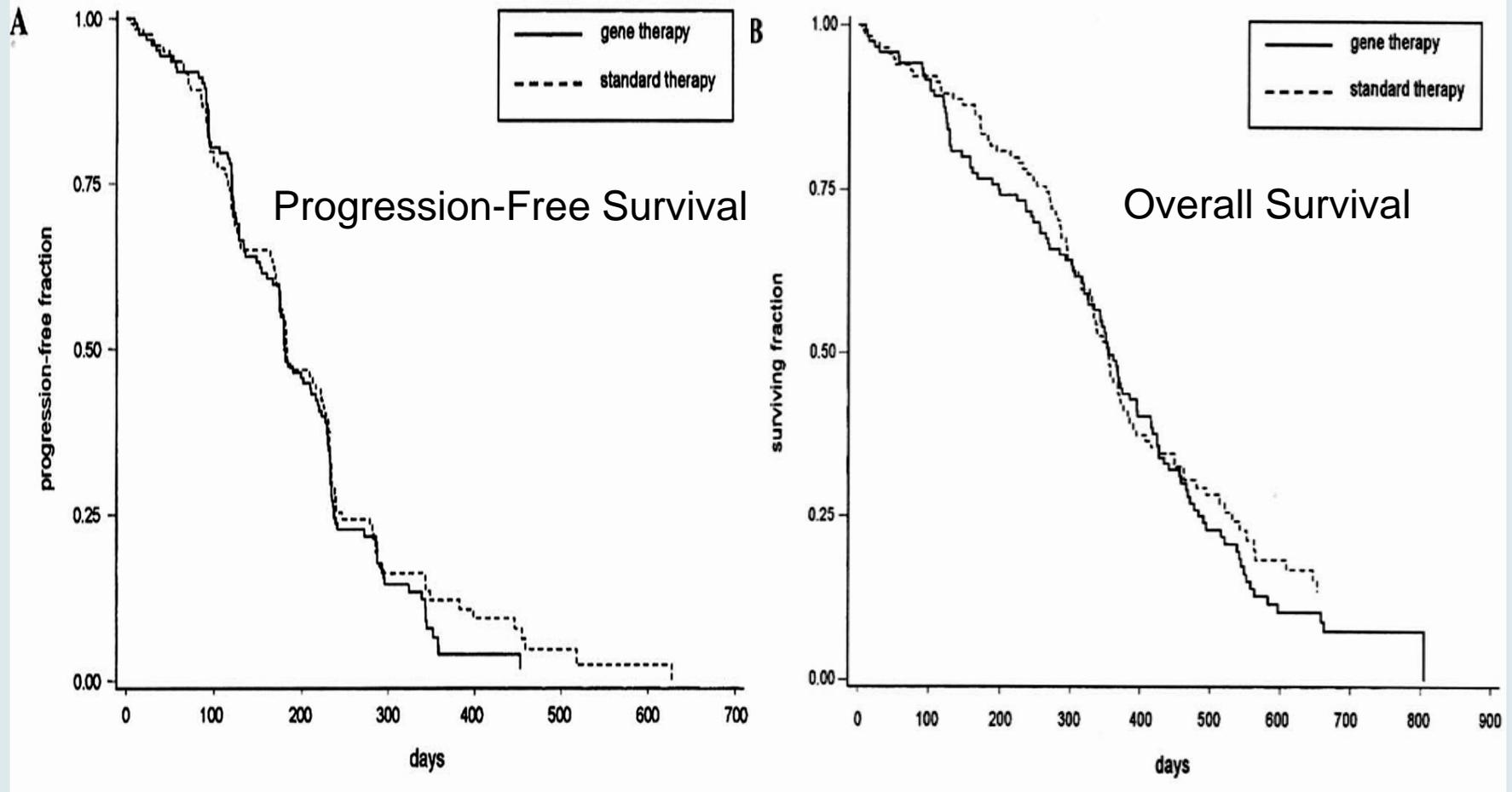


## *Simple replicating retrovirus*

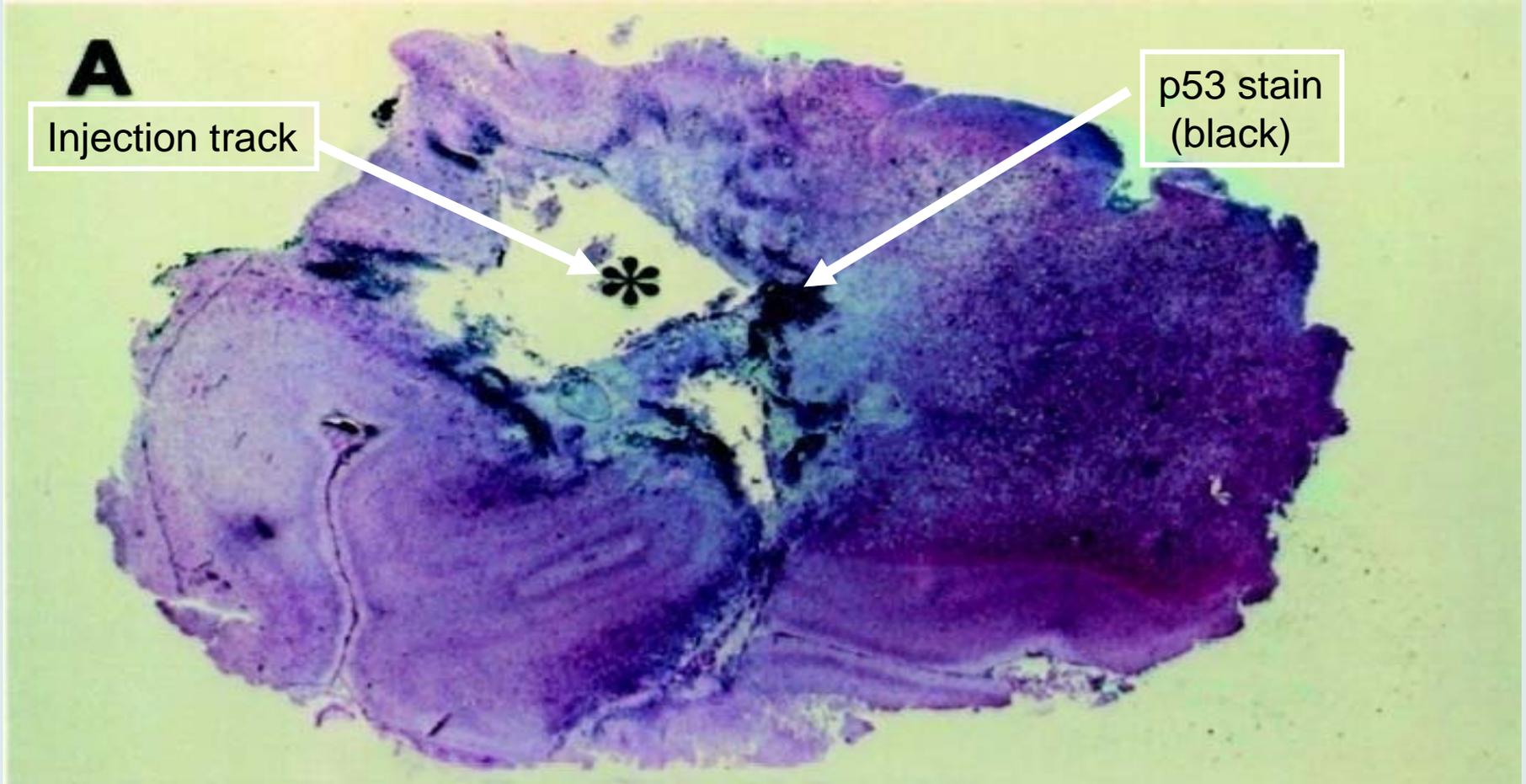


## *1<sup>st</sup> generation non-replicating retrovirus vector*

# Non-replicating retroviral vector failed in Phase III

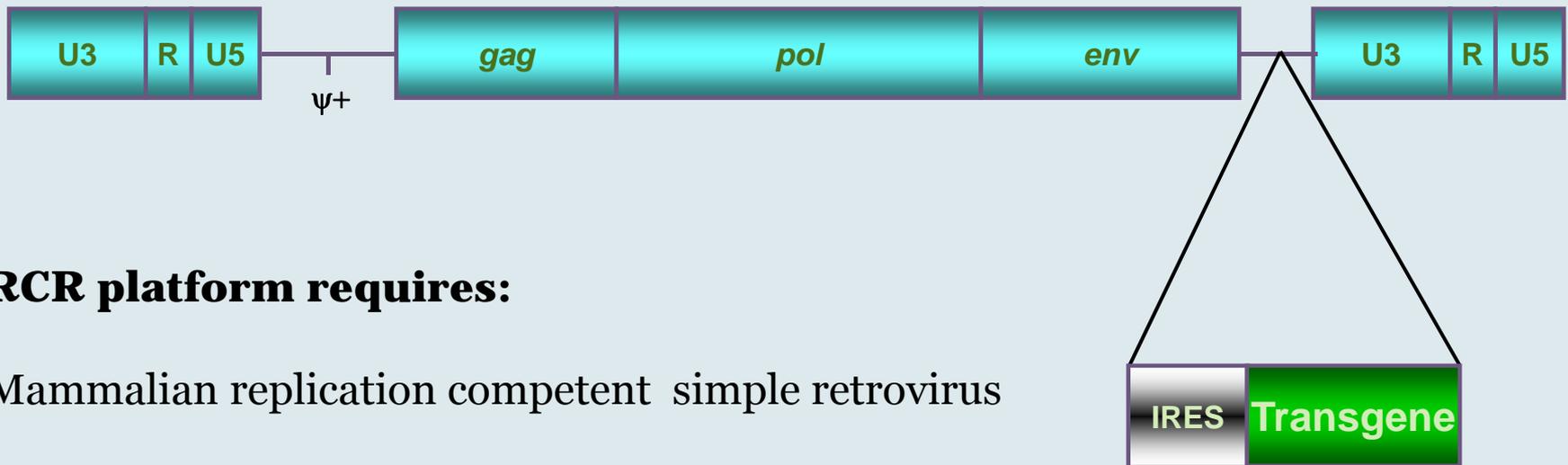


# Non-replicating adenovirus vectors did not spread



Section through a resected human glioma treated with Adp53 vector

# New platform: RCR vector expressing a transgene



## **RCR platform requires:**

Mammalian replication competent simple retrovirus

Insertion in position 3' to env and 5' to LTR for stability

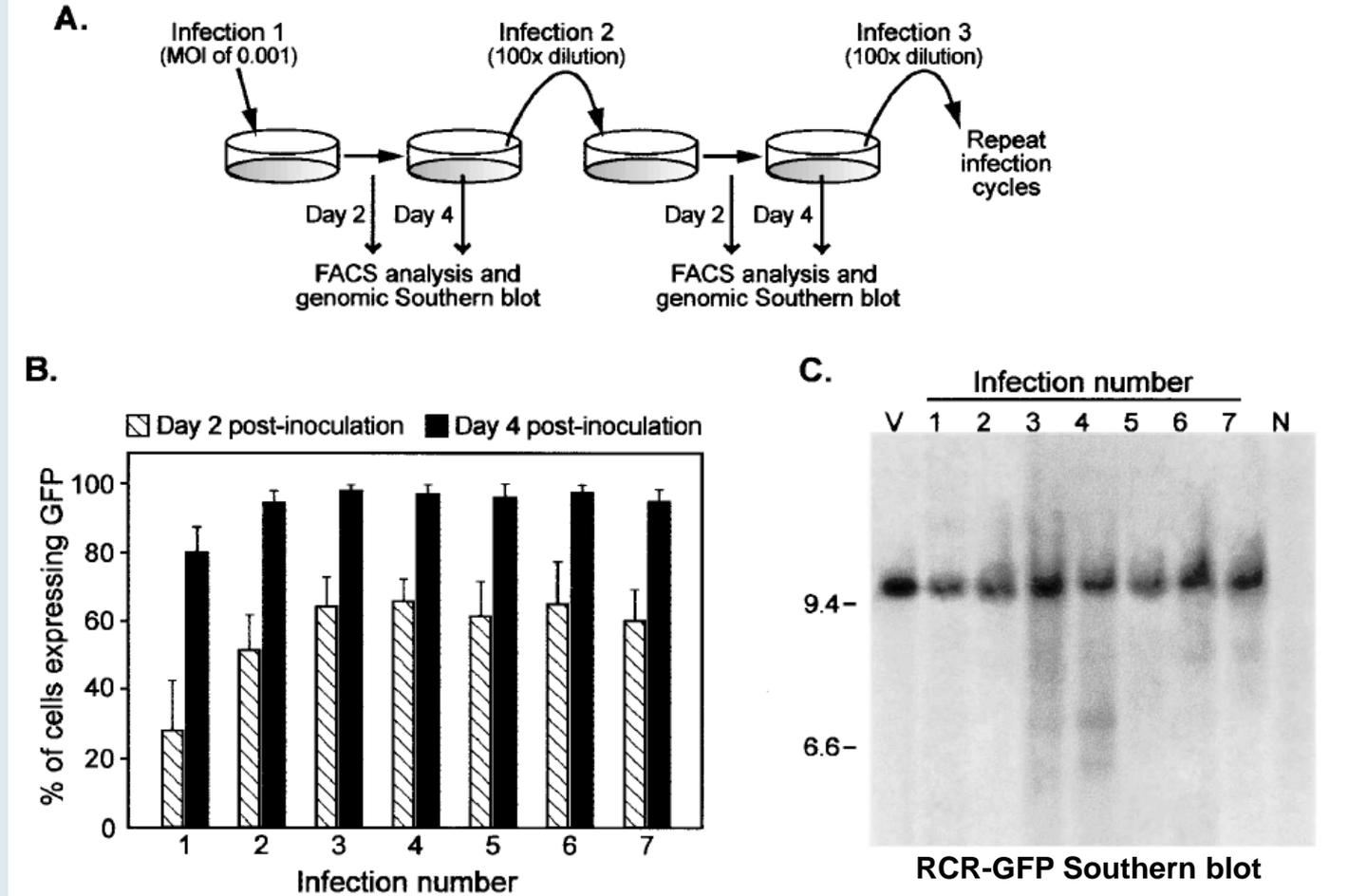
IRES sequence for effective transgene expression

# Effective RCR vector ideally should...



- Be stable during cell-to-cell transfer
- Spread through the tumor
- Exhibit tumor selectivity
- Deliver a therapeutic gene
- Allow for long-term control of tumor

# RCR vector is stable during cell-to-cell transfer



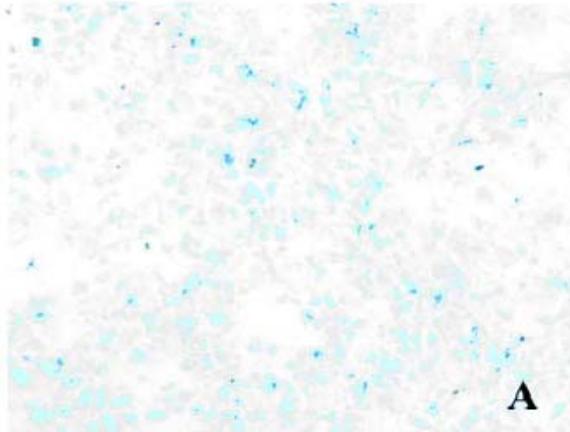
# RCR vector spreads through tumors



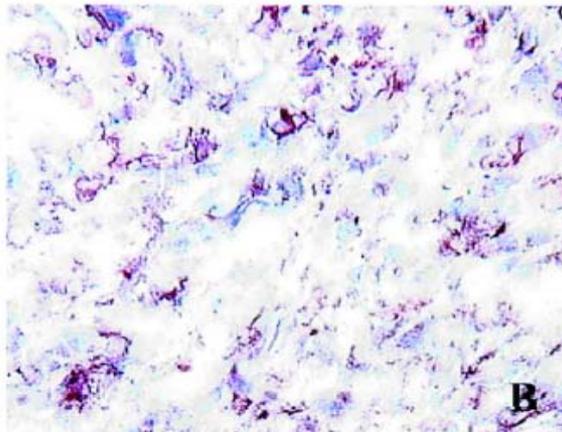
negative control

2 weeks post-infection

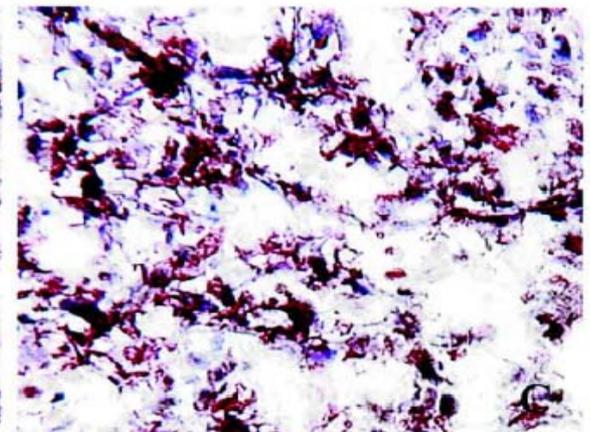
3 weeks post-infection



A

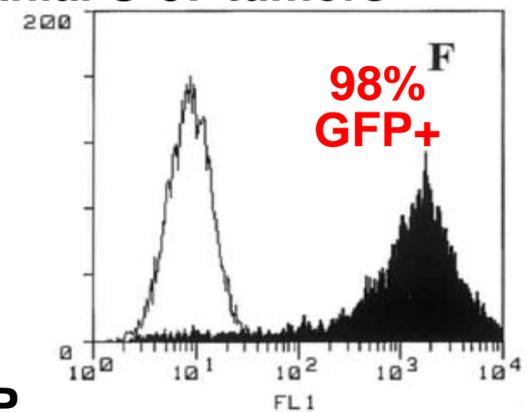
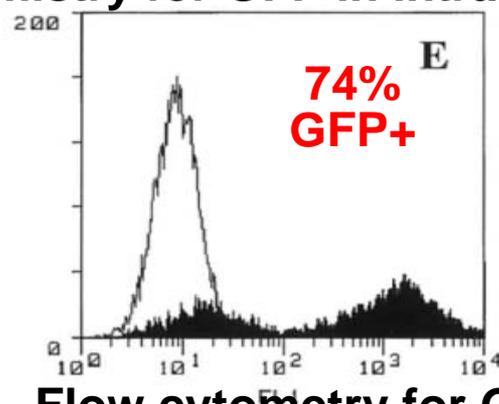
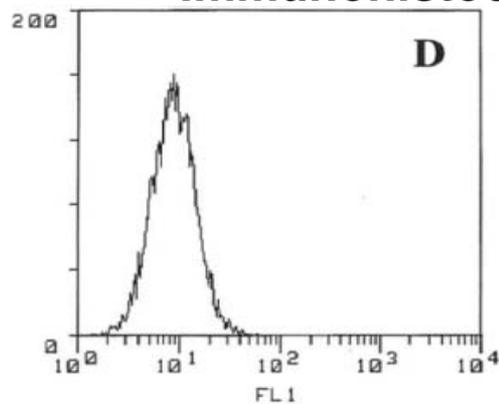


B



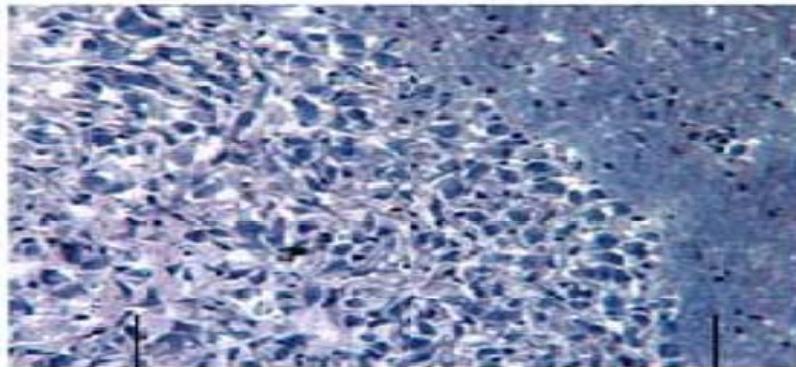
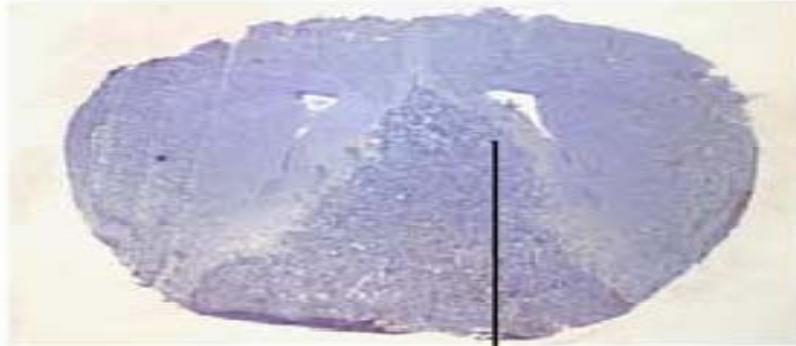
C

Immunohistochemistry for GFP in intracranial U-87 tumors



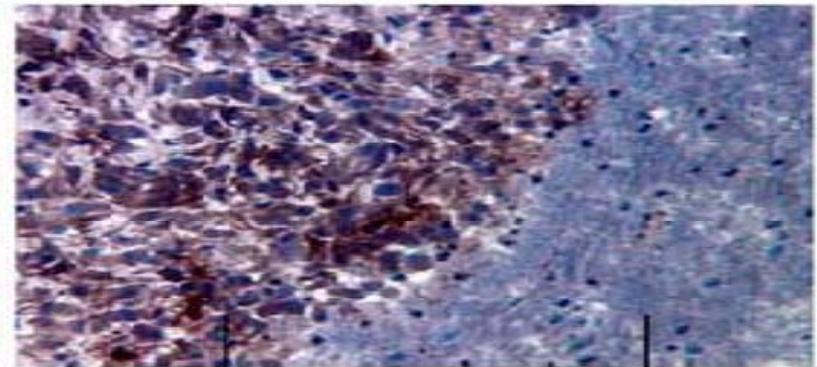
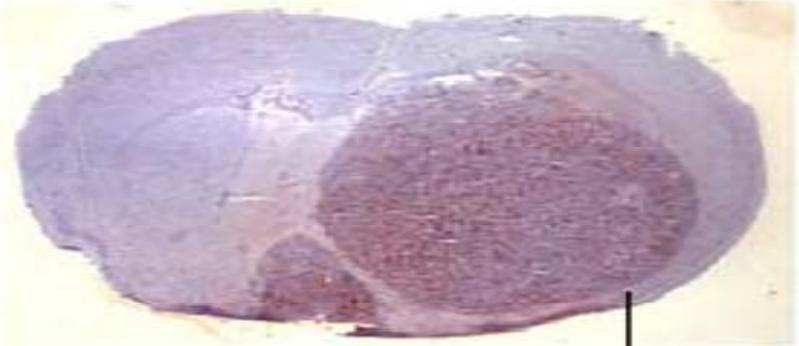
Flow cytometry for GFP

# RCR vector exhibits tumor selectivity



tumor  
(U-87)

brain



tumor  
(anti-env staining)

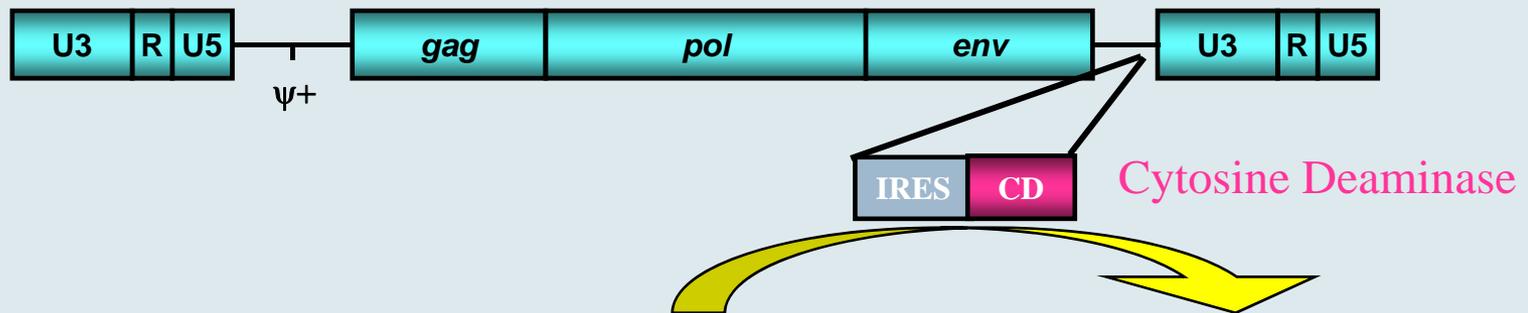
brain

# RCR vector exhibits tumor selectivity

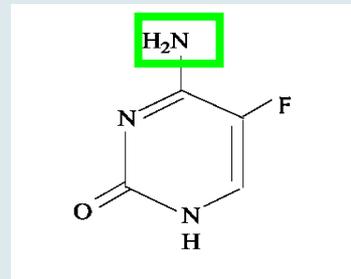


- RCR only infects dividing cells
- RCR “hides” in immune-privileged tumor environment
  - Interferon response pathways are dysregulated
  - Antigen presentation is down-regulated
  - Tumor produces immunosuppressive cytokines
  - RCR is weakly immunogenic

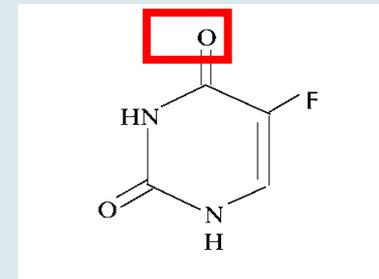
# RCR vector delivers yeast-derived cytosine deaminase gene



- 5-FC is orally available
- 5-FC crosses the BBB
- 5-FC is approved for Rx of fungal brain infections



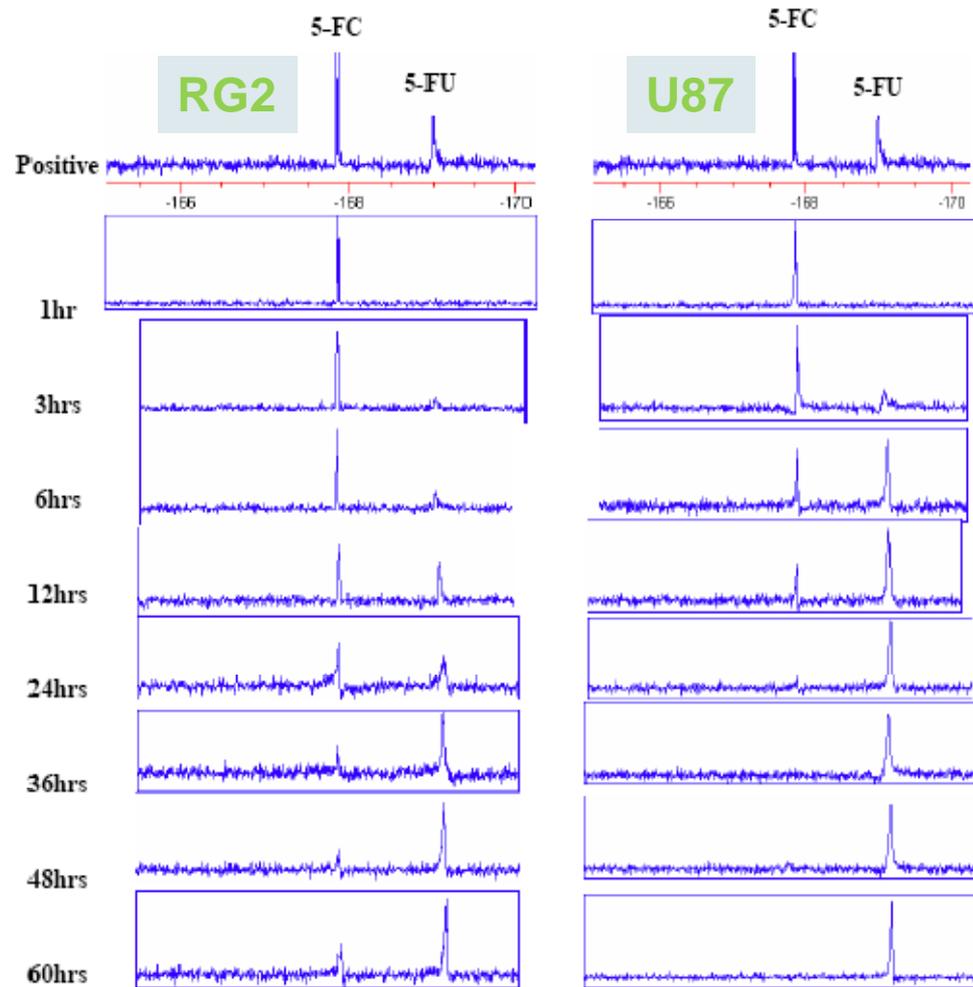
5FC  
(5-fluorocytosine)



5FU  
(5-fluorouracil)

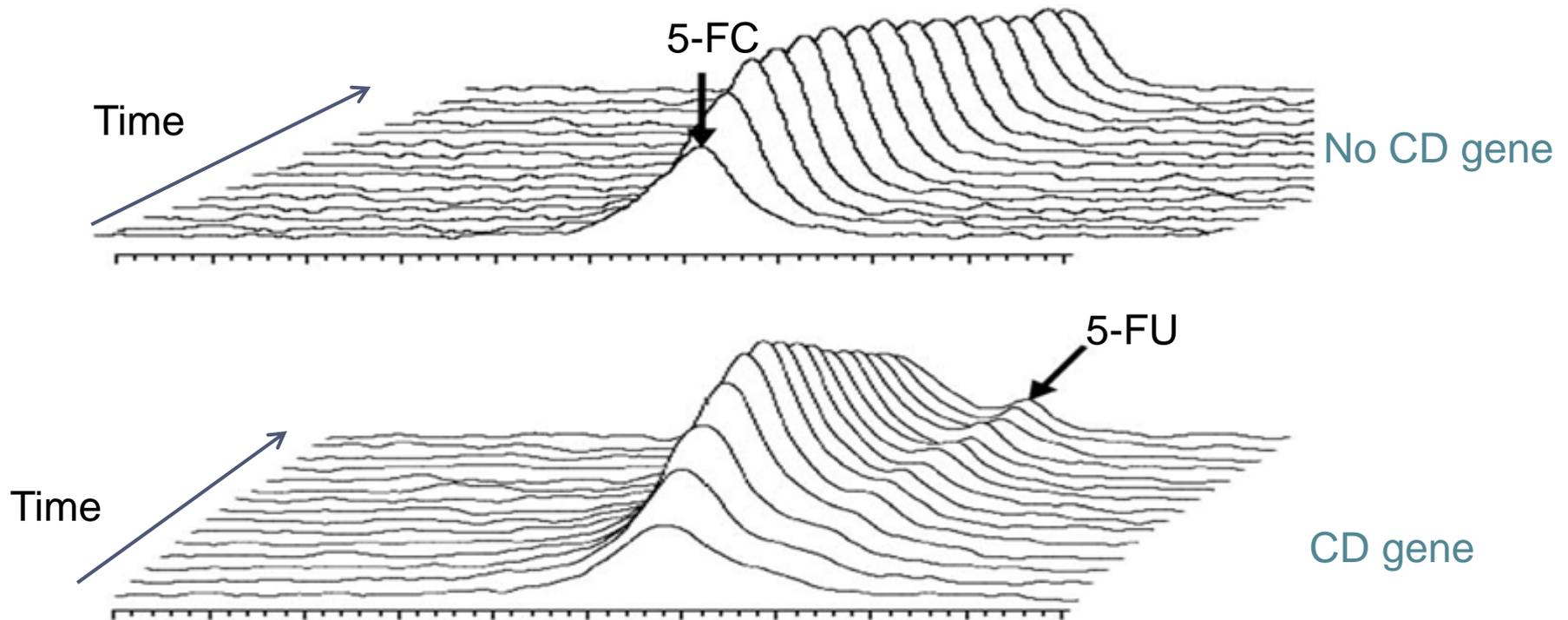
CD converts 5FC prodrug to 5FU anticancer drug

# RCR-CD converts 5-FC to 5-FU in cell culture



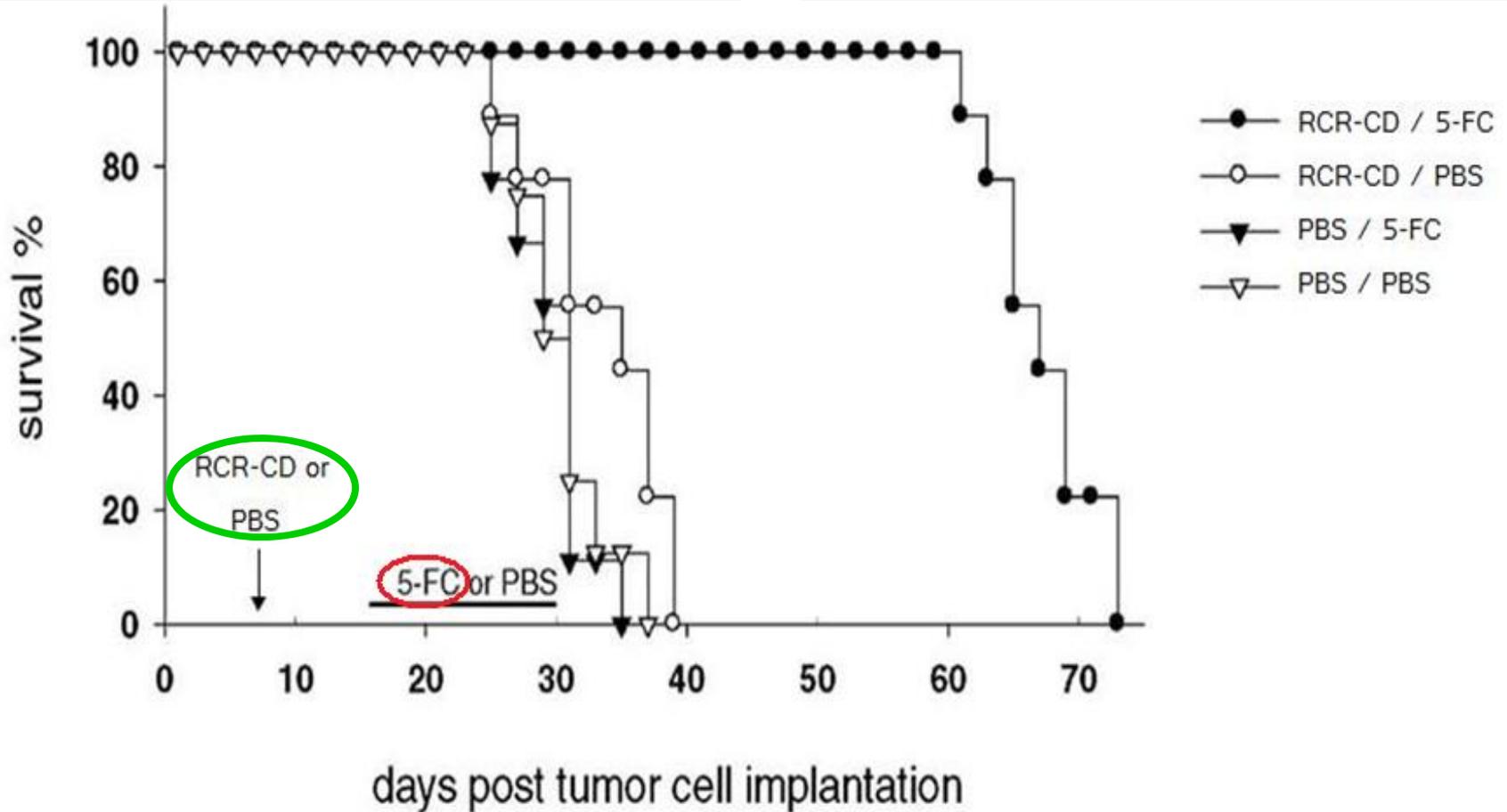
MRS <sup>19</sup>F-Spectroscopy

# Demonstration of CD transgene activity in vivo



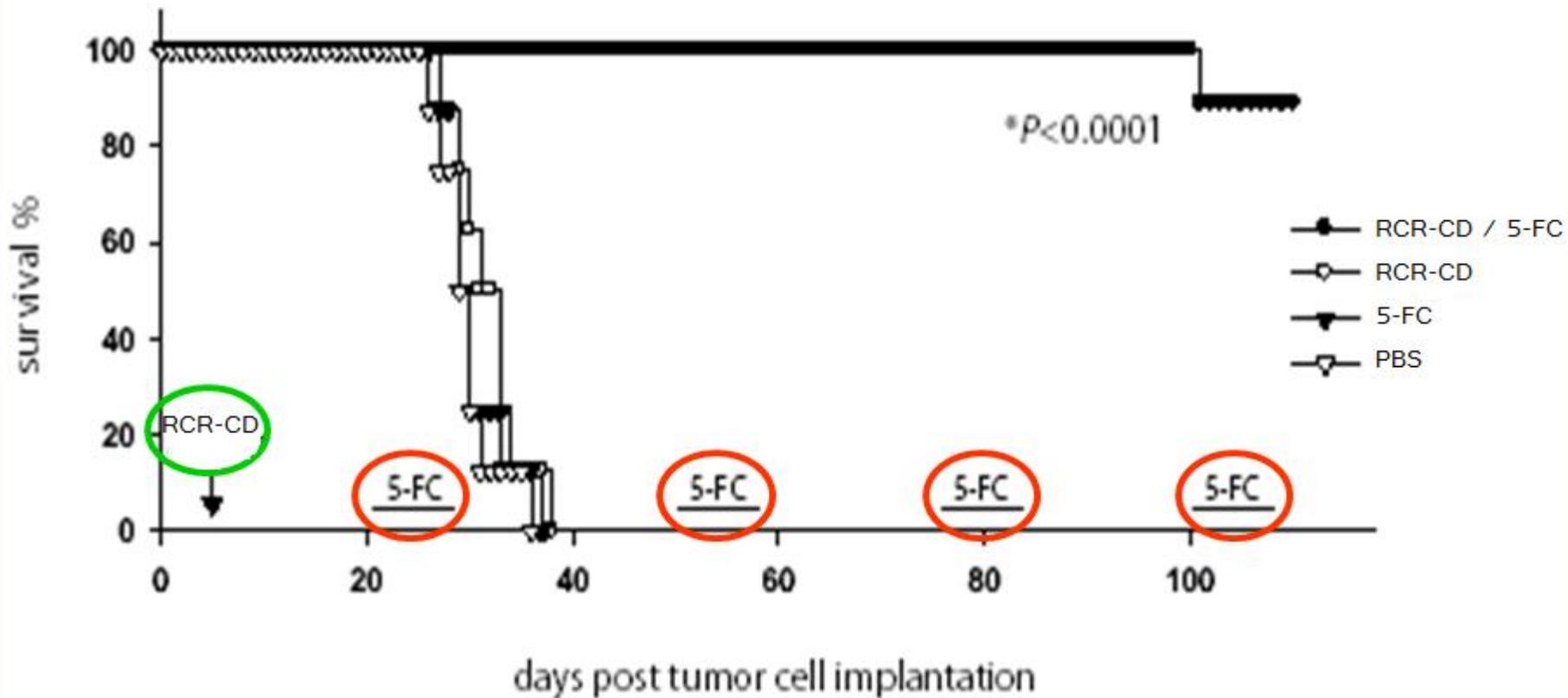
MRS  $^{19}\text{F}$ -Spectroscopy

# RCR-CD doubles survival after a single cycle of 5-FC



Vector dose =  $2 \times 10^4$  TU/g brain

# RCR-CD allows for long-term control of tumor



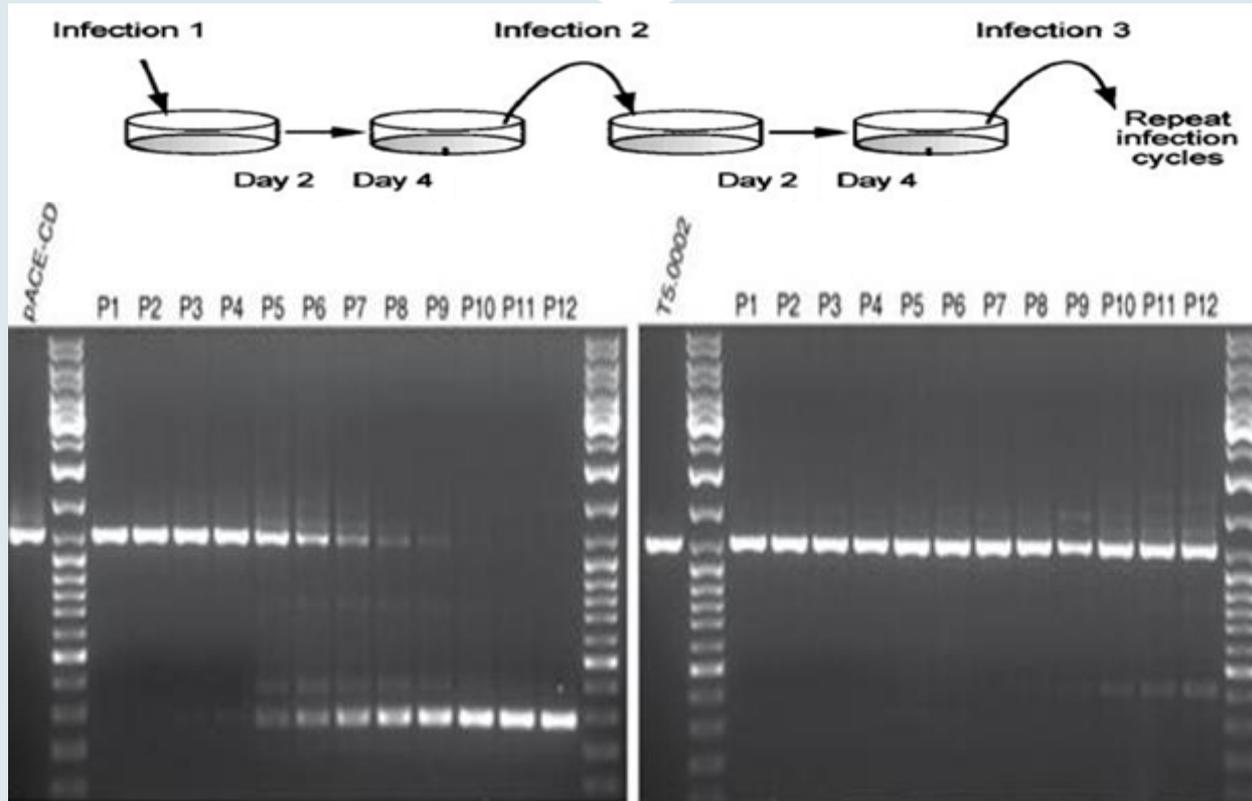
Vector dose =  $2 \times 10^4$  TU/g brain

# Effective RCR vector ideally should...



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# Toca 511 is more stable during cell-to-cell transfer



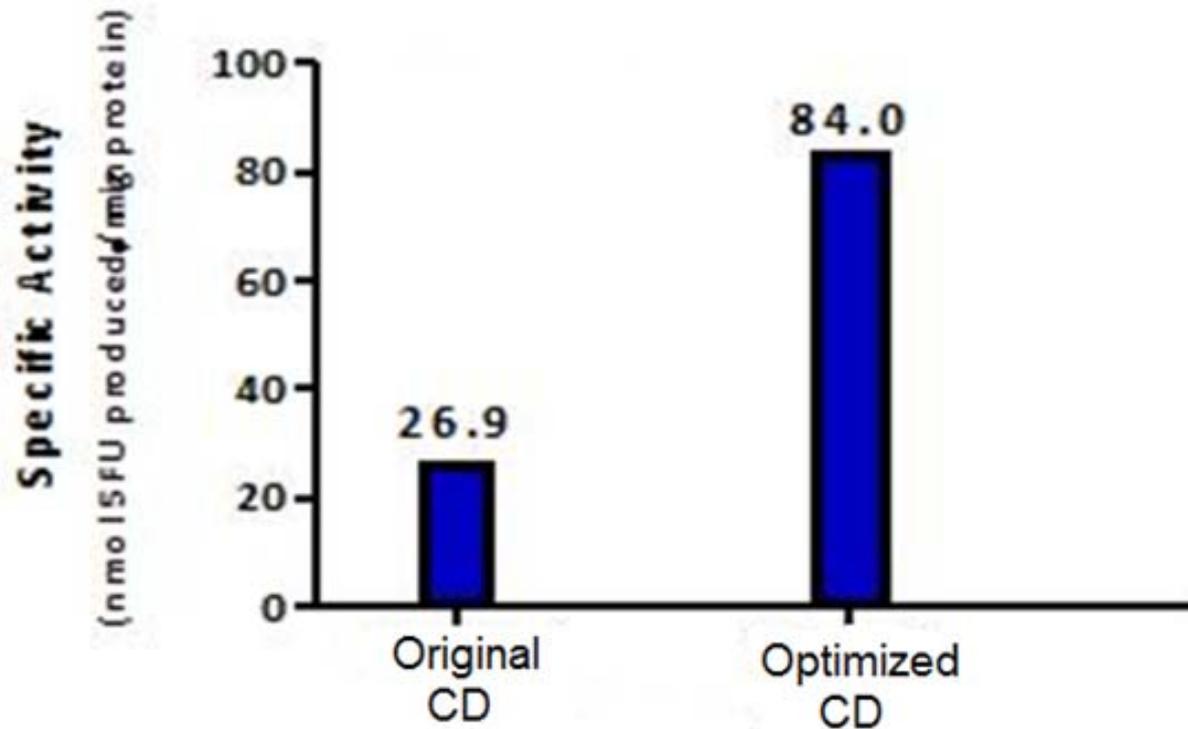
Original Backbone  
Original CD

New Backbone  
Humanized CD codon

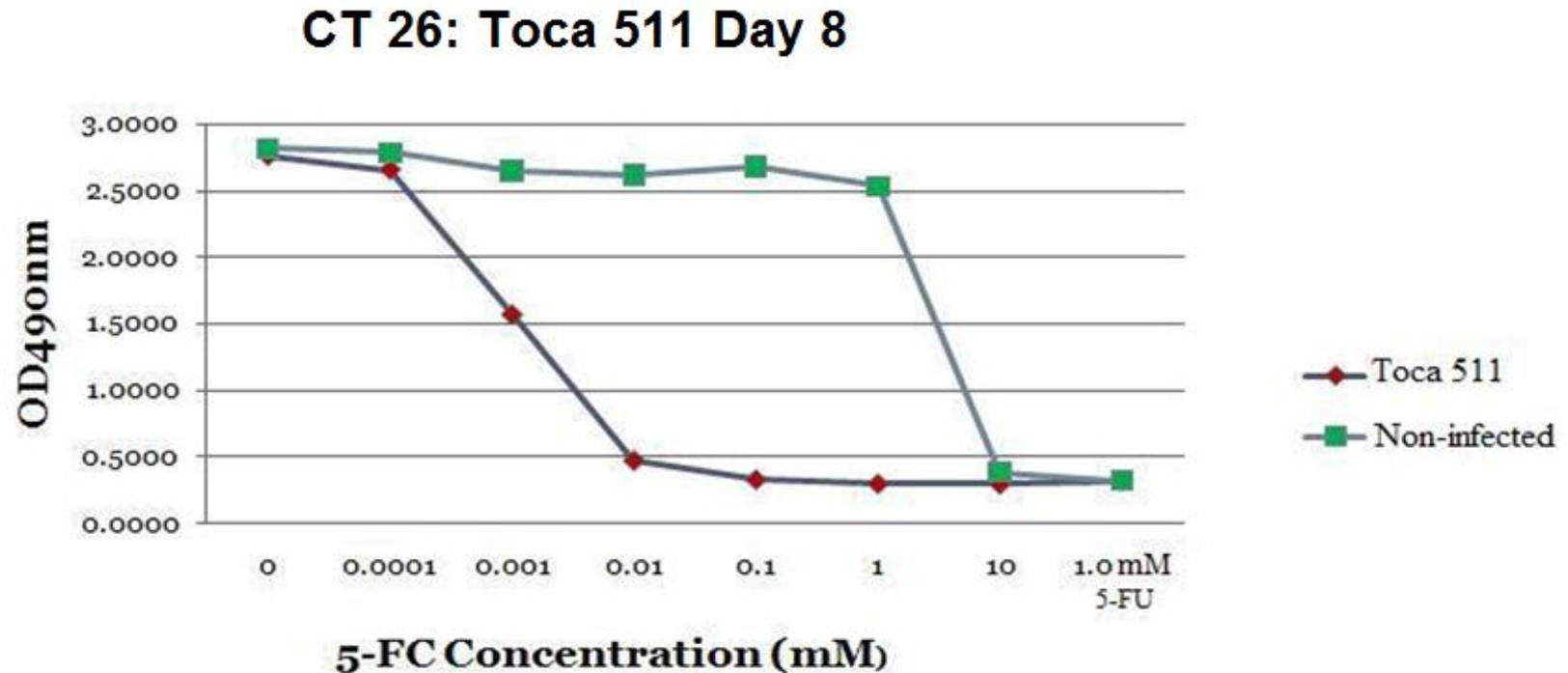
# Toca 511 produces 3-fold more 5-FU in vitro



CD activity after 7 days U-87 infection (100%)



# Toca 511 vector kills tumor cells at attainable 5-FU serum concentrations



$IC_{50} = 0.001 \text{ mM}$  ( $0.13 \mu\text{g/ml}$ )

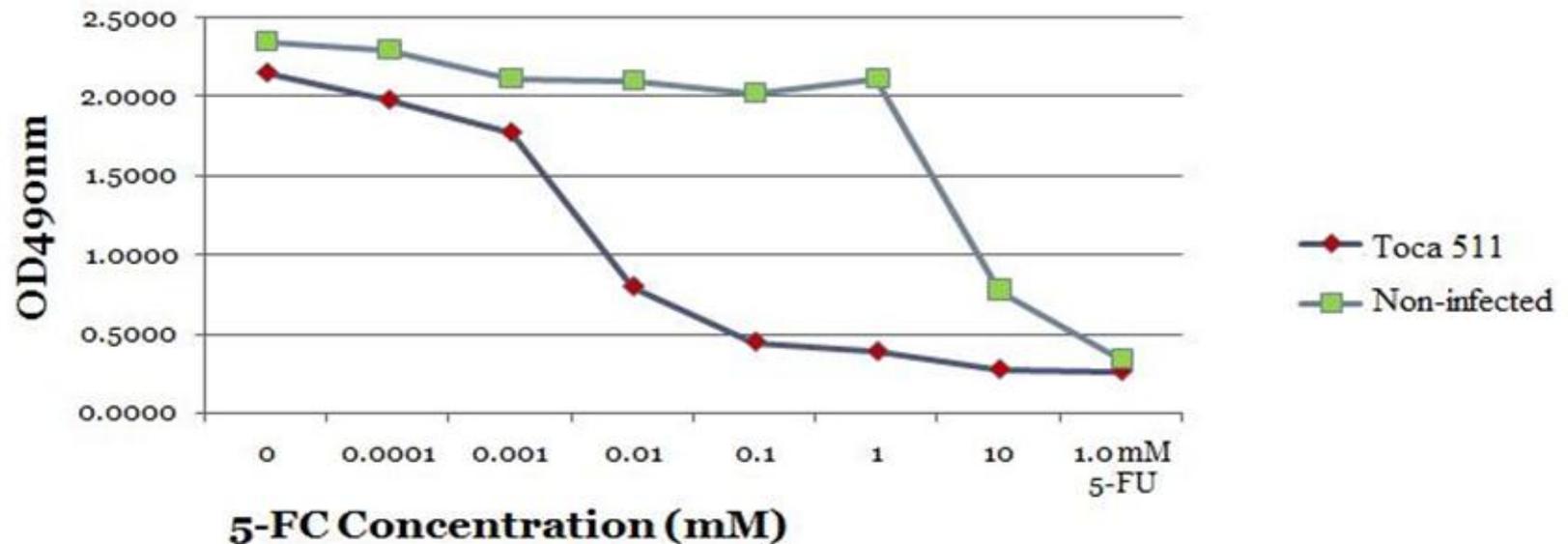
$IC_{90} = 0.006 \text{ mM}$  ( $0.78 \mu\text{g/ml}$ )

Estimated CSF levels in humans: 48-80  $\mu\text{g/ml}$  (>60-fold above  $IC_{90}$ )

# Toca 511 vector kills tumor cells at attainable 5-FU serum concentrations



## U-87: Toca 511 Day 8



$IC_{50} = 0.004 \text{ mM}$  ( $0.49 \mu\text{g/ml}$ )

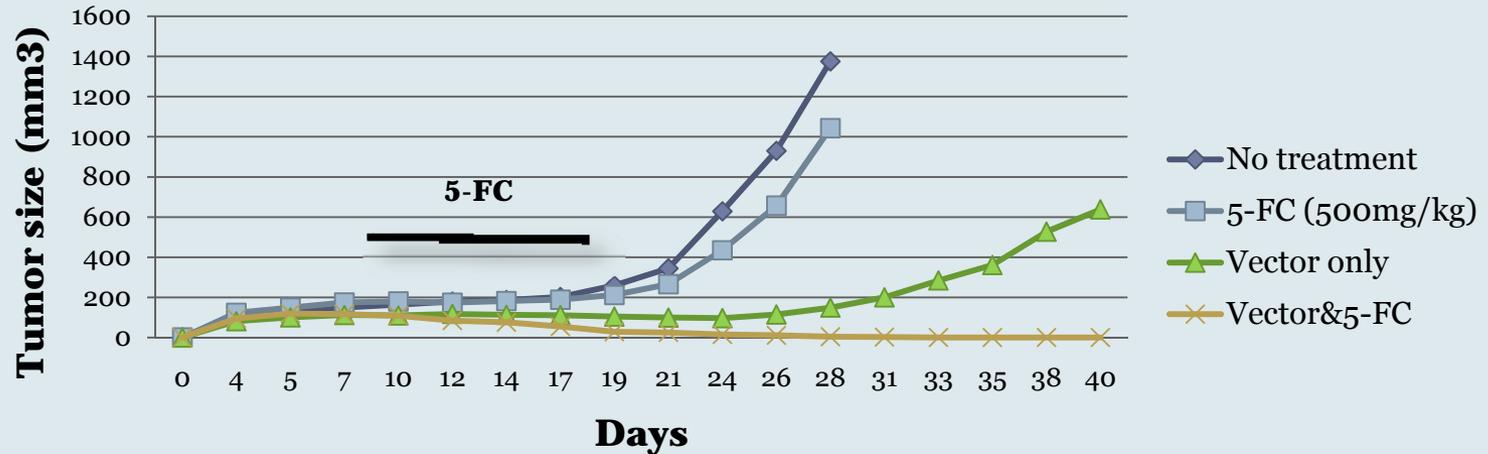
$IC_{90} = 0.034 \text{ mM}$  ( $4.42 \mu\text{g/ml}$ )

Estimated CSF levels in humans:  $48\text{-}80 \mu\text{g/ml}$  (>10-fold above  $IC_{90}$ )

# Toca 511/5-FC combination is effective in vivo



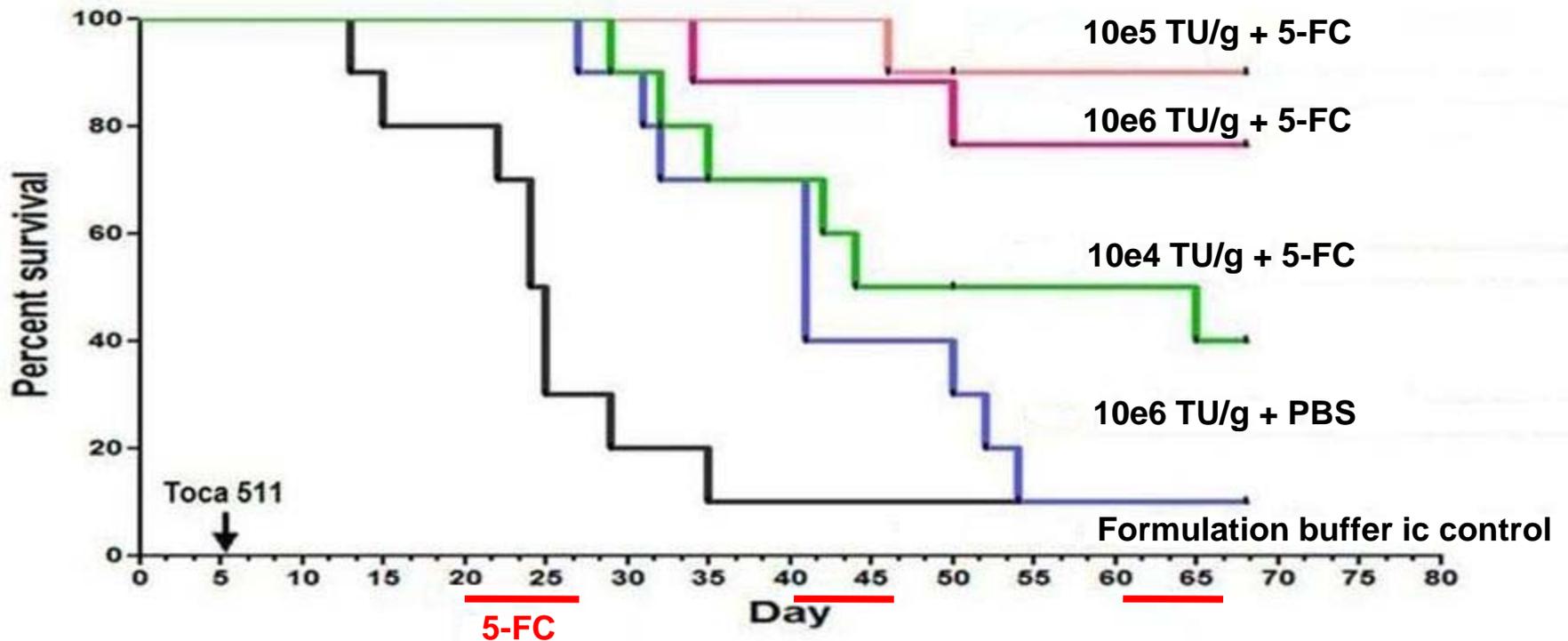
## U87 Sub-Q Tumor model



# Toca 511/5-FC increases survival



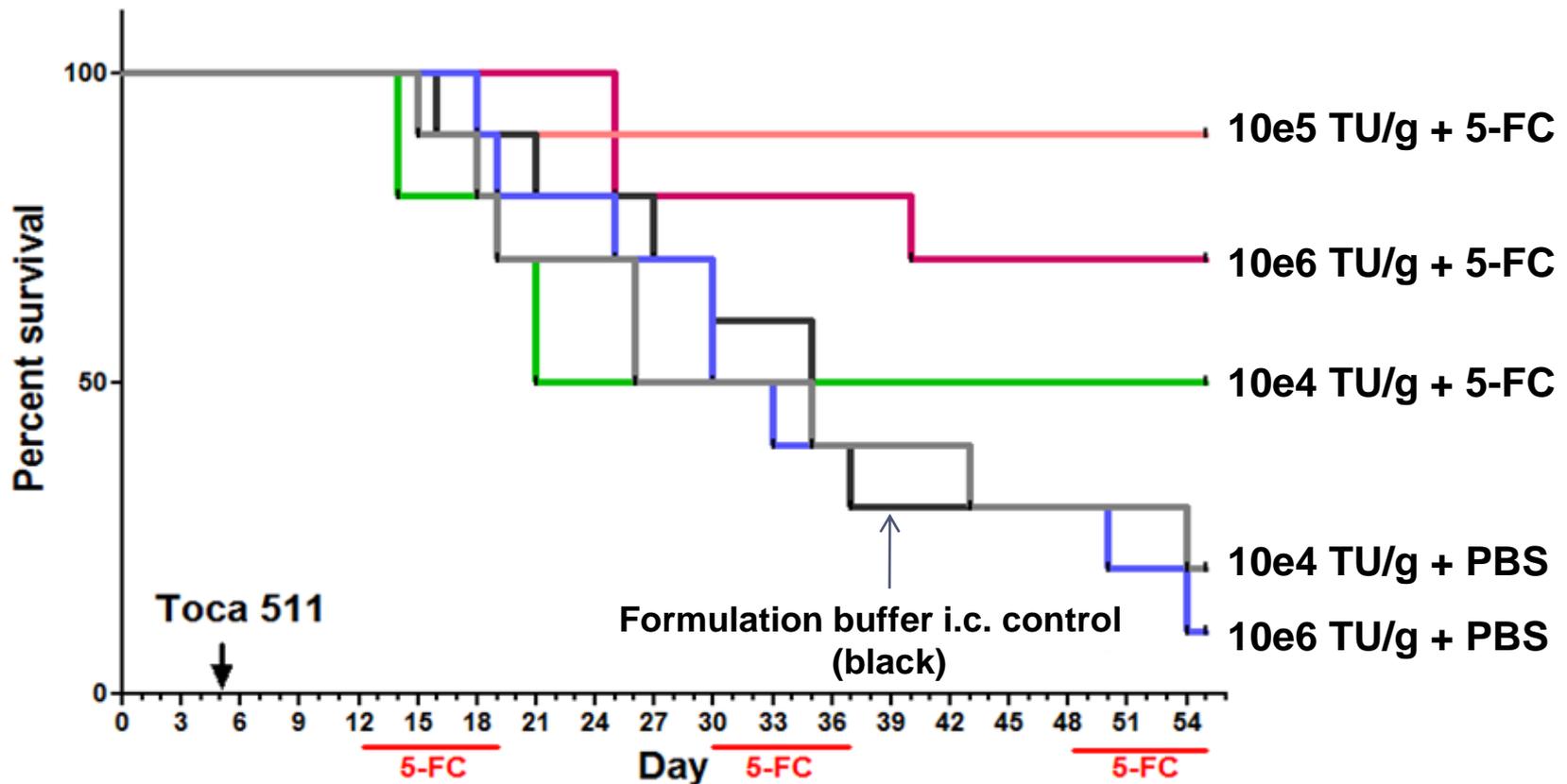
U-87 nude xenograft intracranial model-10 animals/group



# Toca 511/5-FC increases survival



CT26 BALB/c syngeneic intracranial model - 10 animals/group

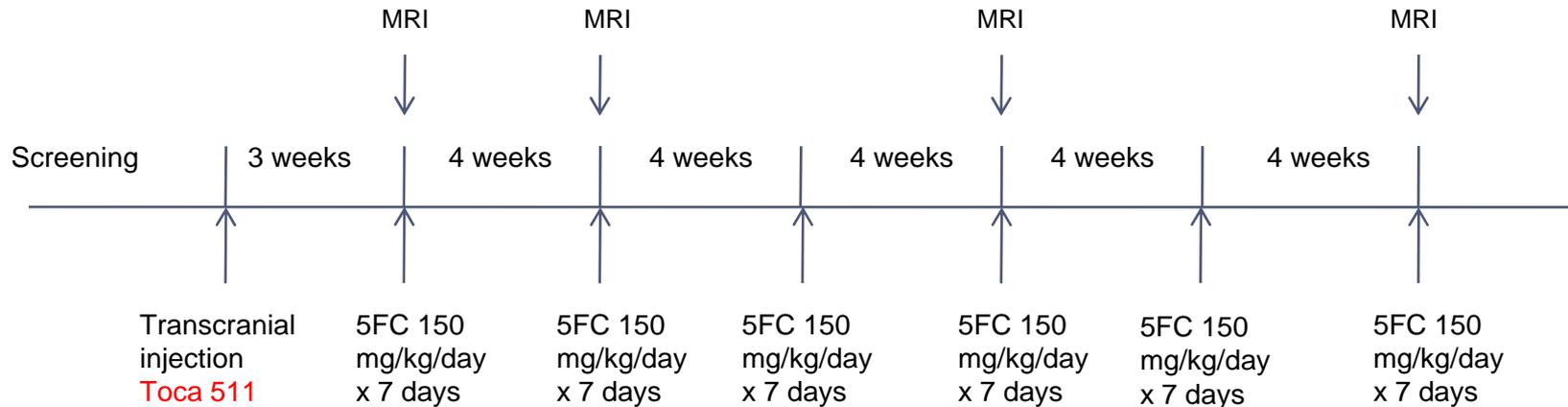


# Proposed Phase 1 Study



- **Indication:**
  - Recurrent GBM (no curative therapies approved-survival ~6 months)
- **Objectives:**
  - Establish maximum tolerated dose (MTD)
  - Assess response rate at MTD
- **Clinical Design:**
  - Open-label, ascending dose study performed at up to 3 centers
  - 3 dose levels (projected):  $10^6$ ,  $10^7$ ,  $10^8$  TU/ml ( $10^2$ ,  $10^3$ ,  $10^4$  TU/g)
  - Standard dose-escalation algorithm: min. of 3 and max of 6 subjects at each dose level
- **Study Protocol:**
  - Stereotactic, transcranial injection 0.4 mL Toca 511 into recurrent tumor
  - 5-FC 150 mg/kg/day started 3 weeks after injection-repeat monthly
  - Gd-MRI to assess objective response (Macdonald criteria)
  - Study ~9 patients in dose-escalation portion of study
  - Study additional 12 patients at MTD (objective tumor response)
- **End Points:**
  - Safety, tolerability, objective tumor response

# Clinical Trial Schematic



# Dose selection for phase 1 study



	<b>Volume injected (mL)</b>	<b>Vector Conc. (TU/mL)</b>	<b>Vector Delivered (TU/g brain)</b>
Mouse effective dose	0.010	10e6	2 x 10e4
Human proposed low dose	0.4	10e6	2.6 x 10e2
Human proposed middle dose	0.4	10e7	2.6 x 10e3
Human proposed high dose	0.4	10e8	2.6 x 10e4

# Key Phase 1 Safety Precautions



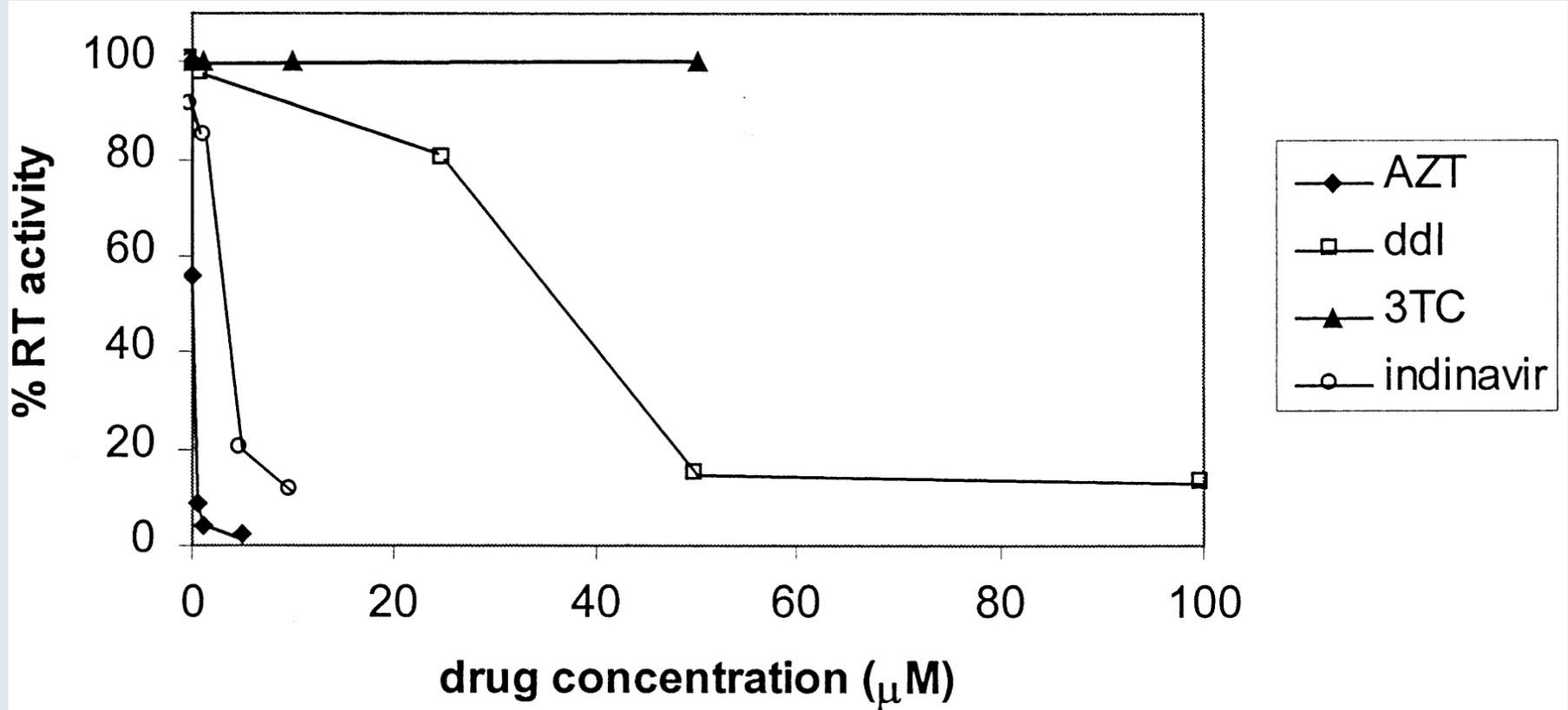
- Select starting dose based on preclinical safety data
- Exclude patients with HIV
- Exclude patients taking  $> 8$  mg dex/day at entry
- Exclude patients with lymphocytes  $< 1,000/\text{mm}^3$
- Exclude patients with abnormal renal function
- Frozen section Bx to confirm needle placement
- Gr 3, 4 AES, uncontrolled seizures, unexplained change neuro status = dose limiting toxicities
- Stopping rules based on viremia + clinical status
- Subjects followed for up to 15 years

# MLV Safety



- Ampho-MLV is classified as RG-2 virus
  - Other common RG-2 viruses: HSV, HBV
- Ampho-MLV is not a known adult human pathogen
- Lab workers have not developed disease despite extensive exposure to MLV and MLV-infected mice
- Biosafety Level 2 precautions will be observed during study
  - Hospital lab already operates at BSL-2
- MLV viremia can be treated with antiretroviral therapies

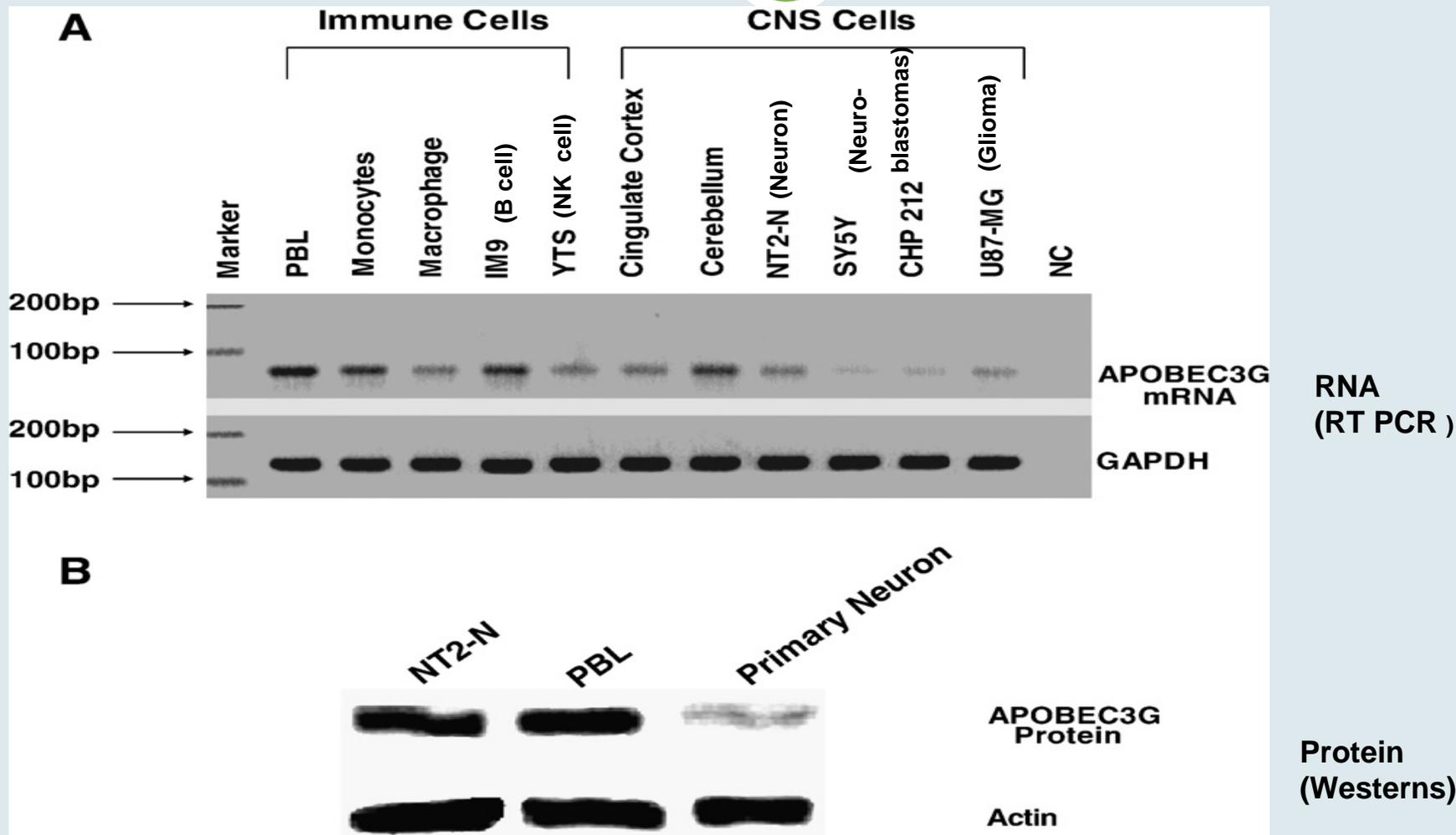
# MLV is susceptible to certain antiretrovirals



**Thank You**

# Questions

# Human immune cells generally express more APOBEC3G than CNS cells



# APOBEC3G is minimally increased in GBM

