

***A Phase I Dose Escalation Study of
Intratumoral Herpes Simplex Virus-1
Mutant rRp450 in Patients with Refractory
Sarcoma or Neuroblastoma***

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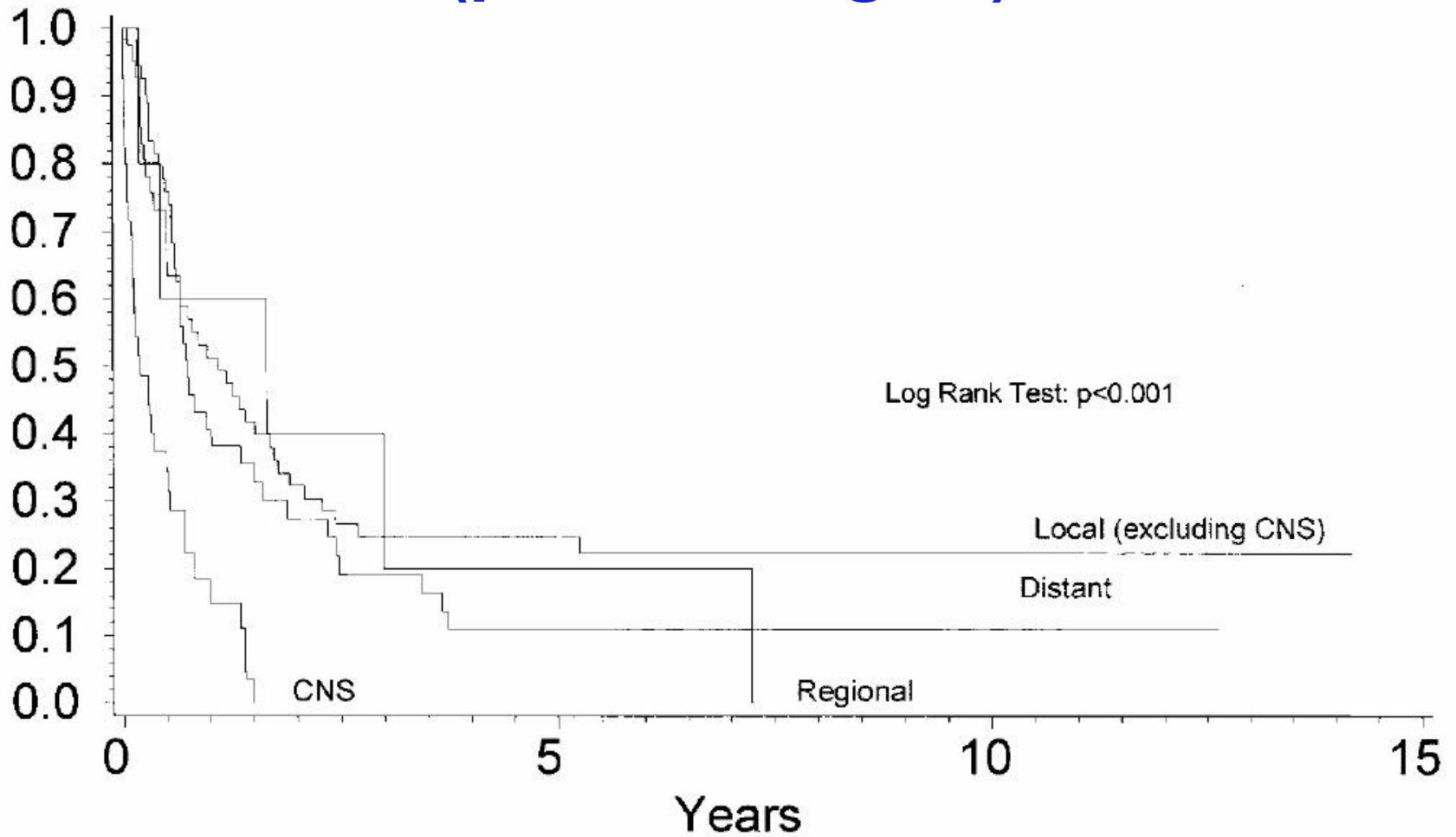
Overview

- Tumor targets
- Oncolytic HSV mutants
- rRp450
 - ◆ Structure
 - ◆ Efficacy
 - Cell lines
 - Human tumor xenografts
 - ◆ Safety
- Clinical trial outline

Tumor Targets

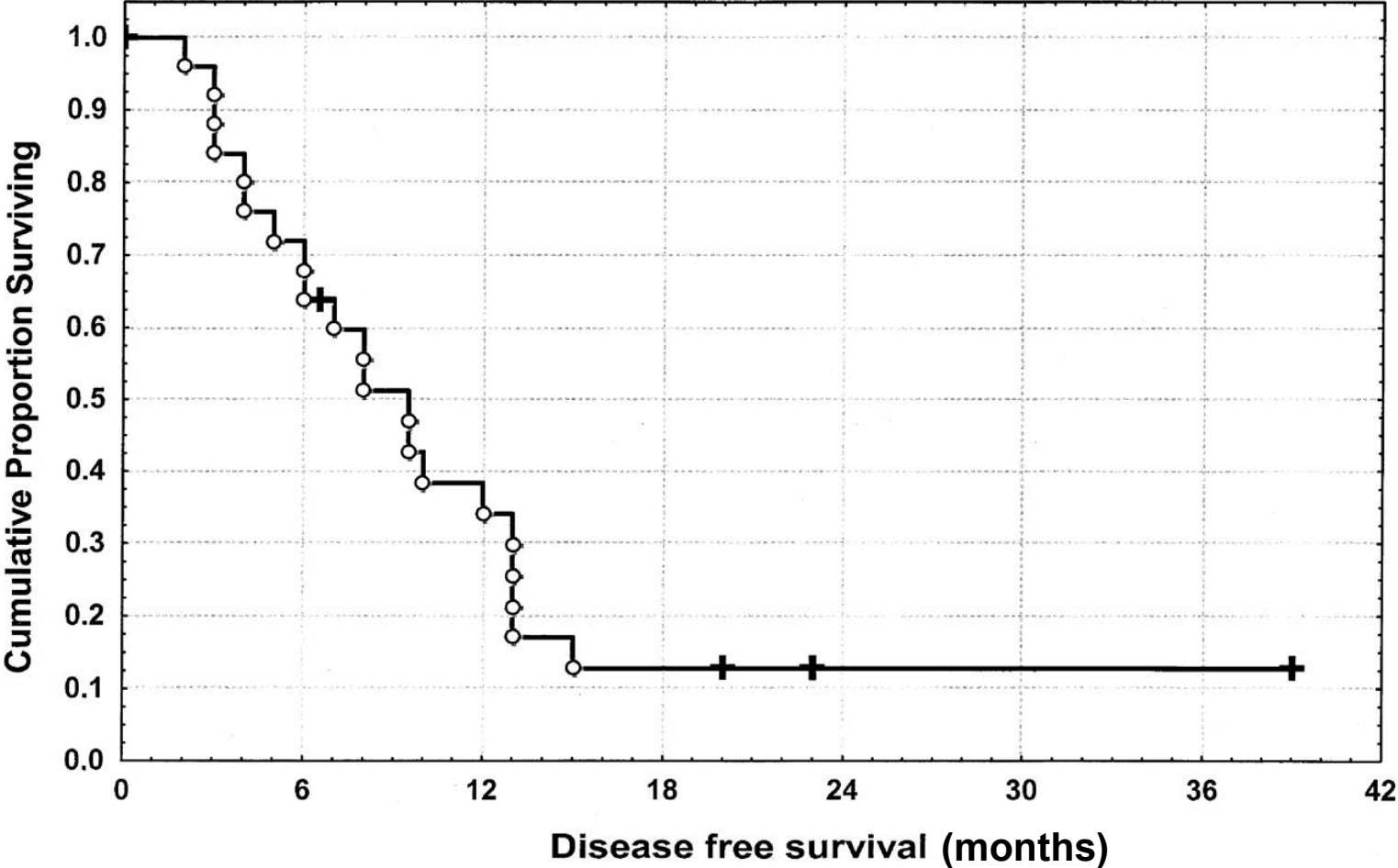
- Relapsed soft tissue sarcomas
 - ◆ Rhabdomyosarcoma (adolescents, young adults)
 - ◆ Malignant Fibrous Histiocytoma (adults)
 - ◆ Malignant Peripheral Nerve Sheath Tumors
 - ◆ Other
- Relapsed bone sarcomas
 - ◆ Osteosarcoma
 - ◆ Ewing's sarcoma family of tumors
- Relapsed neuroblastoma

Survival After Relapsed Rhabdomyosarcoma (parameningeal)



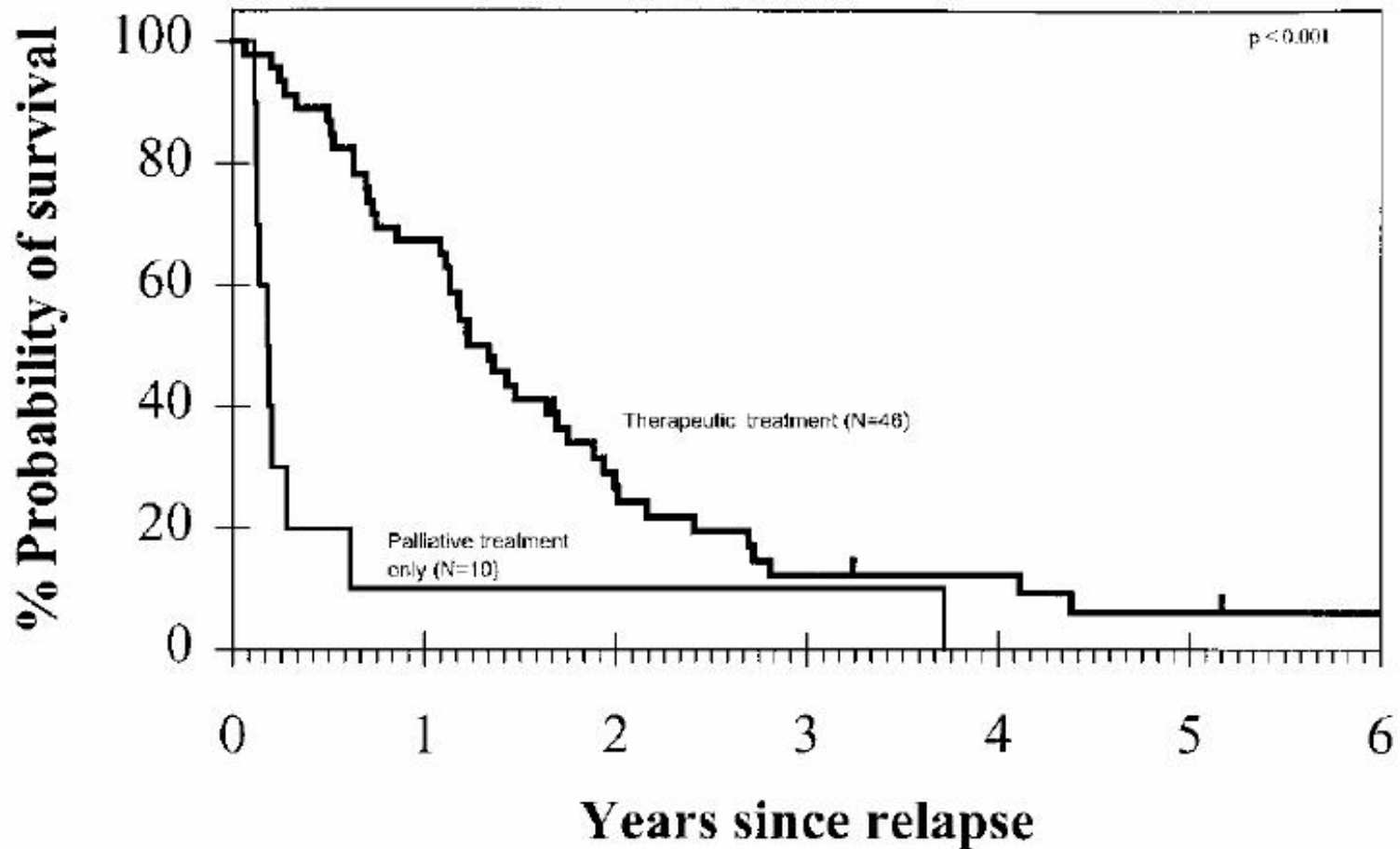
Raney et al., Med Ped Onc 38:22, 2002

Survival After Relapsed Osteosarcoma



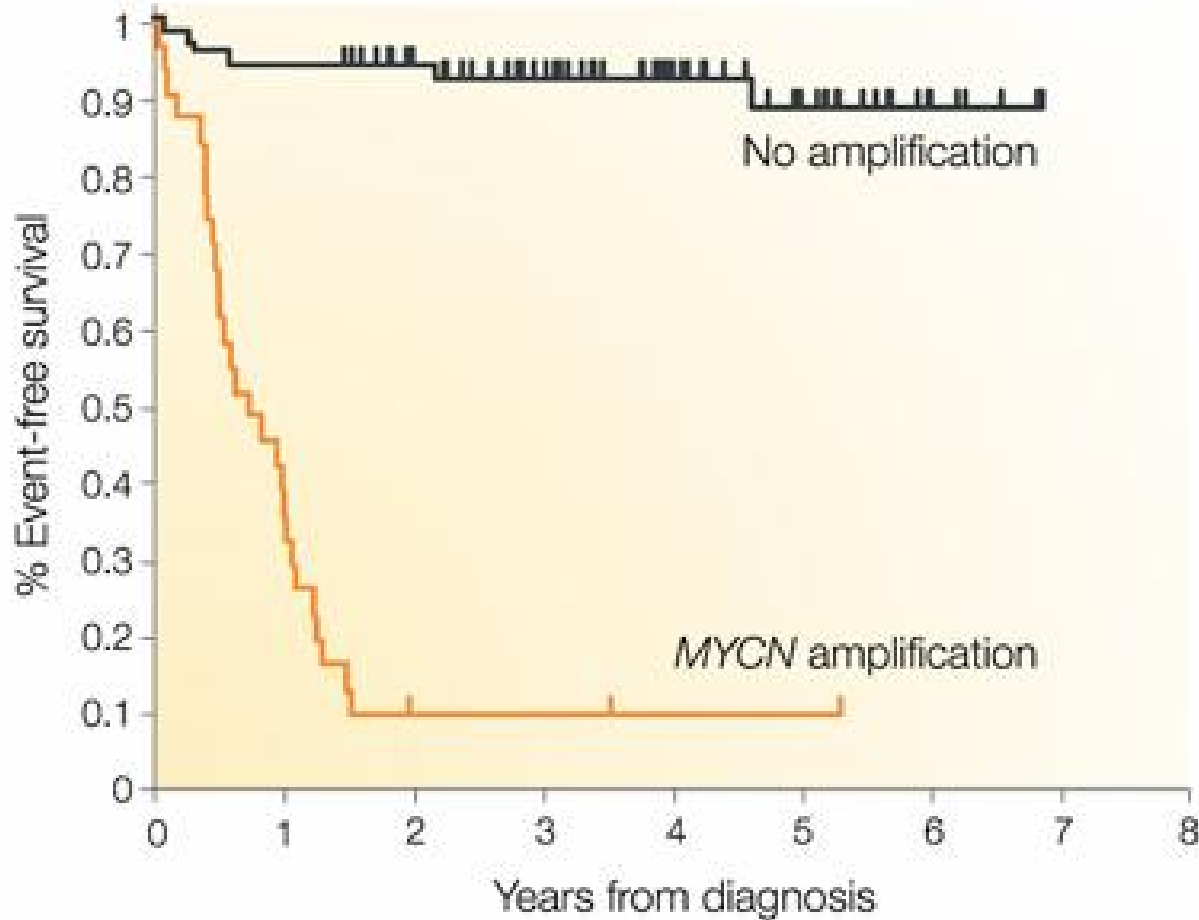
Fagioli et al., J Clin Oncol 20:2150-2156, 2002

Survival After Relapsed Ewing's Sarcoma



Shankar et al., Med Pediatr Oncol 40:141-147, 2003

Survival After Diagnosis of High-Risk Neuroblastoma



Nature Reviews Cancer 3: 203-216, 2003

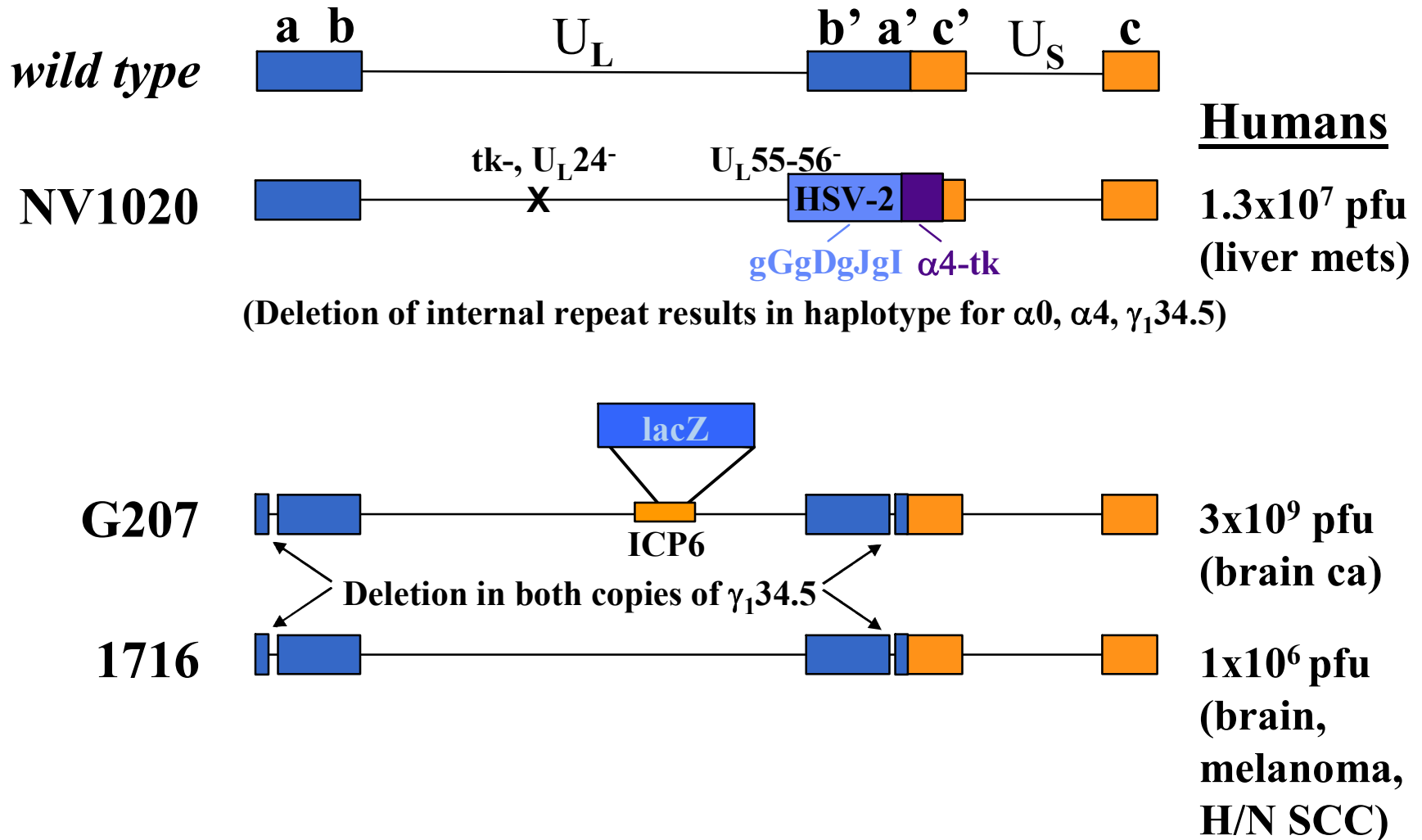
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Oncolytic HSVs as Cancer Therapeutics

- Direct cell lysis (oncolysis)
- Bypass resistance to conventional therapy
- Self-propagation within tumor
- Enhanced delivery of therapeutic genes

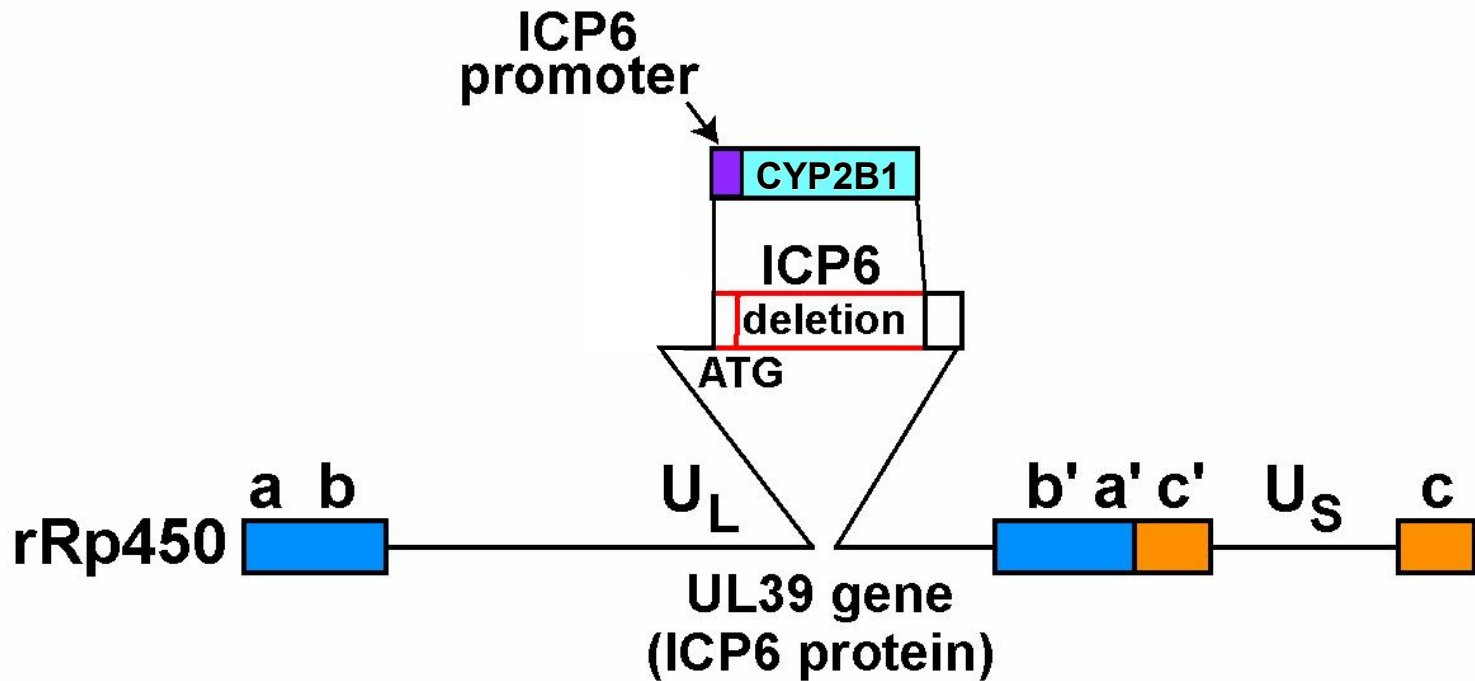
Oncolytic HSV-1 Mutants in Clinical Trials



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- **rRp450**
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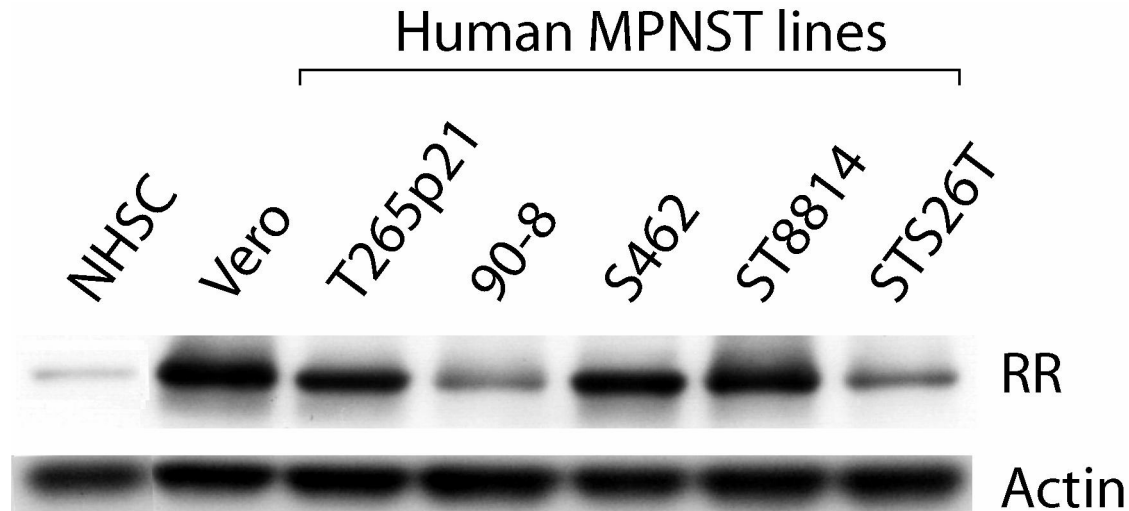
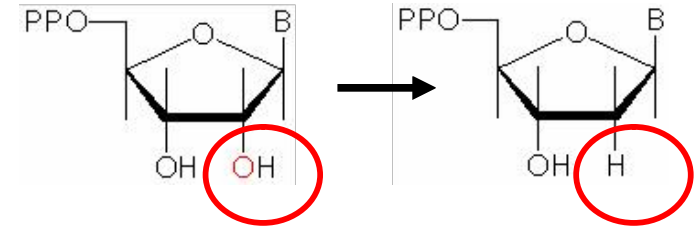
rRp450



Retains native thymidine kinase expression, sensitivity to acyclovir

HSV1 U_L39 Encodes ICP6, the Large Subunit of Ribonucleotide Reductase

- Converts ribonucleotides to deoxyribonucleotides
- Critical for reactivation of latent virus
- Tumor cells express high cellular RR



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Cytotoxicity With Oncolytic HSV Mutants

Each “+” indicates 10-fold increase in sensitivity

	<u>Cancer</u>	<u>Cell Line</u>
	eRMS	RD
<i>Rhabdomyosarcoma</i>	aRMS	A204 Rh18 Rh30 RhRKM-P4
<i>Osteosarcoma</i>	OS	143.98.2 HS899(D)T
<i>Ewing sarcoma</i>	EWS	A673 SK-ES 5838 TC32
<i>Malignant Fibrous Histiocytoma</i>	MFH	MFH1
	NB	SKNBE(2) SMS-SANS LAN5 SKNSH CHLA-20 CHLA-79 IMR32 SHSY5Y
<i>Neuroblastoma</i>		
	MPNST	T265p21 ST8814 90-8 S462 STS26T
<i>Malignant Peripheral Nerve Sheath Tumor</i>		

Cytotoxicity With Oncolytic HSV Mutants

Each “+” indicates 10-fold increase in sensitivity

	<u>Cancer</u>	<u>Cell Line</u>	<u>NV1020</u>	<u>G207</u>
	eRMS	RD	++	+
<i>Rhabdomyosarcoma</i>	aRMS	A204		
		Rh18	+++	+++
		Rh30		
		RhRKM-P4	++	+
<i>Osteosarcoma</i>	OS	143.98.2	++	++
		HS899(D)T	++	++
<i>Ewing sarcoma</i>	EWS	A673	+	+
		SK-ES	+	+
		5838	-	-
		TC32	+	+
<i>Malignant Fibrous Histiocytoma</i>	MFH	MFH1	+++	+++
	NB	SKNBE(2)	+++	
<i>Neuroblastoma</i>		SMS-SANS	+++	
		LAN5	++	
		SKNSH	+++	
		CHLA-20	+++	
		CHLA-79	+++	
		IMR32	+++	
		SHSY5Y	+++	
<i>Malignant Peripheral Nerve Sheath Tumor</i>	MPNST	T265p21		++
		ST8814		++
		90-8		++++
		S462		++++
		STS26T		++

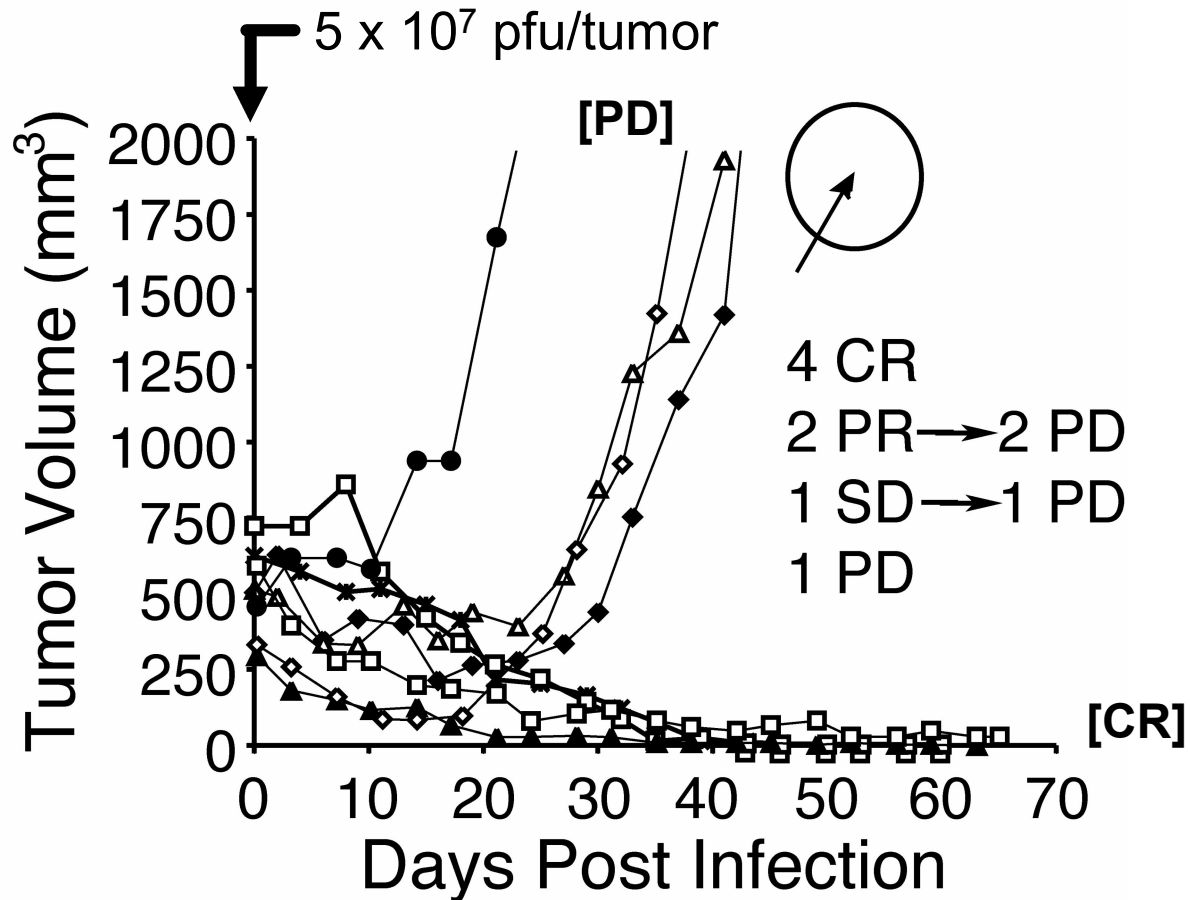
Cytotoxicity With Oncolytic HSV Mutants

Each “+” indicates 10-fold increase in sensitivity

	<u>Cancer</u>	<u>Cell Line</u>	<u>NV1020</u>	<u>G207</u>	<u>rRp450</u>
	eRMS	RD	++	+	++
<i>Rhabdomyosarcoma</i>	aRMS	A204			++
		Rh18	+++	+++	+++
		Rh30			+++
		RhRKM-P4	++	+	
<i>Osteosarcoma</i>	OS	143.98.2	++	++	
		HS899(D)T	++	++	
<i>Ewing sarcoma</i>	EWS	A673	+	+	++
		SK-ES	+	+	++
		5838	-	-	+
		TC32	+	+	++
<i>Malignant Fibrous Histiocytoma</i>	MFH	MFH1	+++	+++	
	NB	SKNBE(2)	+++		
<i>Neuroblastoma</i>		SMS-SANS	+++		++++
		LAN5	++		++++
		SKNSH	+++		
		CHLA-20	+++		++++
		CHLA-79	+++		
		IMR32	+++		
		SHSY5Y	+++		++++
<i>Malignant Peripheral Nerve Sheath Tumor</i>	MPNST	T265p21		++	+++*
		ST8814		++	+++
		90-8		++++	++++
		S462		++++	++++
		STS26T		++	+++

**In MPNST cells, data determined with parent virus of rRp450, hrR3*

Effect of a Single Injection of NV1020 on Human Rh18 RMS Tumors in Nude Mice

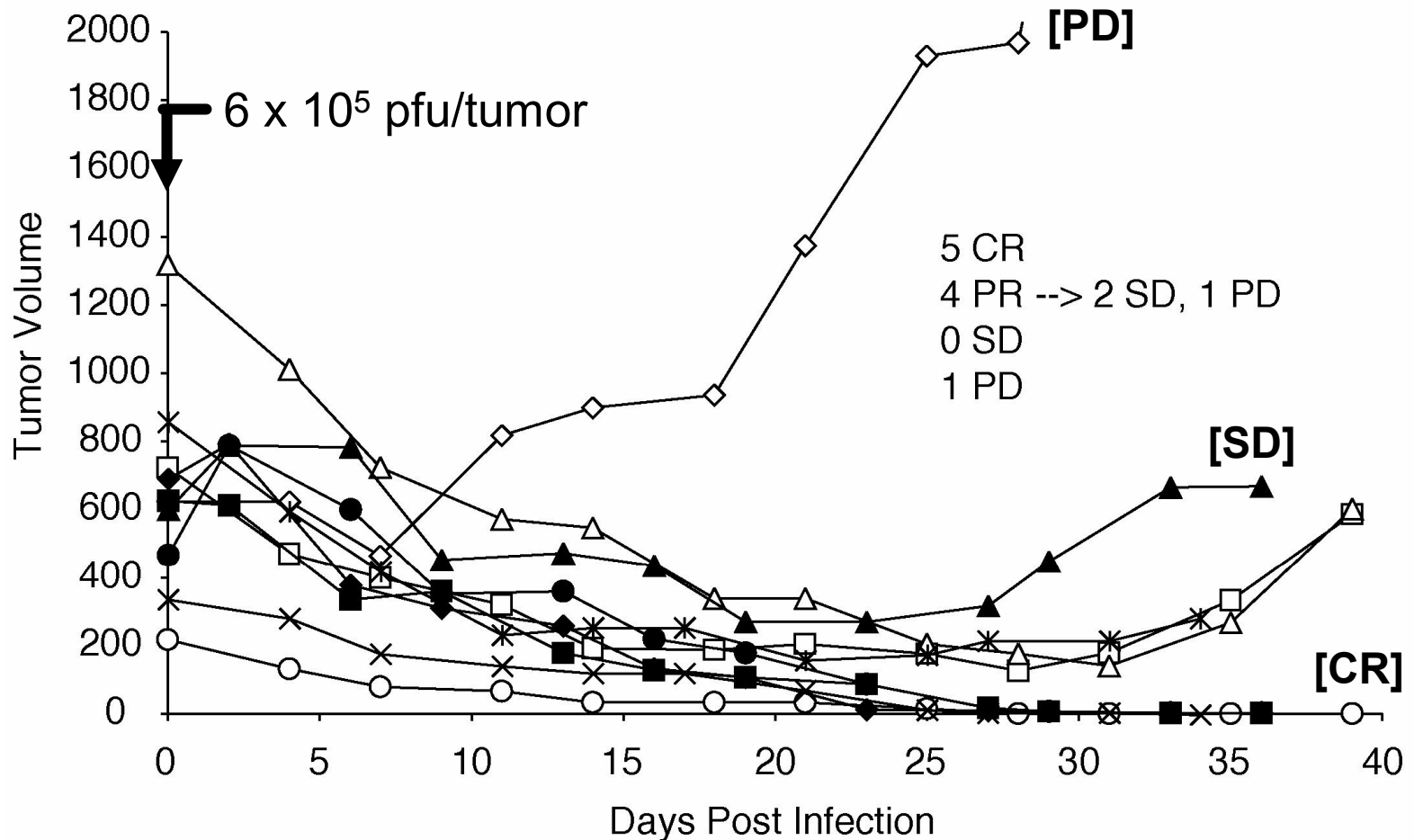


Currier et al., 2005

75% response rate (CR+PR, 6/8)

Similar data in models of neuroblastoma (Parikh et al., 2005)

Effect of a Single Injection of rRp450 on Human Rh18 RMS Tumors in Nude Mice



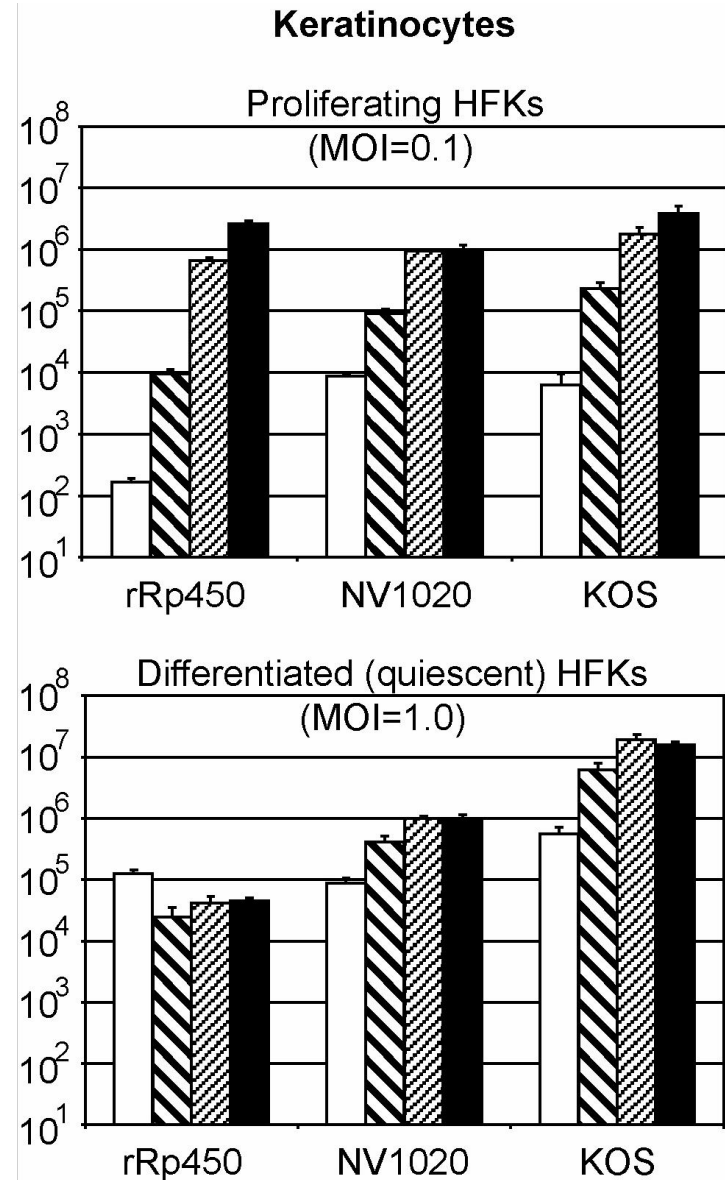
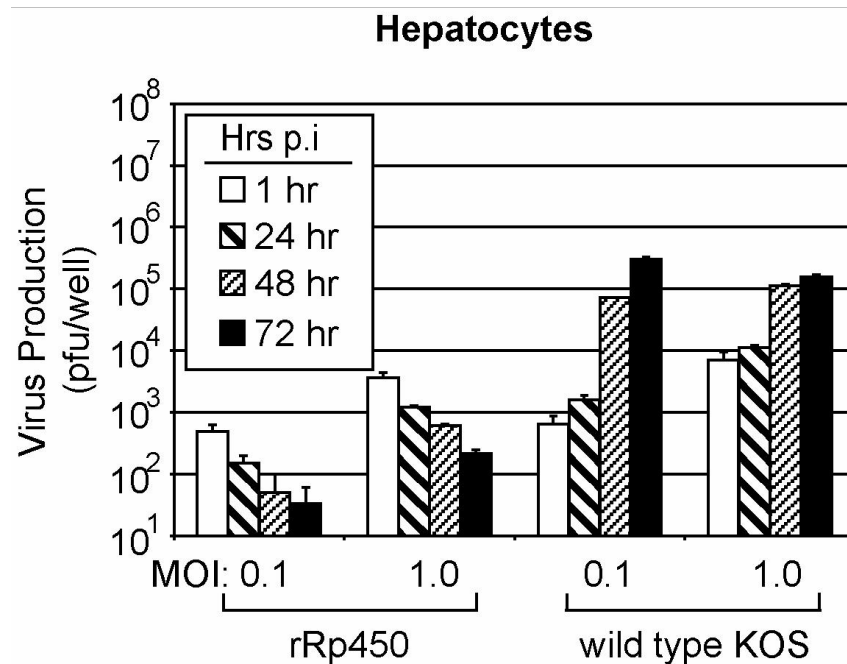
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90% response rate (CR+PR, 9/10)

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Attenuation of rRp450 in Normal Primary Human Cells



In Vivo rRp450 Safety Studies

- FVB/N mice, 10^8 pfu rRp450
- Virus alone, Virus + cyclophosphamide (CYP)
- Clinical studies after intravenous, intratumoral virus
 - ◆ Early (day 3) and late (day 28) timepoints
 - ◆ No clinically significant effects on
 - Weight
 - CBC (WBC, ANC, ALC, Hb, Platelets)
 - Electrolytes (Na, K, Ca, Mg, Phos)
 - Renal function (BUN, creatinine)
 - Liver function (transaminases)
 - ◆ Virus + CYP same as CYP alone

FVB/N Mice Survival Studies: Virus Alone

(# alive/total)

	Wild type KOS					rRp450
Dose (pfu)	10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ⁸	10 ⁸
Intravenous	3/3	9/10*	0/6	0/6	0/6	20/20
Intracerebral	2/10	0/10	nd	nd	nd	18/20

*All had neurologic toxicity, 9 recovered

FVB/N Mice Survival Studies: Virus + Cyclophosphamide (CYP)

(# alive/total)

	rRp450	CYP*	rRp450+CYP**
Intravenous	20/20	9/10	18/20
Intracerebral	18/20	9/10	9/10

*50 mg/m² intraperitoneal cyclophosphamide

**CYP given 24 h after virus

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Study Objectives

- Primary Aims
 - ◆ To estimate the maximum tolerable dose (MTD) of rRp450 administered as a direct intratumoral injection
 - ◆ To determine the dose-limiting toxicities (DLT) of intratumoral rRp450
- Secondary Aims
 - ◆ To measure antiviral immune response
 - ◆ To measure the systemic viremia
 - ◆ To preliminarily define the antitumor activity of rRp450

Eligibility: Inclusion

- Age ≥ 2 years and ≤ 30 years of age
- Histologic diagnosis of sarcoma or neuroblastoma
 - ◆ Relapsed/refractory to conventional therapy
 - ◆ Accessable, measurable disease
- Life Expectancy ≥ 8 weeks

Eligibility: Exclusion

(standard pediatric phase I criteria)

- No acute toxic effects of prior therapy
- ≥ 4 weeks since myelosuppressive chemotherapy
- ≥ 28 days since anti-neoplastic biologic therapy
- Prior radiotherapy
 - ◆ ≥ 2 weeks since local palliative radiotherapy
 - ◆ ≥ 6 months since extensive radiotherapy
- ≥ 6 months since autologous bone marrow transplantation
- ≥ 2 months since different phase I agent
- No allogeneic BMT, prior gene therapy, pregnancy, breast-feeding

Treatment Plan

- Direct CT-guided intratumoral injection
- Three young adults first (22-30 years old)
- Up to four injections, ≥ 3 weeks apart
- Six dose strata
 - ◆ Begin at 1×10^7 pfu
 - ◆ Escalate up to 3×10^9 pfu

Dose Determination

- No MTD in humans yet for HSV vectors
- Max dose of NV1020 tested in intra-hepatic artery was 1.3×10^7 pfu
- Begin at 80% dose = 1.0×10^7 pfu
(Standard practice in pediatric phase I studies)
- On per kg basis, systemic exposure would be 10,000-fold less than max dose tested in mice (10^8 pfu)

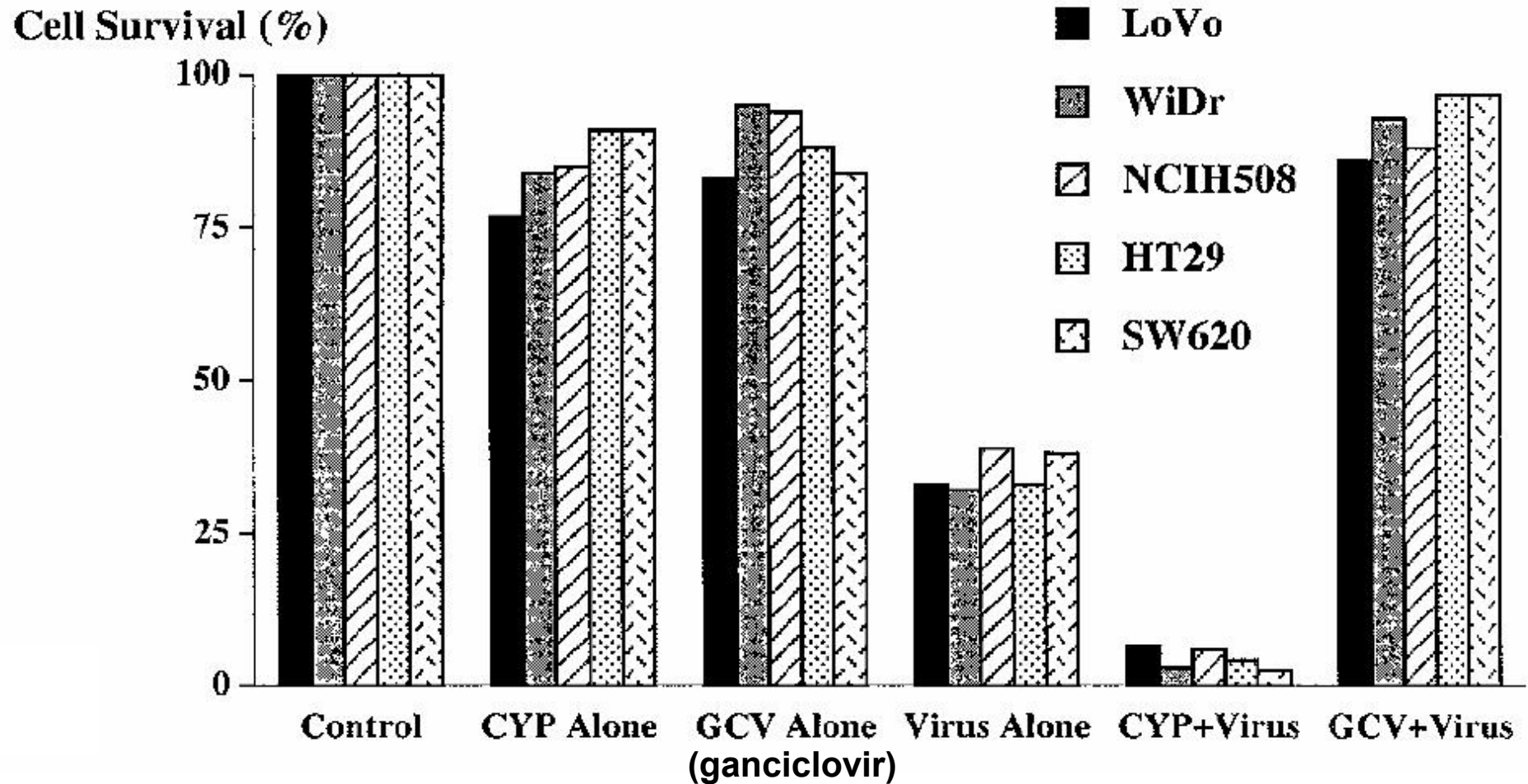
cGMP Vector Production

- NGVL (NCRR program) support provisionally awarded, pending administrative issues
- Human Gene Therapy Applications Lab (HGTAL), University of Pittsburgh
 - ◆ Joseph Glorioso, M.D.
 - ◆ Dave Krisky, M.D., Ph.D.
- Successful pre-manufacturing runs
 - ◆ Column purification
 - ◆ Titters up to 8.5×10^{10} pfu/ml

Conclusions

- Intended participants have incurable cancers
- Cancer models are susceptible to oncolytic HSVs
- Efficacy of rRp450 is attractive
 - ◆ Increased cytotoxicity in cell lines
 - ◆ Platform for future combination with oxazaphosphorines
- rRp450 appears safe
 - ◆ Attenuated by ~10,000-fold vs. wild type in quiescent cells
 - ◆ At least as attenuated as NV1020
 - ◆ High doses tolerated by mice via intratumoral, intravenous, and intracerebral routes
 - ◆ No added toxicity when combined with cyclophosphamide
- rRp450 warrants safety testing in subjects with refractory, potentially HSV-susceptible cancers

rRp450 + Cyclophosphamide



Pawlik et al., Cancer 95:1171, 2002

rRp450 + Cyclophosphamide

Effect on liver metastases in a colon cancer model

<u>Treatment</u>	<u># mets</u>
Mock + PBS	>100
Mock + CYP	>100
rRp450 + PBS	10-15
rRP450 + CYP	<5