Neural Stem Cell-Based Anti-Cancer Gene Therapy for Gliomas

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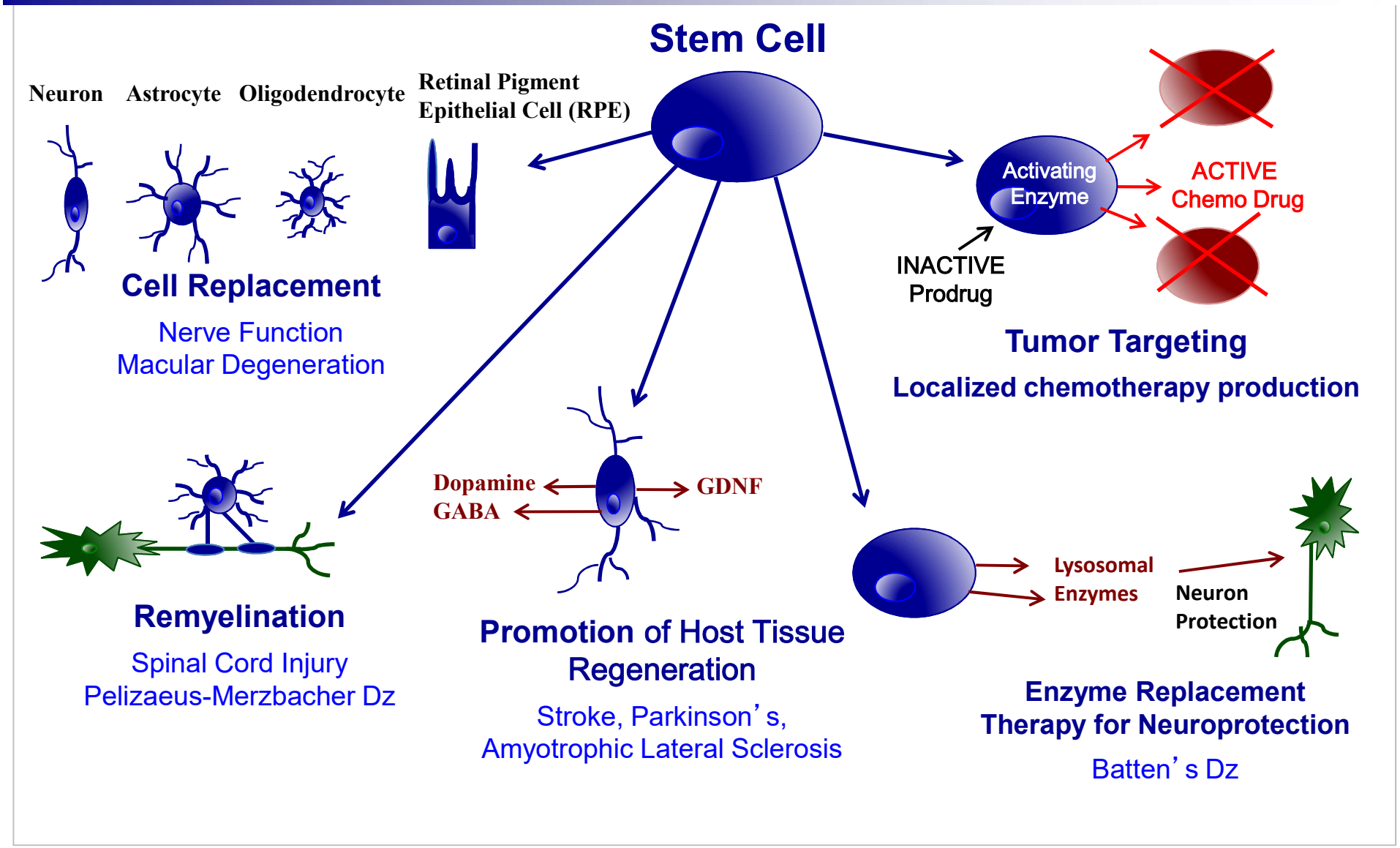
Disclosures

None

Neural Stem Cell-Mediated CNS Therapies in Clinical Trials

Sources of NSCs: autologous; allogeneic: embryonic, adult; genetically-modified



Aboody et al, Neuron May 26;70(4):597-613, 2011
Translating Stem Cell Studies to the Clinic for CNS Repair

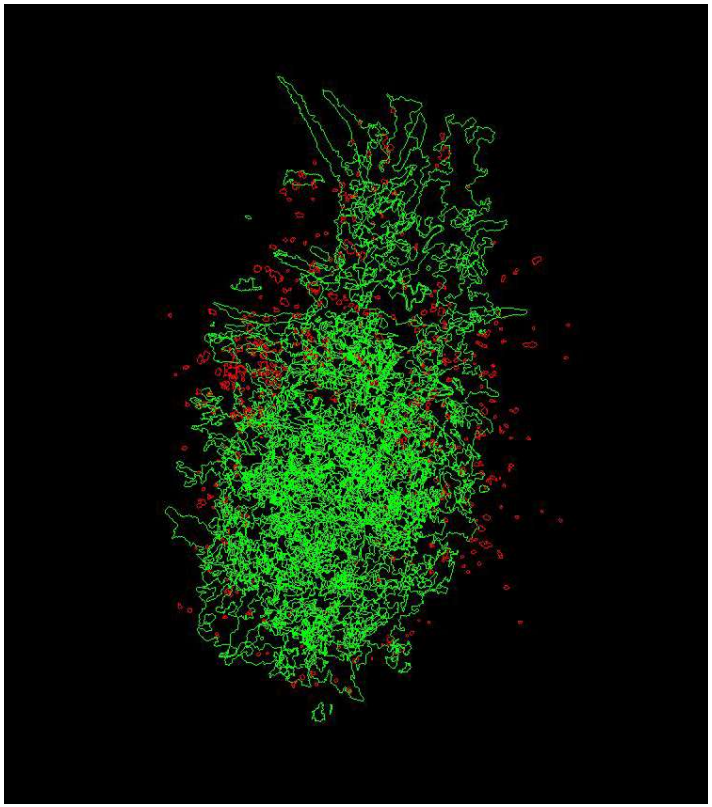
HB1.F3.C21 Neural Stem Cells

- **NSCs inherently tumor-tropic**

  V-*myc* immortalized to maintain stem-like properties, including the ability to migrate and remain undifferentiated

- **Can be modified to deliver a therapeutic agent**

Human NSCs can be used as vehicles for drug delivery to the brain



Green: U251 tumor in mouse brain
Red: Di-I labeled NSCs administered intraventricularly

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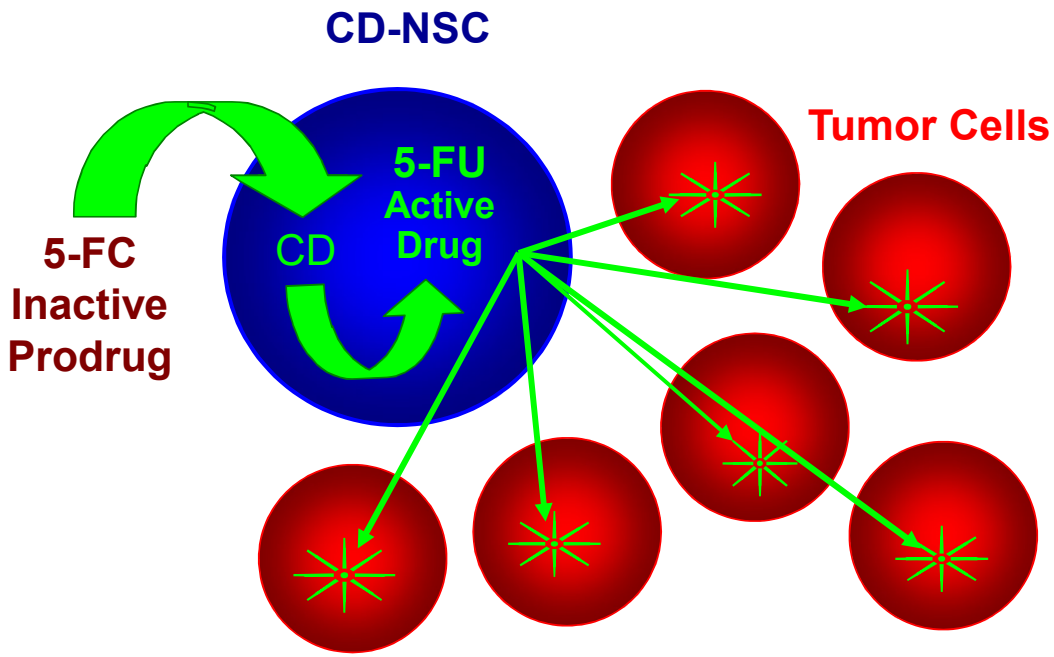
NSC-Mediated Enzyme/Prodrug Gene Therapy

Engineer NSCs to express enzyme that can activate a prodrug to an anti-cancer agent
Administered NSCs localize to tumor sites



CD-NSC + 5-FC → 5-FU
Chemotherapy production localized to tumor sites

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First-in-Human Study of a NSC-Mediated Anti-Cancer Therapy

- **Primary Objective:**

Determine the **safety and feasibility** of intracerebral administration of allogeneic genetically modified NSCs in combination with oral 5-FC in patients with recurrent high-grade gliomas.

- **Secondary Objectives:**

Demonstrate **proof of concept**:

- Conversion 5-FC to 5-FU by CD-expressing NSCs
- NSC migration to tumor foci

Assess the immunogenicity of NSCs

Determine the fate of NSCs

Funding: NIH/NCI R21 CA137639-01A1

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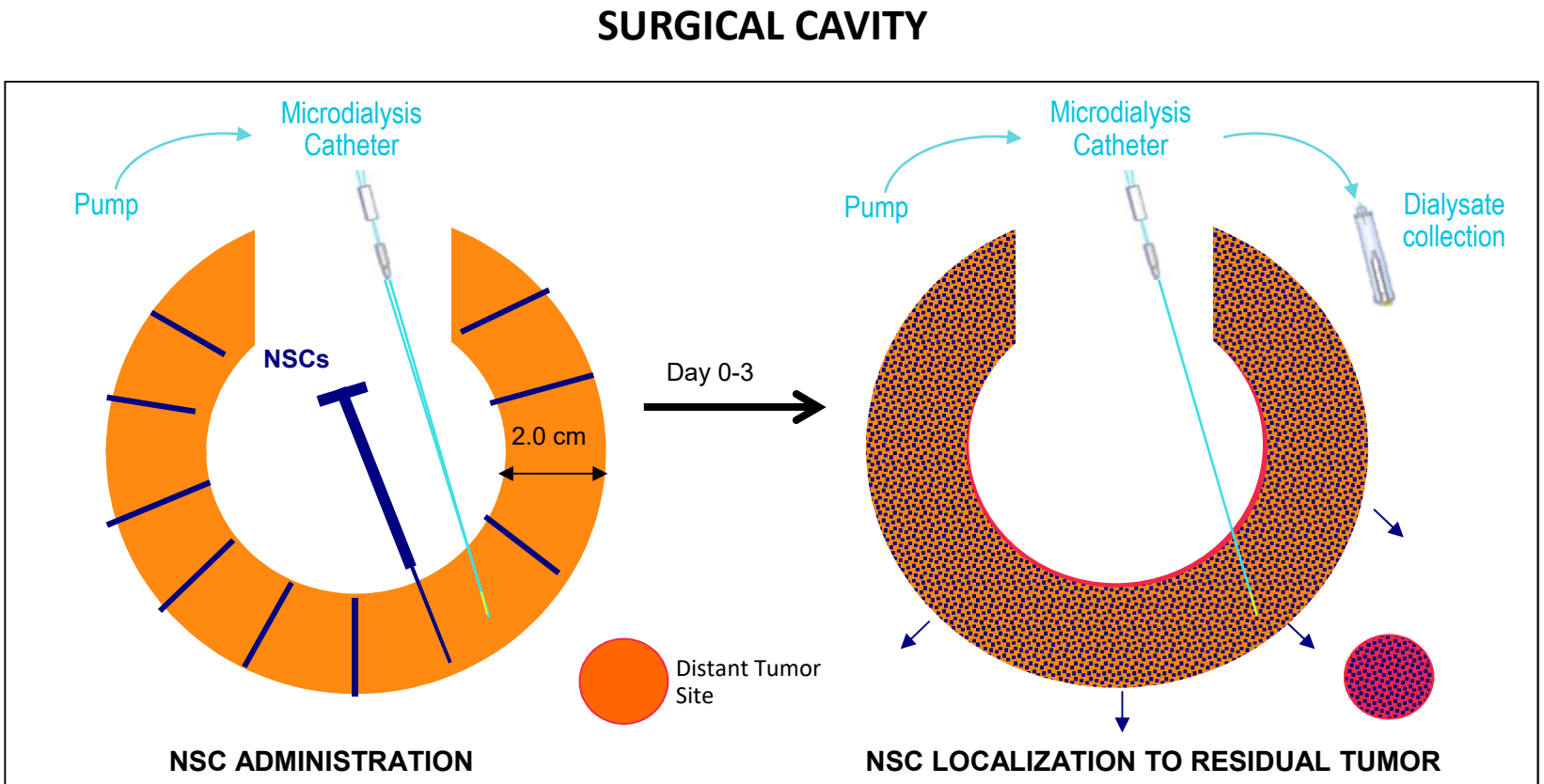
First-in-Human Study of a NSC-Mediated Anti-Cancer Therapy



Day 0: Intracerebral injections of CD-NSCs, placement of microdialysis catheter

Days 4-10: Patients take oral 5-FC, serial dialysate and blood samples drawn for PK analysis

Days 4, 10, 32, 60: Blood samples drawn for immunologic correlative studies

Days 32: Brain MRI

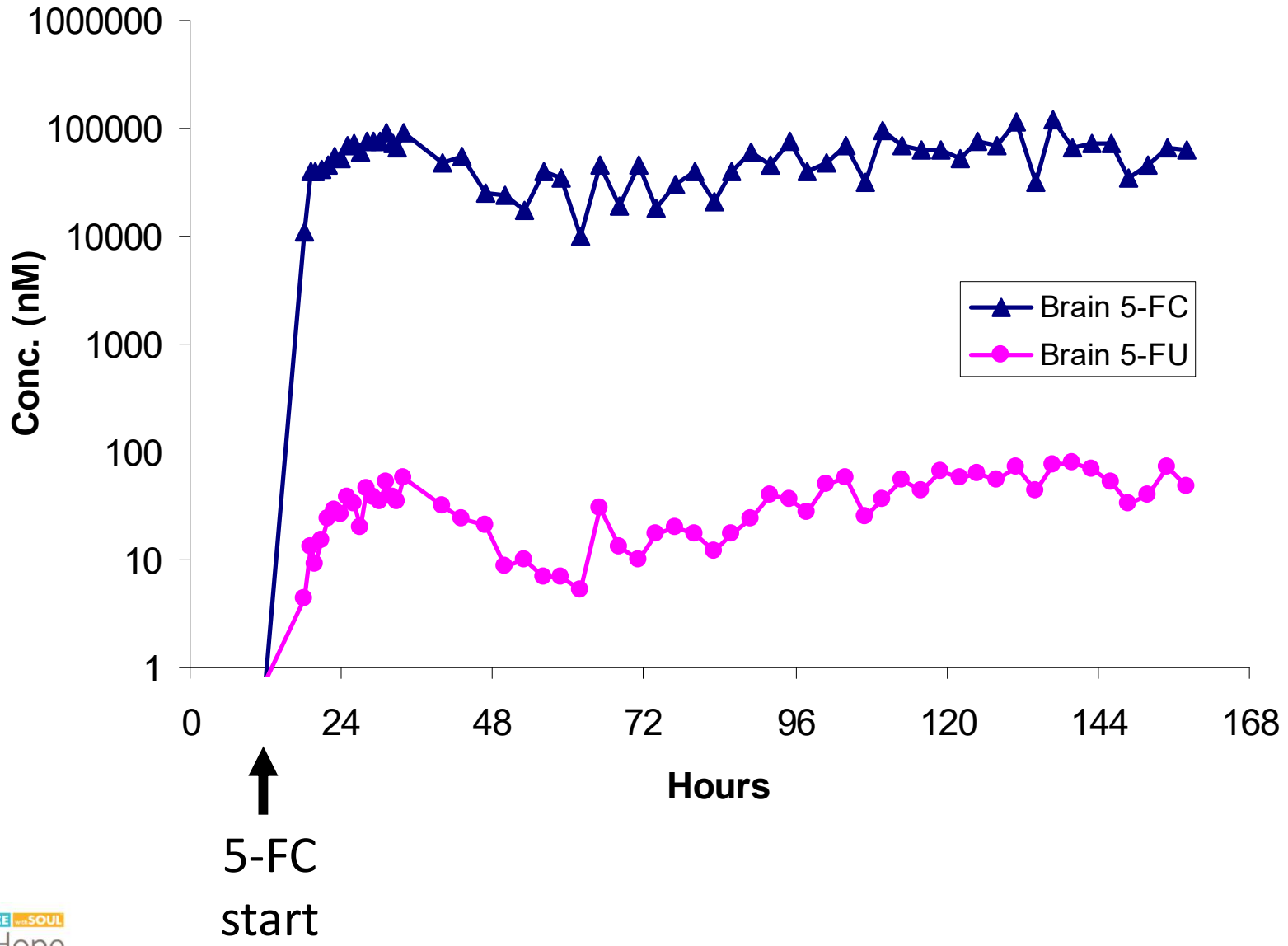
Portnow et al., Clin Cancer Res 2016

First-in-Human Study

Dose Level	CD-NSCs	5-FC (mg/kg /day)
1	10×10^6	75
2	10×10^6	150
3	50×10^6	150

- 12 evaluable patients treated
- 1 dose limiting toxicity possibly related to 5-FC: grade 3 transaminitis
- Best response: stable disease for 5 months

Pt #4 5-FC & 5-FU in Brain



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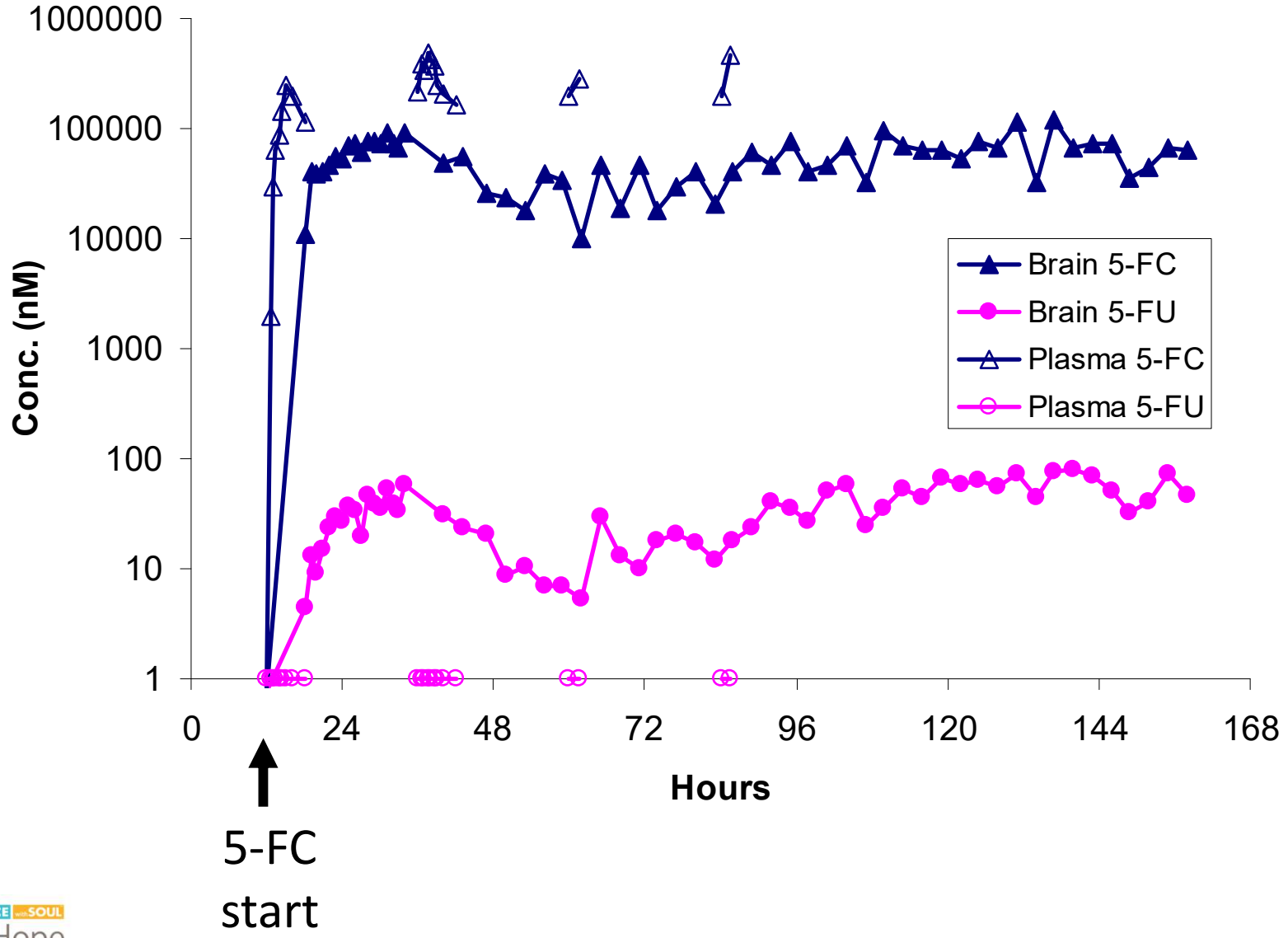
Pt #4 5-FC & 5-FU in Brain and Plasma



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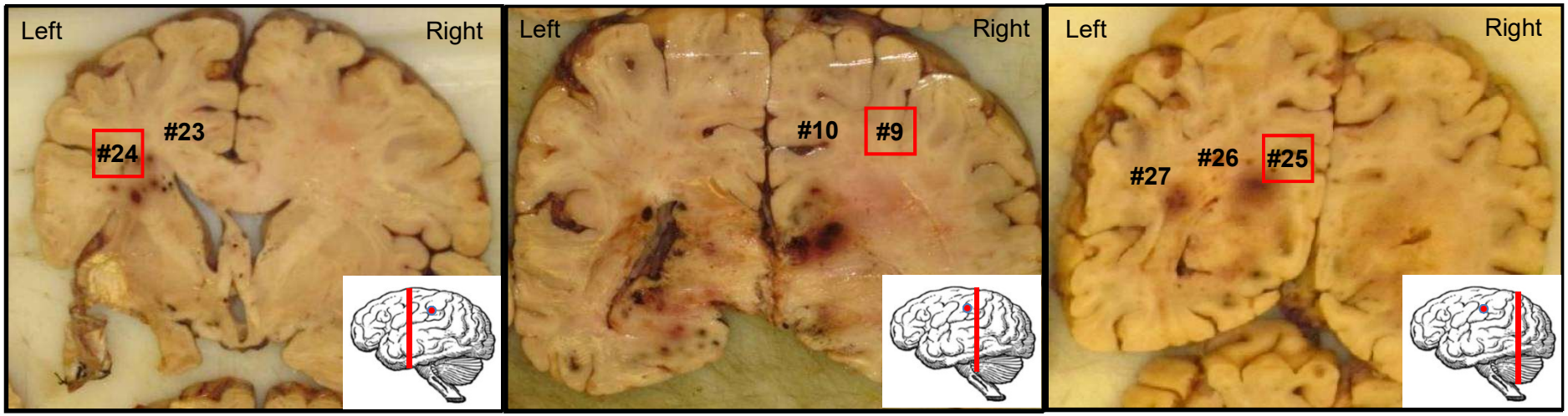
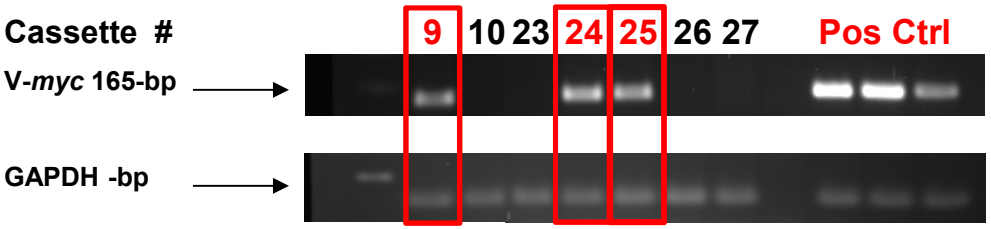
Evidence of NSC Migration to Distant Foci of Tumor



Left frontal lobe | Right parietal lobe | Left occipital lobe



Nested PCR for *v-myc* (a marker for the CD-NSCs)

***V-myc*-positive areas (NSCs) were detected within tumor foci distant from the primary injection site in the right parietal lobe, including the opposite cerebral hemisphere.**

Summary: First-in-Human Study of CD-NSCs + 5-FC

- Safety of 1 dose of intracranial HB1.F3.CD NSCs
- Proof of Concept – conversion of prodrug to active drug by NSCs
- No humoral, CD4/CD8 T-cell or NK immunogenicity on first exposure
- Fate of NSCs (2 brain autopsies):
  - NSCs detected at tumor sites distant from injection site including within the contralateral hemisphere
  - No evidence of secondary tumors

Portnow et al, Clin Cancer Res 2016

Phase I CD-NSC Study

Goals:

- Determine the maximum number (volume) of NSCs that can be administered intracranially.
- Feasibility of administering repeated doses of NSCs through a Rickham.
- Assess for possible immune responses developing after repeated exposure to these allogeneic NSCs.

Funding: R01 grant from the FDA Orphan Products Development Program
City of Hope
Phase One Foundation

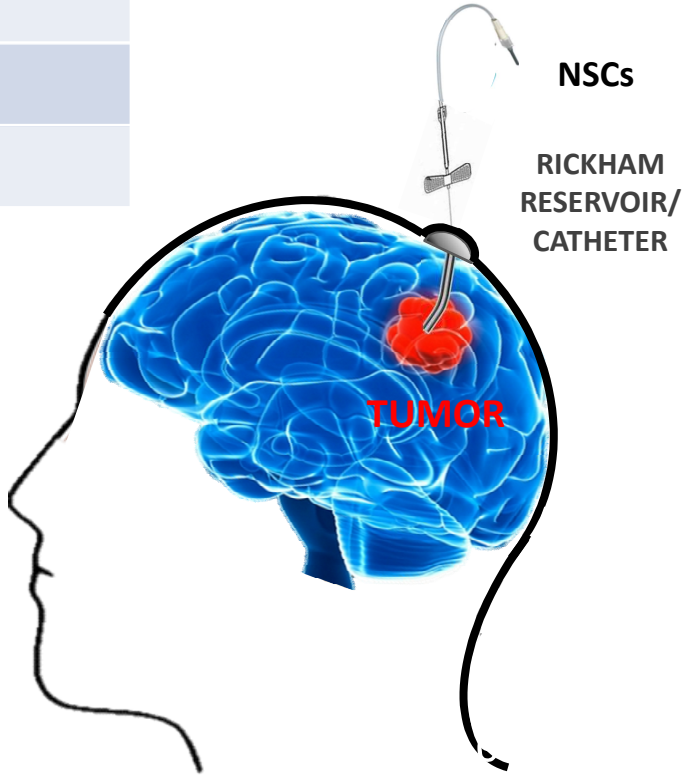
Treatment Schema

3+3 dose escalation design

Dose Level	CD-NSCs	5-FC (mg/kg /day)	Leucovorin (mg q 6h)
1	50 x 10^6	150	---
2	100 x 10^6	150	---
3	150 x 10^6	150	---
4	150 x 10^6	150	25

Treatment cycle: 28 days

- **CD-NSCs administered intracranially via a Rickham on days 1 & 15**
- **3 days after each CD-NSC dose 5-FC (and leucovorin) administered orally every 6 hours x 7 days (on days 4-10 & 18-24)**



Summary of Results

15 evaluable patients treated

Median age: 57

31% had recurrent grade 3 gliomas; 69% recurrent GBM.

Safety

NSC dose of 150 million was well tolerated.

1 dose limiting toxicity: grade 3 catheter-related wound infection.

Most common toxicity: grade 2 fatigue, thrombocytopenia, nausea.

Median # of treatment cycles: 2 (range: 1-5).

PCR for V-*myc*: no evidence of NSCs in systemic circulation.

Testing for replication-competent retrovirus was negative.

Efficacy

2 patients had stable disease for 5 months.

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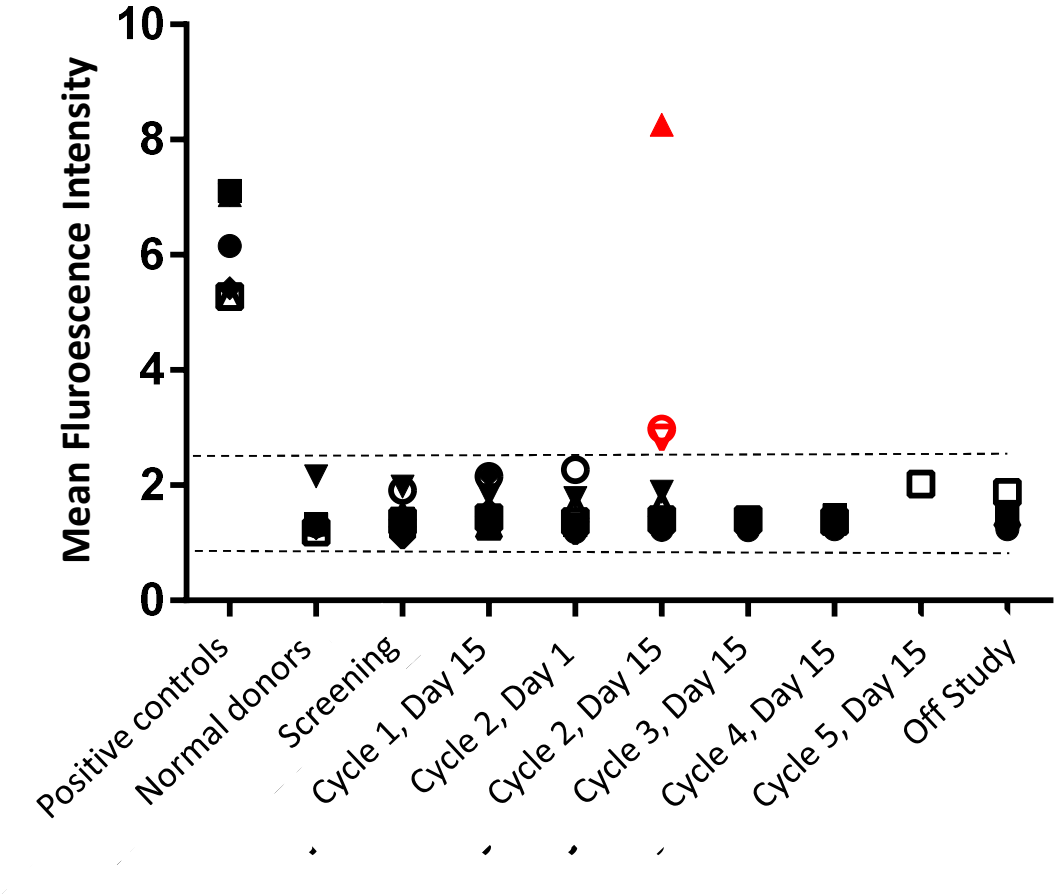
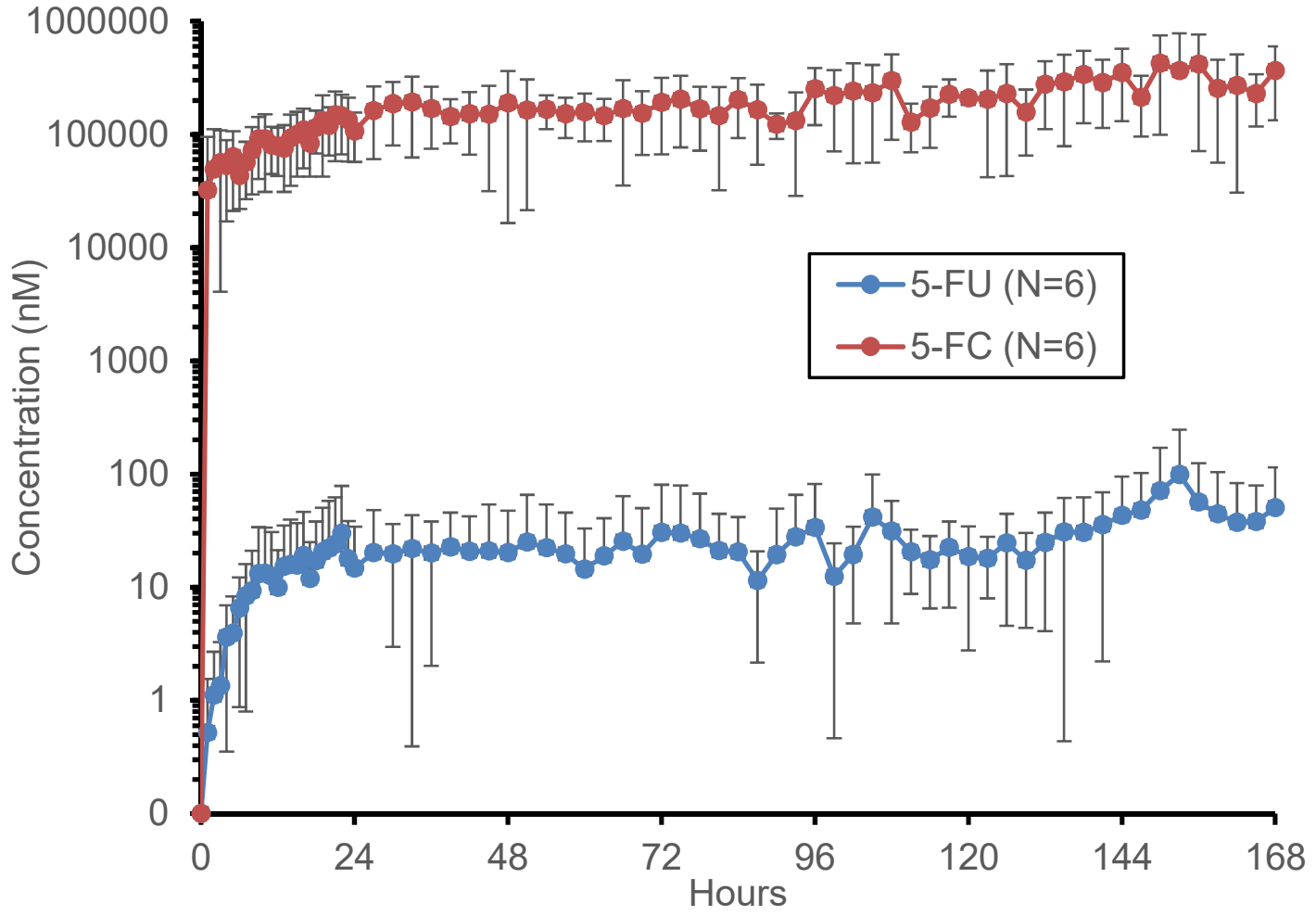
Assessment for Development of Antibodies Against the NSCs



3 patients developed antibodies against the neural stem cells after the 3rd dose.

No correlation with # of cycles administered or use of dexamethasone.

Microdialysis Data From Proximal Catheters



CD-NSCs continue to produce 5-FU in the brain during the entire 7 days of oral 5-FC.

Summary: Phase I Study of CD-NSCs + 5-FC

- Safety of multiple doses of intracranial HB1.F3.CD NSCs
- Feasibility of Rickham catheter to administer NSCs into the brain
- 20% of patients developed anti-NSC antibodies with repeat exposure
- Intracerebral microdialysis data

NSCs converted 5-FC to 5-FU in the brain throughout the entire 5-FC dosing interval.

5-FU IC50 concentrations in brain not achieved at highest NSC dose. However, the cytotoxic effect of 5-FU is time-dependent, and the impact of continuous exposure to lower levels is TBD.

Future Directions

Neural stem cells as a platform technology for targeting anti-cancer agents to tumor cells in the brain

prodrug converting enzymes: CD, carboxylesterase (NCT02192359)

oncolytic viruses: CRAd-S-pk7 (NCT03072134)

Oligonucleotides (Cpg-STAT3)

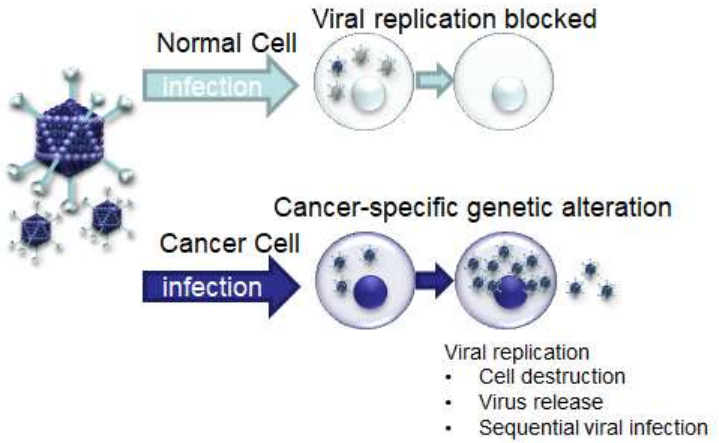
monoclonal antibodies (HER2)

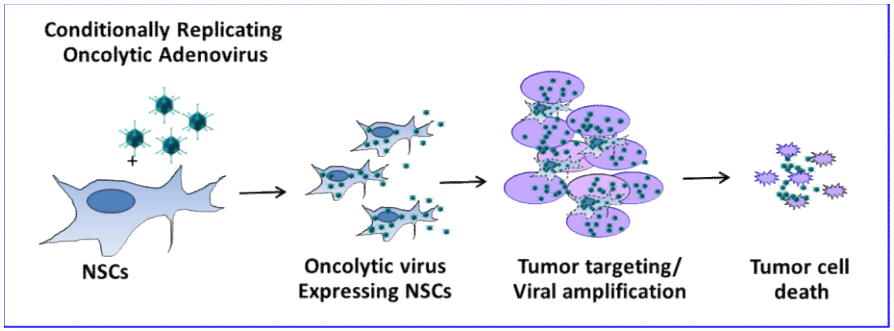
Immune modulators (IL-2)

Differentiating agents (Wnt-11, Twist, Snail)

NSC-Based Oncolytic Virotherapy

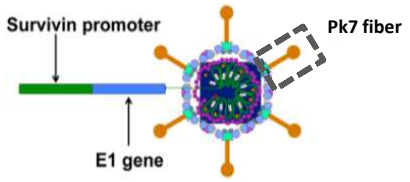
- Oncolytic viruses only infect tumor cells
- Kill infected cells by
  - overwhelming replication
  - eliciting a host anti-tumor immune response
  - Acting as a direct immunostimulant
- Viral progeny spreads throughout the tumor, subsequently infecting and lysing surrounding cancer cells
- CRAds – **C**onditionally **R**eplicative **Ad**enoviruses
- CRAds can activate the immune system to induce anti-tumor immunity



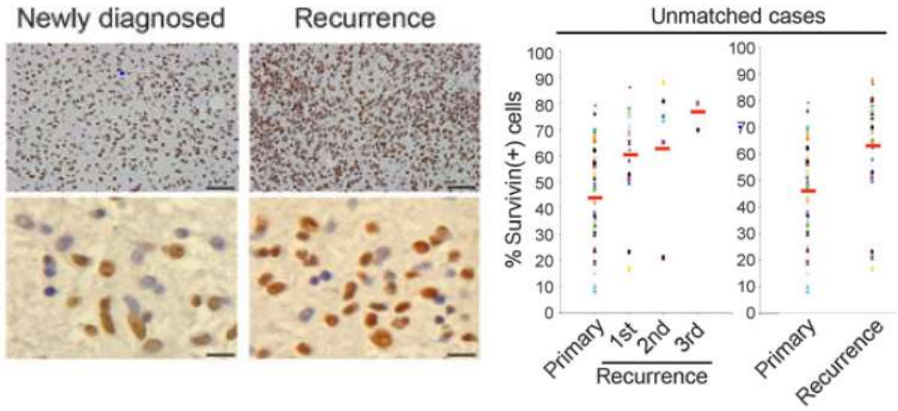


Rationale for CRAd-S-pk7

- CRAds specifically kill cancer cells while sparing normal cells based on exploitation of a tumor specific promoter
- **The CRAd-S-pk7 selectively replicates in tumor cells expressing survivin**
- Survivin expression is upregulated in recurrent GBM



Viral element	Effect
Survivin	Restricts viral replication in tumor cells
E1 gene	Essential gene for viral replication
Pk7 fiber	Enhances infectivity via integrin binding



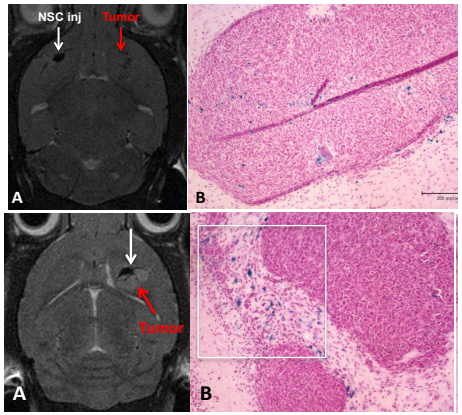
Guvenc, H. et al. 2012 *Clin Cancer Res*

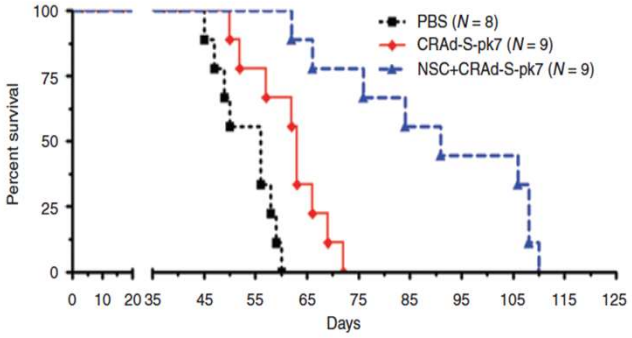
Rationale for NSC Delivery of CRAd-S-pk7

Advantages to using neural stem cells as delivery vehicle for oncolytic virus:

- **NSCs <u>protect the oncolytic virus from neutralizing antibodies</u> en route to tumor sites**

- **NSCs deliver the oncolytic virus to multiple invasive tumor sites (crossing normal tissue) to <u>improve viral biodistribution</u>**





Preliminary Clinical Data

- **First-in-human study in newly diagnosed GBM patients (NCT 03072134)**

Assessing safety of a single dose of NSC-CRAd-S-pk7

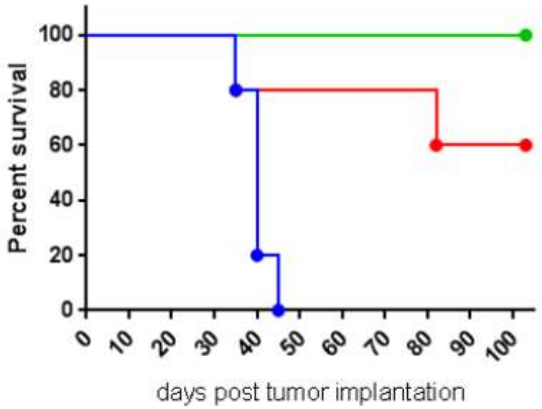
Cohort	Number of NSCs injected	Total Viral Dose (vp/patient)	Number of participants registered	Number of DLTs
1	50×10^6	6.25×10^{10}	3	0
2	100×10^6	1.25×10^{11}	3	0
3	150×10^6	1.875×10^{11}	6	1

Clinical trial currently open at Northwestern University and COH

Preliminary Preclinical Data

Single versus multiple weekly doses of NSC-CRAd-S-pk7

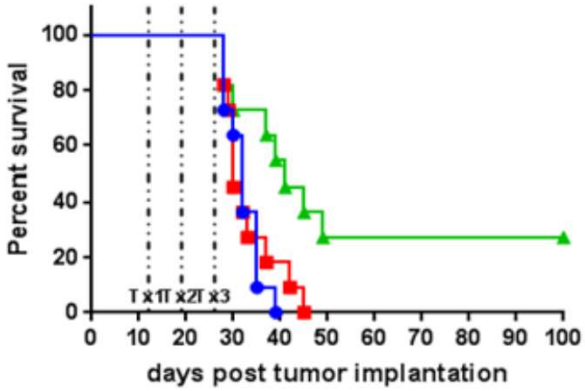
Implanted with 5,000 GL261.dsred tumor cells (n=5 for LTS)



Tumor only
1 round of NSC-CRAd-S-pk7
3 rounds of NSC-CRAd-S-pk7

PBS treatment: all non-treated mice died by day 48
1 Round of treatment: 3/5 mice were alive at day 100
3 Rounds of treatment: 5/5 mice were alive at day 100

Implanted with 10,000 GL261.ffluc tumor cells (n=11 for LTS)

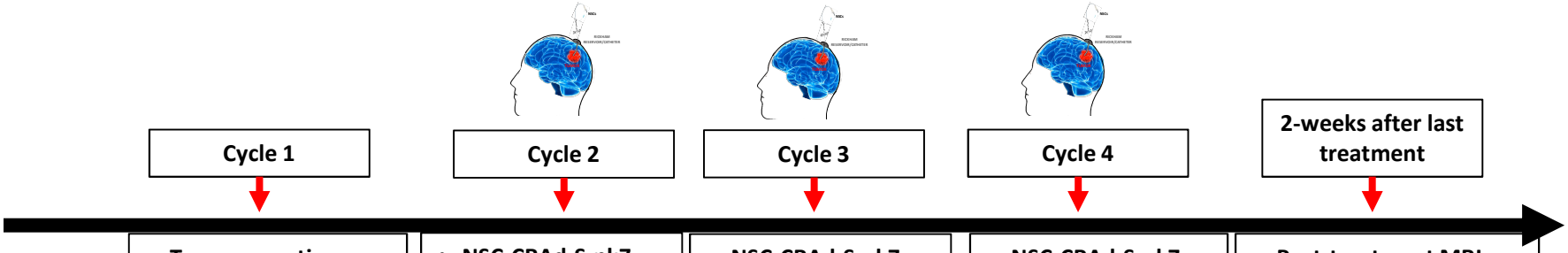


PBS treatment: all non-treated mice died by day 40
1 Round of treatment: all mice died by day 47
3 Rounds of treatment: 4/11 mice alive at day 100

(Aboody, unpublished data)

Next Phase I Study

Assessment of Multiple Weekly Doses of NSC-CRAd-S-pk7 in Recurrent GBM Patients



Cohort 1	+	+	-	-	+
Cohort 2	+	+	+	-	+
Cohort 3	+	+	+	+	+

Cycle = 1 week

City of Hope Brain Tumor Team

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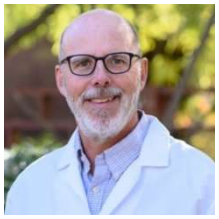

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Neural Stem Cells Migrate to Tumor Cells

NSCs respond to multiple factors produced by tumor cells & the tumor microenvironment

Extracellular Matrix: Human GBM-derived ECM laminin > fibronectin > tenascin

Hypoxia: HIF-1α upregulated factors

VEGF: tumor neovasculature, angiogenic tumors

Soluble Factors: chemokines/cytokines, inflammation (IL-6, IL-8)

Tumor Ligands	NSC Receptors
HGF (SF)	c-Met
EGF	EGFR
VEGF	VEGFR
SCF	c-Kit
SDF-1 (CCL12)	CXCR4
MCP-1 (CCL2)	CCR2
PDGF-A,B	PDGFRa
UPA	uPAR

Aboody et al, PNAS, 2000; Schmidt et al, Neoplasia, 2005; Ziu et al, J Neuro-Onc,2006; Kendall et al, Stem Cells, 2008; Zhao et al, Mol Canc Res, 2008; Gutova et al, Stem Cells, 2008; Zhao et al, Stem Cells, 2012

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