

The patient with a GBM

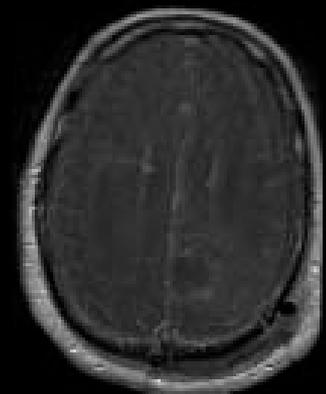
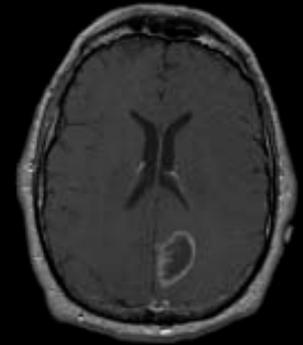
- **Standard therapy:**
 1. Surgical resection OR biopsy
 2. External limited field radiation for 6 weeks
 3. Concomitant chemotherapy with temozolomide/postradiation temozolomide

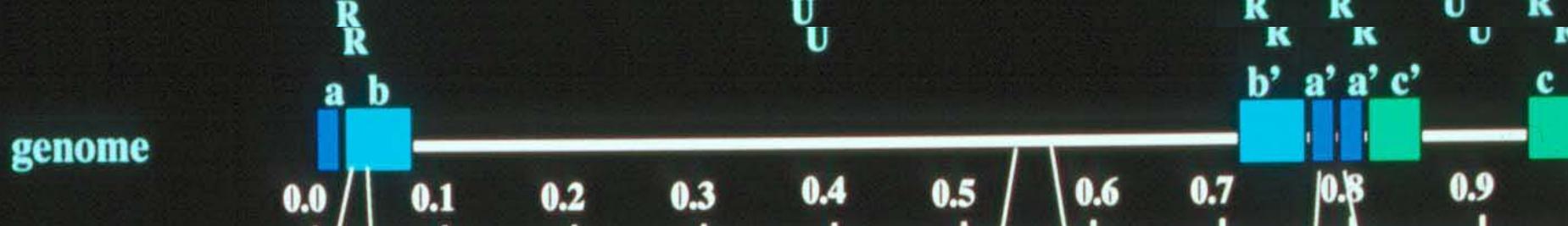
- **4. Almost all recur**



Radiation
←
6-8 wks

Surgery
↓





Cancer Res., April 1, 2005

Research Article

An Oncolytic HSV-1 Mutant Expressing ICP34.5 under Control of a Nestin Promoter Increases Survival of Animals even when Symptomatic from a Brain Tumor

Hirokazu Kambara,¹ Hideyuki Okano,^{2,3} E. Antonio Chiocca,¹ and Yoshinaga Saeki¹

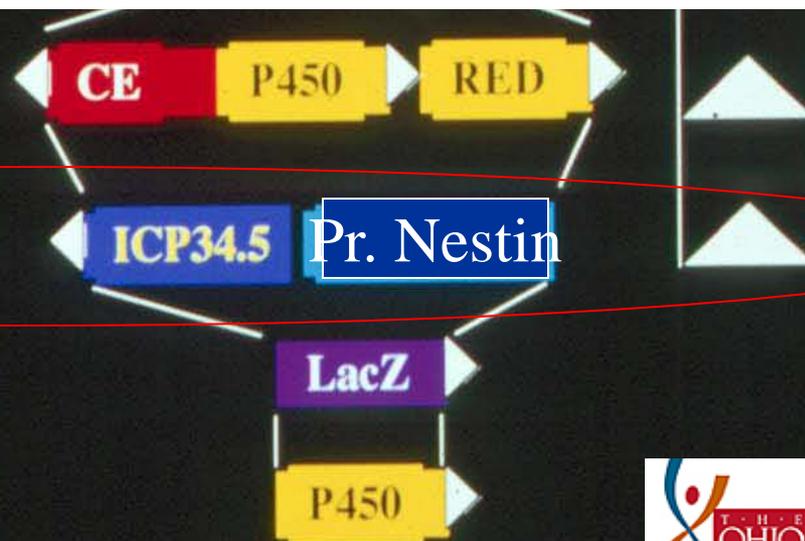
¹Dardinger Center for Neuro-oncology, Department of Neurological Surgery, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio; ²Department of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; and ³Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

MGH2

Nestin34.5

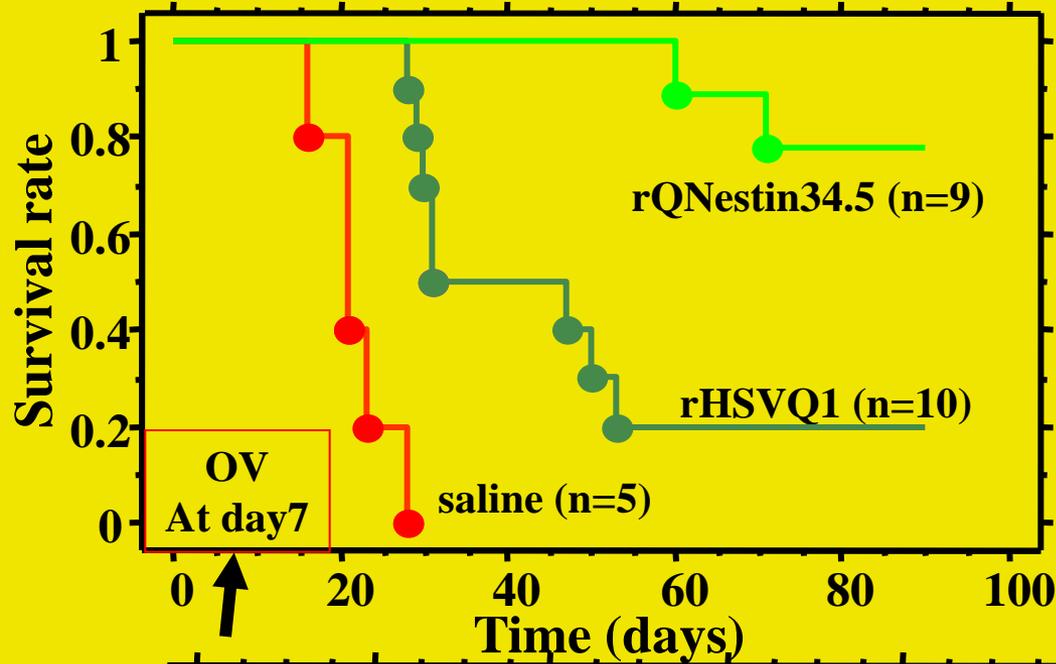
hrR3

rRp450



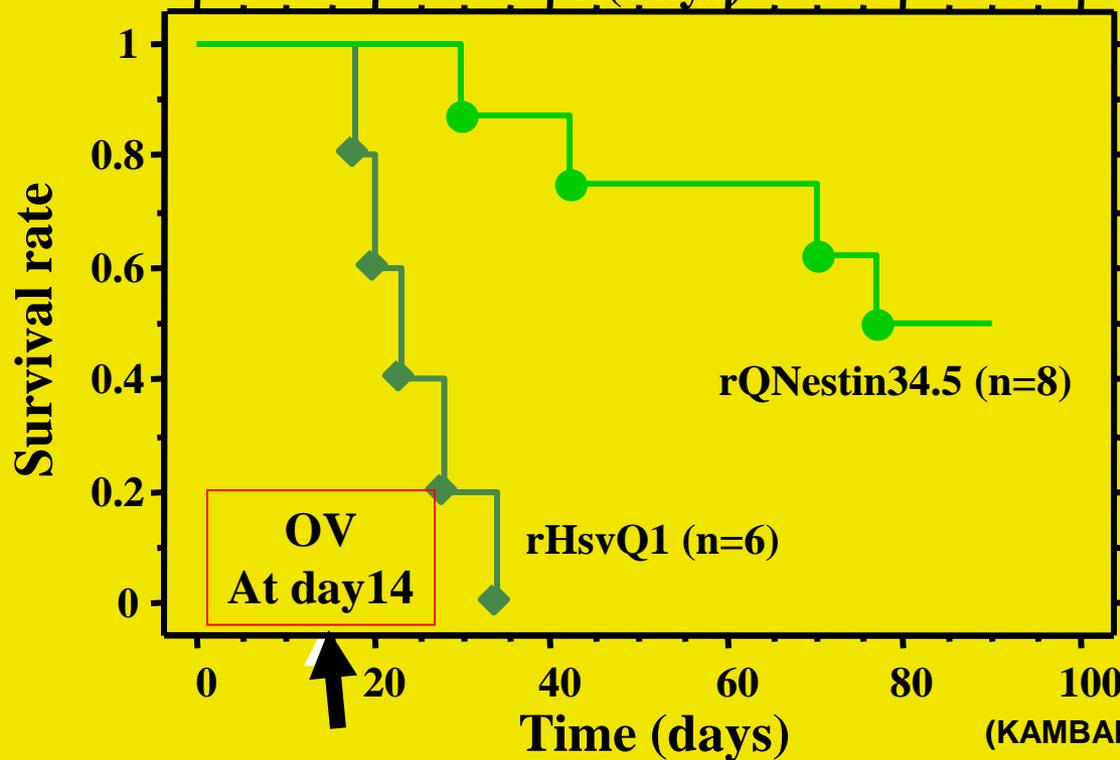
Mechanisms of selectivity/ safety

1. HSV1 strain is attenuated laboratory strain (i.e. ts ICP4 mutation; complement—evading gC mutation).
2. UL39 (ICP6 mutation) restricts replication to mitotic cells or, if quiescent, cells would need *p16* defects to complement replication (Aghi et al., *Oncogene*, 2008).
3. Nestin promoter/enhancer transcriptional cassette restricts expression of viral ICP34.5 gene (needed for robust replication) to nestin-expressing cells (Kambara et al., *Cancer Res.* 2005)



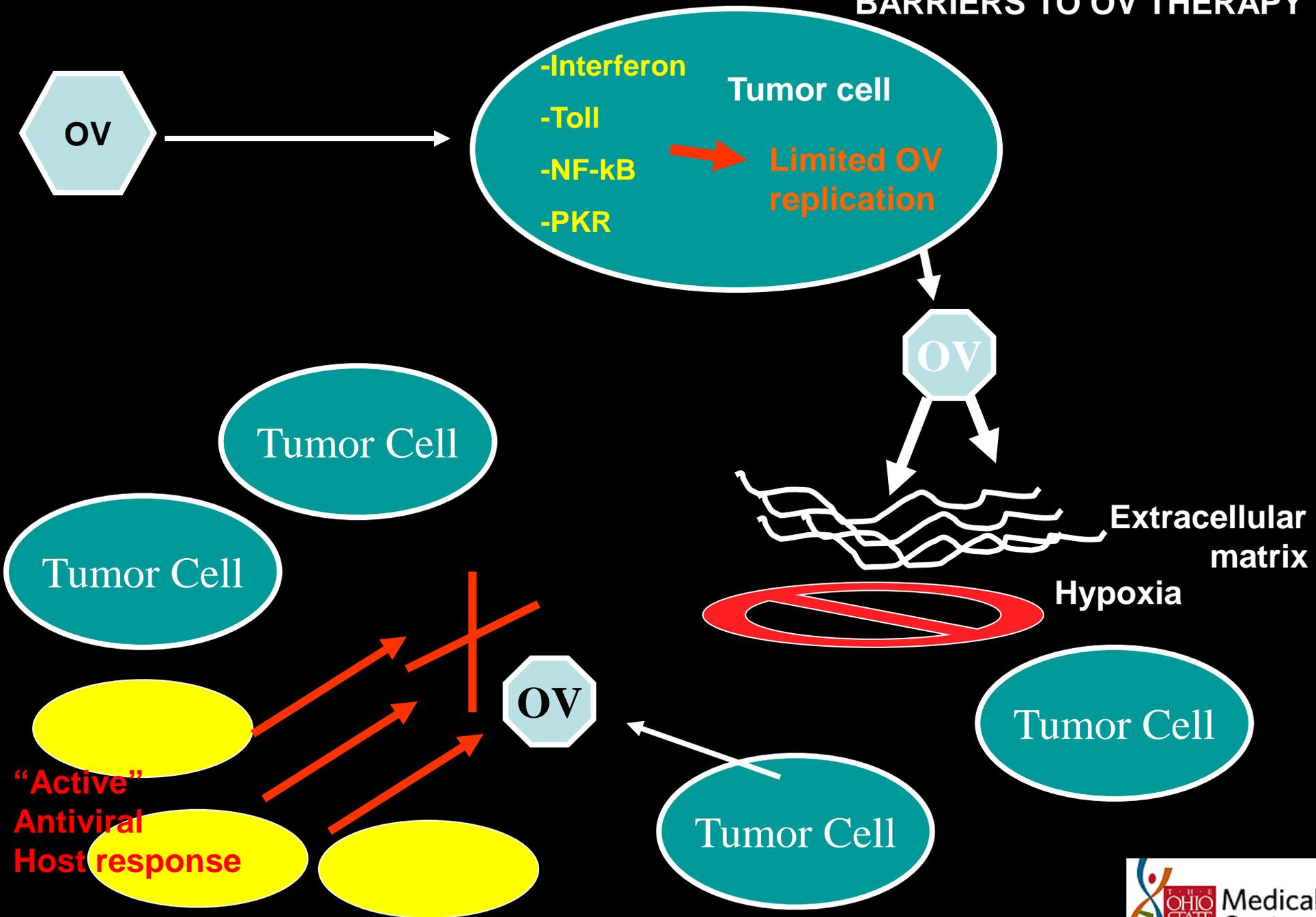
Animal survival is 80% when OV is injected 7 days after tumor implant

(Dose of virus = 3×10^5 pfus)



Animal survival is 50% when OV is injected 14 days after tumor implant

BARRIERS TO OV THERAPY

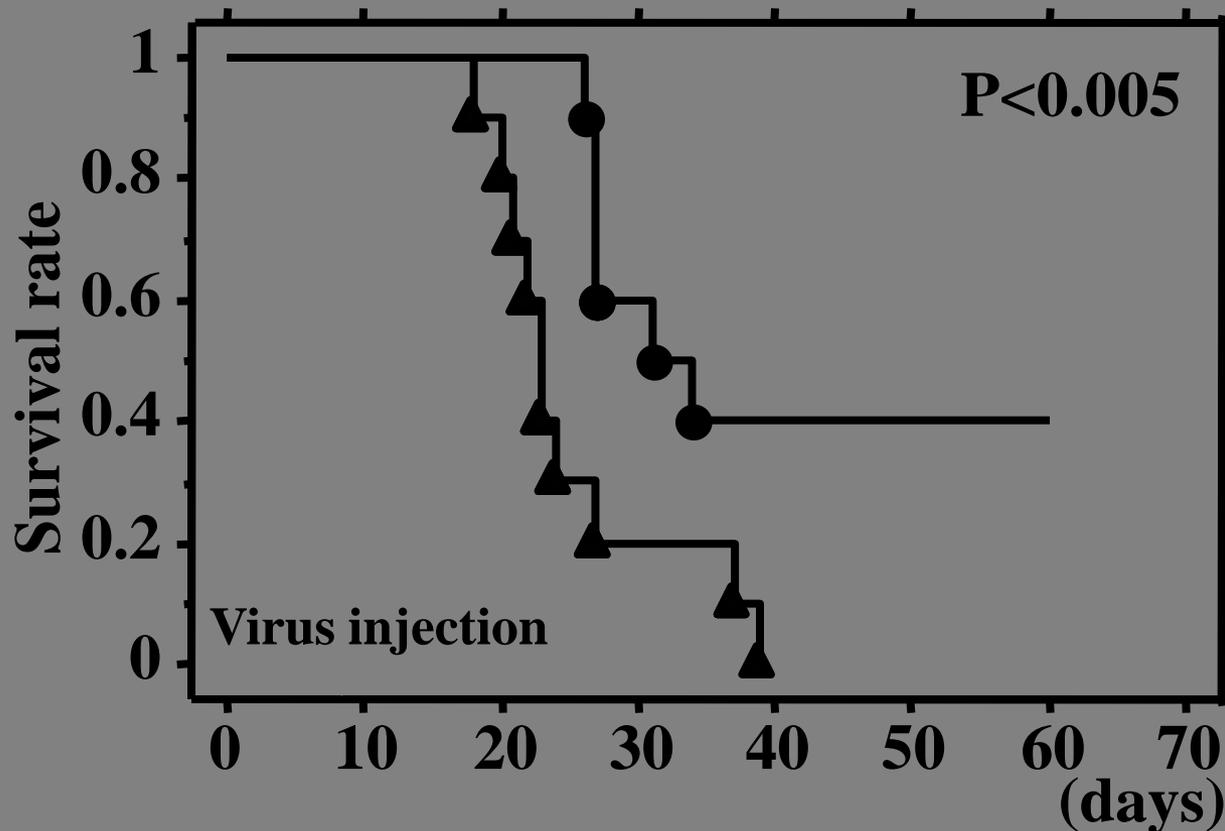


Cyclophosphamide Allows for *In vivo* Dose Reduction of a Potent Oncolytic Virus

Hirokazu Kambara, Yoshinaga Saeki, and E. Antonio Chiocca

Dreadinger Center for Neuro-oncology and Neurosciences, Department of Neurological Surgery, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio

Athymic mice with Intracerebral glioma xenografts



Other OV's where CPA facilitates oncolysis:
 HSV2 (Li et al, Cancer Res, 2007)
 Measles (Ungerechts et al, Mol. Ther., 2007)
 Adenovirus (several papers)

● ● 3x10³ pfu + CPA
 ▲ ▲ 3x10³ pfu + vehicle

Summary (efficacy)

- rQNestin34.5v.2 more efficacious in mouse model of human glioma (3×10^4 - 10^5 pfus) than ICP34.5- virus
- CPA enhances efficacy and allows for dose-reduction (3×10^3 pfus)
- rQNestin34.5v.2 is more cytotoxic and replicates more in human gliomas than normal cells in vitro
- Functionally, Nestin-driven ICP34.5 in rQNestin34.5 leads to less eiF2a-P in glioma compared to normal cells in vitro

Route	Species	Virus	Dose (Pfu)	No CPA	CPA (300 mg/kg)	CPA (200 mg/kg)
intracranial	Balb/C	V2	1 x 10 ⁶		0 / 4	0 / 10 (day-33)
		V2	3 x 10 ⁶		3 / 23 (day-57)	
		V2	1 x 10 ⁷	1 / 28	4 / 24	0 / 9 (day-33) + 0 / 15 (day-14)
		F	1 x 10 ³		4 / 4	
		F	1 x 10 ⁴	3 / 5		
		F	1 x 10 ⁵	15 / 20		
		PBS		0 / 9	1 / 6	0 / 1 (day-33) + 0 / 5 (day-14)
intracranial	Athymic	V2	1 x 10 ³		0 / 5	
		V2	1 x 10 ⁴		1 / 25 (day-43)	0 / 15 (day-20)
		V2	3 x 10 ⁴		0 / 4	
		V2	1 x 10 ⁵		10 / 23	
		V2	3 x 10 ⁵	0 / 11 (day-13)	2 / 15	
		V2	1 x 10 ⁶	11 / 43	0 / 4	
		V2	3 x 10 ⁶	8 / 29		
		V2	1 x 10 ⁷	6 / 27		
		F	1 x 10 ⁴	19 / 19		
		PBS		0 / 2	0 / 2	0 / 5 (day-20)
intrathecal	Balb/C 8-week	V2	1 x 10 ⁶		0 / 5	
		V2	3 x 10 ⁶		0 / 5	
		V2	1 x 10 ⁷	1 / 6	0 / 5	0 / 12 (day-15)
		F	1 x 10 ⁵	1 / 5		
		PBS		0 / 4	1 / 4	0 / 3 (day-15)
intrathecal	Balb/C >6-month	V2	1 x 10 ⁷	0 / 5	0 / 9	0 / 11 (day-12)
		F	1 x 10 ⁵	3 / 5		
		PBS		0 / 4	0 / 4	0 / 3 (day-12)
intrahepatic	Balb/C	V2	1 x 10 ⁷	0 / 10	2 / 10	0 / 12 (day-13)
		F	1 x 10 ⁵	0 / 9	4 / 5	
		PBS		0 / 10	0 / 6	0 / 3 (day-13)
intravenous	Balb/C	V2	1 x 10 ⁷	0 / 10	1 / 10	1 / 12 (day-13)
		F	1 x 10 ⁵	0 / 4	2 / 5	
		PBS		0 / 5	2 / 5	0 / 3 (day-13)

Summary (Safety)

- In Balb/c mice, ic injection of $10^7 > \text{LD05}$ (1/28)
- In Balb/c + 300 mg/kg CPA, 0/4 mice alive at 10^6
- CPA at 200 mg/kg tolerated much better!
- CPA increases toxicity in Balb/c: ic injection $10^7 = \text{LD17}$ (4/24); it $10^7 = 0/13$; ih $10^7 = 2/10$; iv $10^7 = 1/10$

Objectives

- Primary: Assess safety and determine MTD of rQNestin34.5v.2 injected into recurrent MG after CPA immunomodulation
- Secondary:
 - Assess levels of rQNestin34.5V.2 after injection with rQNestin34.5v.2 and any other local tumor response
 - Determine MRI alterations in injected tissue
 - Delineate cellular response in brain tissue
 - Assess shedding/ viremia /antibody responses

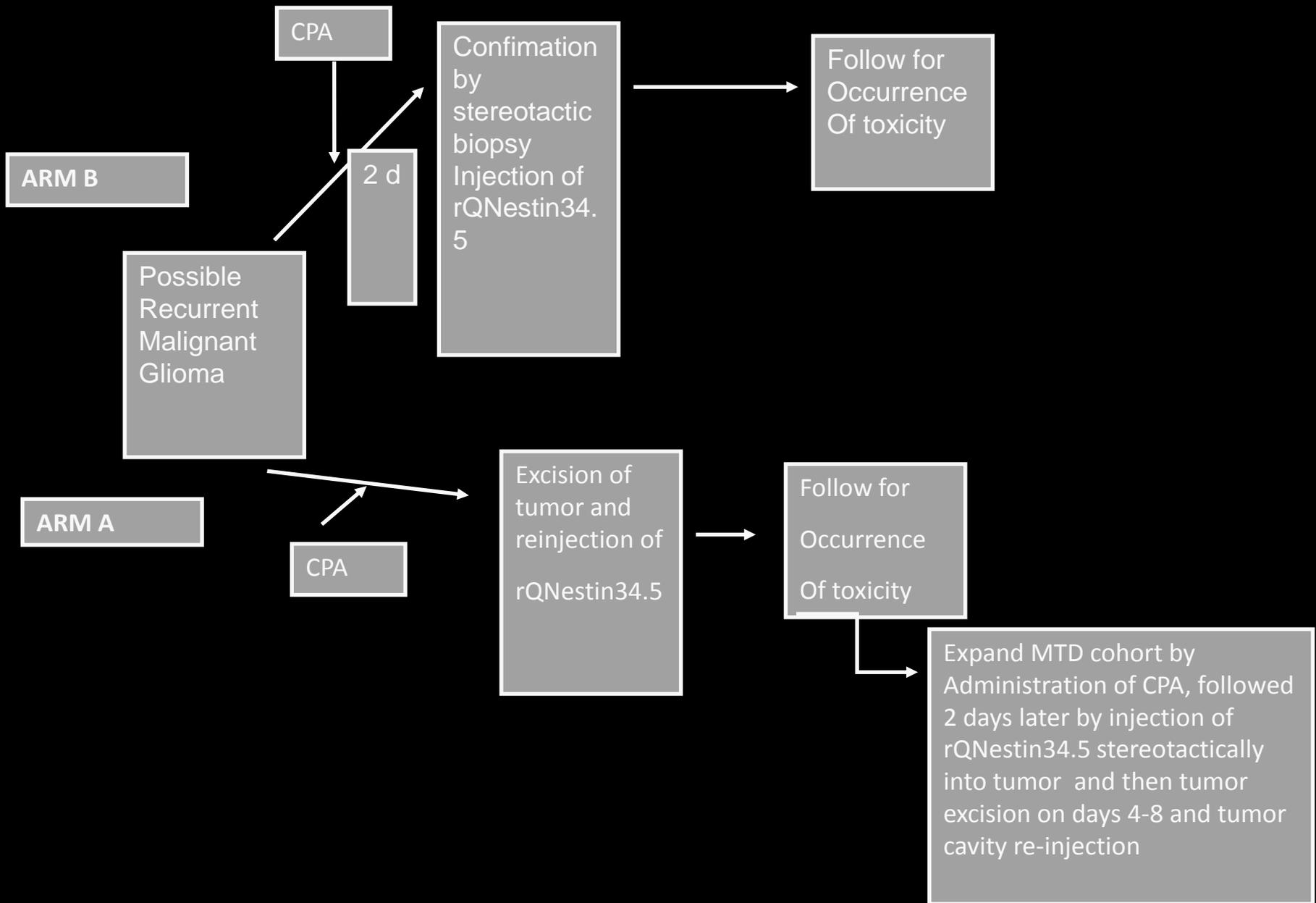
Inclusion criteria

- Frozen tissue confirmation of MG
- Prior diagnosis of glioma (AA, MG, O, AO, Mixed)
- Prior history of chemoradiation per Stupp
- Lesion greater than 1 cm in diam.
- Heme, renal, liver function
- KPS of at least 70
- Age 18-80
- Informed consent
- Birth control
- Steroids stable for one week
- Capable of MRI

Exclusion Criteria

- Significant liver/renal disease
- Hepatitis B/C history
- Progressive systemic malignancy
- HIV and active viral/bacterial/fungal infections
- Active HSV1 infection and on antiherpetics
- Allergy to CPA
- Immunosuppressive disorder
- Anesthesia risk
- Serious cardiopulmonary issues
- Pregnant/lactating women
- Spillage into ventricle
- Other experimental protocol within 6 weeks

Trial Scheme



Rationale for two arms

1. At recurrence, some patients can undergo a re-resection, while in others the recurrent tumor may not be resectable without causing deficits
2. In either case, clinical trials are warranted due to lack of effective therapies
3. It is likely that subject tolerance for rQNestin34.5v.2 injection into an unresected tumor (arm B) will be different than that for a resected tumor (arm A) and that injection into a tumor mass (arm B) may differ from injection into the peritumoral area (arm A)

Dosing

- 5 cohorts (from 10^8 pfu by half-log up to 10^{10} pfus).
- Rationale: In Balb/c mice treated with 10^6 pfu there was 0/4 mortality with 300mg/kg CPA and 0/10 mortality with 200 mg/kg CPA. Weight of mice = 20 grams, which would translate (80 kg human) to safe dose of 4×10^9 pfu (weight).

Patients

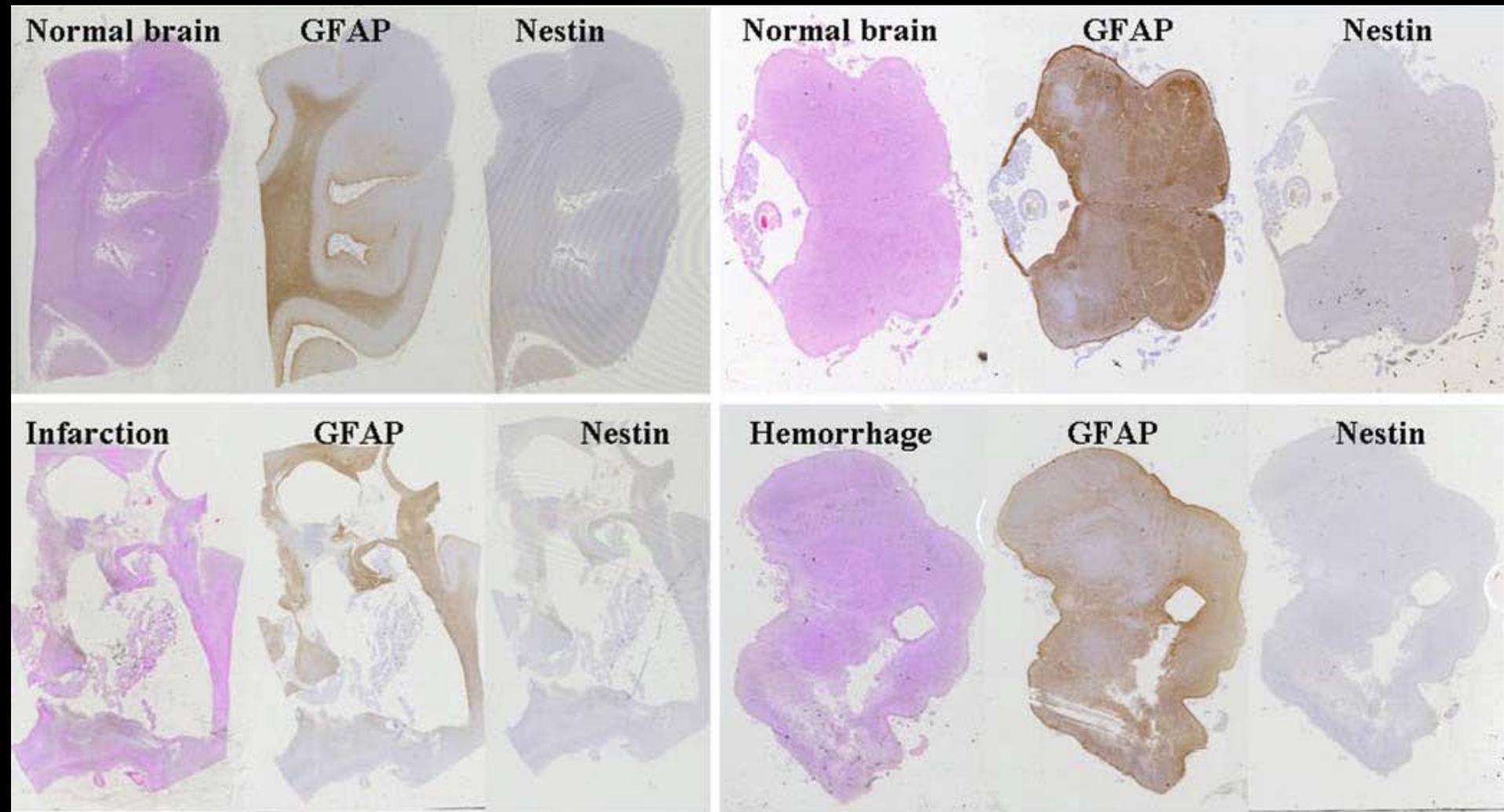
- 18 total (3 per cohort with expansion to 3 additional at MTD or HTD)
- 10 days to pass between first subject accrued to cohort and subsequent 2.
- Inter-cohort escalation to wait 21 days
- MTD = dose immediately preceding the dose of one DLT

DLTs

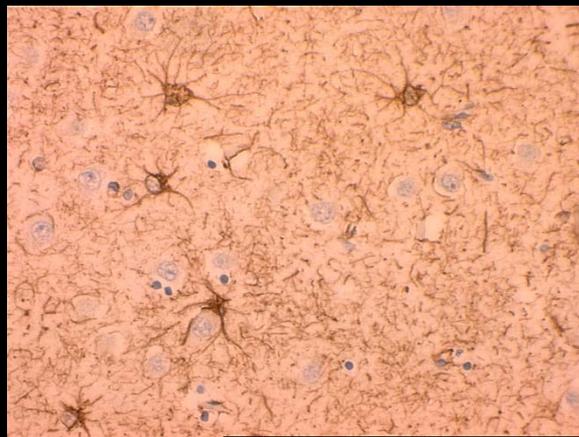
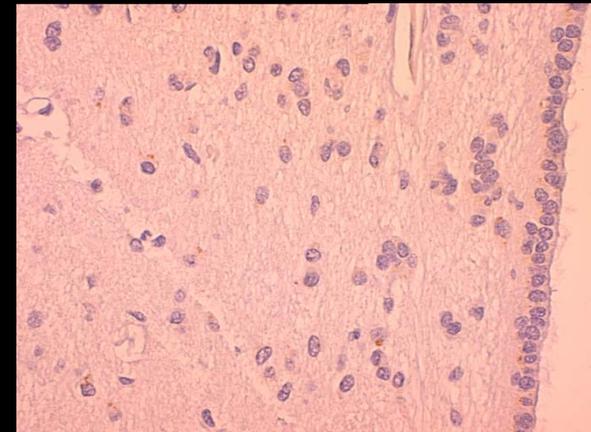
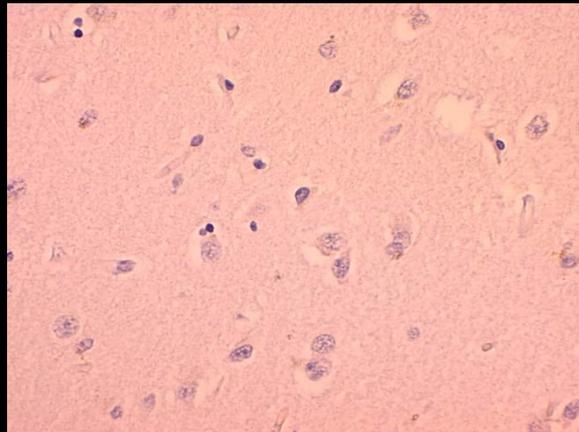
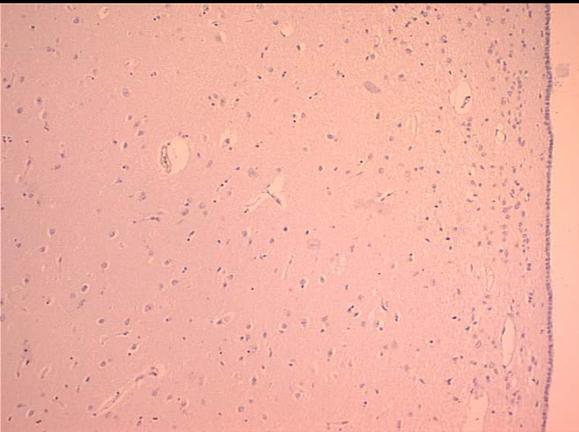
- Grade 4-5 toxicity on CTC AE v.4 except for grade 4 lymphocyte, neutrophil, WBC decrease on investigation category (due to CPA)
- Grade 3 encephalitis/meningitis on infections category
- Grade 3 toxicity for ataxia, LOC, encephalopathy, EP disorder, HC, ICH, LE, myelitis, PTS, stroke, somnolence
- Grade 3 Psych disorder for delirium, hallucinations, psychosis,

Major Questions from Reviewers

Adult human brains (relative lack of nestin Ihc – Kitai et al., 2010)

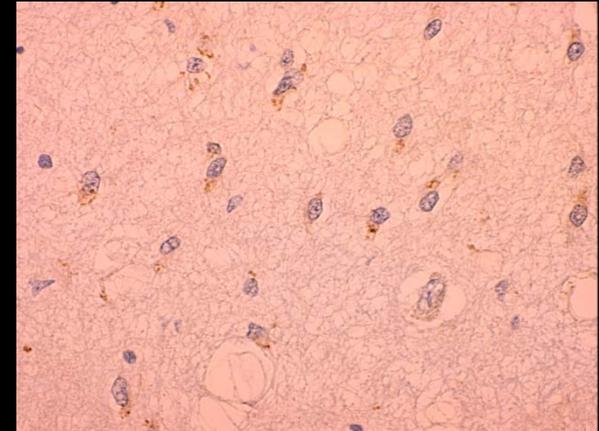
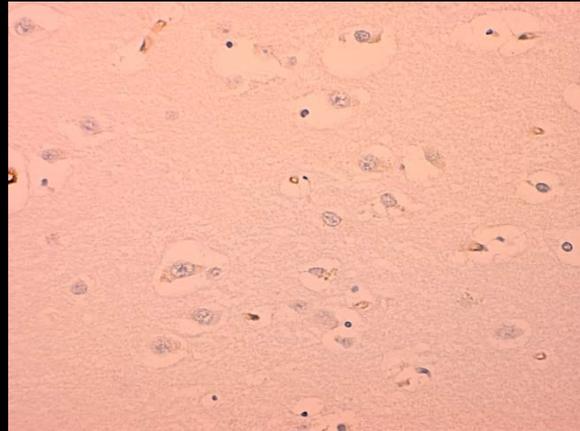
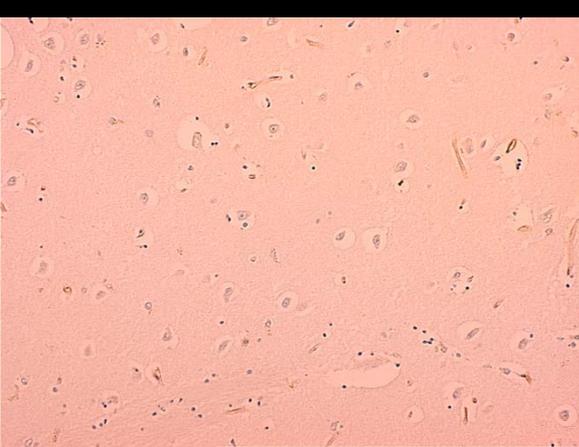


Patient 1 – 50+ year old with GBM. Nestin immunohistochemistry in SVZ (NSC niche)



GFAP positive control

Patient 2 – 50+ year old with previous history of GBM (passed away from causes other than tumor...at post-mortem, no gross tumor found in brain. Nestin IHC in white matter

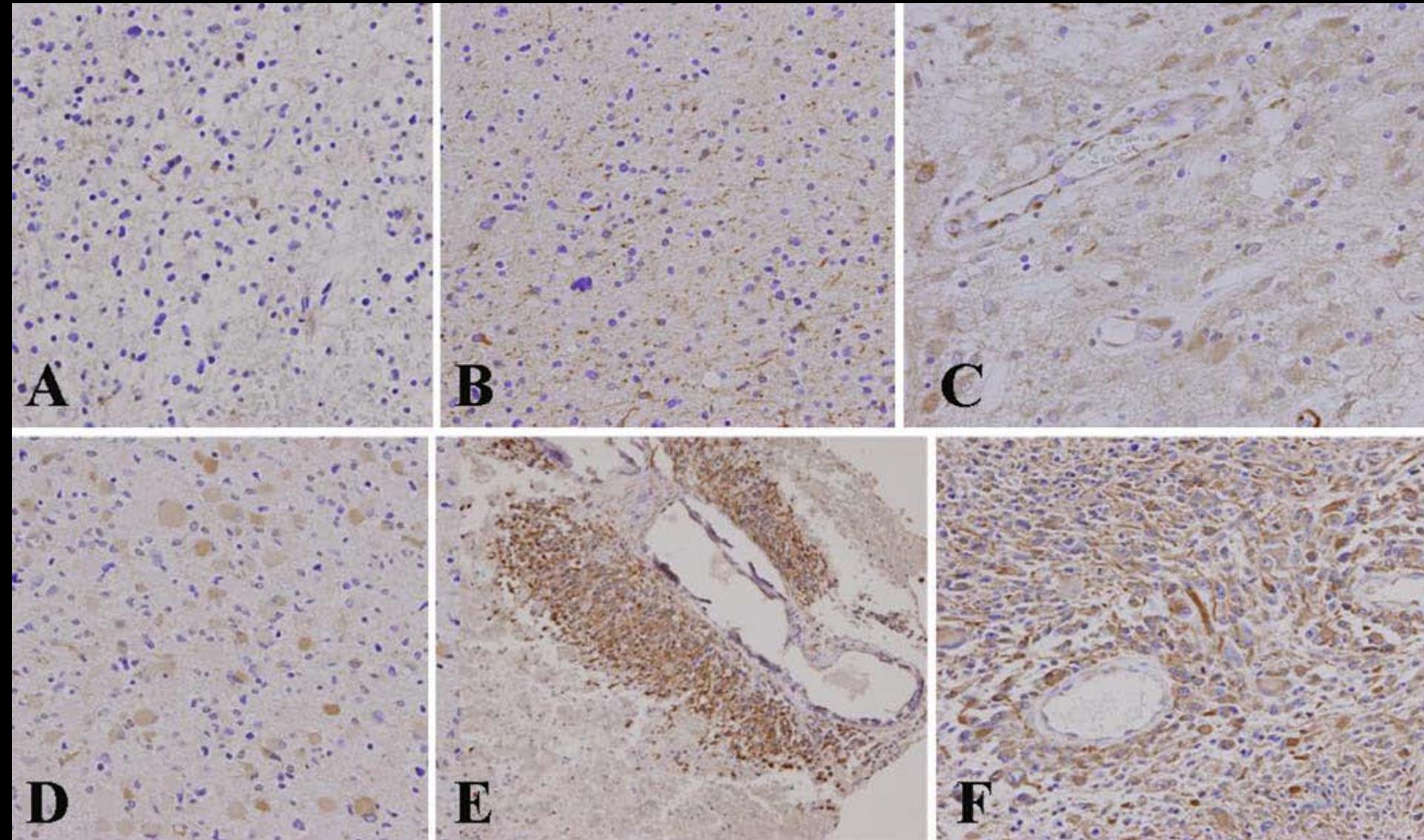


Summary

- Mice brains with reportedly high levels of nestin expression in NSCs niches may be more susceptible to rQNestin34.5v.2 toxicity than older adult human brains with much less nestin expression
- Current therapies (XRT) shown to destroy NSCs in brain.

Questions: In GBM what is the range of the % of nestin-positive tumor cells within a tumor?
(Breakefield)

Range of the % of nestin-positive tumor cells within a tumor?(Breakefield)



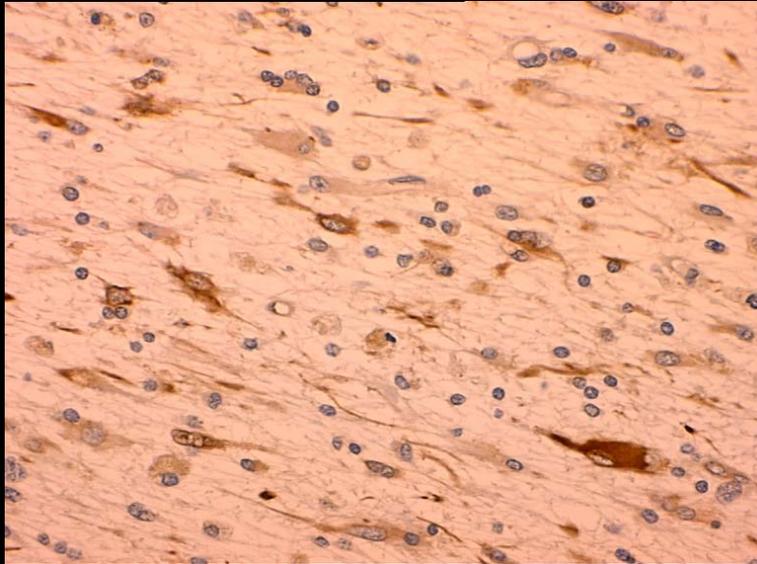
(Kitai et al., 2010)

Table 2 (Zhang et al., 2009)⁴

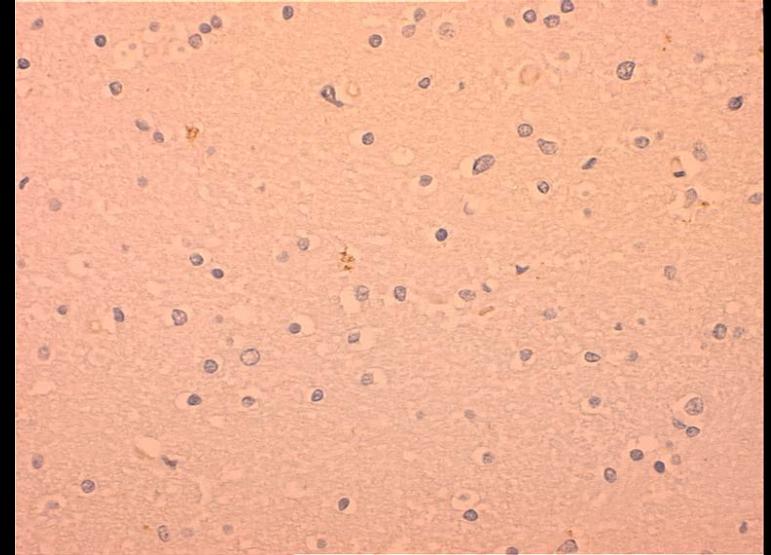
Nestin and CD133 expression in human gliomas tissues with different clinical grading

Clinical Grading	NO.	Nestin (n, %)			CD133 (n, %)		
		0	1+~2+	3	0	1+~2+	3
<i>Low-grade tumors</i>	56	16 (28.6)	32 (57.1)	8(14.3)	20 (37.1)	27 (48.2)	9(16.1)
Astrocytoma	18	7 (38.8)	11 (61.1)	0 (0)	9 (50.0)	9 (50.0)	0 (0)
Ependymoma	15	6 (40.0)	8 (53.3)	1 (6.7)	7 (46.7)	7 (46.7)	1 (6.7)
Oligodendroglioma	11	2 (18.2)	8 (72.7)	1 (9.1)	3 (27.3)	7 (63.6)	1 (9.1)
Oligodendroastrocytoma	4	1 (25.0)	1 (25.0)	2 (50.0)	1 (25.0)	1 (25.0)	2 (50.0)
Pilocytic astrocytoma	8	0 (0)	4 (50.0)	4 (50.0)	0 (0)	3 (37.5)	5 (62.5)
<i>High-grade tumors</i>	69	6(8.7)	36 (52.2)	27 (39.1)	7(10.1)	37 (53.6)	25 (36.2)
GBM	48	4 (8.3)	28 (58.3)	16 (33.3)	5 (10.4)	29 (60.4)	14 (29.2)
Anaplastic astrocytoma	11	2 (18.2)	6 (54.5)	3 (27.3)	2 (18.2)	7 (63.6)	2 (18.2)
Malignant oligodendroglioma	6	0 (0)	1 (16.7)	5 (83.3)	0 (0)	1 (16.7)	5 (83.3)
Malignant ependymoma	4	0 (0)	1 (25.0)	3 (75.0)	0 (0)	0 (0)	4 (100.0)

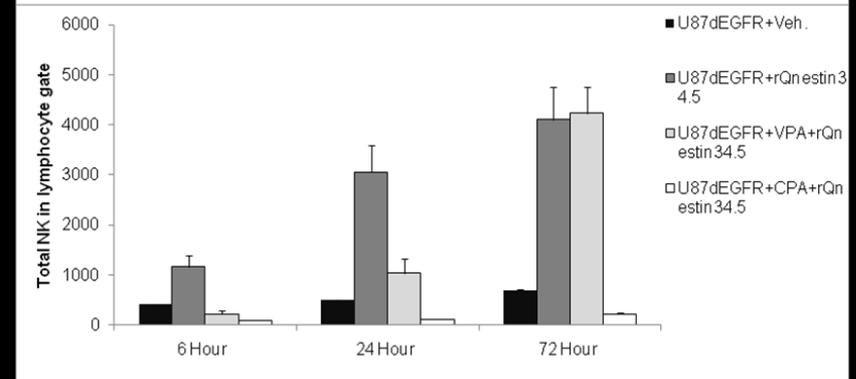
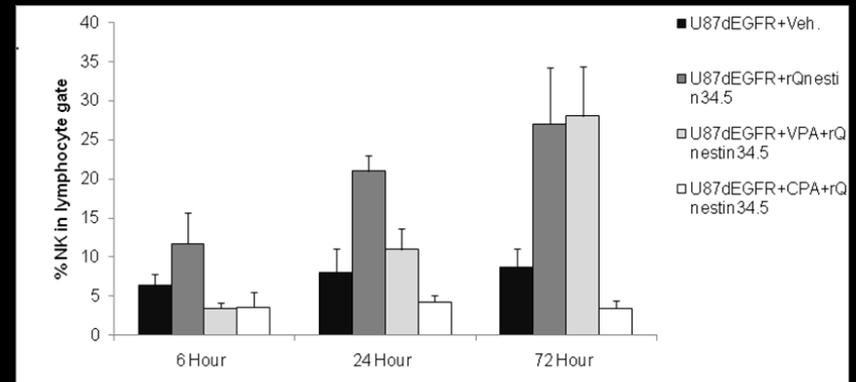
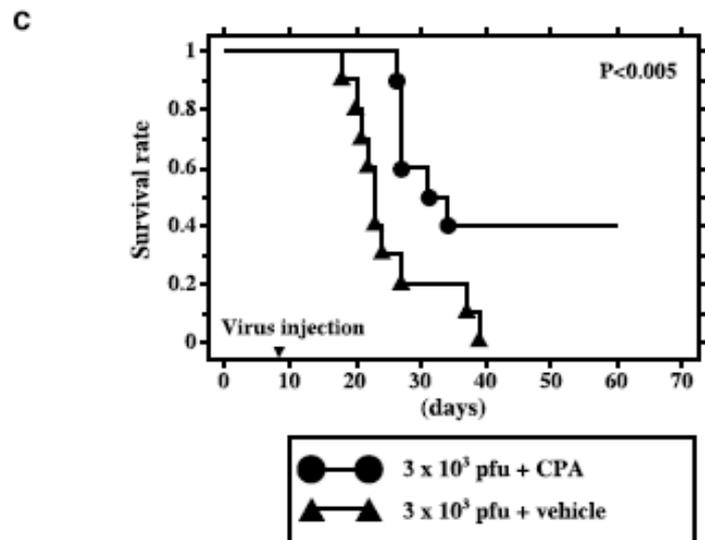
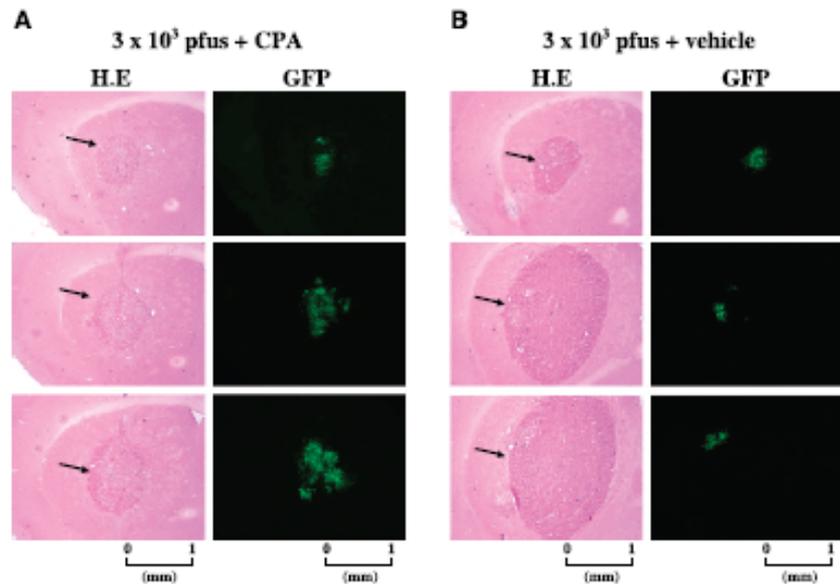
Nestin IHC in patient with recurrent GBM



Nestin IHC in white matter of patient with GBM treated, But without recurrence



What is their current data on effect of CPA treatment prior to virus injection in tumors in animal models? (Breakefield)

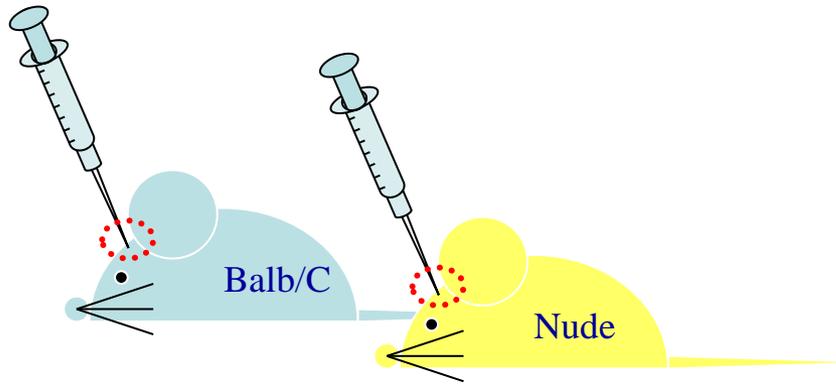


Queries: Do the investigators
have the preclinical toxicity data
for combined use of
rQNestin34.5 and
cyclophosphamide?
(Fong)

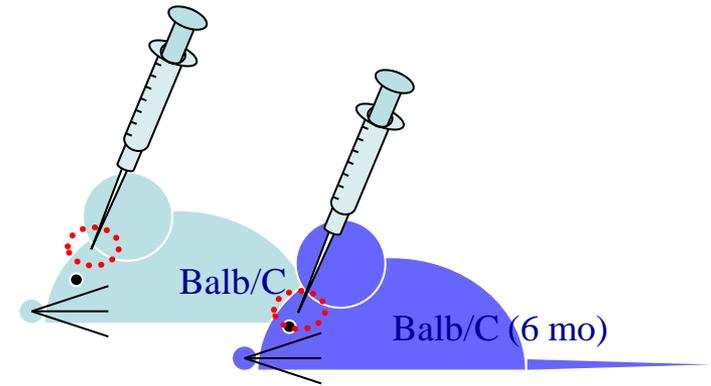
In vivo toxicity assay: rQNestin34.5-v.2

II. Determine toxicity upon in vivo administration of rQNestin34.5-v.2

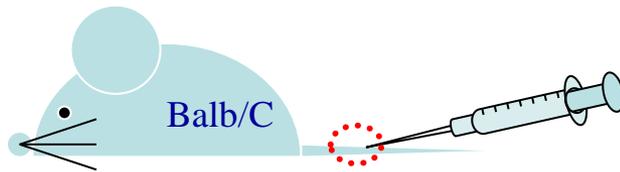
(i) intracerebral



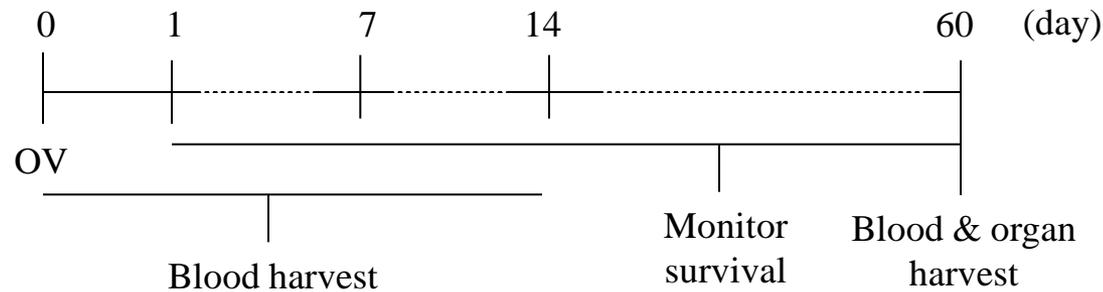
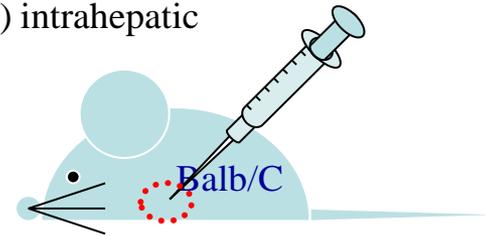
(ii) intrathecal



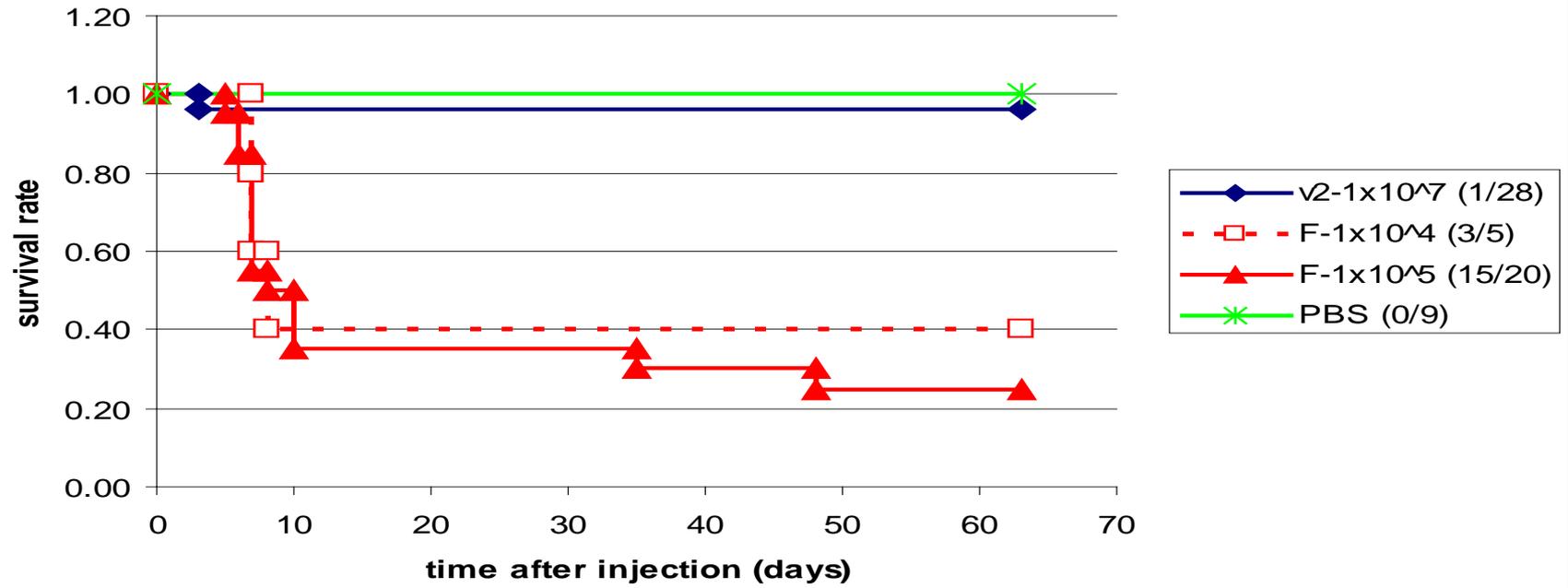
(iii) intravenous



(iv) intrahepatic



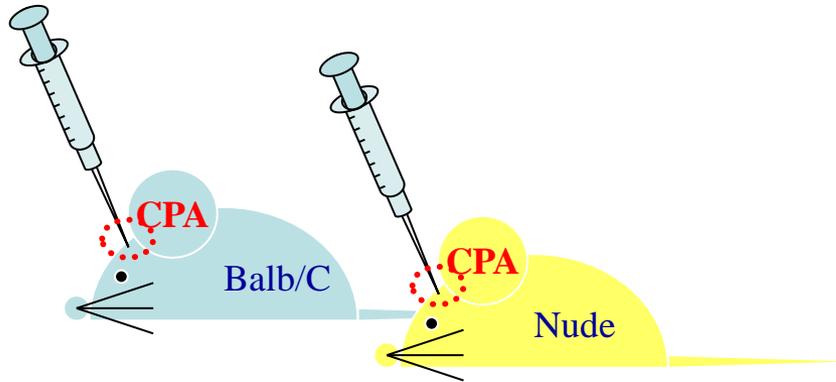
Balb/C-intracranial



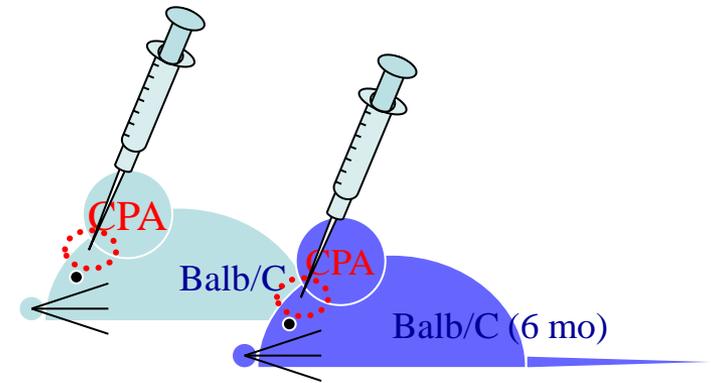
Preclinical trial: rQNestin34.5-v.2

III. Determine toxicity upon in vivo administration of rQNestin34.5-v.2 in the presence of CPA

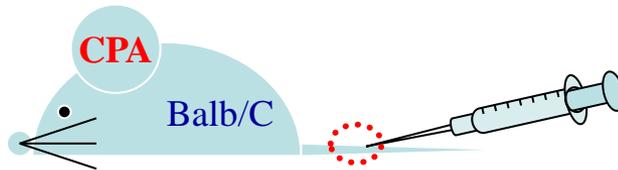
(i) intracerebral



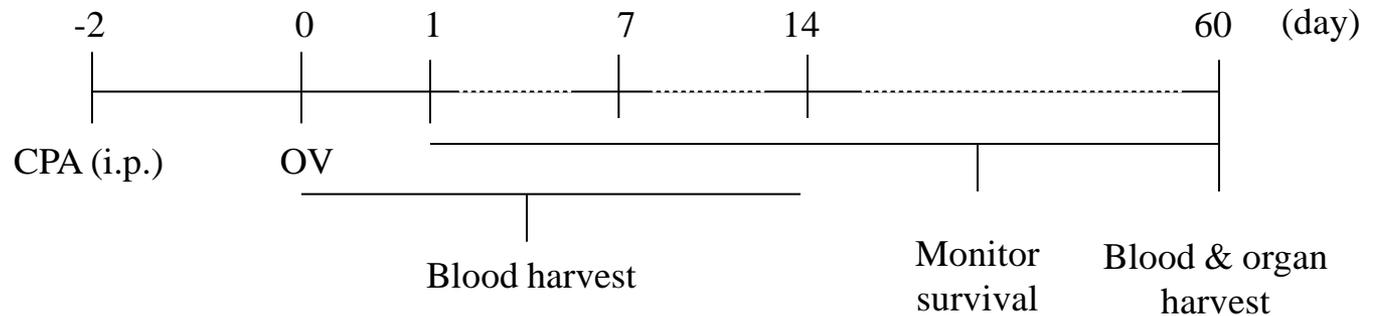
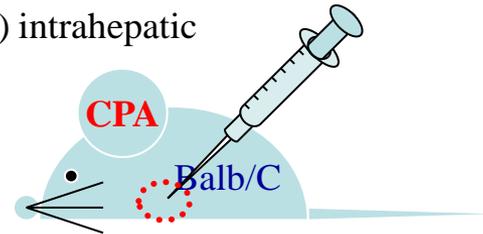
(ii) intrathecal

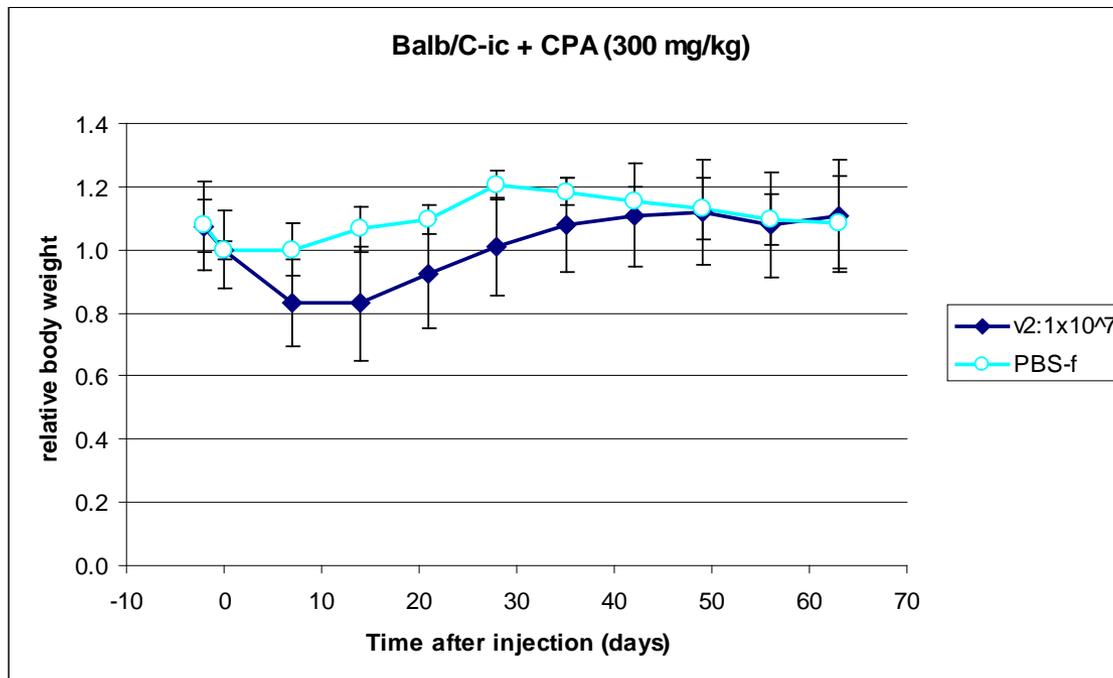
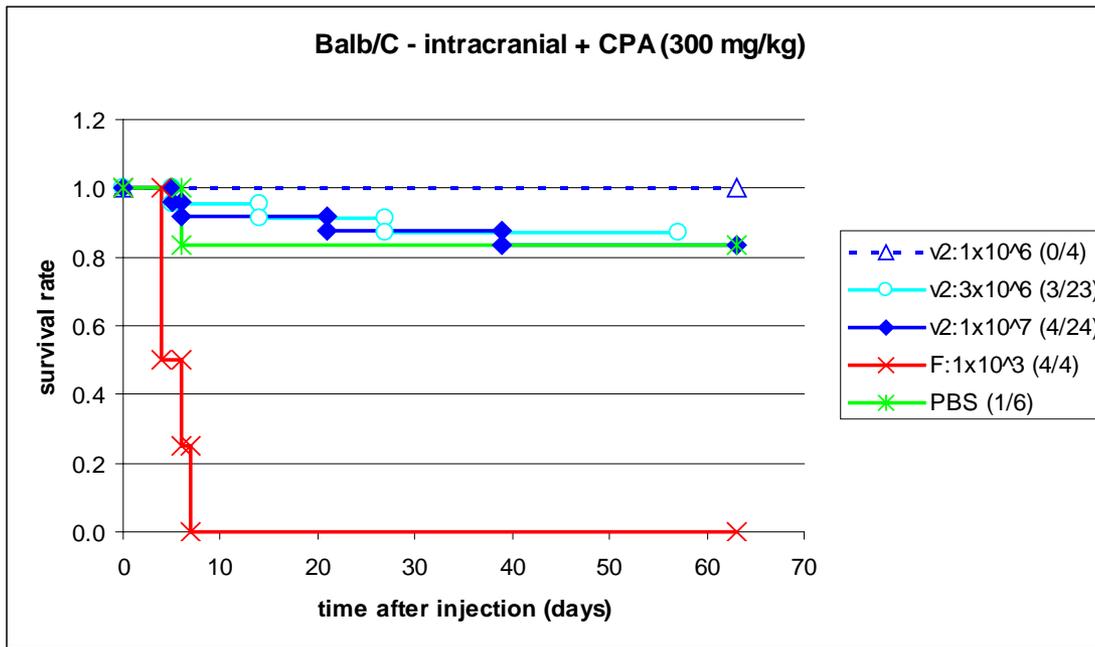


(iii) intravenous



(iv) intrahepatic

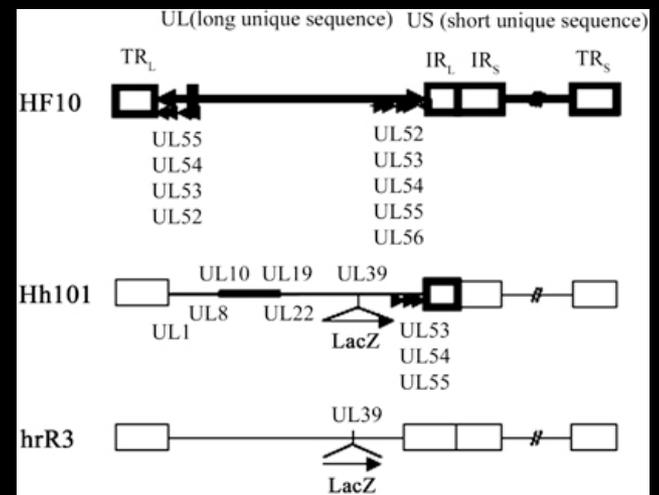
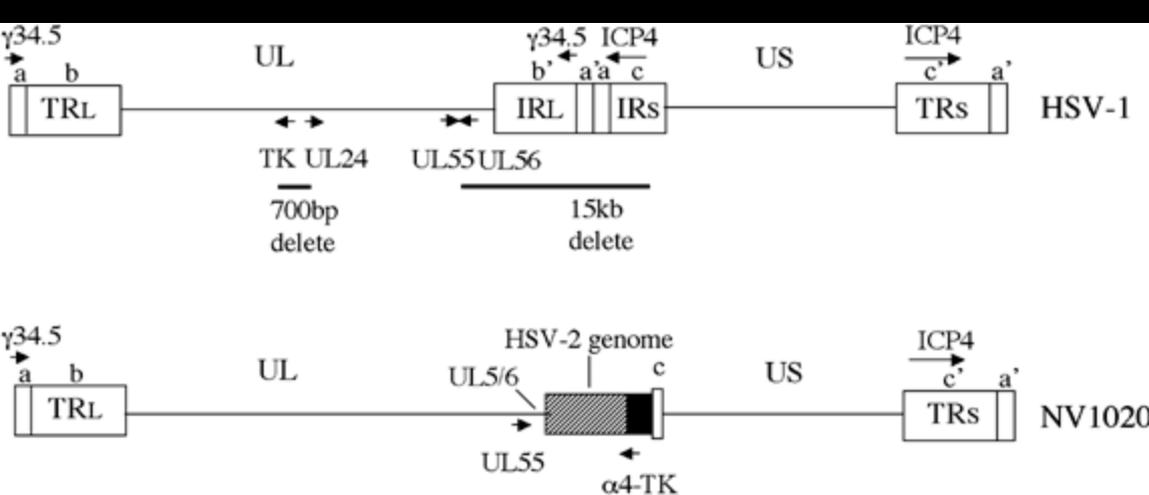




Questions: Are there likely non-tumor tissues in human adults that may trigger Nestin-34.5? (Fong)

3 Clinical Trials with systemic injections of ICP34.5+ α HSV-1s, where ICP34.5 is expressed from viral promoter.

2 with ih injection of NV1020 (Kemeny et al., 2006; Geevarghese et al., 2010 and 1 with ip injection of HF10 (Nagoya Univ.)

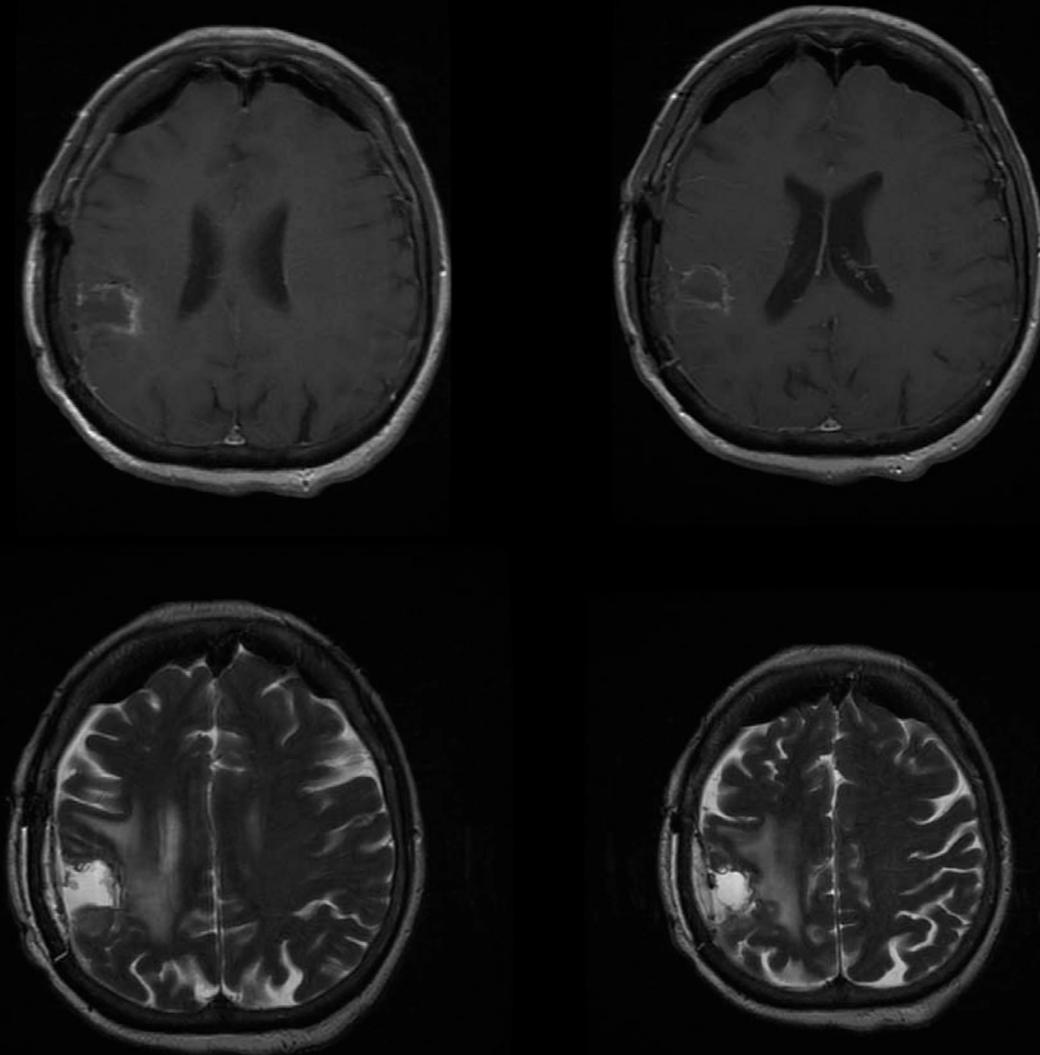


Questions: Should parallel cohorts be performed for HSV Ab+ and HSV AB- subjects? Is there likely to be a difference in toxicity and efficacy between these two groups? Might there be a possibility that toxicity in seronegative subjects not seen in seropositive subjects may prematurely end the trial if both groups are studied together? (Fong)

1. This has not been observed in the glioma trials or in the peripheral trials so far in either glioma or non-glioma trials

(“One HSV-1-seronegative patient was inadvertently enrolled: her postinfusion viral syndrome was mild and no noteworthy other adverse events were observed. Others have treated small numbers of HSV-1-seronegative patients with a similar HSV construct without untoward toxicity (Markert et al., 2009)⁸, suggesting that this eligibility constraint might be lifted in future studies.) – (Geevarghese et al., 2010)

How will the investigators distinguish between physical effect of injection from viral infection in the MRI studies? What is the data in preclinical studies of MR changes directly attributable to viral infection?



Questions: Cyclophosphamide (PHA) will be given 2 days before tumor biopsy or resection. The virus rQNestin34.5v.2 will be injected if the biopsy demonstrates high grade or malignant tumor. Is PHA administration the standard of care for patients who may not receive the virus if the biopsy does not identify tumor? Is the potential HPA toxicity justified in these individuals? (Yankaskas)

CPA has and is still used as salvage chemotherapy for patients with Recurrent MG.